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Women in Alzheimer's disease and related dementias 2022

Edited by
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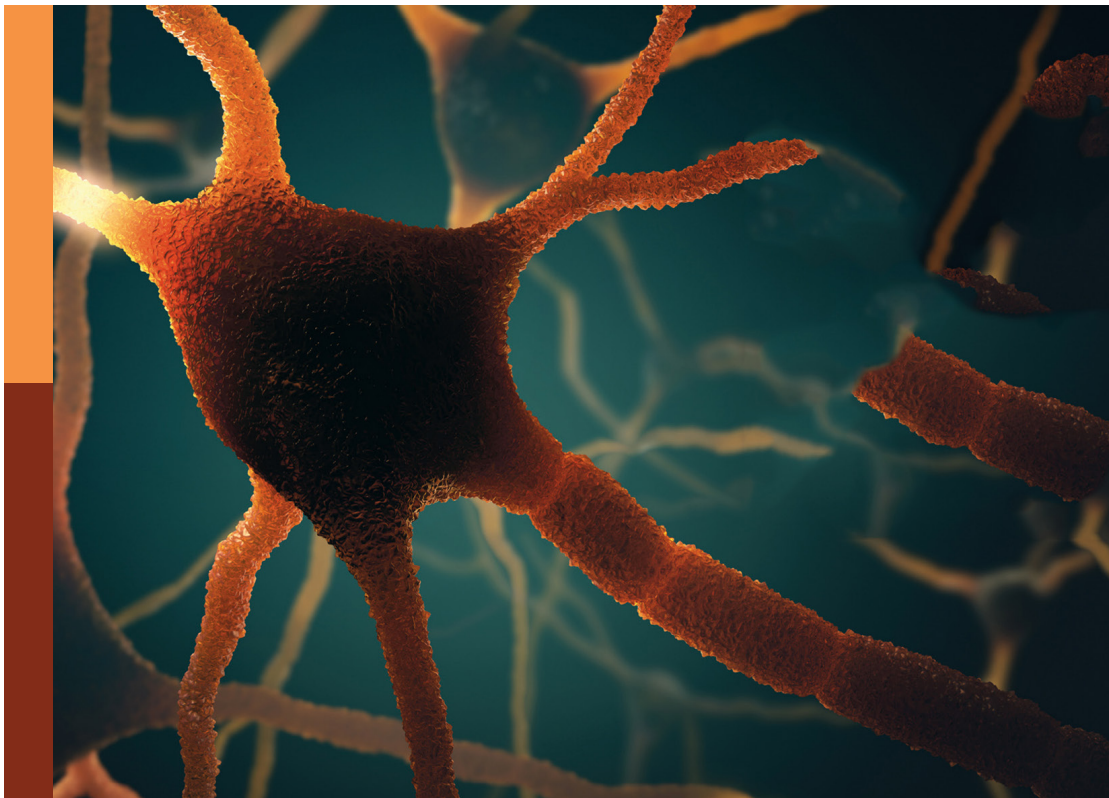

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Women in Alzheimer's disease and related dementias: 2022

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What does a "good life" mean for people living with dementia? A protocol for a think-aloud study informing the value of care

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Introduction: Economic evaluation currently focuses almost exclusively on the maximization of health, using the Quality-Adjusted Life-Year (QALY) framework with instruments such as the EQ-5D, with a limited number of health-focused dimensions providing the assessment of health benefit. This evaluative framework is likely to be insufficient for setting priorities in dementia care because of its exclusive concern with health. Data are also often collected from the perspective of a proxy, limiting the voice of those living with dementia in decision-making. This protocol describes a research project that aims to gather the perspectives of people living with dementia, their insights, and preferences for assessing their quality of life to inform economic evaluation outcome measurement and design with a goal of creating a more robust evidence base for the value of healthcare services. Specifically, this study will elucidate what a "good life" means to people living with dementia and how well instruments currently used in economic evaluation meet this description. This project will further test the acceptability of capability wellbeing instruments as self-report instruments and compare them to generic and dementia-specific preference-based instruments.

Methods and analysis: People living with dementia, diagnosed, or waiting to receive a formal diagnosis and with the capacity to participate in research, will be invited to participate in an hour "think aloud" interview. Participants will be purposefully selected to cover a range of dementia diagnoses, age,

and sex, recruited through the integrated care, geriatric, and post-diagnostic clinics at St James' and Tallaght University Hospitals and dementia support groups in the Ireland. During the interview, participants will be invited to reflect on a "good life" and "think aloud" while completing four economic quality of life instruments with a perspective that goes beyond health (AD-5D/QOL-AD, AQOL-4D, ICECAP-O, ICECAP-SCM). An interviewer will then probe areas of difficulty when completing the instruments in a semi-structured way. The analysis will identify the frequency of errors in comprehension, retrieval, judgment, and response from verbatim transcripts. Qualitative data will be analyzed using constant comparison.

Ethics: The St James's Hospital and Tallaght University Hospital Joint Research Ethics Committee approved the study (Approval Date: 11 April 2022).

KEYWORDS

dementia, outcome measurement, preference-based health-related quality of life, wellbeing, capability approach, interview, economic evaluation

1 Introduction

The challenging questions of how healthcare systems can address the needs of patients efficiently and deliver equitable and cost-effective care remain ever pertinent as we continue navigating through the COVID-19 pandemic. The field of health economics and outcomes research offers tools to conduct clinical and economic evaluations of health interventions. These tools serve as an essential foundation to derive information on efficiency and value across the healthcare system. While these evaluations are vitally important, the experience of the past years has surfaced the importance of patient-reported outcome measures (PROMs) to inform investment in services and support person-centered and value-based care by providing a way of measuring health outcomes from the patient's perspective (Churruca et al., 2021).

Patient-reported outcome measures provide an opportunity for people living with dementia to share through questionnaires their perceptions of health and wellbeing, quality of life, daily functioning, and symptoms, as well as experiences of care. Responses to PROMs questions enable healthcare services to tailor the care that patients need and want. These measures aim to fill a vital gap in the knowledge about outcomes that matter to people living with dementia.

A number of dementia-specific PROMs exist, yet none are used in dementia registries, and the majority of studies utilize PROMs *via* a proxy (Ayton et al., 2020). Measuring the quality of life of people with dementia is complex. Despite strong indication within the literature that people with dementia are willing (Hirschman et al., 2005) and able to express views and participate in decision-making (Kim et al., 2002) as well as respond consistently to questions about preferences and choices related to daily living (Feinberg and Whitlatch, 2001; Kane et al., 2003; Moyle et al., 2012a), their participation is often limited (Tyrrell et al., 2006; Fetherstonhaugh et al., 2013; Daly et al., 2018).

Denied or tokenistic participation in decision-making could be attributed to several reasons. Traditionally, PROM data is collected using conventional techniques, including surveys, questionnaires, or interviews, all of which require adequate cognitive and communication skills. Dementia may lead to progressive difficulties with memory, expressive and receptive language, and reading comprehension (Caramelli et al., 1998), making the traditional PROMs increasingly difficult for people living with dementia to complete. Consequently, proxies, family members or care partners are asked to complete the assessment on their behalf. While proxy ratings are considered valid, they are based on observable behavior rather than subjective experience. This mismatch of views could cause significant disparities in rating between the proxy and the person's experiencing the condition (Karlawish et al., 2008; Gräske et al., 2012; Moyle et al., 2012b; Arons et al., 2013; O'Shea et al., 2020).

Augmentative and alternative communication (AAC) is a set of tools and strategies used to support people with multiple and complex cognitive and physical difficulties. The AAC methods provide alternative access to the preferences of people living with dementia in the form of single pages and phrases, pictorial tools, and other visual aids. The most recent literature review by Haroon et al. (2022) confirmed that quality of life information could be elicited more effectively from people living with dementia through pictorial tools. Yet, the application of the AAC methods to economic evaluation outcomes measurement and design is limited (Broomfield et al., 2019).

Another set of reasons for the complex nature of the PROM assessment in dementia could be attributed to the choice of instruments used to assess this construct. The

EQ-5D is the most frequently chosen instrument in economic evaluations internationally (Szende et al., 2014). In the EQ-5D, the quality of life is defined by mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Hence, the EQ-5D assesses only physical and psychological aspects of quality of life and does not capture many critical psychosocial aspects that may influence the quality of life of people with dementia (Reilly et al., 2020).

Recent studies on how people with dementia might live well with the condition indicate that factors beyond health for example, self-efficacy and humor contribute significantly to their overall quality of life (Clarke et al., 2020; Lamont et al., 2020). Economic quality of life assessment could be incomplete without accounting for elements like the quality of personal and social relationships, effective communication, feeling valued and respected by others, and independence (Reilly et al., 2020). Instruments such as the EQ-5D may not capture aspects of quality of life beyond health that people living with dementia themselves consider essential.

An alternative approach has been proposed to capture broader wellbeing called the capability approach. Sen (1987) developed the capability approach with a core focus on what individuals are free and able to do and be (i.e., are capable of). It is a theoretical framework with a growing range of applications (Helter et al., 2020). For example, it has been highlighted as a way of helping people with mental health difficulties engage with their values and priorities, for instance, by influencing the design and delivery of mental health services (Helter et al., 2022).

Capability instruments like the ICECAP for Older people (ICECAP-O) and ICECAP Supportive Care Measure (ICECAP-SCM) have been developed to supplement the EQ-5D and are increasingly used in economic evaluations and trials, particularly among older people. The ICECAP-O uses a broader scope of quality of life domains identified by older people as important to wellbeing (attachment, security, role, enjoyment, and control) (Coast et al., 2008). The ICECAP-SCM covers seven domains (love and affection, choice, physical suffering, emotional suffering, dignity, support, and preparation) (Bailey et al., 2016).

Assessment of the quality of life of people living with dementia using the capability wellbeing approach is emerging (Helter et al., 2020). To date, two studies have elicited responses directly from community-dwelling people living with dementia using the ICECAP-O (Nyman et al., 2021; Bibi et al., 2022). In comparison, three other studies surveyed proxies using either ICECAP-O (Makai et al., 2014; Sarabia-Cobo et al., 2017) or ICECAP-O and ICECAP-SCM (Froggatt et al., 2020). The ICECAP-O was found to be both a reliable and valid measure of the quality of life for use with people living with mild/moderate dementia without a proxy (Nyman et al., 2021; Bibi et al., 2022). It is not entirely clear yet, however, which ICECAP (ICECAP-O for older people or ICECAP-SCM for those at the end of life) is to be used when assessing the quality of life of people living with dementia; or whether a dementia-specific capability wellbeing instrument(s) co-designed with people living with dementia is warranted to support the evidence base for the effectiveness and value of healthcare services at different stages and within the distinct contexts in which they experience life.

1.1 Aims

The overarching aim of this study is to gather perspectives of people living with dementia, their insights, and preferences on the question of assessing their quality of life in economic evaluation. Specifically, this study aims to address two research questions (RQ):

> **RQ1:** What does a "good life" mean to someone living with dementia?

> **RQ2:** How well do instruments currently used in economic evaluation reflect this construct?

Four different types of preference-based PROMs with a perspective that goes beyond health will be tested in this study. These comprise a dementia-specific quality of life PROM (*AD-5D/QOL-AD*), a generic health-related quality of life PROM (*AQOL-4D*), and two capability PROMs of broader wellbeing (*ICECAP-O* and *ICECAP-SCM*).

Specific objectives of this study include:

(1) To define themes of a "good life" or quality of life from the perspective of people living with dementia as well as explore differences and similarities in the type of dementia, age and sex.
(2) To gather perspectives of people living with dementia, their insights, and preferences for assessing their quality of life using different preference-based patient-reported instruments, their design and administration mode.
(3) To explore differences in the completion of the PROMs in terms of *comprehension* (e.g., any misunderstanding of a word, phrase, or response option); *retrieval* (e.g., a recall error); *judgment* (e.g., recalled experiences are irrelevant or inadequate); *response* (e.g., participant's response is inconsistent with the personal experience expressed or the desired response is missing from the response choices); and *completion time*.

2 Methods and analysis

This protocol adheres to the Consolidated Criteria for Reporting Qualitative Studies (COREQ) (Tong et al., 2007).

2.1 Theoretical framework

This research project comprises a "think-aloud" study followed by a semi-structured interview. A think-aloud study is a cognitive interview method that asks participants to verbalize their thoughts and actions as they perform a task (Willis, 2005). Think-aloud interviews enable examining comprehension, retrieval, judgment, and response difficulties when completing a questionnaire. The interviewer remains silent so long as individuals continue to think aloud. This process allows for a more realistic picture of the problems that individuals face when completing questionnaires than more direct interview methods that may interrupt task completion (Kuusela and Paul, 2000).

2.2 Participant selection and setting

2.2.1 Sampling

A purposeful sample will be recruited for this study. Potential participants will be recruited through the integrated care, geriatric and post-diagnostic clinics at Tallaght University Hospital and St James' Hospital in Dublin, Ireland, and supplemented with additional recruitment *via* dementia support groups. Participants will be required to satisfy the following inclusion criteria:

- being diagnosed or waiting to receive a formal diagnosis of dementia;
- have the capacity to participate in research, i.e., sufficiently well to be able to provide informed consent and participate in research;
- wishing to participate; and,
- able to communicate in the English language.

Patients who are medically or psychiatrically unstable or acute, as assessed by the clinical team, will not be eligible to participate. All of the inclusion criteria must be satisfied for a person to be eligible to participate.

2.2.2 Method of approach

For clinical recruitment, a clinician, during a routine appointment, will approach an eligible patient with an opportunity to participate in this study. If interested, potential participants will be handed a participant information leaflet to read in their own time. If they agree to participate, they can leave their contact details (name, email address, or phone number) with a research team member present at the time or contact the research team directly to discuss the study further, obtain informed consent and arrange for study participation.

For recruitment *via* dementia support groups, a group facilitator would approach potential participants using their judgment of the person's capacity to participate in research and ability to provide informed consent. They will approach potential participants with an invitation to participate in the study. If interested, potential participants will be given a participant information leaflet to read in their own time. If they are interested in participating, they can leave their contact details with the group facilitator or contact the research team directly.

2.2.3 Setting of data collection and consent

Interviews will be completed at a time and place convenient for the participants, either face-to-face in the participant's home or any other convenient location or remotely *via* platforms such as Teams. If the participant opts for a home visit, a research team member will revisit the information in the participant information leaflet, and proceed to obtain written, informed consent from the participant. If the participant opts for remote participation, the consent forms will be sent by email or post at least 1 week before the interview. Once connected remotely, a research team member will go through the consent form and the participant information sheet and address any questions relevant to participation. If agreeing to consent, participants will be asked to sign the form on camera, if available, witnessed by the researcher remotely and by their care partner. Participants will be able to withdraw from the study at any point following consent, with no explanation required. Their participation or withdrawal from the study will not impact their regular care in any way.

2.2.4 Sample size

The study will aim to recruit about 30 people living with dementia of different types and demographics in order to achieve a heterogeneous sample across different types of dementia, age and sex. There is no clear sample size guidance for cognitive interviewing. Previous think-aloud studies have used sample sizes in the region of 18–36 (Al-Janabi et al., 2013; Horwood, 2014; Bailey et al., 2016; Mitchell et al., 2020). The proposed sample size should be sufficient to reach "information power" in identifying important themes arising from interviews and enable the quantitative "scoring" element of the analysis (Malterud et al., 2016).

2.3 Instruments

2.3.1 Cognitive assessment

PROMIS-Cognitive Function- Short Form 4a (PROMIS-SF 4a) (Physician Health Organization [PHO], 2020) is a 4-item instrument used to capture self-reported cognitive complaints. The PROMIS-SF 4a in this study will provide a subjective assessment of cognitive function by a person with dementia. This brief tool includes individual reviews of processing speed, working memory and executive function cognitive domains. Each question has five response options ranging in value from one to five. The sum of the response values to each question

is added to generate a total raw score ranging from 4 to 20. Raw scores ranging between 15 and 20 are considered normal, mild complaints are between 12 and 14, moderate complaints are between 6 and 11, and severe complaints are less than 6 (P® Scoring Manuals, 2022).

General Practitioner Assessment of Cognition (GPCOG) (Brodaty et al., 2002) is a screening tool for dementia, comprising two components: (1) cognitive test items conducted with the person and (2) historical questions asked of an informant. Results > 8 or < 5 on the GPCOG patient section indicate cognitively intact or impaired, respectively. For patients requiring an informant questionnaire, a score of 3 or less out of 6 in this section suggests cognitive impairment (Brodaty et al., 2002).

The Dementia Communication Difficulties Scale (DCDS) (Murphy et al., 2007) in this study will help further define a dementia stage, such as early, moderate, or late. DCDS comprises 13 statements based on existing definitions of the communication problems commonly experienced by people as dementia progresses. The DCDS requires a third party who knows the person with dementia well to assess various aspects of their communication on a 5-option scale. Each DCDS option is assigned a score: "Never" = 0, "Sometimes" = 1, "Often" = 2, "Always" or "Says too little for me to judge" = 3. A person's DCDS rating is obtained by totaling their scores for all 13 statements. DCDS ratings can range from 0 to 39, with a higher rating indicating greater communication difficulty. Stages of dementia group definitions include: early stage (DCDS ratings between 0 and 10.5); moderate stage (DCDS ratings between 11 and 19.5); late stage (DCDS rating between 20 and 39) (Murphy et al., 2007).

2.3.2 Quality of life

The Quality of Life in Alzheimer's Disease (QOL-AD)/AD-5D (Logsdon et al., 2002) is a condition-specific instrument for assessing health-related quality of life for people living with dementia and the Alzheimer's Disease Five Dimensions (AD-5D) (Comans et al., 2020) is a preference-based scoring algorithm for QOL-AD that calculates quality-adjusted life years and enables its use in economic evaluation. The QOL-AD/AD-5D comprises 13 attributes (physical health, energy, mood, living situation, memory, family, marriage, friends, self as a whole, ability to do chores, ability to do things for fun, money and life as a whole). Response options include 1 (poor), 2 (fair), 3 (good), and 4 (excellent), with higher scores indicating better quality of life. The patients' ratings are performed in an interview, with standardized instructions to avoid influencing the results. It is worth noting that while the QOL-AD was originally developed as a disease-specific scale for Alzheimer's, it has been used to assess the quality of life of people living with other types of dementia (Larsson et al., 2011).

The Australian quality of life (AQOL-4D) (Hawthorne et al., 1999) is a generic health-related quality of life instrument that consists of 12 attributes covering four dimensions, including independent living–self-care, household tasks and mobility; relationships–friendships, isolation and family role; mental health–sleeping, worrying and pain; and senses–seeing, hearing and communication. Each dimension has four response levels. It is an abbreviated version of AQOL questionnaire that could be completed in 1–2 min by a participant without an accompanying instructions (Hawthorne et al., 1999).

The ICECAP-O (Coast et al., 2008) is a capability wellbeing instrument developed for self-completion by older adults, specifically for the economic evaluation of health and care interventions. It consists of five dimensions relating to a person's capability to have attachment, security, role, enjoyment, and control. Each dimension has four response levels ranging from no capability to full capability (Coast et al., 2008).

The ICECAP-SCM (Bailey et al., 2016) is a capability wellbeing instrument developed for use in evaluation of palliative and supportive care interventions. The instrument has seven attributes derived from qualitative data collected from those at various stages along the trajectory toward death. Participants are asked to indicate their wellbeing "at the moment" in terms of choice (being able to have a say), love and affection (being able to be with people who care about you), freedom from physical suffering, freedom from emotional suffering, dignity (being able to maintain dignity and self-respect), support (able to have help and support), and preparation (having the opportunity to make preparations). There are four response levels to each attribute ranging from no capability (1), a little capability (2), some capability (3), full capability, generally expressed as experiencing a lot of an attribute (4) (Huynh et al., 2017).

2.4 Data collection

The interview will begin with a recap of the study aims, interview format and data confidentiality, consent for participation and recording of the interview. In order to "warm up," a preliminary task will ask participants to "think aloud" as they count windows at the place they live. Participants will also be asked a few basic questions about their age, dementia diagnosis, and their family circumstances. Participants will then be asked to reflect on what "good life" means to them and asked to verbalize their thoughts while completing the four PROMs, containing 37 questions in total, thinking aloud when reading and answering these questions. The order of the PROMs will be randomly determined prior to the interview, using a random number generated by RAND function in Microsoft Excel. Lastly, participants will be asked to self-rate their cognitive health with four standardized questions (Physician Health Organization [PHO], 2020); while a close person will be asked to complete DCDS and GPCOG.

Participants will not be interrupted unless they are silent for longer than 10 s when they will be asked to "keep thinking aloud." A close person will be present and may offer support with interpretation during the interview if required, but they will be asked not to provide their own views. An interviewer will facilitate this interaction.

If the participant is able to provide consent but does not appear to be able to complete any of the PROMs, such as unable to directly participate, the interviewer will shorten the interview by thanking the participant for their interest in this study and sensitively terminating the interview. In such cases, we will not seek consent to interview a close person on behalf of a patient as the purpose of this study is specifically to examine the ability of people living with dementia directly to self-complete these PROMs and express their preferences.

After completing each PROM and after completing all four PROMs, there will be a short discussion to clarify the participants' thoughts. The interviewer will seek to clarify any areas where there is uncertainty about the recorded answer and briefly discuss the participant's opinions more generally.

Interviews could last anywhere between 1 and 1.5 h and will be audio recorded. The semi-structured part of the interview will be conducted using a topic guide to help ensure a consistent approach across interviews and between interviewers. However, the research team will use the guide in a responsive way tailored to individual experiences. This means that the topics covered and the order in which they are discussed could vary, especially between interviews. Interviewers will use open, non-leading questions, and answers will be fully probed (for example, asking "How?" and "In what way?").

If participants become upset during the interview for any reason, the issue will be handled sensitively, and participants will be asked whether they would like to stop the interview. If the participant's preference is to stop the interview, this will, of course, be done, but if they wish to continue, this preference will also be met. Following the facilitation of an interview, the researcher will be debriefed by another research team member. They will not share any information which can potentially identify the participant during the debrief.

2.5 Data analysis

All interviews will be transcribed verbatim. Key topics will be identified through familiarization with the transcripts and discussion among the research team to create a list of themes and sub-themes called "nodes." The transcripts will be managed using the software package NVivo 10.

A central chart will be created to give an overview of each interview in terms of the key sampling characteristics. The final analytical stage will involve working through the coded data both within and across cases and themes, identifying similarities and differences and interrogating the data to seek to explain emergent patterns and findings (Spencer et al., 2013).

To explore differences in the completion of the PROMs, three raters will independently assess each transcript for errors in terms of:

- comprehension (understanding the question in the way intended);
- retrieval (retrieving information – in general, this is assumed to be the ability to retrieve information from long-term memory, but, for this case, with proxy respondents, it will also be used to indicate errors where the respondent is unable to retrieve information that they were unaware of);
- judgment (judging how the retrieved information should be used to answer the question);
- response (participant's response is inconsistent with the personal experience expressed or the desired response is missing from the response choices); and,
- completion time.

Kappa scores will be calculated to assess inter-rater agreement, applying the following set of rules, used in the previous research by Froggatt et al. (2020), to determine whether a response should be classified as an error and, if so, of what type:

- If no error is identified by any rater, then no error will be recorded.
- If an error type is identified by all raters, then an error of that type will be recorded.
- If an error is identified by one or two raters, then the raters collectively will come to a final decision through discussion.
- If an error is identified by all three raters, but there will be disagreement about the nature of the error, then the raters will collectively come to a final decision through discussion.
- If raters cannot come to a collective decision through discussion, then the final assessment will be made based on majority choice.

2.6 Ethics

This study received approval by the St James's Hospital and Tallaght University Hospital Joint Research Ethics Committee. Approval was granted on 11 April 2022.

2.7 Patient and public involvement (PPI)

In preparation for this study, we conducted extensive patient and public consultations. First, we engaged with the Dementia Community Research Advisory Panel members (DC-RAP)

TABLE 1 Synopsis of the PPI/PPV feedback received and corresponding changes made.

	ICECAP-O original	ICECAP-O "smiley version"	AQOL-4D	DEMQOL-U	AD-5D (QOL-AD)
Like most	Symmetrical, clean, consistent, easy to follow [PPI1–4]	Easy, simple, not busy, 1 page, quick [PPI1–4]	"Comprehensive" [PPI1]; "liked examples" [PPI1]; talked about "personal day to day tasks – easy to answer" [PPI1 and 4]; "gets down to emotional level. . . loneliness for example" [PPI2]	"colours delineate text" [PPI1]; "relatively simple" [PPI2]; "talked about feelings" [PPI4]	"covers QOL succinctly and add additional categories as marriage and money" [PPI2]; "not long" [PPI3]; "very clear" [PPI4]
Liked least	A lot of words and lack of space [PPI1–4]; "hard to answer" [PPI3 and 4] "emotionally draining" [PPI4]	"Colour – visually too much" [PPI2]; "too simple" [PPI1]	"a lot of words" [PPI1 and 4] and "acronyms" [PPI2]; "long" [PPI4]	"negatively worded questions (lonely, frustration)" [PPI2]; "kept forgetting about last week" reference [PPI1]; confusing words, i.e., cognition and forgetfulness, relationship defined as verbal communication [PPI3]; many questions are covered by other PROMs [PPI2]	"too many instructions" [PPI2 and 3]
Other comments	"Why can? It takes a minute to register; talking about yourself or your ability? If someone dependent – would they know that – depends on how advanced they are. Not all will be able to admit they lost independence" [PPI4]	Color and smile – double message (red-bad; green-good) what if a person prefers being dependent, but the associated color was red? [PPI4]	"If facilitated this would invite dialog, if given right away might scare them" [PPI1]; "Unlikely will answer over the past week, they will answer at the moment" [PPI4]		Questions about choirs and marriage may not apply to everyone (e.g., old fashioned gentleman, nursing home residents, widowed)
Changes made	Based on feedback, the lead author developed two alternative design options, i.e., visual, using bars and tabular; applied to both ICECAP-O and ICECAP-SCM, with the latter accompanied by interviewer instructions. A decision was made to use a tabular design in this study	A decision was made not to use this design in the present study	A decision was made to change some of the wording of questions, font size and spacing; removed abbreviations and acronyms. Approval was sought and granted to use this version in the present study	A decision was made not to use this PROM	No changes were made; a decision was made to use the original interviewer-led version in the present study

(Global Brain Health Institute [GBHI], 2022) established as part of the Global Brain Health Institute (GBHI) Person and Public Voice (PPV) project. We ran five one-on-one cognitive interviews with care partners of people living with dementia. Cognitive interviewing is an evidence-based, qualitative method specifically designed to investigate whether a questionnaire fulfill their intended purpose; often used as part of pretesting and before the main data collection (Willis, 2005).

During the cognitive interviewing, we obtained feedback on the topic guide, PROMs [ICECAP-O original and ICECAP-O "smiley version" (Kinghorn et al., 2021), AQOL-4D, DEMQOL-U, and QOL-AD], including their design, wording, and administration mode. **Table 1** provides a synopsis of the questions asked, feedback received, and corresponding changes made to the instruments. Subsequently, an adapted version, underpinned by the AAC principles and accompanied by interviewer instructions was developed for the ICECAP-SCM and ICECAP-O.

After the initial round of changes, we conducted another cognitive interview with a person living with dementia. We confirmed the chosen instruments, their design, and the likelihood of completing 37 questions across the four PROMs in an interview. Patient and public consultations during the analysis stage and dissemination will continue for the duration of this project.

Ethics statement

The studies involving human participants were reviewed and approved by the St. James's Hospital and Tallaght University Hospital Joint Research Ethics Committee.

The patients/participants provided their written informed consent to participate in this study.

Author contributions

IK wrote the initial draft, edited, and organized the final version of the manuscript. All authors provided expertise from their respective fields, involved in writing, review, and editing, and read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Al-Janabi, H., Keeley, T., Mitchell, P., and Coast, J. (2013). Can capabilities be self-reported? A think aloud study. *Soc. Sci. Med.* 87, 116–122. doi: 10.1016/j.socscimed.2013.03.035

Arons, A. M., Krabbe, P., Schölzel-Dorenbos, C., van der Wilt, G., and Rikkert, M. (2013). Quality of life in dementia: a study on proxy bias. *BMC Med. Res. Methodol.* 13:110. doi: 10.1186/1471-2288-13-110

Ayton, D. R., Gardam, M., Pritchard, E., Ruseckaite, R., Ryan, J., Robinson, S., et al. (2020). Patient-reported outcome measures to inform care of people with dementia—a systematic scoping review. *Gerontologist* 61, e185–e194. doi: 10.1093/geront/gnz179

Bailey, C., Kinghorn, P., Orlando, R., Armour, K., Perry, R., Jones, L., et al. (2016). 'The ICECAP-SCM tells you more about what I'm going through': a think-aloud study measuring quality of life among patients receiving supportive and palliative care. *Palliat. Med.* 30, 642–652. doi: 10.1177/0269216315624890

Bibi, I., Polman, R., and Nyman, S. R. (2022). Reliability of the ICECAP-O quality of life scale with community-dwelling people with dementia. *Gerontol Geriatr Med.* 8:23337214221086802. doi: 10.1177/23337214221086802

Brodaty, H., Pond, D., Kemp, N. M., Luscombe, G., Harding, L., Berman, K., et al. (2002). The GPCOG: a new screening test for dementia designed for general practice. *J. Am. Geriat. Soc.* 50, 530–534. doi: 10.1046/j.1532-5415.2002.50122.x

Broomfield, K., Harrop, D., Judge, S., Jones, G., and Sage, K. (2019). Appraising the quality of tools used to record patient-reported outcomes in users of augmentative and alternative communication (AAC): a systematic review. *Quality Life Res.* 28, 2669–2683. doi: 10.1007/s11136-019-02228-3

Caramelli, P., Mansur, L. L., and Nitrini, R. (1998). *Language and communication disorders in dementia of the alzheimer type, in handbook of neurolinguistics*. Cambridge, MA: Academic Press, 463–473. doi: 10.1016/B978-012666055-5/50036-8

Churruca, K., Pomare, C., Ellis, L., Long, J., Henderson, S., Murphy, L., et al. (2021). Patient-reported outcome measures (PROMs): a review of generic and condition-specific measures and a discussion of trends and issues. *Health Expect.* 24, 1015–1024. doi: 10.1111/hex.13254

Clarke, C., Woods, B., Moniz-Cook, E., Mountain, G., Øksnebjerg, L., Chattat, R., et al. (2020). Measuring the well-being of people with dementia: a conceptual scoping review. *Health Quali. Life Outcomes* 18:249. doi: 10.1186/s12955-020-01440-x

Coast, J., Flynn, T., Natarajan, L., Sproston, K., Lewis, J., Louviere, J., et al. (2008). Valuing the ICECAP capability index for older people. *Soc. Sci. Med.* 67, 874–882. doi: 10.1016/j.socscimed.2008.05.015

Comans, T. A., Nguyen, K., Ratcliffe, J., Rowen, D., and Mulhern, B. (2020). Valuing the AD-5D dementia utility instrument: an estimation of a general population tariff. *Pharm. Econ.* 38, 871–881. doi: 10.1007/s40273-020-00913-7

Daly, R. L., Bunn, F., and Goodman, C. (2018). Shared decision-making for people living with dementia in extended care settings: a systematic review. *BMJ Open* 8:e018977. doi: 10.1136/bmjopen-2017-018977

Feinberg, L. F., and Whitlatch, C. J. (2001). Are persons with cognitive impairment able to state consistent choices? *Gerontologist* 41, 374–382. doi: 10.1093/geront/41.3.374

Fetherstonhaugh, D., Tarzia, L., and Nay, R. (2013). Being central to decision making means I am still here!: the essence of decision making for people with dementia. *J. Aging Stud.* 27, 143–150. doi: 10.1016/j.jaging.2012.12.007

Froggatt, K., Best, A., Bunn, F., Burnside, G., Coast, J., Dunleavy, L., et al. (2020). *A group intervention to improve quality of life for people with advanced dementia living in care homes: the namaste feasibility cluster RCT in health technology assessment*. London: National Institute for Health Research. doi: 10.3310/hta24060

Global Brain Health Institute [GBHI] (2022). *The dementia community research advisory panel*. Available online at: https://www.gbhi.org/dementia-community-research-advisory-panel. (accessed October 03, 2022).

Gräske, J., Fischer, T., Kuhlmey, A., and Wolf-Ostermann, K. (2012). Quality of life in dementia care – differences in quality of life measurements performed by residents with dementia and by nursing staff. *Aging Mental Health* 16, 819–827. doi: 10.1080/13607863.2012.667782

Haroon, M., Dissanayaka, N., Angwin, A., and Comans, T. (2022). How effective are pictures in eliciting information from people living with dementia? A systematic Review. *Clin. Gerontol.* 2022, 1–14. doi: 10.1080/07317115.2022.2085643

Hawthorne, G., Richardson, J., and Osborne, R. (1999). The assessment of quality of life (AQoL) instrument: a psychometric measure of health-related quality of life. *Quality Life Res.* 8, 209–224. doi: 10.1023/A:1008815005736

Helter, T. M., Coast, J., Łaszewska, A., Stamm, T., and Simon, J. (2020). Capability instruments in economic evaluations of health-related interventions: a comparative review of the literature. *Qualit Life Res* 29, 1433–1464. doi: 10.1007/s11136-019-02393-5

Helter, T. M., Coast, J., Łaszewska, A., Stamm, T., and Simon, J. (2022). Comparison of capability and health-related quality of life instruments in capturing aspects of mental well-being in people with schizophrenia and depression. *Br. J. Psych. Open.* 8:e117. doi: 10.1192/bjo.2022.514

Hirschman, K. B., Joyce, C., James, B., Xie, S., and Karlawish, J. (2005). Do Alzheimer's disease patients want to participate in a treatment decision, and would their caregivers let them? *Gerontologist* 45, 381–388. doi: 10.1093/geront/45.3.381

Horwood, J. (2014). Evaluating the face validity of the ICECAP-O capabilities measure: a "think aloud" study with hip and knee arthroplasty patients. *Appl. Res. Quality Life* 9, 667–682. doi: 10.1007/s11482-013-9264-4

Huynh, E., Coast, J., Rose, J., Kinghorn, P., and Flynn, T. (2017). Values for the ICECAP-supportive care measure (ICECAP-SCM) for use in economic evaluation at end of life. *Soc. Sci. Med.* 189, 114–128. doi: 10.1016/j.socscimed.2017.07.012

Kane, R. A., Kling, K., Bershadsky, B., Kane, R., Giles, K., Degenholtz, H., et al. (2003). Quality of life measures for nursing home residents. *J. Gerontol. Biol. Sci. Med. Sci.* 58, 240–248. doi: 10.1093/gerona/58.3.M240

Karlawish, J. H., Zbrozek, A., Kinosian, B., Gregory, A., Ferguson, A., Low, D., et al. (2008). Caregivers' assessments of preference-based quality of life in Alzheimer's disease. *Alzheimer's Dementia* 4, 203–211. doi: 10.1016/j.jalz.2007.11.018

Kim, S. Y., Karlawish, J. H., and Caine, E. D. (2002). Current state of research on decision-making competence of cognitively impaired elderly persons. *Am. J. Geriatr. Psychiatry* 10, 151–165. doi: 10.1097/00019442-200203000-00006

Kinghorn, P., Duggal, M. R., and Tew, J. (2021). Selecting & piloting appropriate tools for capturing outcomes related to adult social care in the UK. *Paper presented at the Oral presentation to the International Health Economics Association Conference*.

Kuusela, H., and Paul, P. (2000). A comparison of concurrent and retrospective verbal protocol analysis. *Am. J. Psychol.* 113, 387–404. doi: 10.2307/1423365

Lamont, R. A., Nelis, S., Quinn, C., Martyr, A., Rippon, I., Kopelman, M., et al. (2020). Psychological predictors of 'living well' with dementia: findings from the IDEAL study. *Aging Ment Health* 24, 956–964. doi: 10.1080/13607863.2019.1566811

Larsson, V., Engedal, K., Aarsland, D., Wattmo, C., Minthon, L., Londos, E., et al. (2011). Quality of life and the effect of memantine in dementia with lewy bodies and Parkinson's disease dementia. *Dement. Geriatr. Cogn. Dis.* 32, 227–234. doi: 10.1159/000334523

Logsdon, R. G., Gibbons, L., McCurry, S., and Teri, L. (2002). Assessing quality of life in older adults with cognitive impairment. *Psychosom. Med.* 64, 510–519. doi: 10.1097/00006842-200205000-00016

Makai, P., Beckebans, F., van Exel, J., and Brouwer, W. (2014). Quality of life of nursing home residents with dementia: validation of the german version of the ICECAP-O. *PLoS One* 9:e92016. doi: 10.1371/journal.pone.0092016

Malterud, K., Siersma, V. D., and Guassora, A. D. (2016). Sample size in qualitative interview studies: guided by information power. *Qualit. Health Res.* 26, 1753–1760. doi: 10.1177/1049732315617444

Mitchell, P. M., Caskey, F., Scott, J., Sanghera, S., and Coast, J. (2020). Response process validity of three patient reported outcome measures for people requiring kidney care: a think-aloud study using the EQ-5D-5L, ICECAP-A and ICECAP-O. *BMJ Open.* 10:e034569. doi: 10.1136/bmjopen-2019-034569

Moyle, W., Gracia, N., Murfield, J., Griffiths, S., and Venturato, L. (2012a). Assessing quality of life of older people with dementia in long-term care: a comparison of two self-report measures. *J. Clin. Nurs.* 21, 1632–1640. doi: 10.1111/j.1365-2702.2011.03688.x

Moyle, W., Murfield, J., Griffiths, S., and Venturato, L. (2012b). Assessing quality of life of older people with dementia: a comparison of quantitative self-report and proxy accounts. *J. Adv. Nurs.* 68, 2237–2246. doi: 10.1111/j.1365-2648.2011.05912.x

Murphy, J., Gray, C. M., and Cox, S. (2007). *Communication and dementia. How talking mats can help people with dementia to express themselves*. New York: York Publishing Services Ltd.

Nyman, S. R., Casey, C., and Polman, R. (2021). Psychometric properties of the ICECAP-O quality of life measurement tool when self-reported by community-dwelling older people with mild and moderate dementia. *Alzheimer Dis. Assoc. Dis.* 35:4. doi: 10.1097/WAD.0000000000000430

O'Shea, E., Hopper, L., Marques, M., Gonçalves-Pereira, M., Woods, B., Jelley, H., et al. (2020). A comparison of self and proxy quality of life ratings for people with dementia and their carers: a European prospective cohort study. *Aging Mental Health* 24, 162–170. doi: 10.1080/13607863.2018.1517727

P® Scoring Manuals (2022). *PROMIS ® Cognitive Function Scoring Manual*. Available from: http://www.healthmeasures.net/administrator/components/com_instruments/uploads/PROMIS%20Cognitive%20Function%20Scoring%20Manual.pdf. (accessed May 18, 2022).

Physician Health Organization [PHO], (2020). *Cognitive function- short form 4a*.

Reilly, S. T., Harding, A., Morbey, H., Ahmed, F., Williamson, P., Swarbrick, C., et al. (2020). What is important to people with dementia living at home? A set of core outcome items for use in the evaluation of non-pharmacological community-based health and social care interventions. *Age Ageing* 49, 664–671. doi: 10.1093/ageing/afaa015

Sarabia-Cobo, C. M., Parás-Bravo, P., Amo-Setién, F., Alconero-Camarero, A., Sáenz-Jalón, M., Torres-Manrique, B., et al. (2017). Validation of the spanish version of the ICECAP-O for nursing home residents with dementia. *PLoS One* 12:e0169354. doi: 10.1371/journal.pone.0169354

Sen, A. (1987). *Commodities and capabilities*. New Delhi: Oxford University Press.

Spencer, L., Ritchie, R., O'Conner, W., Morrell, G., and Ormston, R. (2013). "Analysis in practice," in *Qualitative research practice*, eds J. L. Ritchie, J. McNaughton, C. Nicholls, and R. Ormston (London: Sage).

Szende, A., Janssen, B., and Cabases, J. (2014). *Self-reported population health: an international perspective based on EQ-5D*. Dordrecht (NL): Springer. doi: 10.1007/978-94-007-7596-1

Tong, A., Sainsbury, P., and Craig, J. (2007). Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *Int. J. Qualit. Health Care* 19, 349–357. doi: 10.1093/intqhc/mzm042

Tyrrell, J., Genin, N., and Myslinski, M. (2006). Freedom of choice and decision-making in health and social care: views of older patients with early-stage dementia and their carers. *Dementia* 5, 479–502. doi: 10.1177/1471301206069915

Willis, G. B. (2005). *Cognitive interviewing: a tool for improving questionnaire design*. Thousand Oaks, CA: Sage Publications. doi: 10.4135/9781412983655

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Health disparities in aging: Improving dementia care for Black women

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In the United States, 80% of surveyed Black patients report experiencing barriers to healthcare for Alzheimer's disease and related dementias (ADRD), delaying the time-sensitive treatment of a progressive neurodegenerative disease. According to the National Institute on Aging, Black study participants are 35% less likely to be given a diagnosis of ADRD than white participants, despite being twice as likely to suffer from ADRD than their white counterparts. Prior analysis of prevalence for sex, race, and ethnicity by the Centers for Disease Control indicated the highest incidence of ADRD in Black women. Older (≥65years) Black women are at a disproportionately high risk for ADRD and yet these patients experience distinct inequities in obtaining clinical diagnosis and treatment for their condition. To that end, this perspective article will review a current understanding of biological and epidemiological factors that underlie the increased risk for ADRD in Black women. We will discuss the specific barriers Black women face in obtaining access to ADRD care, including healthcare prejudice, socioeconomic status, and other societal factors. This perspective also aims to evaluate the performance of intervention programs targeted toward this patient population and offer possible solutions to promote health equity.

KEYWORDS

Alzheimer's disease, Alzheimer's disease and related dementias, discrimination, outreach, healthcare equity

1. Introduction

"It's a social justice and bioethics issue," Carl V. Hill, Ph.D., MPH, Chief Diversity, Equity, and Inclusion Officer for the Alzheimer's Association, remarks while reflecting on the current state of dementia care for Black women. Having established the framework for health disparities at the National Institute on Aging (NIA) before joining the Alzheimer's Association, Hill is no stranger to tackling some of the toughest problems in health equity (Hill et al., 2015). The current state of affairs in Alzheimer's disease and related dementias (ADRD) care for Black patients, particularly Black women, resides toward the top of such a list. As the fifth leading cause of death in older Black Americans, ADRD poses a great risk to patient health in this demographic (Us Against Alzheimer's, 2021). Current estimates indicate that Black individuals are at 2–3 times greater risk of developing AD than their White counterparts with the highest risk for those with familial history of AD (Green et al., 2002; Younan et al., 2022). Black Americans could face up to four times the amount of ADRD

cases over present-day estimates by 2060, as the aging population balloons to more than double by 2030 (Matthews et al., 2019; Alzheimer's Association, 2022). As such, the elevated risk of ADRD for Black individuals will only continue to worsen in the coming years (Figure 1).

Women are approximately twice as likely to develop ADRD than men across all races and ethnicities (Lim et al., 2022). The CDC found the highest prevalence in women and Black individuals when examining ~3.2 million Medicare Fee-For-Service beneficiaries diagnosed with ADRD in 2014 (Matthews et al., 2019). Research examining age-standardized diagnostic incidence rate and relative risk of late-onset ADRD also found the highest risk for Black women (Lim et al., 2022). Black women live at the crossroads of the most vulnerable populations for ADRD. Yet, research efforts tend to focus more broadly on either race or sex, giving little credence to the compounding effect that has led Black women to have the highest amount of ADRD cases. To that end, this perspective article will review the current understanding of contributing factors for increased ADRD risk in Black women, including ADRD healthcare access barriers, and evaluate current interventional programs and strategies to address this issue.

2. Chronic discrimination as a neurobiological insult in black patients

The biological and environmental pathways that lead to poorer health outcomes in Black patients are complex and intertwined. Researchers have long established that extended periods of stress can wreak havoc on patient's physical and mental health and brain function (Kloet et al., 2005; Blair and Cybele Raver, 2016). A lifetime of exposure to systemic racism and discrimination for Black patients places them in a vulnerable biological standing due to chronic psychological stress and impacts to mental health (Geronimus, 1992; Geronimus et al., 2010; Blair and Cybele Raver, 2016). Anxiety, depression, and other psychiatric conditions can increase risk for ADRD and negatively impact patient health outcomes (Richmond-Rakerd et al., 2022). Prior studies show that Black Americans have a higher allostatic load, referring to the cumulative damage done to the body under chronic stress. Black women had the highest allostatic load scores compared to Black men and White persons, regardless of income level (Geronimus et al., 2006). Evidence may also support that Black women between 49 and 55 years of age are roughly 7.5 years older biologically than their White counterparts, as assessed by telomere length – a repetitive sequence of DNA found at the end of chromosomes – from peripheral blood mononuclear cells (Geronimus et al., 2010). These sequences are used to protect chromosomes during cell division and replication. Telomere length is a well-established biomarker with an inverse relationship to age, as telomeres shorten throughout the lifespan (Houben et al., 2008). Stress pathways chronically activated by psychosocial stressors can increase the rate of telomere shortening and, in turn, indicate biological aging (Epel et al., 2004; Cherkas et al., 2006; Rewak et al., 2014). Data support longer telomere length at birth in Black patients than White patients, with accelerated shortening observed most specifically in Black women (Roux et al., 2009; Rewak et al., 2014; Brown et al., 2016).

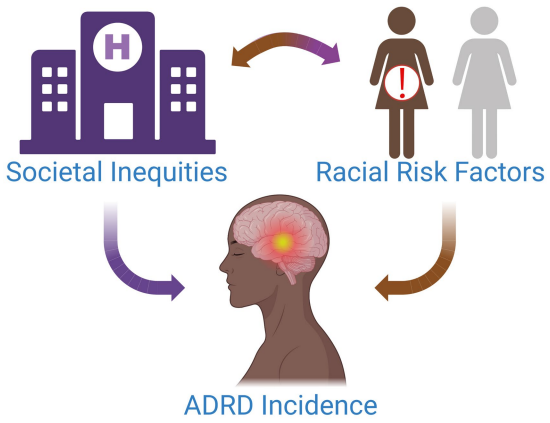

FIGURE 1
Multiple factors contribute to the increased incidence of ADRD in Black women. Societal inequities and discrimination drive chronic stress and inhibit access to high-quality healthcare for this patient population. Over time, this can have serious biological and health implications for patients, placing them at an elevated risk for modifiable diseases associated with ADRD and potentiating their risk for ADRD.

The biological consequences of discrimination for Black patients are also observed in ADRD. The onset of ADRD symptoms typically occurs at an earlier age and with greater severity for Black Americans compared to their White counterparts (Barnes and Bennett, 2017). Black Americans with high brain amyloid deposition showed a more severe cognitive decline over a 20-year follow-up than White patients with similar amyloid load (Gu et al., 2015; Barnes and Bennett, 2017; McDonough, 2017). Similar studies demonstrate that Black patients with high brain amyloid exhibit smaller hippocampal volumes and decreased cortical thickness compared to matched White patients (McDonough, 2017). As participants were matched on several parameters, these findings may indicate a faster neurodegenerative rate in Black patients. Recent studies have focused on biological mechanisms involved in both stress and ADRD, such as inflammation and oxidative damage pathways. One pilot study demonstrated an association between high interleukin-10 levels and impaired executive function but yielded mixed results on cognitive measures (Patel et al., 2020). Reduced AD risk in Black women that utilize statins has also been observed, possibly supporting that metabolic dysfunction and inflammation act as primary biological drivers for increased ADRD incidence in Black women (Zissimopoulos et al., 2017). The elevation of these driving factors is in line with previously reported chronic stress and cardiovascular disease in this patient population (Stampfer, 2006; Geronimus et al., 2010; Centers for Disease Control and Prevention, 2018). However, pilot studies such as these are limited by their sample sizes and possible sampling bias due to the lack of recruitment of Black patients in ADRD research (Barnes and Bennett, 2017). Current knowledge on the driving force of neurological vulnerability and possible accelerated biological aging in Black women remains limited and warrants further study.

3. Institutional discrimination drives ADRD health disparity in black women

Joyce Balls-Berry, Ph.D., Associate Professor at Washington University in St. Louis and the Health Disparities and Equity Core at the Knight Alzheimer's Disease Research Center (ADRC), emphasizes that "one of the big problems that we have, especially for women of color and even just communities of color regardless of self-identity on race or ethnicity, is a delay in diagnosis." Several barriers contribute to this delay

and prevent Black patients from accessing the highest quality care, including healthcare prejudice and disparities, lack of culturally competent care, and systemic racism.

Only 20% of surveyed Black Americans feel they have no barriers to quality healthcare for ADRD (Alzheimer's Association, 2021). The highest levels of discrimination in dementia health care are experienced by Black Americans, according to two national surveys from the Alzheimer's Association (Alzheimer's Association, 2021). Healthcare prejudice impacts all facets of care and can have serious implications for the health of Black patients with ADRD. A recent study from the NIA found that Black participants were 35% less likely to receive a diagnosis than White participants, despite having a well-established higher incidence of ADRD (Lennon et al., 2022). Black patients with an ADRD diagnosis are also less likely to have been told by a doctor that they have a "memory-related disease" (Alzheimer's Association, 2021). These findings support possible underdiagnosis of ADRD in Black patients and may have larger implications for the current understanding of ADRD incidence in this patient population.

Disparities in patient treatment for modifiable risk factors of ADRD may contribute to their increased incidence for Black women. Obesity, diabetes, and hypertension are all modifiable risk factors for ADRD (Omura et al., 2022). Black women experience a greater incidence of all three conditions than Black men and White individuals (Agyemang and Powell-Wiley, 2013). In the United States, Black women are 70% more likely to experience obesity in their lifetime than White women (Agyemang and Powell-Wiley, 2013; Williams et al., 2015). The increased incidence of obesity in Black women likely also contributes to their disproportionate rate of diabetes. An estimated 1 in 4 Black women (≥ 55 years old) has diabetes, occurring at roughly twice the rate of White women (Beckles and Thompson-Reid, 2001; Agency for Healthcare Research and Quality, 2016; Centers for Disease Control and Prevention, 2022). Diabetes and obesity tend to co-occur with hypertension, which affects 58% of Black women in the U.S. compared to only 41% of White and Hispanic women (Virani et al., 2021). Almost half (46%) of Black women suffer from the highest level of hypertension (Stage 2), comprising a greater portion of the population than Black men (42%) (Abel et al., 2021). Hypertension is also a major risk factor for cardiovascular disease, the leading cause of death in Black women, and a significant risk factor for ADRD (Stampfer, 2006; Centers for Disease Control and Prevention, 2018). The higher prevalence of these conditions and discrimination in health care, especially for Black female patients, likely contribute to their unequal burden of ADRD prevalence.

Healthcare discrimination against Black patients extends beyond the scope of ADRD and has implications for patient health outcomes. Patients of racial and ethnic minorities in the U.S. are less likely to receive preventative healthcare and are more likely to experience a lower quality of care (Smedley et al., 2003; Institute of Medicine, 2012; Agency for Healthcare Research and Quality, 2021). The same report showed that Black patients' health outcomes remain worse than for White patients even when accounting for income, neighborhood status, comorbid illnesses, and health insurance type. One study also demonstrated a strong link between healthcare discrimination based on race, sex, age, education, or income level and the health outcomes of diabetic patients (Piette et al., 2006). Black patients are less likely to receive recommended services for diabetes and less likely to have their hemoglobin A1c and blood pressure under control (Millett et al., 2008; Satcher, 2008; Agency for Healthcare Research and Quality, 2016). The unequal treatment of Black patients could have implications for health outcomes long before their ADRD diagnosis.

Tantamount to discrimination is a lack of access to culturally competent care for Black ADRD patients. Only 48% of surveyed Black persons report feeling confident they can access culturally competent care (Alzheimer's Association, 2021). Their fears are not unfounded, as a growing amount of evidence supports that common diagnostic tools are less accurate for non-White patients. Many cognitive tests commonly utilized in ADRD clinical evaluation, like the Stroop Color and Word Test or the Mattis Dementia Rating Scale, include race-norming adjustments that assume lower performance scores for Black patients and thus complicate the interpretation of low performance scores that may result from cognitive impairment (Gasquoine, 2009; Vyas et al., 2020; Barnes, 2022). Race-norming in cognitive tests may prevent Black patients experiencing dementia symptoms from getting the proper diagnosis. Other cognitive tests included in the ADRD battery, such as the Mini-Mental State Exam, are known to suffer from racial bias that results in the misdiagnosis of Black patients. These neuropsychological tests can yield staggering false-positive results between races and ethnicities, up to 42% in Black patients compared to 6% in their White counterparts (Stephenson, 2001). Race-norming obfuscates the true prevalence of ADRD in Black patients and creates a significant barrier for Black patients to obtain sufficient dementia care.

Biomarker evaluations may provide a reprieve from implicit and racial bias in cognitive testing but are not without limitations. Previous studies have reported lower levels of cerebrospinal fluid biomarkers for ADRD in Black patients compared to White patients, including tau and phosphorylated tau (Howell et al., 2017; Morris et al., 2019). The patient's race also impacted the interaction of apolipoprotein e4, a genetic biomarker for ADRD, and tau (Morris et al., 2019). Recently, a study also found that differences in tau levels for Black Americans can impair the accuracy of biomarker models predicting brain amyloidosis commonly used in ADRD evaluation (Schindler et al., 2022). These results led the authors to recommend future analyses of molecular ADRD biomarkers to adjust for race differences. Balls-Berry, authored on the article, proposes that methods for biological screening of ADRD need to be thought of "in terms of ancestry and in terms of social and structural determinants of health, which are often not looked at as being a biomarker for disease."

4. Outreach and intervention programs for black women with ADRD

The disconnect between healthcare providers and Black patients can have a resounding impact on ADRD care. Black patients are twice as likely not to seek out healthcare when experiencing thinking or memory problems than White patients (Alzheimer's Association, 2021). The reasoning behind the avoidance of healthcare intervention may be partly due to a lack of community educational outreach, as Black Americans are more likely to attribute ADRD symptoms to normal aging than White Americans (Glover et al., 2019). As cultural education among physicians remains subpar, these nuances in recognizing symptoms and treating different populations of patients impair the quality of care for ADRD (Assistant Secretary For Planning and Evaluation (ASPE), 2022). This divide widens as other factors such as socioeconomic status, level of education, and psychological stress not only inhibit access to high-quality dementia care but also put Black patients at greater risk for

ADRD (Geronimus et al., 2010; Hill et al., 2015; Blair and Cybele Raver, 2016).

Historical mistrust of the medical community also impacts the likelihood that Black patients will seek out dementia care and other healthcare services (Green-Harris et al., 2019). Long-standing discrimination and ethical misconduct from medical and academic institutions toward Black Americans and minority communities have significantly damaged the relationship between patient and provider. Surveys indicate that 62% of Black Americans feel medical research is biased against people of color, and only 53% believe a cure for AD would be distributed fairly without discrimination (Alzheimer's Association, 2021). Healthcare providers must first recognize and give credence to past misconduct and present mistrust in the relationship with Black patients. As Ball-Berry remarks, "What I would also like to see happen is for us to really acknowledge our historical hurts that we have done as institutions… and how that has further marginalized and impacted communities of color, especially the Black community, from being able to participate in studies because they felt like they were not heard when these things were happening."

Lack of inclusion for Black patients in ongoing clinical research has limited scientific understanding and impacted the ability to generalize scientific findings and treatments to the larger community. "We have so much work to do— with less than 10% of African Americans being represented among Alzheimer's clinical trials," says Hill. Low recruitment of Black participants also occurs in complementary intervention programs and studies of ADRD risk factors (Alegria et al., 2021). Reliance on passive untailored recruitment strategies and implicit bias act as primary barriers to effectively reaching Black patients and underserved communities (Green-Harris et al., 2019). State-level efforts to rethink current outreach strategies have yielded key insights to the path forward for healthcare providers. The Wisconsin Registry for Alzheimer's Project (WRAP) utilizes an asset-based community development approach that has improved access and care for Black patients with ADRD (Green-Harris et al., 2019). Their outreach efforts focused on identifying key community stakeholders, creating culturally-tailored programming, and providing educational resources for providers, patients, and caregivers. WRAP leaders concluded that by meeting the community's needs first, Black patients were more likely to participate in ADRD research through WRAP.

Balls-Berry works to cultivate the same dynamic with patients for the Knight ADRC. She encourages "creating shared visions with our community and patient partners in a way that opens up doors for new places to recruit, to collect data, to provide health education, and showing up and giving our time to these communities because it's going to advance the mission of those groups, not just our mission." Knight ADRC partners with several organizations and key stakeholders in the community to facilitate outreach efforts toward Black patients and unserved communities. Similar efforts are underway at Southern Illinois University School of Medicine (SIUSOM), where the Smith Alzheimer's Center has launched health equity initiatives through the Beyond the Medical Center Program in collaboration with the National Association for the Advancement of Colored People. SIUSOM also recently launched a Center for Equity in Professional Development to amplify community engagement and health education. Collectively, university ADRD outreach works to provide value to the community and encourage participation in clinical research.

Acting at the state and national level, Hill emphasized key takeaways that the Alzheimer's Association encourages for outreach efforts. Ensuring cultural relevance, appropriateness, and effectiveness in all outreach materials and healthcare resources is paramount, according to Hill. The Alzheimer's Association focuses on a community-based participatory model to accomplish this goal, emphasizing strategic partners in the community that are active at national and local levels, such as religious groups and healthcare workers organizations. Outreach efforts must go beyond just establishing community connections, but also listening and incorporating feedback from those partners. "We have to think critically and from an innovative perspective about how to get our free resources to the communities that are disproportionately affected, underrepresented, and underserved."

5. Discussion

Black women seek help from a healthcare system that is fundamentally not built to serve them. From preventative care to delayed diagnosis and treatment, Black patients experience chronic discrimination inside and outside the healthcare system that contributes to a higher incidence of long-term health issues and places them at greater risk for ADRD. Implementing a training structure that actively recruits students from underserved communities and instills culturally competent care among healthcare providers is paramount to combating this issue. Such training must address implicit racial and ethnic bias and an understanding of the racial limitations of diagnostic tools. Further, more research is needed to understand the racial biases that may exist in ADRD biomarker and physiological evaluations of Black patients and to discover more culturally competent protocols for diagnosis. The diagnosis and treatment of ADRD require healthcare providers to become more aware of variations in the clinical presentation of ADRD by race and to customize their care and approach to their patients.

Improving dementia care for Black women also requires healthcare providers to connect with organizations and representative members of their communities. These members could serve as liaisons for communication that will help to build a trusting relationship within the Black community. Increasing access to healthcare services and education will also be required to help Black female patients to receive all-encompassing care. Following, the healthcare pathway prior to requiring ADRD care needs to improve. Establishing key contacts within the community can facilitate organizational strategies toward recruitment and dissemination of educational resources. A central tenet of any recruitment strategy must be to serve the community and provide preventative healthcare and education. By doing so, the organization can establish itself as a resource in the community and encourage patients to participate in clinical research. Underdiagnosis in this patient population may partially explain difficulties in recruiting for clinical trials. Increasing diversity in trial participation is a big push for both the Alzheimer's Association and the NIA. The successful recruitment of more Black women to clinical research will aid in developing a deeper understanding of ADRD and facilitate the creation of more culturally competent dementia care for the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

CF proposed the idea for the perspective article, conducted interviews, and acted as lead writer and project manager. MC assisted with project management, conducted interviews, and performed manuscript editing. AL and RB provided clinical thought partnership and revised the manuscript. KH, CY, and EH supervised project completion and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abel, W. M., Spikes, T., and Greer, D. B. (2021). A qualitative study: hypertension stigma among black women. *J. Cardiovasc. Nurs.* 36, 96–103. doi: 10.1097/JCN.0000000000000759

Agency for Healthcare Research and Quality. (2016). Part 2: trends in priorities of the heckler report—Care for Diabetes. *Chartbook on Health Care for Blacks*. Available at: https://www.ahrq.gov/research/findings/nhqrdr/chartbooks/blackhealth/part2-diabetes.html.

Agency for Healthcare Research and Quality. (2021). 2021 National Healthcare Quality and disparities report. Department of Health and Human Services. Available at: https://www.ahrq.gov/research/findings/nhqrdr/nhqdr21/index.html

Agyemang, P., and Powell-Wiley, T. M. (2013). Obesity and black women: special considerations related to genesis and therapeutic approaches. *Curr. Cardiovasc. Risk Rep.* 7, 378–386. doi: 10.1007/S12170-013-0328-7

Alegria, M., Sud, S., Steinberg, B. E., Gai, N., and Siddiqui, A. (2021). Reporting of participant race, sex, and socioeconomic status in randomized clinical trials in general medical journals, 2015 vs 2019. *JAMA Netw. Open* 4:e2111516. doi: 10.1001/jamanetworkopen.2021.11516

Alzheimer's Association (2021). 2021 Alzheimer's disease facts and figures. *Alzheimers Dement.* 17, 327–406. doi: 10.1002/ALZ.12328

Alzheimer's Association (2022). 2022 Alzheimer's disease facts and figures. *Alzheimers Dement.* 18, 700–789. doi: 10.1002/alz.12638

Assistant Secretary For Planning and Evaluation (ASPE) (2022). *Federal Efforts to address racial and ethnic disparities in Alzheimer's disease and related dementias* Washington, DC, Assistant Secretary for Planning and Evaluation.

Barnes, L. L. (2022). Alzheimer disease in African American individuals: Increased incidence or not enough data? *Nat. Rev. Neurol.* 18, 56–62. doi: 10.1038/s41582-021-00589-3

Barnes, L. L., and Bennett, D. A. (2017). Alzheimer's disease in African Americans: risk factors and challenges for the future. *Health Aff.* 33, 580–586. doi: 10.1377/HLTHAFF.2013.1353

Beckles, Gloria L. A., and Thompson-Reid, Patricia E.. (2001). "Diabetes & Women's health across the life stages: a public health perspective." Centers for Disease Control and Prevention. Available at: https://stacks.cdc.gov/view/cdc/6426.

Blair, C., and Cybele Raver, C. (2016). Poverty, stress, and brain development: new directions for prevention and intervention. *Acad Pediatr.* 16, S30–S36. doi: 10.1016/j.acap.2016.01.010

Brown, L., Needham, B., and Ailshire, J. (2016). Telomere length among older U.S. adults: differences by race/ethnicity, gender, and age. *J. Aging Health* 29, 1350–1366. doi: 10.1177/0898264316661390

Centers for Disease Control and Prevention. (2018). "Leading causes of death–females–Non-Hispanic black–United States, 2018." CDC health equity. 2018. Available at: https://www.cdc.gov/women/lcod/2018/nonhispanic-black/index.htm.

Centers for Disease Control and Prevention. (2022). National Diabetes Statistics Report. Available at: https://www.cdc.gov/diabetes/data/statistics-report/diagnosed-diabetes.html.

Cherkas, L. F., Aviv, A., Valdes, A. M., Hunkin, J. L., Gardner, J. P., Surdulescu, G. L., et al. (2006). The effects of social status on biological aging as measured by White-blood-cell telomere length. *Aging Cell* 5, 361–365. doi: 10.1111/J.1474-9726.2006.00222.X

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., et al. (2004). Accelerated telomere shortening in response to life stress. *Proc. Natl. Acad. Sci. U. S. A.* 101, 17312–17315. doi: 10.1073/PNAS.0407162101/SUPPL_FILE/07162SUPPTEXT.HTML

Gasquoine, P. G. (2009). Race-norming of neuropsychological tests. *Neuropsychol. Rev.* 19, 250–262. doi: 10.1007/s11065-009-9090-5

Geronimus, A. T. (1992). The weathering hypothesis and the health of African-American women and infants: evidence and speculations. *Ethn. Dis.* 2, 207–221.

Geronimus, A. T., Hicken, M., Keene, D., and Bound, J. (2006). 'Weathering' and age patterns of Allostatic load scores among blacks and whites in the United States. *Am. J. Public Health* 96, 826–833. doi: 10.2105/AJPH.2004.060749

Geronimus, A. T., Hicken, M. T., Pearson, J. A., Seashols, S. J., Brown, K. L., and Cruz, T. D. (2010). Do US black women experience stress-related accelerated biological aging?: a novel theory and first population-based test of black-White differences in telomere length. *Hum. Nat.* 21, 19–38. doi: 10.1007/S12110-010-9078-0/TABLES/2

Glover, C. M., Cocroft, S., James, B. D., and Barnes, L. L. (2019). Perceptions of risk factors for Alzheimer disease among community-dwelling, nondemented older African Americans. *Alzheimer Dis. Assoc. Disord.* 33, 254–259. doi: 10.1097/WAD.0000000000000314

Green, R. C., Adrienne Cupples, L., Go, R., Benke, K. S., Edeki, T., Griffith, P. A., et al. (2002). Risk of dementia among White and African American relatives of patients with Alzheimer disease. *JAMA* 287, 329–336. doi: 10.1001/JAMA.287.3.329

Green-Harris, G., Coley, S. L., Koscik, R. L., Norris, N. C., Houston, S. L., Sager, M. A., et al. (2019). Addressing disparities in Alzheimer's disease and African-American participation in research: an asset-based community development approach. *Front. Aging Neurosci.* 11:125. doi: 10.3389/FNAGI.2019.00125

Gu, Y., Razlighi, Q. R., Zahodne, L. B., Janicki, S. C., Ichise, M., Manly, J. J., et al. (2015). Brain amyloid deposition and longitudinal cognitive decline in nondemented older subjects: results from a multi-ethnic population. *PLoS One* 10:e0123743. doi: 10.1371/journal.pone.0123743

Hill, C. V., Pérez-Stable, E. J., Anderson, N. A., and Bernard, M. A. (2015). The National Institute on Aging health disparities research framework. *Ethn. Dis.* 25, 245–254. doi: 10.18865/ED.25.3.245

Houben, J. M. J., Moonen, H. J. J., van Schooten, F. J., and Hageman, G. J. (2008). Telomere length assessment: biomarker of chronic oxidative stress? *Free Radic. Biol. Med.* 44, 235–246. doi: 10.1016/J.FREERADBIOMED.2007.10.001

Howell, J. C., Watts, K. D., Parker, M. W., Junjie, W., Kollhoff, A., Wingo, T. S., et al. (2017). Race modifies the relationship between cognition and Alzheimer's disease cerebrospinal fluid biomarkers. *Alzheimer's Res. Ther.* 9:88. doi: 10.1186/s13195-017-0315-1

Institute of Medicine (2012). "Healthy people 2010: how far have we come in reducing health disparities" in *How Far Have We Come in Reducing Health Disparities? — Progress Since 2000: Workshop Summary* (Washington, DC: National Academies Press)

Kloet, E. R., De, M. J., and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* 6, 463–475. doi: 10.1038/nrn1683

Lennon, J. C., Aita, S. L., Del Bene, V. A., Rhoads, T., Resch, Z. J., Eloi, J. M., et al. (2022). Black and White individuals differ in dementia prevalence, risk factors, and symptomatic presentation. *Alzheimer's Dement.* 18, 1461–1471. doi: 10.1002/alz.12509

Lim, U., Wang, S., Park, S. Y., Bogumil, D., Wu, A. H., Cheng, I., et al. (2022). Risk of Alzheimer's disease and related dementia by sex and race/ethnicity: the multiethnic cohort study. *Alzheimers Dement.* 18, 1625–1634. doi: 10.1002/ALZ.12528

Matthews, K. A., Wei, X., Gaglioti, A. H., Holt, J. B., Croft, J. B., Mack, D., et al. (2019). Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015–2060) in adults aged ≥65 years. *Alzheimer's Dement.* 15, 17–24. doi: 10.1016/J.JALZ.2018.06.3063

McDonough, I. M. (2017). Beta-amyloid and Cortical thickness reveal racial disparities in preclinical Alzheimer's disease. *Neuroimage Clin.* 16, 659–667. doi: 10.1016/j.nicl.2017.09.014

Millett, C., Gray, J., Bottle, A., and Majeed, A. (2008). Ethnic disparities in blood pressure Management in Patients with Hypertension after the introduction of pay for performance. *Ann. Fam. Med.* 6, 490–496. doi: 10.1370/AFM.907

Morris, J. C., Schindler, S. E., McCue, L. M., Moulder, K. L., Benzinger, T. L. S., Cruchaga, C., et al. (2019). Assessment of racial disparities in biomarkers for Alzheimer disease. *JAMA Neurol.* 76, 264–273. doi: 10.1001/JAMANEUROL.2018.4249

Omura, J. D., McGuire, L. C., Patel, R., Baumgart, M., Lamb, R., Jeffers, E. M., et al. (2022). Modifiable risk factors for Alzheimer disease and related dementias among adults aged ≥45 years — United States, 2019. *MMWR Morb. Mortal. Wkly Rep.* 71, 680–685. doi: 10.15585/mmwr.mm7120a2

Patel, R. A., Wharton, W., Bay, A. A., Ni, L., Barter, J. D., and Hackney, M. E. (2020). Association between anti-inflammatory Interleukin-10 and executive function in African American women at risk for Alzheimer's disease. *J. Clin. Exp. Neuropsychol.* 42, 647–659. doi: 10.1080/13803395.2020.1798879

Piette, J. D., Bibbins-Domingo, K., and Schillinger, D. (2006). Health care discrimination, processes of care, and diabetes patients' health status. *Patient Educ. Couns.* 60, 41–48. doi: 10.1016/J.PEC.2004.12.001

Rewak, M., Buka, S., Prescott, J., De Vivo, I., Loucks, E. B., Kawachi, I., et al. (2014). Race-related health disparities and biological aging: does rate of telomere shortening differ across blacks and whites? *Biol. Psychol.* 99, 92–99. doi: 10.1016/J.BIOPSYCHO.2014.03.007

Richmond-Rakerd, L. S., D'Souza, S., Milne, B. J., Caspi, A., and Moffitt, T. E. (2022). Longitudinal associations of mental disorders with dementia: 30-year analysis of 1.7 million New Zealand citizens. *JAMA Psychiat.* 79, 333–340. doi: 10.1001/jamapsychiatry.2021.4377

Roux, D., Ana, V., Ranjit, N., Jenny, N. S., Shea, S., Cushman, M., et al. (2009). Race/ethnicity and telomere length in the multi-ethnic study of atherosclerosis. *Aging Cell* 8, 251–257. doi: 10.1111/J.1474-9726.2009.00470.X

Satcher, D. (2008). Examining racial and ethnic disparities in health and hypertension control. *Ann. Fam. Med.* 6, 483–485. doi: 10.1370/AFM.927

Schindler, S. E., Karikari, T. K., Ashton, N. J., Henson, R. L., Yarasheski, K. E., West, T., et al. (2022). Effect of race on prediction of brain amyloidosis by plasma Aβ42/Aβ40, phosphorylated tau, and Neurofilament light. *Neurology* 99, e245–e257. doi: 10.1212/WNL.0000000000200358

Smedley, Brian D., Stith, Adrienne Y., and Nelson, Alan R.. (2003). *Unequal Treatment: Confronting Racial and Ethnic Disparities in Health Care*. Washington, DC: National Academies Press, 1–764.

Stampfer, M. J. (2006). Cardiovascular disease and Alzheimer's disease: common links. *J. Intern. Med.* 260, 211–223. doi: 10.1111/J.1365-2796.2006.01687.X

Stephenson, J. (2001). Racial barriers may hamper diagnosis, Care of Patients with Alzheimer Disease. *JAMA* 286, 779–780. doi: 10.1001/JAMA.286.7.779-JMN0815-3-1

Us Against Alzheimer's. (2021). "2021 Annual Report." Washington, DC. Available at: https://www.usagainstalzheimers.org/networks/african-americans.

Virani, S. S., Alonso, A., Aparicio, H. J., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., et al. (2021). Heart disease and stroke statistics—2021 update. *Circulation* 143, E254–E743. doi: 10.1161/CIR.0000000000000950

Vyas, D. A., Eisenstein, L. G., and Jones, D. S. (2020). Hidden in plain sight — reconsidering the use of race correction in clinical algorithms. *N. Engl. J. Med.* 383, 874–882. doi: 10.1056/NEJMms2004740

Williams, E. P., Mesidor, M., Winters, K., Dubbert, P. M., and Wyatt, S. B. (2015). Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. *Curr. Obes. Rep.* 4, 363–370. doi: 10.1007/S13679-015-0169-4

Younan, D., Wang, X., Gruenewald, T., Gatz, M., Serre, M. L., Vizuete, W., et al. (2022). Racial/ethnic disparities in Alzheimer's disease risk: role of exposure to ambient fine particles. *J. Gerontol. Ser. A* 77, 977–985. doi: 10.1093/GERONA/GLAB231

Zissimopoulos, J. M., Barthold, D., Brinton, R. D., and Joyce, G. (2017). Sex and race differences in the association between statin use and the incidence of Alzheimer disease. *JAMA Neurol.* 74, 225–232. doi: 10.1001/jamaneurol.2016.3783

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Experiences with and perspectives on advance care planning in young- and late-onset dementia: A focus group study with physicians from various disciplines

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Introduction: Despite the relevance of advance care planning (ACP) for people with dementia, its uptake in this population is particularly low. Several challenges for ACP in dementia have been identified from physicians' perspectives. However, the literature available mainly includes general practitioners and focuses exclusively on the context of late-onset dementia. This is the first study to inquire physicians from four highly relevant specialisms in dementia care, with a focus toward potential specificities based on patients' age. The research question of this study is: "What are physicians' experiences with and perspectives on discussing ACP with people with young- and/or late-onset dementia?".

Methods: Five online focus groups were conducted with 21 physicians (general practitioners, psychiatrists, neurologists and geriatricians) in Flanders, Belgium. Verbatim transcripts were analyzed through the qualitative method of constant comparative analysis.

Results: Physicians believed that the societal stigma related to dementia influences people's reaction to their diagnosis, at times characterized by catastrophic expectations for the future. In this regard, they explained that the topic of euthanasia is sometimes addressed by patients very early in the disease trajectory. Respondents paid ample attention to actual end-of-life decisions, including DNR directives, when discussing ACP in dementia. Physicians felt responsible for providing accurate information on both dementia as a condition, and the legal framework of end-of-life decisions. Most participants felt that patients' and caregivers' wish for ACP was more driven by who their personality than by their age. Nonetheless, physicians identified specificities for a younger dementia population in terms of ACP: they believed that ACP covered more domains of life than for older persons. A high consistency regarding the viewpoints of physicians from differing specialisms was noted.

Discussion: Physicians acknowledge the added value of ACP for people with dementia and especially their caregivers. However, they face several challenges for engaging in the process. Attending to specific needs in young-onset, in comparison to late-onset dementia, requires ACP to entail more than solely medical domains. However, a medicalized view on ACP still appears to be dominant in practice as opposed to its broader conceptualization in academia.

KEYWORDS

advance care planning (ACP), young-onset dementia (YOD), focus group (FG), physicians, late-onset dementia

Introduction

Advance care planning (ACP) is defined as a process of communication between patients, family caregivers and professionals to explore patients' preferences for future (medical) care, including at the end of life (Sudore et al., 2017). The concept has evolved considerably over time, now focusing on an ongoing process that also helps prepare people for "in the moment decision making" when necessary, rather than focusing on the completion of advance directives (Van den Block, 2019; Tishelman et al., 2021). In general, dementia leaves people with a relatively long timeframe of loss of ability to self-manage care and diminishing cognitive function (Gaster et al., 2017). Despite ongoing discussions about the value of ACP (Tishelman et al., 2021), it is argued that ACP can be particularly relevant for people with dementia and their caregivers as the condition eventually precludes patients from taking part in their own treatment decisions (Alam et al., 2022). In case of Alzheimer's Disease, diagnosis can be made during stages of mild cognitive impairment. The larger timeframe for planning care, due to earlier diagnosis, increases the opportunity for and importance of ACP (Porsteinsson et al., 2021). Nonetheless, the uptake of ACP in dementia is low with less than 40% of patients worldwide undertaking ACP (Sellars et al., 2019). Research showed that having dementia, in comparison to other conditions, is negatively associated with discussing treatment preferences, indicating that there are certain specific challenges related to engaging in ACP in dementia (Evans et al., 2014).

Particularly in dementia, discussing future care is considered difficult due to uncertainties regarding the future and due to the jeopardized decisional capacity of people with dementia (Tilburgs et al., 2018a; Sellars et al., 2019). More specifically, a recent meta-review of systematic reviews and primary studies (Keijzer-van Laarhoven et al., 2020) showed that physicians feel responsible for providing high-quality end-of-life care to people with dementia but face moral dilemmas that may cause them to behave avoidantly toward initiating ACP. Among others, these dilemmas arise from not wanting to emotionally burden patients, trying to maintain hope, dealing with uncertainties in patients' prognoses and having ethical concern regarding patients' declining capacity (Keijzer-van Laarhoven et al., 2020). Fearing a shift in patients' preferences as the condition progresses was also identified as causing reluctance for physicians to make advance decisions with people with dementia (De Vleminck et al., 2014). Conversely, a qualitative study also found that beliefs about the perceived benefits of ACP can motivate physicians to engage people with dementia in the process, such as the belief that ACP would align patients', family caregivers' and clinicians' care goals (Alam et al., 2022).

For a more inclusive understanding of physicians' attitudes and challenges in terms of ACP in dementia, several physician specialties that are essential in dementia diagnosis and care should be inquired. Although there is literature available, these studies mainly include general practitioners and focus exclusively on the context of late-onset dementia (De Vleminck et al., 2014; Tilburgs et al., 2018b; Alam et al., 2022). There is a dearth of studies that inquire physicians from various specialisms. Moreover, research in which physicians are questioned about their perspectives not only regarding late-onset, but also young-onset dementia (YOD) is absent. Globally, it is estimated that 370,000 people younger than 65 develop dementia symptoms before the age of 65 annually, defined by the term YOD (Hendriks et al., 2021). The very limited number of studies focusing on people with YOD and their family caregivers, showed that they barely engage in ACP, yet have clear preferences for how to do so (Van Rickstal et al., 2019). Among others, these include their wish for physicians to timely initiate and flexibly approach the process, provide accurate information and pay attention to more than only the medical aspects of care (Van Rickstal et al., 2019, 2022).

To the best of our knowledge, this is the first inquiry of physicians from four highly relevant specialisms in dementia care (GP's, psychiatrists, neurologists and geriatricians) regarding ACP, with a specific interest toward the potential specificities depending on patients' age at diagnosis. The research question of this study is: "What are physicians' experiences with and perspectives on discussing ACP with people with young- and/or late-onset dementia?".

Materials and methods

Design

This exploratory study used the qualitative research method of focus groups, as this approach allows for open discussion and interaction between participants. Conducting focus groups online was necessary due to the COVID-regulations at the time yet was also an attempt to minimize participation burden for already challenged healthcare providers. In adherence with a recent guideline for virtual qualitative data-collection (Dos Santos Marques et al., 2021), the maximum participants per focus

group was lowered (n = 5) to facilitate in-depth discussion. This paper follows the COREQ-criteria for reporting qualitative research.

Participants

To answer our research question, we aimed for a heterogeneous sample in terms of physicians' specialism within focus groups, to allow for in-depth insights. We included general practitioners, neurologists, psychiatrists and geriatricians as these specialties are crucial in the care for people with dementia. Physicians were purposively sampled through a personal email of the main researcher (RVR) or through a general recruitment mail spread within several organizations (Belgian Dementia Council, and the Flemish Associations for Psychiatry, Geriatrics, and Neurology). After physicians expressed their willingness to participate, they were sent a doodle in which they could indicate suitable moments for the focus group to take place.

Data-collection

For these focus groups, an interview guide consisting of open-ended questions was developed within the research team (see **Box 1**). Participants were informed about some important "ground rules" at the start of each focus group, such as no talking across each other, respecting confidentiality regarding others' participation, the content of discussions, etc. Each focus group was moderated and observed by two researchers (four by RVR and ADV, one by RVR, and LVdB). The focus groups took place online through secured Zoom-meetings in November and December 2021. The focus groups were conducted in Dutch, were video- and audiotaped with participants' consent and were transcribed verbatim. After the fifth focus group, researchers reached consensus that data-saturation had been reached and no additional focus groups needed to be organized.

Data-analysis

Verbatim transcripts of the focus groups were analyzed through the qualitative method of constant comparative analysis (Hewitt-Taylor, 2001; Dierckx de Casterlé et al., 2012). In this inductive approach, a code is assigned to a certain idea or concept (usually one or two sentences). These codes are subsequently compared within and between transcripts, identifying broader themes or concepts. Two transcripts were read and coded in full independently by two researchers. After discussion and agreement on a coding structure, the remaining three transcripts were coded and analyzed by RVR. Once coding was completed and codes were added to the coding framework, RVR and ADV together revised the transcripts and the obtained coding structure.

Ethics

The study was approved by the Ethics Committee of the University Hospital Brussels (B.U.N. 143201939497) as the central commission and by Hospital Network Antwerp (ZNA, approval no 5208) and GasthuisZusters Antwerp (GZA, 190304ACADEM) as local commissions. A signed informed consent was obtained by all participants prior to the start of the focus group.

BOX 1 Focus group topic guide.

1. Introduction

Description of ACP provided by researchers:

"Advance care planning is a process of communication between patients, their family caregivers and professionals in which patients' views, values and preferences for future (medical) care are explored. This process should enable patients to help guide future decisions (also at those times when they are no longer able to make or express choices). ACP can, but does not necessarily, result in the documentation of wishes in advance directives"

To what extent is this description similar to how you conceptualize ACP/your understanding of the concept?

Throughout the following questions, respondents were systematically asked if there were any specificities in case of young- vs. late-onset dementia.

2. Experience with ACP

To what extent do you engage in ACP in your clinical practice?

If you engage in ACP with patients/family caregivers:
Who usually initiates the communication?
If at physician's initiative: How do you usually initiate ACP?

Is there, in your experience, **a right time** to initiate ACP?

Who is usually involved in ACP? (patients, family caregivers, other care professionals,. . .)

What are important **topics** to discuss within ACP?

Are there specific **hindering factors** when it comes to engaging in ACP in case of dementia?

3. Wish to engage in ACP from patients/caregivers

In your experience, to what extent do you feel there is a **need/wish for ACP** from patients and their family caregivers?

What is the **added value** of engaging in ACP in dementia? Is there a difference in this value, in your perspective, for patients vs. for family caregivers?

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Results

The average duration of a focus group was 95 min. A total of 21 physicians took part in one of five focus groups (two $n = 5$, two $n = 4$, one $n = 3$). Of these 21 physicians, five were general practitioners, three were specialized in psychiatry, six in neurology, and five in geriatrics. Except for one last-year neurology resident, all were board-certified specialists. Five women and 16 men participated.

Six major themes were identified from our data: (1) stigmatic image related to dementia as a specificity for ACP in this population, (2) physicians' focus on specific end-of-life decisions when discussing ACP in dementia, (3) physicians feeling responsible for providing information on dementia and on the law regarding end-of-life decisions, (4) the age of patients and caregivers as an influence on the content of ACP, (5) physicians seeing more benefits of ACP for family carers, and (6) congruency between medical professions. Several of our findings are generally related to dementia as a condition and can therefore be interpreted as applicable to both the young- and late-onset variant.

Stigmatic image related to the condition as a specificity for ACP in dementia

A factor that physicians believed to negatively influence patients' fears and concerns about the future, was the stigma related to dementia. In this regard they discussed how the popular media is at times responsible for diminishing nuances in people's image of dementia: the last phase of disease progression is portrayed as representative for the entire disease trajectory.

> *"That one quickly thinks that it's only about that last vegetative stage and that one would also end up there very soon etcetera. In the beginning, that's something that strongly traverses those conversations. One doesn't know that there are many years preceding that" (FG 24, 138–140).*

Despite patients' initial expectations regarding their disease progression, physicians referred to people with dementia who, along the way, sometimes find their trajectory more manageable than initially expected. From their perspective, this posed a difficulty for engaging in ACP, since the evolution of patients' wishes was felt to be too unpredictable to offer guidance for future care decisions.

> *"If they say 'I don't ever want to be in a wheelchair,' or 'I always want to be able to feed myself,' or something like that, then eventually, when push comes to shove, they don't mind being wheeled around or they don't mind that they're being cooked for. So, it changes so much that it's not fully predictable" (FG23, 170).*

Some physicians explained that the "catastrophic" image of dementia at times caused patients to drastically react to receiving their diagnosis and that they, and especially younger patients, quite impulsively expressed a wish for euthanasia the moment of or soon after hearing their diagnosis.

> *"When disclosing the probable diagnosis or the results, people very often or at least several times show a catastrophic reaction and then they immediately start thinking about that last stage" (FG24, 146).*

> *"Yes, and with people with young-onset dementia." There are a few patients who at the moment of diagnosis nearly immediately say "okay, I have said that I want euthanasia in that case" (FG65, 89).*

This moment was said to be grasped by physicians as an opportunity for further exploration, explanation and broader discussion of preferences.

> *"If you then assess 'what motivates that (euthanasia) question?' or 'what is truly behind it?'. . . Then you actually arrive at a much broader framework of care planning that basically no longer entails what the initial question for euthanasia was, but more about care and planning and those things. . ." (FG24, 75/76).*

Physicians' focus on specific end-of-life decisions when discussing ACP in dementia

All participating physicians were familiar with the description of ACP provided at the beginning of the focus groups. However, it became apparent that physicians mostly elaborated on or re-directed the conversation to a specific aspect of ACP, namely to anticipatory end-of-life decision-making, such as DNR-orders (do not resuscitate) and euthanasia.

Physicians' perceived motives behind euthanasia requests

According to our participants, the request for euthanasia was usually a request for something else in terms of future care. In most cases, it turned out to be the patient's expression of a concern for which they sought guidance rather than an actual wish for euthanasia.

> *"In many cases it turns out that it (euthanasia request) is about other concerns that can easily be addressed in a different way and then the question disappears" (FG24, 84).*

> *"Actually they are not asking for euthanasia, they are asking the question 'if I end up in circumstances that I don't find dignified, are you still going to help me?'" (FG43, 94).*

This was also explained by physicians through the motives on which they thought these patients' comments on euthanasia or euthanasia requests were based. Participants mentioned that these could stem from agitation about what the future will bring, unwillingness to move to a residential care facility and fear of the unknown.

> *"What is said frequently, is 'Yes, if I would have to go to a nursing home, then I'm done. I don't want to live like a vegetable. I've seen it with my mother or my father. Then, I would actually prefer euthanasia and I want you to write that down in my file like that'" (FR44, 171).*

Physicians felt responsible for providing information on dementia and on the legal framework of end-of-life decisions

Many physicians also felt that media had contributed to both the public's awareness about euthanasia as an end-of-life option and had contributed to confusion about what is possible or impossible under Belgian law. Explaining patients about the legal framework was said to be an important task in clinical practice in terms of ACP.

> *"So, a big part of the time or a big part of the energy goes out to just explaining what's possible and what isn't possible" (FG44, 186).*

Additionally, it was mentioned that providing information (in terms of for instance law or prognosis) could function as a care intervention itself.

> *"I often notice that by discussing and explaining it (the legal framework) and by defining it, they sometimes find some peace already. That that request (euthanasia) sometimes stems from fear of the unknown and that informing them is at times already sufficient to find peace. That the questions then sometimes also fade away to the background" (FG 24, 72–74).*

According to our participants, patients tended to hold a "catastrophic" view of (young-onset) dementia, characterized by drastically declining functional and cognitive abilities. Driven by this alarming image, patients at times initiate ACP or euthanasia discussions according to physicians. In this regard, participants underscore a clear need for education in the sense of prognostic information.

> *"If we get the question (euthanasia), it's usually indeed a question for, yes. . . that has a whole lot to do with the stigma around dementia, I think. Many people regard someone with Alzheimer's disease as someone who sits in a wheelchair, drooling, in a nursing home, as a figure of speech. But of course that's not always the stage that everyone progresses to. So, I think that it's important to educate a bit in terms of what the possible patterns and expectations can be" (FG43, 101–104).*

In terms of discussing prognosis, physicians explained they typically use "vague" terms and "averages" when describing a patient's medical future. This manner of communication was based on both clinical uncertainty about the dementia trajectory according to participants, and physicians' wish to safeguard patient's hope and positive emotions.

> *"General terms are averages: but I try to avoid making individual predictions" (FG44, 109).*

> *"One of the biggest problems from my experience is that, often, we are also not honest toward our patients with dementia" (FG43, 171–172).*

Although patients' image of dementia might be "catastrophic" at times and in need of nuanced information, some physicians emphasized that one cannot deny the inevitable negative aspects when going through the entirety of a dementia trajectory. Participants felt that these aspects are difficult to disclose openly to patients.

> *"It doesn't always have to be as bad as dying drooling in a nursing home, but well, the cases in which the older man, the grumpy old man becomes the endearing father, those are less frequent than the other story" (FG43, 202).*

> *"If we take good care of them and place them in a decent nursing home, then they die of, well, what do they actually die of? Do they starve? Do they have a spontaneous fracture because they have been lying in bed for years?" (FG43, 176–177).*

They expressed that a longitudinal and trusting relationship between patient and physician increased their "openness and honesty" in terms of disclosing prognostic information, for instance about the speed of disease progression or expected difficulties ahead.

> *"The way in which you get more concrete in terms of prognosis, that's also an advancing insight. After the diagnosis, the progression, the first two years that always gives an indication of how quickly it could evolve" (FG65, 166–167).*

> *"And you don't name it with, yeah, terms that are hurtful, but yeah. . . sometimes we have known these people for years. Yes, then I dare to be honest about it (prognosis). I'm quite straightforward and the people who continue to come into consultations with me, are the ones who can tolerate that and even expect it" (FG43, 180–181).*

The age of patients and caregivers influences the content of ACP discussions

It was noted that both people with young-, as well as people with late-onset dementia are heterogeneous groups. The extent to which people wish to engage in ACP was generally regarded as connected to who the patient was as a person, rather than associated to the patient's age.

> *"There are people, both among younger patients, but also among older patients, who are very set on their autonomy and from that perspective can also be very verbal and have a clear request for ACP or other things. Just as well, there are younger patients who would rather avoid that type of conversation" (FG24, 98–99).*

Although some physicians said that younger vs. older people with dementia are usually more "articulate," "assertive," and "have a higher need for control", the majority of physicians saw an equal amount of younger and older patients wanting to discuss ACP. However, they noted that the life context of younger people, with younger children and spouses, might make their questions about the end of life more salient.

> *"I can imagine that under those circumstances the questions about wishes for the end of life are much more prominently present and that one contemplates it much more at that age compared to at an older age. With these younger people, they (wishes for the end of life) will be brought up sooner or later" (FG65, 109–110).*

Several physicians talked about how YOD, in comparison to late-onset dementia, might lead to diminished acceptance of the diagnosis, higher grief and to more conflict within families, among others about financial matters.

> *"Older people already let go of life a bit more and accept that there they are at a high age at which things will end sooner or later" (FG65, 81).*

> *"There is also much more sadness of people with young-onset dementia, for so many good years lost" (FG65, 139).*

Respondents explained that caregivers at times had a higher wish to engage in ACP than patients. Examples provided were when patients did not grasp the implications of their condition, were no longer cognitively competent or when patients had expressed a death-wish to their caregiver, who wanted to discuss this further with the physician. Several physicians explained that during their consultations, caregivers of younger as opposed to older, people showed more tendency to bring up ACP.

> *"Of course I have people who have no illness awareness, and especially in that case that question will arise through the caregiver. Especially if there is no awareness of illness, then it all appears very 'far off' for the patient, and that can be difficult at times" (FG65, 57–58).*

> *"Yes, it regularly occurs that some type of death wish was expressed by the patient and that that actually is the impetus for the partner or the children to initiate that conversation. They often refer back to it like 'you remember that you've said that, what do you actually mean by that?'" (FG65, 64–65).*

Additionally, physicians explained that ACP discussions usually cover more domains in YOD due to the challenges the diagnosis brings along in multiple areas of patients' and caregivers' lives.

> *"Evidently, with younger people there is often the difficulty of the partner still working, that the children are still young, still studying, at times still living at home, which actually complicates it even more. Then that is a broader conversation, because it becomes even more difficult with caregivers themselves, that conversation" (FG65, 135).*

> *"If there are children who are still young and who, just to give an example, become scared of their father or mother, or where their relationship changes entirely. Or a professional situation, people who are still working. You simply come across many more problems, which obliges one to consider at least a mid-long timeframe" (FG44, 133–134).*

ACP was believed to especially benefit family caregivers

Several advantages of ACP engagement were discussed by physicians, for the majority relating to family caregivers. ACP was told to lead to an 'emotional relief', less conflict and less suffering since family caregivers were enabled to fulfill their need to provide care to their loved-one.

> *"And I think that for family it's also very important to have that feeling like 'we are doing well, we have done well'" (FG24, 33).*

Physicians believed that both patients and caregivers would assess the care provided as more positive, due to ACP.

> *"The bottom line is of course that people, the caregiver as well as the patient, will evaluate the care received more positively in the sense that they feel it is more closely aligned to what they wish" (FG24, 23).*

From patients' perspective, physicians hypothesized that not wanting to be a burden to others might be a motivating factor ACP, aside from keeping their own best interest for the future in mind.

> *"By some (patients) it is indeed addressed that they somehow do it (ACP) for the caregivers, but it's not an 'or-or story', it's a combination of how they themselves feel about it" (FG65, 188).*

Patients' need and desire to take care of their family and ACP as a means to fulfill that need, was noted as well.

> *"That's also partly taking care of my children. That's drafting a care plan, so that my children know that it's okay what they do or not do with me" (FG43, 249).*

Congruency between medical professions

There were no divergent themes when comparing between physicians from differing specialties. Moreover, there appeared to

be a consensus amongst respondents that general practitioners are usually able to play a key role in ACP, due to their usually longstanding relationship with the patient and his/her family, and their professional context in which they are more likely to have frequent consultations with patients, possibly including home visits. It was noted that systematic sharing of ACP information between the various physicians involved in a patient's care was desirable, yet that such information flow was not sufficiently common.

Discussion

Summary of results

This study shows that physicians believe that the societal stigma related to dementia impacts how people react to their diagnosis, including catastrophic expectations for their future. In this regard, they mentioned that the topic of euthanasia is at times addressed early in the disease trajectory by patients. Physicians themselves paid ample attention to actual end-of-life decisions, including DNR directives, when discussing ACP in dementia. As part of ACP, physicians felt it was their responsibility to provide accurate information on both dementia as a condition, and the legal framework of end-of-life decisions. Most participants felt that patients' and caregivers' wish for ACP was more driven by who they are as people than by their age. Physicians did identify specificities for a younger dementia population in terms of ACP: they believed that ACP covered more domains of life than for older persons. A high consistency regarding the viewpoints of physicians from differing specialisms was noted.

Strengths and limitations

The main strength of this study is that it assembled focus groups heterogeneously in terms of specialisms crucial in dementia care, allowing for in-depth insights from and for various medical disciplines. Our research question focused on people with late-onset, as well as with young-onset dementia. This led to findings that are insightful for clinicians, when caring for this underexposed group. A limitation of this study is that we did not observe actual practices, but analyzed what respondents shared about these practices. Also, certain results might be less or not generalizable to other legal contexts besides those with physician- assisted dying laws. In this regard, however, we deem our results to be informative within the current internationally evolving landscape of physician-assisted dying legislation. Future comparative research in countries with varying legislative frameworks would be insightful for understanding the possible impact of the law on ACP and on ACP communication.

Interpretation of findings

Physicians explained that, at times, they struggled with disclosing prognostic information due to clinical uncertainty characteristic to dementia. The difficulty or even inability to provide accurate prognostic information experienced by our participants, has also been reported by patients and family caregivers in different countries (Sellars et al., 2019). It has been shown that patients and family caregivers felt a distrust toward clinician's mastery and knowledge of dementia (Groen-van de Ven et al., 2017). Physicians communicating openly to their patients about their uncertainty, might counter such feeling of distrust and contribute to a relationship of mutual confidence and trust. This could in turn facilitate ACP, as a sense of rapport was previously identified as a prerequisite for ACP in dementia by patients, their caregivers and general practitioners (Tilburgs et al., 2018b; Van Rickstal et al., 2019). Attending to not only patients' and caregivers' uncertainty in decision-making (Sellars et al., 2019), but also to that of physicians, might empower all parties when it comes to initiating ACP. Comparing our findings with existing literature, showed that there is an important commonality between barriers identified by professional caregivers on the one hand, and barriers identified by family caregivers and patients on the other.

Physicians explained that disclosing prognostic information might also be hampered by constraints they experience in openly and honestly communicating about disease progression. Although participants acknowledged that a dementia trajectory undeniably has negative elements, they simultaneously pleated for a more nuanced image of dementia, with a sometimes more steadily progression than expected or feared. Qualitative studies showed that people with dementia and their caregivers tend to oscillate between "wanting to know" and "not wanting to know" (Wawrziczny et al., 2016) and prefer to take it "one day at a time" (Van Rickstal et al., 2019; Keijzer-van Laarhoven et al., 2020). Additionally, people with late- and young-onset dementia and their caregivers have previously highlighted that, regardless of being diagnosed with dementia, there is still room for enjoyment (Dening et al., 2017; Van Rickstal et al., 2019). Moreover, previous research showed that focusing on the present as opposed to worrying about the future, is associated with experiencing fewer unmet needs and therefore is an effective coping strategy (Millenaar et al., 2018). Having a realistic and truthful view on the future, yet also allowing hope and positivity to co-exist with this, appears a useful balancing act to be undertaken by all those involved when engaging in ACP. When placing our finding in the context of findings with patients and caregivers, it appears that physicians' moral threshold to engaging in ACP, also described in previous research (Keijzer-van Laarhoven et al., 2020), is at times justified. According to participants, the societal negative image that is related to dementia increases the need for realistic information provision. Physicians in our study described how patients at times demonstrate catastrophic reactions to receiving a diagnosis, also based on the common, stigmatic image of dementia. If grasped by physicians, these reactions might function as a steppingstone to discuss ACP more broadly, according to them.

In the current study, ACP was considered by physicians to be a means or an opportunity for people with dementia of fulfilling a caretaking role toward their family. It has been previously stated by patients and caregivers that if people with dementia undertake ACP, one of their main purposes is to take care of their loved-ones (Van Rickstal et al., 2022). The relational, as opposed to purely individual, nature of ACP appears evident from the viewpoint of all parties involved. As such, particularly in the context of dementia, a family- rather than a solely patient-centered approached to ACP

could be desirable. As physicians also expressed that their wish to safeguard patients' emotional wellbeing shapes their own behavior in terms of ACP, the previous idea of a mutual protective role between people with dementia and their family caregiver (Van Rickstal et al., 2022) could be extended from a dyad to a protective triad which also includes the professional caregiver.

Physicians identified specificities for the content of ACP in YOD. The desire for ACP was put forward by our respondents as person- rather than age- and/or generation- related, depending on someone's personality. Nonetheless, several specificities in terms of age were mentioned. Young-onset dementia usually affects people in the prime of life, with possibly children still living at home, financial commitments, work, and at times caring for older relatives themselves (Withall, 2013; Draper and Withall, 2016). According to our respondents, ACP in YOD was indeed considered to cover a broader range of domains due to the plurality of life-areas affected by the condition. Additionally, if the topic arose, it was told to be more prominently present in consultations with younger as opposed to older patients and caregivers. The general hypothesis that younger people with dementia and their caregivers have a higher need for ACP due to a higher wish for autonomy (Koopmans et al., 2015), appears not to correspond with patients', family caregivers' (Van Rickstal et al., 2019, 2022) or professionals' narratives. However, our former and current research shows that all parties do acknowledge that the content of ACP shows distinctions based on patients' younger vs. older age, mainly due to stage of life. Through insights of patients with YOD and their carers it was previously recommended to conceptualize ACP as holistic (Van Rickstal et al., 2022), consistent with respondents of the current study who explain that ACP in YOD can entail a broader range of topics. Overall consensus seems to exist that clinicians need to dedicate heightened attention to non-medical domains to adequately address ACP in this younger population. However, it was formerly shown that Flemish people with YOD and their carers spontaneously incorporate euthanasia in their thought framework on end of life (Van Rickstal et al., 2020), and as such, it can also be regarded as a sensitivity from our participating physicians toward their patients that they pay adequate attention to end-of-life decisions. It appears recommended to find a balance between broadening ACP to medical, social and relational domains (Tilburgs et al., 2018b; Van Rickstal et al., 2022), yet simultaneously elaborating on specific concerns patients have, such as euthanasia, if this were the case.

Conclusion

Overall, physicians acknowledge the benefits of ACP for people living with dementia and particularly for their family yet describe several challenges for actually engaging in the process. Some of these difficulties are related to dementia as a condition, others are associated with constraints for engaging in such conversations. Attending to specificities in terms of ACP for people with young-onset, compared to late-onset, requires physicians to pay attention toward non-medical domains. The finding that participants elaborated on actual end-of-life decisions, such as euthanasia and Do Not Resuscitate- directives, shows that the medicalized concept of ACP is still dominant in practice.

Data availability statement

The datasets presented in this article are not readily available because we are unable to provide raw data as these include identifiable information from respondents (focus groups with physicians). Requests to access the datasets should be directed to RV, romy.van.rickstal@vub.be.

Ethics statement

This study was approved by the Ethics Committee of the University Hospital Brussels (B.U.N. 143201939497) as the central commission and by Hospital Network Antwerp (ZNA, approval no. 5208) and GasthuisZusters Antwerpen (GZA, 190304ACADEM) as local commissions. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alam, A., Barton, C., Prathivadi, P., and Mazza, D. (2022). Advance care planning in dementia: a qualitative study of Australian general practitioners. *Aust. J. Prim. Health* 28, 69–75. doi: 10.1071/PY20307

De Vleminck, A., Pardon, K., Beernaert, K., Deschepper, R., Houttekier, D., Van Audenhove, C., et al. (2014). Barriers to advance care planning in cancer, heart failure and dementia patients: a focus group study on general practitioners' views and experiences. *PLoS One* 9:e84905. doi: 10.1371/journal.pone.0084905

Dening, K. H., King, M., Jones, L., and Sampson, E. L. (2017). Healthcare decision-making: past present and future, in light of a diagnosis of dementia. *Int. J. Palliat. Nurs.* 23, 4–11. doi: 10.12968/ijpn.2017.23.1.4

Dierckx de Casterlé, B., Gastmans, C., Bryon, E., and Denier, Y. (2012). QUAGOL: a guide for qualitative data analysis. *Int. J. Nurs. Stud.* 49, 360–371. doi: 10.1016/j.ijnurstu.2011.09.012

Dos Santos Marques, I. C., Theiss, L. M., Johnson, C. Y., McLin, E., Ruf, B. A., Vickers, S. M., et al. (2021). Implementation of virtual focus groups for qualitative data collection in a global pandemic. *Am. J. Surg.* 221, 918–922. doi: 10.1016/j.amjsurg.2020.10.009

Draper, B., and Withall, A. (2016). Young onset dementia. *Int. Med. J.* 46, 779–786. doi: 10.1111/imj.13099

Evans, N., Pasman, H. R., Donker, G. A., Deliens, L., Van den Block, L., Onwuteaka-Philipsen, B., et al. (2014). End-of-life care in general practice: A cross-sectional, retrospective survey of 'cancer', 'organ failure' and 'old-age/dementia' patients. *Palliat. Med.* 28, 965–975. doi: 10.1177/0269216314526271

Gaster, B., Larson, E. B., and Curtis, J. R. (2017). Advance directives for dementia: meeting a unique challenge. *JAMA* 318, 2175–2176. doi: 10.1001/jama.2017.16473

Groen-van de Ven, L., Smits, C., Oldewarris, K., Span, M., Jukema, J., Eefsting, J., et al. (2017). Decision trajectories in dementia care networks: decisions and related key events. *Res. Aging* 39, 1039–1071. doi: 10.1177/0164027516656741

Hendriks, S., Peetoom, K., Bakker, C., van der Flier, W. M., Papma, J. M., Koopmans, R., et al. (2021). Global prevalence of young-onset dementia: a systematic review and meta-analysis. *JAMA Neurol.* 78, 1080–1090. doi: 10.1001/jamaneurol.2021.2161

Hewitt-Taylor, J. (2001). Use of constant comparative analysis in qualitative research. *Nurs. Std* 15, 39–42. doi: 10.7748/ns2001.07.15.42.39.c3052

Keijzer-van Laarhoven, A. J., Touwen, D. P., Tilburgs, B., van Tilborg-den Boeft, M., Pees, C., Achterberg, W. P., et al. (2020). Which moral barriers and facilitators do physicians encounter in advance care planning conversations about the end of life of persons with dementia? A meta-review of systematic reviews and primary studies. *BMJ Open* 10:e038528. doi: 10.1136/bmjopen-2020-038528

Koopmans, R. T., van der Steen, J. T., and Bakker, C. (2015). Palliative care in people with young-onset dementia (yod): an undiscovered area! *J. Am. Med. Direct. Assoc.* 16, 1008–1009. doi: 10.1016/j.jamda.2015.07.001

Millenaar, J. K., Bakker, C., van Vliet, D., Koopmans, R. T. C. M., Kurz, A., Verhey, F. R. J., et al. (2018). Exploring perspectives of young onset dementia caregivers with high versus low unmet needs. *Int. J. Geriatr. Psychiatry* 33, 340–347. doi: 10.1002/gps.4749

Porsteinsson, A. P., Isaacson, R. S., Knox, S., Sabbagh, M. N., and Rubino, I. (2021). Diagnosis of Early Alzheimer's disease: clinical practice in 2021. *J. Prevent. Alzheimer's dis.* 8, 371–386. doi: 10.14283/jpad.2021.23

Sellars, M., Chung, O., Nolte, L., Tong, A., Pond, D., Fetherstonhaugh, D., et al. (2019). Perspectives of people with dementia and carers on advance care planning and end-of-life care: A systematic review and thematic synthesis of qualitative studies. *Palliat. Med.* 33, 274–290. doi: 10.1177/0269216318809571

Sudore, R. L., Lum, H. D., You, J. J., Hanson, L. C., Meier, D. E., Pantilat, S. Z., et al. (2017). Defining advance care planning for adults: a consensus definition from a multidisciplinary delphi panel. *J. Pain Sympt. Manag.* 53, 821–832.e1. doi: 10.1016/j.jpainsymman.2016.12.331

Tilburgs, B., Vernooij-Dassen, M., Koopmans, R., van Gennip, H., Engels, Y., and Perry, M. (2018a). Barriers and facilitators for GPs in dementia advance care planning: A systematic integrative review. *PLoS One* 13:e0198535. doi: 10.1371/journal.pone.0198535

Tilburgs, B., Vernooij-Dassen, M., Koopmans, R., Weidema, M., Perry, M., and Engels, Y. (2018b). The importance of trust-based relations and a holistic approach in advance care planning with people with dementia in primary care: a qualitative study. *BMC Geriatr.* 18:184. doi: 10.1186/s12877-018-0872-6

Tishelman, C., Eneslätt, M., Menkin, E. S., and Van Den Block, L. (2021). Tishelman et al's response to morrison: advance directives/care planning: clear. Simple, and wrong *J. Palliat. Med.* 24, 16–17. doi: 10.1089/jpm.2020.0540

Van den Block, L. (2019). Advancing research on advance care planning in dementia. *Palliat. Med.* 33, 259–261. doi: 10.1177/0269216319826411

Van Rickstal, R., De Vleminck, A., Aldridge, M. D., Morrison, S. R., Koopmans, R. T., van der Steen, J. T., et al. (2019). Limited engagement in, yet clear preferences for advance care planning in young-onset dementia: An exploratory interview-study with family caregivers. *Palliat. Med.* 33, 1166–1175. doi: 10.1177/0269216319864777

Van Rickstal, R., De Vleminck, A., Morrison, S. R., Koopmans, R. T., van der Steen, J. T., Engelborghs, S., et al. (2020). Comparing advance care planning in young-onset Dementia in the USA vs Belgium: Challenges partly related to societal context. *J. Am. Med. Direct. Assoc.* 21, 851–857. doi: 10.1016/j.jamda.2020.01.007

Van Rickstal, R., Vleminck, A., Engelborghs, S., Versijpt, J., and Van den Block, L. (2022). A qualitative study with people with young-onset dementia and their family caregivers on advance care planning: A holistic, flexible, and relational approach is recommended. *Palliat. Med.* 36, 964–975. doi: 10.1177/02692163221090385

Wawrziczny, E., Pasquier, F., Ducharme, F., Kergoat, M. J., and Antoine, P. (2016). From 'needing to know' to 'needing not to know more': an interpretative phenomenological analysis of couples' experiences with early-onset Alzheimer's disease. *Scand. J. Caring Sci.* 30, 695–703. doi: 10.1111/scs.12290

Withall, A. (2013). The challenges of service provision in younger-onset dementia. *J. Am. Med. Direct. Assoc.* 14, 230–232. doi: 10.1016/j.jamda.2013.01.012

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Sex and gender considerations in Alzheimer's disease: The Women's Brain Project contribution

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The global population is expected to have about 131.5 million people living with Alzheimer's disease (AD) and other dementias by 2050, posing a severe health crisis. Dementia is a progressive neurodegenerative condition that gradually impairs physical and cognitive functions. Dementia has a variety of causes, symptoms, and heterogeneity concerning the influence of sex on prevalence, risk factors, and outcomes. The proportion of male-to-female prevalence varies based on the type of dementia. Despite some types of dementia being more common in men, women have a greater lifetime risk of developing dementia. AD is the most common form of dementia in which approximately two-thirds of the affected persons are women. Profound sex and gender differences in physiology and pharmacokinetic and pharmacodynamic interactions have increasingly been identified. As a result, new approaches to dementia diagnosis, care, and patient journeys should be considered. In the heart of a rapidly aging worldwide population, the Women's Brain Project (WBP) was born from the necessity to address the sex and gender gap in AD. WBP is now a well-established international non-profit organization with a global multidisciplinary team of experts studying sex and gender determinants in the brain and mental health. WBP works with different stakeholders worldwide to help change perceptions and reduce sex biases in clinical and preclinical research and policy frameworks. With its strong female leadership, WBP is an example of the importance of female professionals' work in the field of dementia research. WBP-led peer-reviewed papers, articles, books, lectures, and various initiatives in the policy and advocacy space have profoundly impacted the community and driven global discussion. WBP is now in the initial phases of establishing the world's first Sex and Gender Precision Medicine Institute. This review highlights the contributions of the WBP team to the field of AD. This review aims to increase awareness of potentially important aspects of basic science, clinical outcomes, digital health, policy framework and provide the research community with potential challenges and research suggestions to leverage sex and gender differences. Finally, at the end of the review, we briefly touch upon our progress and contribution toward sex and gender inclusion beyond Alzheimer's disease.

KEYWORDS

Alzheimer's disease, sex differences, digital therapeutics, precision medicine, Women's Brain Project, artificial intelligence, gender

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia. It is now well established that the natural history of the disease involves several stages that precede full-blown dementia. Amyloid plaques, composed of aggregated beta-amyloid (Aβ) protein, start accumulating in the brains of AD patients up to 20 years before symptoms (Fagan et al., 2014; Palmqvist et al., 2017). Initially, the brain can cope and compensate for the neurotoxicity of this process in what is called "preclinical AD." However, often linked to the spreading of tau pathology in cortical regions, symptoms start to appear in what is called "mild cognitive impairment" or "prodromal AD." The cognitive function progressively deteriorates, leading to dementia in a few years. When considering the AD continuum, hence also including preclinical and prodromal phases, global figures on the number of people suffering from AD were projected to be 32, 69, and 315 million in AD dementia, prodromal AD, and preclinical AD, respectively (Gustavsson et al., 2022). Considering these numbers preclinical AD stage accounts for 17% of all persons aged 50 and above and 52% of those were women (Gustavsson et al., 2022).

In fact, AD has been shown to be more frequent in women than in men (Martin Prince et al., 2015). Since aging is one of the most critical risk factors in developing AD, the higher female frequency has often been associated with men's shorter life span than women (Kim et al., 2015; Podcasy and Epperson, 2016); however, increasing evidence indicates that this is not the only reason; biological as well as socio-cultural mechanisms are probably at play.

The biological traits that differ between men and women are referred to as sex. These are genetically defined physical features that result from the expression of sex chromosomes and are generated throughout puberty by hormonal stimulation. On the other hand, gender is a socio-cultural concept that includes behaviors attributed to being feminine and masculine that are specific to a given culture. Each society has culturally imposed behavioral and temperamental features that are deemed proper for males and females, resulting in gender norms, roles, stereotypes, and, consequently, disparities that impact aspects such as education, employment, and income.

Both sex and gender are determinants of health, according to the World Health Organization [WHO], 2021. However, when WBP was founded in 2016, the role of sex and gender was seldom acknowledged, and their study was considered a niche topic. For instance, the higher frequency among women living with AD was rarely acknowledged, and most scientists considered it negligible in clinical research. Furthermore, the sex and the gender of individuals involved in clinical development were often not described nor analyzed explicitly with regard to the disease's characteristics. In the same way, the sex of the animals used in preclinical studies was often not reported or discussed. In terms of dementia care, the burden of caregivers (including emotional and financial), which mainly falls on the female population, was not recognized or addressed by specific policy actions at the time.

WBP was founded in Switzerland in 2017 as a non-profit association to study sex and gender determinants of the brain and mental health as the gateway to precision medicine. It is composed of professionals hailing from different disciplines with strong female leadership.

At the time of writing, WBP has contributed to more than 50 papers and 20 policy-led documents, published 6 books, given over 150 talks and lectures, and engaged in more than 50 collaborations with different stakeholders. Its work has had a profound impact on the community and has inspired several other organizations, resulting in the strengthen of *ad hoc* working groups, such as the Coordinating Panel of Diversity Equity and Inclusion at the European Academy of Neurology (EAN), the Gender mainstreaming group at OECD and the Center for Gender Medicine (CfGM) at Karolinska Institutet. Currently, WBP is now in the initial phases of establishing the world's first Sex and Gender Precision Medicine Institute.

Since its conception, WBP has been focused on pushing the boundaries of sex and gender in AD, as our organization grew over the years, we have helped and worked with experts from other areas apart from AD. Therefore, the scientific work we have done in AD in terms of basic research, clinical applications, outreach, and policy was cross-applied by our collaborators and us to other diseases with success. Therefore, in this article we first highlight our main contributions to the field of AD in terms of basic science, clinical outcomes, digital health and policy framework. From our findings, we provide the research community with practical suggestions to leverage sex and gender differences in research studies and finally, we briefly touch upon our contribution toward sex and gender inclusion beyond AD.

2. The Women's Brain Project

Over the past 5 years, WBP has contributed to the identification of profound differences in brain and mental diseases at large, and in AD in particular. The studies have revealed that such differences are complex and multifaceted, involving both biological (sex) and socio-cultural (gender) aspects; they interest all levels of research, from basic to clinical, and can also be found in novel technologies. Therefore, to properly address the complexity of the topic, the activity of the WBP has developed around four main pillars of interest: basic science, clinical science, digital biomarkers, and socio-economic determinants of health.

Biological sex differences, from genetics to hormones, can profoundly affect disease mechanisms as well as drug development. Unfortunately, the sex of the animals used in preclinical studies is often not reported or discussed. Such lack of consideration of the sex of animals in preclinical studies leads to a knowledge gap and has likely hindered therapeutic innovation. The basic science study of sex differences in brain physiology and drug mechanism of action is the first pillar of WBP's work.

Clinical differences between men and women are crucial areas of interest in the WBP's work. These refer to physiology, pharmacokinetics and pharmacodynamics interactions as well as symptoms (e.g., Butlen-Ducuing et al., 2021); identifying such differences calls for tailored approaches in diagnosis, care and patient journeys in AD but also in other diseases (Liberale et al., 2018).

Thanks to the advent of high-throughput advanced technologies, statistical models and computational tools, we now have novel potential digital biomarkers for early diagnosis of AD. The role of sex and gender in novel digital health technologies particularly for AD, is another pillar of WBP activities.

Finally, the study of differences between men and women cannot neglect the role of gender, meant as the socio-economic and socio-cultural construct of being a man or a woman in society. As socio-economic determinants of health are modifiable, a significant line of WBP activities has focused on policy-related projects to highlight existing gender differences and gender-based inequity in these determinants of health and how policymakers could address them.

The results gathered in the past 5 years based on these four pillars have been collected in a first textbook on the topic, "Sex and Gender Differences in Alzheimer's Disease" (Ferretti et al., 2021). In the following sections, we highlight some of the major contributions and lessons learned by the team with useful recommendations.

3. Importance of sex differences in preclinical Alzheimer's research

The presence of gonadal hormones and hormone cycles represents the first crucial biological difference between men and women, which are genetically driven. Different studies have shown the underpinning role of the sex chromosome complement, X chromosome inactivation, and environmental and epigenetic regulators in sex differences and their role in brain diseases, as we described in Pallier et al. (2022). The X chromosome transcriptome accounts for a significant fraction of the genome in both men and women. Still, it is often excluded from GWAS studies due to the complexity of its statistical analysis (Ferretti and Santuccione Chadha, 2021). For this reason, little is known about the involvement of sex chromosomes in AD and only recently have studies started to highlight its role (Davis et al., 2021). Taking AD as an example, we argue that a greater need to account for the interaction between sex and X-linked gene expression is required (Ferretti and Santuccione Chadha, 2021).

When it comes to studies in animal models, it is mistakenly believed that data from preclinical studies using female animals are complicated to analyze due to the higher variance associated with the estrous cycle; as a result, there have been comparatively few preclinical investigations done using female mice, or both male and female, in many fields of neuroscience (Beery and Zucker, 2011; Karp et al., 2017; Karp and Reavey, 2019). However, it is now known that female mice are not more variable than male mice, and both should be used.

Sex-related differences in AD have implications for developing drug targets and should therefore be carefully characterized in the context of preclinical studies and drug development. To raise awareness on this topic we have dedicated a special issue in Ferretti and Galea (2018); while more studies are looking into this, many issues still remain. We suggest that mixed-sex cohorts be the starting point for preclinical research, and data should be checked for sex differences. The next step should be mixed clinical trials if there are no obvious sex differences. In preclinical and clinical contexts, examining cohorts of each sex may be acceptable if the findings indicate a sex difference, as highlighted in Butlen-Ducuing et al. (2021). It is important to note that drug pharmacokinetics (PK) and pharmacodynamics (PD) can potentially differ among the sexes and it may be crucial in these circumstances to gather PK/PD data for both sexes already in preclinical studies and then assess whether or not to incorporate a design for potential sex differences in human dosage-finding trials to determine the ideal dose.

Finally, beyond *in vivo* studies, neuroscience is quickly embracing the use of new and complex *in vitro* models of disease mechanisms and drug response. Taking AD as a case study, we currently examine how sex differences can be accounted for *in vitro*. We argue that *in vitro* models of increasing complexity should account for a sex as an experimental variable. Therefore, we propose practical recommendations as to how to investigate sex differences (if not known) or address (if known) them (Castro-Aldrete, Einsiedler et al., in preparation).

4. Sex and gender differences and clinical outcomes in Alzheimer's disease

Several differences in clinical phenotypes have been described among men and women living with AD. We have summarized such differences in several reviews (Ferretti et al., 2018, 2020; Martinkova et al., 2021), a dedicated special issue (Mielke et al., 2018), as well as in the different textbooks on the topic (Abdelnour et al., 2021; Moro et al., 2022). In sum, the body of data suggests that sex is an essential component in the phenotypic heterogeneity of AD and should not be overlooked in clinical practice or preclinical research. Therefore, the analysis and reporting of sex differences in clinical investigations must be significantly improved to create strong enough data to guide clinical practice and policy (Ferretti et al., 2018; Ferretti and Galea, 2018; Hampel et al., 2018b).

Interestingly, the available evidence indicates significant sex and gender differences along the AD continuum, which are disease-stage dependent. Women appear to be relatively protected than males during the prodromal stages; interestingly, women display better cognitive performance for the same amount of hippocampal neurodegeneration (Sundermann et al., 2016). In our own work in collaboration with Alzheimer's Disease Precision Medicine Initiative" (APMI), we have found sex differences in AD biomarkers of amyloidosis, neurodegeneration, and rsFC in cognitively intact individuals older than 70 years. In particular, male compared with female sex have higher accumulation of *in vivo* brain amyloid load in the anterior cingulate cortex. This indicates that a greater amyloid load is necessary before men manifest symptoms (Cavedo et al., 2018). This clinical difference in prodromal phases is not unique to AD and we have contributed to its study also in other fields; for instance, in the behavioral variant of frontotemporal dementia (bvFTS), women show larger behavioral and executive reserve than men, and neurodegeneration in women must be more severe to cause symptoms comparable to those in males (Illán-Gala et al., 2021).

In contrast with the female protection observed at early stages, it has been shown that after clinical diagnosis, women with mild-cognitive impairment (MCI) progress twice as fast as men (Lin et al., 2015), a result with potential implications for the management of patients as well as for clinical trial design. A very interesting line of research indicates that women in the early stages of AD might miss diagnosis based on standard neuropsychological tests. As women outperform men in verbal memory, these scales

tend to be too easy for women and miss the beginning of the pathological process; women would therefore be diagnosed at later stages, which might explain the faster decline observed after diagnosis, as women are more advanced in the disease trajectory (Sundermann et al., 2021). This sex and gender difference is only one, but a powerful example of patient heterogeneity.

To address patient heterogeneity, the field of medicine is moving in the direction of biomarker-based precision medicine. While well advanced in other fields such as oncology, addressing patient heterogenicity is still in its infancy in neurology. In AD, several biomarkers, including imaging, fluid and digital ones, have been developed and are starting to be employed more and more in research and the clinical context in recent years. The diagnostic and prognostic value of such biomarkers can differ for men and women, including in preclinical stages (WBP, manuscript in preparation). Together with the APMI and cohort program (APMI-CP), the WBP is advocating for a new, AI-powered, biomarker-based clinical framework, which should be implemented to close the sex gap (Hampel et al., 2018a). However, a sex-sensitive clinical diagnosis of AD dementia based on biomarkers is still needed.

Alzheimer's disease risk factors can be different for men and women. Evidence for sex-specific vulnerability to APOE4 is growing (Farrer et al., 1997; Altmann et al., 2014; Neu et al., 2017), and the effects of sex-genotype interactions on responses to hormone replacement therapy and cholinesterase inhibitors have been observed (Macgowan et al., 1998; Holland et al., 2013; Jack et al., 2015). Midlife cardiovascular risk factors, for instance, are linked to a higher risk of developing dementia in women than men (Huo et al., 2022).

Although the WBP has emphasized largely on APOE4 studies as a risk factor with sex and gender considerations in AD, we would like to highlight that due to the multifactorial and heterogeneous nature that characterizes AD, apart from APOE4, there might be other risk factors that can affect females or males risk of developing AD. Such genetic and modifiable risk factors have been described elsewhere in excellent detail (Baumgart et al., 2015; Bellenguez et al., 2022).

In addition to genetic risk factors, modifiable, life-style related risk factors are well known to affect risk (as much as 40%) of AD (Livingston et al., 2020). It is important to highlight that most of such modifiable risk factors are known to occur differently across sexes and genders since inequities are not only linked to biological sex but also to gender, as summarized by Ferretti et al. (2020).

According to Zhang et al. (2021), factors such as psychological state, level of engagement in society, pre-existing conditions such as diabetes and traumatic brain injury (TBI), and lifestyle habits can change a person's risk levels to develop AD. For example, different studies have demonstrated the risk between diabetes and AD (Winkler et al., 2014; Espeland et al., 2018; Marseglia et al., 2018). Furthermore, a positive correlation between TBI and AD has been described in a Danish study that explored different levels of injury and their impact on AD (Nordström and Nordström, 2018).

Regarding modifiable vascular and lifestyle-related risk factors, the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) has demonstrated that a multidomain intervention could improve or maintain cognitive functioning in at-risk elderly people from the general population (Ngandu et al., 2015).

The occurrence of sex and gender differences in risk factors calls for tailored preventative campaigns for men and women and might also be a key element to consider in patient stratification for clinical trials and overall study design.

The emergence of highly diverse subgroups of patients with distinct risk factors, comorbidities, illness trajectories and likely specific neurobiology would require a new approach where the generated data can be multiplexed in a combinatorial approach with clinical, biomarker and other omic data to produce algorithms for the prediction, diagnosis, prognosis and treatment of AD, analogous to the situation in oncology (Ferretti and Santuccione Chadha, 2021). The lack of consideration of such heterogeneity has severely hindered our ability to identify at-risk individuals early and discover successful treatments in clinical trials. Therefore, if criteria for patient admittance into trials are created, sex may be taken into account alongside other variables (genetic, biomarker status) to generate a biologically homogeneous sample (Ferretti et al., 2020).

In our meta-analysis of 56 randomized clinical trials for AD, we have shown that only 12.5% of articles reported data stratified by sex (Martinkova et al., 2021); therefore, we advocate for more transparent reporting of results, even when negative. In the same study, we determined that of the 39 575 total participants with AD included in the clinical trials examined, women represented 59.0% of patients, a number that lowered to 57.9% of women when we examined only experimental drugs that had not been approved. As women represent up to 65% of the real-world population living with AD, these data indicated that female patients might have specific barriers to accessing clinical trials. Some might be due to specific criteria that systematically exclude women, such as lower educational level (Rosende-Roca et al., 2021), however, additional barriers might exist and need to be identified across the patient journey, as discussed in the next sections.

5. Inclusion of sex and gender in digital health applications for Alzheimer's disease

As in all areas of our lives, novel technologies are emerging also in AD and will most likely become crucial tools to support the patient journey in AD.

In fact, technological innovations in digital technologies and data analytics provide an umbrella of opportunities to estimate health variables to improve personalized health outcomes. Digital Health refers to the use of data and communication technologies to promote wellness and, in some instances, manage illnesses. Digital health technologies use computing platforms, software and sensors that span many uses. Examples of Digital Health technologies are mobile medical apps intended to improve clinical decisions to diagnose and treat diseases. These apps collect users' data which are subsequently stored and analyzed to enhance health status. Digital biomarkers and predictive algorithms promise to dramatically change the landscape of medicine, greatly improving and streamlining health management, from risk factors monitoring to diagnosis and treatment.

The field is developing today the technologies that will be used in the next decades and it is important to be aware of sex and gender aspects also in this field. Bias is a hidden issue of most databases

used to generate algorithms; as such the risk, as highlighted in other fields using artificial intelligence (AI), is to generate biased tools that do not serve the whole population. The AD community needs to investigate whether gender biases might affect the efficiency of AI tools for health. At the same time, sex-specific characteristics might be leveraged to improve the efficiency of such digital tools. For this reason, the WBP has a dedicated working group focusing on this topic and has contributed to our understanding of how even such technologies must consider sex and gender aspects.

The popularization of digital biomarkers could change the course of clinical research, especially concerning AD. In a first article, we gathered experts in novel technologies and identified sex as a factor that should be included in all digital biomarker algorithms (Cirillo et al., 2020). This is particularly relevant also in AD risk assessment, diagnosis, and clinical treatment and promises to allow more personalized and accurate care leading to better disease management (Ferretti et al., 2020; Sundermann et al., 2021).

Most studies in the past have used pooled data (male and female) to generate predictive algorithms for risk prediction based on genetic or phenotypic and clinical patient data. Importantly, recent studies have shown that sex is a key aspect of such algorithms (van Maurik et al., 2019); in some cases, one can even increase the predictive value by creating stratified algorithms by sex (Fan et al., 2020). There is, therefore, a strong case for a careful analysis of sex differences in datasets, as these can be leveraged to make digital tools more powerful. This is what we called in our paper a "desirable bias" (Cirillo et al., 2020).

On the other hand, until last year, it was not known whether sex differences would be also present also in digital biomarkers. To study this, the WBP has partnered with Altoida Inc., which has created the Neuro-Motor Index (NMI), a digital biomarker application dedicated to early AD diagnosis. The NMI measures cognition *via* augmented reality (AR) and motor skills in the fingers with an aim to replicate daily activities and tasks (Buegler et al., 2020).

The WBP-led study analyzed data from clinical settings (N = 438 patients from Italy, Greece, and Spain) and data from the Japanese population (N = 130 patients) (Harms et al., 2022). In this study, the clinical data consists of controlled tests of elderly subjects with MCI, AD or Aβ (+) biomarkers or young healthy controls. Each patient completed a series of tests on hand-held electronic devices supplied with the Altoida Inc., application to produce data points. We used all of this data (which was devoid of anagraphical information) to train an algorithm to determine the sex of the patient based on the data received. The sex classifier successfully determined the sex of the patient among healthy patients, but its power faded in MCI and AD populations (Harms et al., 2022). Notably, the successful differentiation in healthy individuals shows that males and females have distinct differences in their neurocognition as detected by the NMI. These results prove that the sex of a patient can affect digital biomarkers; more studies will be needed to confirm and explain these findings. Based on these results, we advocate for digital biomarker programs to factor in sex when gathering data, in this case, for AD diagnosis and treatment design.

Digital biomarkers are only one example of the power of AI-driven solutions in healthcare. AI-powered data analysis can detect specific patterns that can be leveraged to improve therapeutic and preventative measures against diseases at large. While this field it's just at its inception in AD, it is much more developed in other branches of medicine.

For example, the value of AI in medicine has been highlighted during the Coronavirus Disease 2019 (COVID-19) pandemic, which has revealed how unprepared healthcare systems are. At the WBP, we believe that AI is the key to preparing the healthcare system for future pandemics and, in general, addressing unmet medical needs such as AD. AI aims to incorporate patient data to make informed decisions concerning patient care and treatment plans.

Integrated AI could improve, among other categories, the triage, diagnosis and risk prevention in the scenario of a pandemic (Santus et al., 2021). A major benefit of AI is its ability to examine large volumes of information being published in various scientific journals and determine what is "good" and what is "bad" according to a previously established set of criteria within a short time frame. AI can also help drug repurposing by developing algorithms that identify and analyze protein-protein interactions. Recently, using AI, baricitinib was identified and has shown success in fighting against COVID-19 (Richardson et al., 2020).

The WBP supports the idea that by using desirable and undesirable bias exclusion, AI can accurately help reduce unnecessary sex and discrimination among genders (Cirillo et al., 2020; Santus et al., 2021). Using desirable bias AI can help in more precise and effective diagnostics for females and male (Stanovsky et al., 2020; Castaneda et al., 2022). For example, the training of AI algorithms can potentially increase accuracy if sex is considered (Straw and Wu, 2022).

6. Sex and gender policy framework in Alzheimer's disease

Constant legislative and policy adaptation to current technological innovation in a rapidly evolving field such as AD is vital for trustworthy relationships. Policy includes many facets of healthcare, and policy framework in clinical trials is only one of them. At the core of WBP we are working toward bridging science and society, educating policymakers and raising awareness on important societal and economic aspects linked to brain health, including sex and gender differences.

The work of the WBP in policy and advocacy includes response to the global policy agenda by contributing to ongoing initiatives as well as driving WBP-led projects and deliverables with evidence generation.

Indeed, gender (meant here as the socio-cultural construct of being a man or a woman in the society) influences a number of crucial health determinants, including wealth, education, access to healthcare and behaviors. An example of a gendered expectation that profoundly affects health in AD is the caregiving burden, which is currently overwhelmingly carried by women worldwide. In papers, books as well as policy reports (Erol et al., 2015; Alzheimer's Disease International and McGill University, 2022) we have highlighted the role of women as caregivers and the need for *ad hoc*, tailored support measures for men and women as caregivers, based on their specific needs.

Specifically, in AD, we have co-authored several policy reports on the role of sex and gender, including sexuality

(Gove et al., 2022). In an invited chapter of the World Alzheimer's report (Alzheimer's Disease International and McGill University, 2022) we have described how sex and gender differences can influence the diagnostic pathway. In particular, gender can affect the speed of diagnosis. Our research and the cumulative evidence in the past years indicate that sex and gender can affect the patient journey even beyond diagnosis, beginning with arranging an appointment for a routine checkup and ending with receiving treatment for an illness or injury. To further our understanding of sex and gender impact on AD patients, in a recent WBP-led survey-based study, we have found key differences in symptom detection, support attitude, treatment regimens, and disease management in the AD patient journey in men and women (Quevenco et al., 2023). This is important for policymakers as tailoring patient journeys for each sex can help the healthcare systems at several levels, from reducing the gap in disease awareness and health-seeking behavior, developing strong preventative routines, encouraging early diagnosis and allowing patients to stay as healthy as possible for as long as feasible. WBP has also contributed to the WHO blueprint for dementia research by highlighting the importance of incorporating sex and gender dimension at all stages of research (World Health Organization [WHO], 2022a).

Alzheimer's disease is one of the most prevalent and costly disease for society and many WBP-led initiatives for evidence generation are geared to address this issue. WBP members have acted as expert advisors in a study detailing neurological disorders' economic burden (Economist Impact, 2022). However, to drive future policy changes, it is important to understand also the economic dimension of gender medicine in neurology. To do so, the WBP has commissioned a health economic study focusing on 5 main disorders (AD, PD, MS, stroke and migraine) to identify major sex and gender differences that can affect healthcare costs and how their consideration could lead to more cost-effective health systems (Economist Impact, 2023).

Given the socio-economic costs of psychiatric and neurological disorders, it is important to characterize further and understand these differences in the larger context of brain health. To do this, we have contributed to the study describing the results of the largest survey to date on perceptions of brain health. This study documented that, in general, the lay public has a low level of awareness of brain health and its risks; differences in awareness of diseases in general and risk among men and women were observed. For example, men are less likely than women to consider factors such as substance use, sleeping habits and diet as having an influence on the brain health (Budin-Ljøsne et al., 2022). These results are important for driving *ad hoc* communication campaigns to educate and raise awareness of brain health in the population, which is key for disease prevention in society.

The importance of brain health in the global agenda has been underscored recently by the, WHO position paper on brain health, to which WBP has contributed (World Health Organization [WHO], 2022c). A particularly important topic, also relevant for AD, is the intersection between brain health and aging. Prevention of age-related disorders is increasingly becoming a key global goal, and to support the discussion in this field WBP is working alongside other partners to understand longevity in the context of brain health.

Together with the OECD the WBP is demonstrating the importance of building and protecting our own brain health, which we call "late-life Brain Capital." We have argued that investing in late-life Brain Capital can help older persons retain, engage, and empower themselves (Dawson et al., 2022). A combination of public health strategies targeting tobacco use, blood pressure control, cardiovascular disease management and prevention, among others, can reduce the likelihood of cognitive decline by making an investment in late-life brain capital. To be effective, these actions need to consider specific needs of segments of the population, including sex and gender differences.

The study of sex and gender differences is highly relevant for policymakers as it can support the development of strategies for prevention, early detection and better treatment of diseases that present a huge medical need and socio-economic cost. However, we realize that sex and gender-sensitive medicine is still in its infancy in neurology and psychiatry. The WBP has therefore convened a dedicated series of regulatory roundtables, now at its third edition, to discuss the best way to integrate sex and gender-sensitive medicine in developing solutions for neurological patients.

To obtain a comprehensive picture, in collaboration with the Task Force on gender and diversity of the EAN, we have mapped current European research, financing, and teaching initiatives that incorporate sex and gender considerations in neuroscience and neurology (Hentzen et al., 2022). We demonstrated that there is a rising demand and interest in neurological domains, both from funding organizations and researchers. However, most activities, particularly in education, are linked to individual researcher motivation and are rarely organically embedded into the curriculum and strategic research goals (Hentzen et al., 2022).

For this reason, the WBP is currently in the process of establishing a foundation and a dedicated research institute to strive for innovation in sex and gender-sensitive precision medicine for brain and mental disorders.

7. Future challenges and recommendations

Although researchers have become increasingly aware of the need to consider the impact of sex and gender on the development and progression of AD, the growing body of research in this area would benefit from carefully addressing current and potential future challenges. In this regard, we have graphically summarized in **Figure 1** recommendations from which AD research will benefit to accurately capture the impact of sex and gender. Furthermore, in **Table 1** we provide key resources that can be a starting point in conducting sex and gender research in AD.

Hormonal differences between men and women can play a significant role in the development and progression of AD (Mosconi et al., 2018; Rahman et al., 2019, 2020). Therefore, understanding how these differences contribute to the disease and how they can be used to develop gender-specific treatments is essential but at the moment we lack proper data. As a future direction, we need to capture hormonal related data in all clinical studies. Equally important, commonly used biomarkers, such as those found in brain scans, blood tests, and spinal fluid used to provide valuable information about the progression of AD, should evaluate whether they may differ between men and women and how hormonal shifts can impact

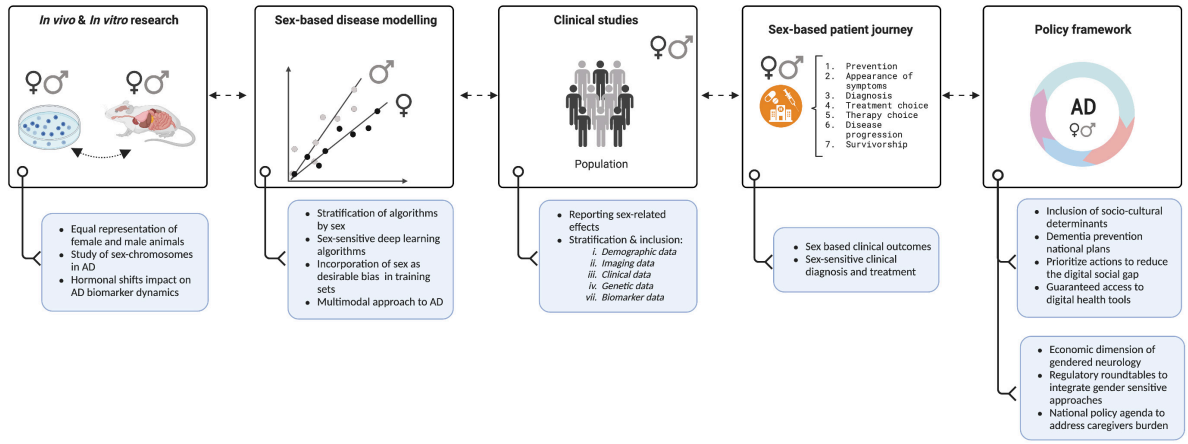

FIGURE 1
The Women's Brain Project toward a sex-sensitive Alzheimer's disease (AD) approach. Figure shows key recommendations (blue boxes) from which Alzheimer's and brain research milestones (white boxes) will benefit in constant feedback to accurately capture the impact of sex and gender and develop precision medicine agendas. Created with Biorender.com.

AD biomarker-dynamics to develop gender-specific treatments (Herman et al., 2022).

Incorporating sex and gender dimensions to training sets of deep learning algorithms should become a priority. If a deep learning model is trained on a dataset that is not representative of the population, it may not accurately determine the effect of sex and gender. To address this issue, it is important that training sets account for a balanced representation of sex and gender in the desired target population. This is especially important in deep learning platforms that simulate the structure of "orphan" proteins, which are proteins for which the structure is unknown. This is important because many disease-related proteins fall into this category, including AD (Varadi et al., 2022). To the best of our knowledge, sex and gender are not disclosed nor included in the algorithm's training set (e.g., AlphaFold). The algorithm is trained using a huge dataset of known protein structures and their matching amino acid sequences, however, this dataset does not include information about the sex of the species from which the proteins were acquired (Senior et al., 2020; Jumper et al., 2021; Varadi et al., 2022). When examining the results of these algorithms, it is critical to include sex and gender, especially when researching disease-related proteins that are known to be impacted by sex and gender. Furthermore, these algorithms may be used to discover prospective therapeutic targets that might be specific to one sex or to investigate sex-based variations in protein structure and function in the human genome (Tunyasuvunakool et al., 2021).

We envision that due to the high level of complexity, exploring the impact of social and cultural factors and its sex-related outcomes on a certain population will be one of the main challenges in AD research. Social and cultural factors are difficult to study due to the lack of standardization in their quantification making it difficult to compare results or perform a meta-analysis. Therefore, the challenges in controlling for confounding variables can influence the accuracy in determining the impact of sex and gender in AD. This is starting to be addressed by developing a multi-feature multimodal approach to neurodegeneration that includes among other variables, sociodemographic information (Moguilner et al., 2022).

With the progression of AD, patients became heavily dependent on their caregivers for everyday functions, which have significant implications not only for them but also for their caregivers. The correlation between low education levels and the burden of caregiving appears to affect mainly women and has been reviewed elsewhere in excellent detail (Mielke, 2018). This burden on caregivers has been associated with the development of AD and other neuropsychiatric disorders, as highlighted in different studies (Shoukr et al., 2022; Hellis and Mukaetova-Ladinska, 2023). According to Figueroa et al. (2021) digital health disadvantages women, particularly those from racial or ethnic minority backgrounds, due to limited access and exclusion from app creation, gender imbalance in digital health leadership, and detrimental gender stereotypes. We believe that societies can benefit from digital health and AI approaches by developing national dementia frameworks that prioritize actions to reduce the digital gap between digitally disadvantaged and advantaged individuals. Guaranteed access to early and accurate diagnosis - including digital health-, medications, and equity in the actions needed to diminish caregiver psychological and financial burden must have high priority.

8. Sex and gender differences beyond Alzheimer's disease

The WBP team has made important contributions to the study of sex and gender differences in AD, as well as its awareness in society and its consideration at the policy level. Sex and gender differences occur and are important in neurology and psychiatry well beyond AD and this is captured by the work of our group in other fields of medicine.

To give a few examples, WBP has contributed to the characterization of sex and gender aspects in traumatic brain injury (Rauen et al., 2021), neuropathic pain Parkinson's disease

TABLE 1 Useful resources for conducting sex and gender research.

Type of resource	Name	Key aspect	References
Courses	Bench to bedside: integrating sex and gender to improve human health course	Free online course by the US National Institutes of Health. It is divided into 6 thematic modules: immunology cardiovascular disease pulmonary disease neurology endocrinology mental health	National Institutes of Health, 2023
	Sex as a biological variable (SABV): a primer	Free online course by the US National Institutes of Health.	Dance, 2019
	Canadian Institutes of Health Research	Includes courses about sex and gender in health research	CIHR, 2019
	Statistical considerations for sex inclusion in basic science research	A recorded presentation explaining statistical and sample-size considerations for including sex as a biological variable.	BlueJeans, 2016
Online tool	Gendered innovations	This tool helps in developing methods to do analysis on the basis of sex and gender, how sex and gender analysis helpful in innovations	European Union, 2011
	Sex and gender research methods	Canadian Institutes of Health Research, is a series of methods articles aimed at equiping researchers with practical tips and tools from prominent researchers on integrating sex, gender, and other identity factors into various fields of health research.	Canadian Institutes of Health Research, 2022
	Genderedinnovations.se	It is developed at Karolinska Institute, is the Swedish version of Stanford's Gendered Innovations and contains useful content in the form of Swedish expertise, experience, tools, videos, and case studies.	Gendered Innovations, 2019
Database	Janusmed sex and gender	It has information related to sex and gender aspects for drug treatment	Janusinfo, 2022
	Drug trial snapshot	It provide information to consumers and healthcare professionals about participation in clinical trials	Drug Trials Snapshots, 2022
	GenderMed database	It provide literature research that addresses sex and gender differences	Oertelt-Prigione et al., 2014
	Gender experts	Database related to women experts on gender equality.	Gender Experts, 2021
Organizations	Gender equality academy	Horizon 2020 project developing and implementing a high-quality capacity-building programme on gender equality in research, innovation, and higher education.	Ge-academy, 2019
	LIBRA	EC-funded project that brings together ten research institutes in ten European countries to promote gender equality in life sciences.	LIBRA, 2021
	PORTIA	UK not-for-profit organization with extensive expertise in EU gender and STEM policy.	PORTIA, 2020
	NIH ORWH	US National Institutes of Health's office for research on women's health	Research on Women's Health, 2023
	Institute of gender in medicine	Focuses on interventions to promote healthy behaviors and how such interventions can be designed in a gender and diversity sensitive way.	Institute of Gender in Medicine, 2023
Recommendations	The promises and pitfalls of sex difference research	Discuss issues related to inclusion of both sexes to specialization of sex differences with attention paid to statistics and the need for sex-specific treatments.	Galea et al., 2020
	Sex and gender differences research design for basic, clinical and population studies: essentials for investigators	Compilation of sex and gender studies to excrete basic causes of diseases and avoid a reflexive attribution of seeming sex differences solely to biology.	Rich-Edwards et al., 2018
	Biomedical research falls short at factoring in sex and gender	Equity, accuracy, and transparency in both the conduct and reporting of research in subjects of both sexes.	Shansky and Anne, 2021
	The impact of sex and gender on the multidisciplinary management of care for persons with Parkinson's disease	Potential impact of sex and gender on care for people with PD, and identify key knowledge gaps that hamper immediate implementation of sex- or gender-sensitive approaches.	Göttgens et al., 2020
	The sex and gender dimensions of COVID-19: a narrative review of the potential underlying factors	Sex is a significant risk factor for severe disease and mortality due to coronavirus disease 2019 (COVID-19).	Taslem Mourosi et al., 2022
Books	Sex and gender differences in neurological disease	Each chapter includes the latest information on sex and gender differences in neurological disease	Moro et al., 2022
	Sex and gender bias in artificial intelligence and healthcare	Highlights the relevance of sex and gender differences and bias in the development of novel technologies for health.	Cirillo et al., 2022
	Sex and gender differences in Alzheimer's disease	The first academic book on sex and gender differences in Alzheimer's disease.	Abdelnour et al., 2021

and schizophrenia in a dedicated, WBP-led special issue in the journal Frontiers in Neuroendocrinology (Szoeke et al., 2020), stroke (Sandset and Ferretti, 2021) and in brain health (de Lange et al., 2021).

As an example of WBP response to the ongoing policy actions, we have contributed, as members of the OneNeurology group, to the Global Action Plan on Epilepsy and other neurological disorders by the WHO, to make sure that sex and gender aspects in neurology are part of the research agenda (World Health Organization [WHO], 2022b).

The specific needs of female migraine patients have been highlighted in several *ad hoc* policy and awareness projects, including communications campaigns, such as #notallinherhead social media campaign, directed at the lay public. Awareness campaigns and communication programs are run by the WBP team also for psychiatric disorders such as depression, anxiety and ADHD.

Interestingly, the COVID-19 pandemic has revealed the importance of sex and gender differences and the WBP has been particularly active in documenting such differences and advocating for their consideration in clinical trials (Grisold et al., 2021; Jensen et al., 2021, 2022).

Finally, we have advocated for the proper consideration of sex and gender in the context of drug development (Ferretti and Galea, 2018), particularly for neurological and psychiatric disorders that present an unmet medical need (Butlen-Ducuing et al., 2021).

9. Conclusion

The work done by the team at WBP showcases the power of female leadership, a diverse and multidisciplinary team in the field of Alzheimer's research and beyond. Collaboratively the WBP have helped to change perceptions, increase visibility and reduce sex biases in preclinical research, clinical science and policy framework. Incorporating specific individual needs (including those driven by sex and gender aspects) will be key to reaching a precision medicine approach in AD, as well as personalized patient management for a more sustainable healthcare.

Author contributions

LC-A conceived the manuscript. LC-A, MM, and MTF wrote the manuscript. LC-A, MM, and GP collected and cataloged the literature. LC-A, MTF, and ASD contributed substantially to the content discussion and reviewed/edited the manuscript before submission. ASC, MTF, and ASD provided the resources and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MTF is the co-founder and Chief Scientific Officer of the non-profit organization the WBP. In the past 2 years she has received personal fees from Eli Lilly, Lundbeck and Roche, for projects not directly related to the present paper. ASC is an employee of Altoida Inc. and also co-founder and *pro bono* CEO of the WBP. ASD is also co-founder of the non-profit organization the WBP. This position paper represents LC-A and ASC personal view only and not the position of any group or entity with which they are associated.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abdelnour, C., Abela, A., Arnaldi, D., Au, R., Balma, M., Barbarino, P., et al. (2021). in *Sex and Gender Differences in Alzheimer's Disease*, eds M. T. Ferretti, A. S. Dimech, and A. S. Chadha (Amsterdam: Elsevier), doi: 10.1016/B978-0-12-819344-0.09984-1

Altmann, A., Tian, L., Henderson, V. W., and Greicius, M. D. (2014). Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann. Neurol.* 75, 563–573. doi: 10.1002/ANA.24135

Alzheimer's Disease International, and McGill University (2022). *Life after Diagnosis: Navigating Treatment, Care and Support*. Available online at: https://www.alzint.org/resource/world-alzheimer-report-2022/ (accessed November 19, 2022).

Baumgart, M., Snyder, H. M., Carrillo, M. C., Fazio, S., Kim, H., and Johns, H. (2015). Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. *Alzheimers Dement.* 11, 718–726. doi: 10.1016/J.JALZ.2015.05.016

Beery, A. K., and Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neurosci. Biobehav. Rev.* 35, 565–572. doi: 10.1016/j.neubiorev.2010.07.002

Bellenguez, C., Küçükali, F., Jansen, I. E., Kleineidam, L., Moreno-Grau, S., Amin, N., et al. (2022). New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nat. Genet.* 54, 412–436. doi: 10.1038/s41588-022-01024-z

BlueJeans (2016). *BlueJeans Network*. Available online at: https://bluejeans.com/playback/s/0Ra60FQEledQlOf0P9Wq3fe1R8Qsj9iu8XleSivjLiTGhTYgYJ3pHOFFUTxotdyB (accessed February 18, 2023).

Budin-Ljøsne, I., Mowinckel, A. M., Friedman, B. B., Ebmeier, K. P., Drevon, C. A., Carver, R. B., et al. (2022). Public perceptions of brain health: an international, online cross-sectional survey. *BMJ Open* 12:e057999. doi: 10.1136/BMJOPEN-2021-057999

Buegler, M., Harms, R. L., Balasa, M., Meier, I. B., Exarchos, T., Rai, L., et al. (2020). Digital biomarker-based individualized prognosis for people at risk of dementia. *Alzheimer's Dement.* 12:e12073. doi: 10.1002/dad2.12073

Butlen-Ducuing, F., Balkowiec-Iskra, E., Dalla, C., Slattery, D. A., Ferretti, M. T., Kokras, N., et al. (2021). Implications of sex-related differences in central nervous system disorders for drug research and development. *Nat. Rev. Drug Discov.* 20, 881–882. doi: 10.1038/d41573-021-00115-6

Canadian Institutes of Health Research (2022). *Sex and Gender Research and Methods*. Available online at: https://cihr-irsc.gc.ca/e/49629.html (accessed February 7, 2023).

Castaneda, J., Jover, A., Calvet, L., Yanes, S., Juan, A. A., and Sainz, M. (2022). Dealing with gender bias issues in data-algorithmic processes: a social-statistical perspective. *Algorithms* 15:303. doi: 10.3390/A15090303

Cavedo, E., Chiesa, P. A., Houot, M., Ferretti, M. T., Grothe, M. J., Teipel, S. J., et al. (2018). Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints. *Alzheimer's Dement.* 14, 1204–1215. doi: 10.1016/J.JALZ.2018.05.014

CIHR (2019). *IGH Learning - CIHR*. Available online at: https://cihr-irsc.gc.ca/e/49347.html (accessed January 29, 2023).

Cirillo, D., Catuara Solarz, S., and Guney, E. (2022). *Sex and Gender Bias in Technology and Artificial Intelligence: Biomedicine and Healthcare Applications*. Cambridge, MA: Academic Press.

Cirillo, D., Catuara-Solarz, S., Morey, C., Guney, E., Subirats, L., Mellino, S., et al. (2020). Sex and gender differences and biases in artificial intelligence for biomedicine and healthcare. *NPJ Digit. Med.* 3:81. doi: 10.1038/s41746-020-0288-5

Dance, A. (2019). Why the sexes don't feel pain the same way. *Nature* 567, 448–450. doi: 10.1038/d41586-019-00895-3

Davis, E. J., Solsberg, C. W., White, C. C., Miñones-Moyano, E., Sirota, M., Chibnik, L., et al. (2021). Sex-specific association of the X chromosome with cognitive change and tau pathology in aging and Alzheimer Disease. *JAMA Neurol.* 78, 1249–1254. doi: 10.1001/JAMANEUROL.2021.2806

Dawson, W. D., Smith, E., Booi, L., Mosse, M., Lavretsky, H., Reynolds, C. F., et al. (2022). Investing in late-life brain capital. *Innovat. Aging* 6, 1–7. doi: 10.1093/GERONI/IGAC016

de Lange, A. M. G., Jacobs, E. G., and Galea, L. A. M. (2021). The scientific body of knowledge: Whose body does it serve? A spotlight on women's brain health. *Front. Neuroendocrinol.* 60:100898. doi: 10.1016/J.YFRNE.2020.100898

Drug Trials Snapshots (2022). *Drug Approvals and Databases*. Available online at: https://www.fda.gov/drugs/drug-approvals-and-databases/drug-trials-snapshots (accessed February 9, 2023).

Economist Impact (2022). *The Value of Action: Mitigating the Global Impact of Neurological Disorders*. Available online at: https://impact.economist.com/perspectives/healthcare/mitigating-global-impact-neurological-disorders?utm_source=linkedin&utm_medium=organic-ei-social&utm_campaign=Roche-neuro&utm_content=native_size/&linkId=100000155658551 (accessed November 19, 2022).

Economist Impact (2023). *Sex, gender and the brain: towards an inclusive research agenda*. Available online at: https://impact.economist.com/perspectives/sites/default/files/womensbrainproject_report_230306.pdf

Erol, R., Brooker, D., and Peel, E. (2015). *Women and Dementia A Global Research Review*. Available online at: https://www.alzint.org/resource/women-and-dementia-a-global-research-review/ (accessed November 19, 2022).

Espeland, M. A., Carmichael, O., Yasar, S., Hugenschmidt, C., Hazzard, W., Hayden, K. M., et al. (2018). Sex-related differences in the prevalence of cognitive impairment among overweight and obese adults with type 2 diabetes. *Alzheimers Dement.* 14, 1184–1192. doi: 10.1016/j.jalz.2018.05.015

European Union (2011). *Gendered Innovations | Stanford University*. Available online at: https://genderedinnovations.stanford.edu/ (accessed January 29, 2023).

Fagan, A. M., Xiong, C., Jasielec, M. S., Bateman, R. J., Goate, A. M., Benzinger, T. L. S., et al. (2014). Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. *Sci. Transl. Med.* 6:226ra30.

Fan, C. C., Banks, S. J., Thompson, W. K., Chen, C. H., McEvoy, L. K., Tan, C. H., et al. (2020). Sex-dependent autosomal effects on clinical progression of Alzheimer's disease. *Brain* 143, 2272–2280. doi: 10.1093/brain/awaa164

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer Disease: a meta-analysis. *JAMA* 278, 1349–1356. doi: 10.1001/JAMA.1997.03550160069041

Ferretti, M. T., Dimech, A. S., and Chadha, A. S. (2021). *Sex and Gender Differences in Alzheimer's Disease*. Cambridge, MA: Academic Press, 1–2. doi: 10.1016/B978-0-12-819344-0.00016-8

Ferretti, M. T., and Galea, L. A. M. (2018). Improving pharmacological treatment in brain and mental health disorders: the need for gender and sex analyses. *Front. Neuroendocrinol.* 50:7. doi: 10.1016/J.YFRNE.2018.06.007

Ferretti, M. T., Iulita, M. F., Cavedo, E., Chiesa, P. A., Dimech, A. S., Chadha, A. S., et al. (2018). Sex differences in Alzheimer disease — the gateway to precision medicine. *Nat. Rev. Neurol.* 14, 457–469. doi: 10.1038/s41582-018-0032-9

Ferretti, M. T., Martinkova, J., Biskup, E., Benke, T., Gialdini, G., Nedelska, Z., et al. (2020). Sex and gender differences in Alzheimer's disease: current challenges and implications for clinical practice. *Eur. J. Neurol.* 27, 928–943. doi: 10.1111/ene.14174

Ferretti, M. T., and Santuccione Chadha, A. (2021). The missing X factor in Alzheimer disease. *Nat. Rev. Neurol.* 17, 727–728. doi: 10.1038/s41582-021-00573-x

Figueroa, C. A., Luo, T., Aguilera, A., and Lyles, C. R. (2021). The need for feminist intersectionality in digital health. *Lancet Digit. Health* 3, e526–e533. doi: 10.1016/S2589-7500(21)00118-7

Galea, L. A. M., Choleris, E., Albert, A. Y. K., McCarthy, M. M., and Sohrabji, F. (2020). The promises and pitfalls of sex difference research. *Front. Neuroendocrinol.* 56:100817. doi: 10.1016/j.yfrne.2019.100817

Ge-academy (2019). *Gender Equality Training Materials*. Available online at: https://ge-academy.eu/ (accessed February 20, 2023).

Gender Experts (2021). *Gender ExpertsA Global Database*. Available online at: https://genderexperts.org/ (accessed February 19, 2023).

Gendered Innovations (2019). *Sex and Gender Methods for Research*. Available online at: https://genderedinnovations.stanford.edu/ (accessed February 3, 2023).

Göttgens, I., van Halteren, A. D., de Vries, N. M., Meinders, M. J., Ben-Shlomo, Y., Bloem, B. R., et al. (2020). The impact of sex and gender on the multidisciplinary management of care for persons with Parkinson's Disease. *Front. Neurol.* 11:576121. doi: 10.3389/FNEUR.2020.576121

Gove, D., Capstick, A., Ettenes, P., Georges, J., Gzil, F., Harper, P., et al. (2022). *Sex, Gender and Sexuality in the Context of Dementia: a Discussion Paper*. Available online at: https://www.alzheimer-europe.org/sites/default/files/2022-02/2022-02-25%20Alzheimer%20Europe%20ethics%20report%202021.pdf (accessed November 6, 2022).

Grisold, W., Moro, E., Teresa Ferretti, M., Hege Aamodt, A., Arabia, G., Lebedeva, E. R., et al. (2021). Gender issues during the times of COVID-19 pandemic. *Eur. J. Neurol.* 28, e73–e77. doi: 10.1111/ENE.14815

Gustavsson, A., Norton, N., Fast, T., Frölich, L., Georges, J., Holzapfel, D., et al. (2022). Global estimates on the number of persons across the Alzheimer's disease continuum. *Alzheimers Dement.* 19, 658–670. doi: 10.1002/ALZ.12694

Hampel, H., Toschi, N., Babiloni, C., Baldacci, F., Black, K. L., Bokde, A. L. W., et al. (2018a). Revolution of Alzheimer precision neurology. passageway of systems biology and neurophysiology. *J. Alzheimers Dis.* 64, S47–S105. doi: 10.3233/JAD-179932

Hampel, H., Vergallo, A., Giorgi, F. S., Kim, S. H., Depypere, H., Graziani, M., et al. (2018b). Precision medicine and drug development in Alzheimer's disease: the importance of sexual dimorphism and patient stratification. *Front. Neuroendocrinol.* 50:31–51. doi: 10.1016/J.YFRNE.2018.06.001

Harms, R. L., Ferrari, A., Meier, I. B., Martinkova, J., Santus, E., Marino, N., et al. (2022). Digital biomarkers and sex impacts in Alzheimer's disease management – potential utility for innovative 3P medicine approach. *EPMA J.* 13, 299–313. doi: 10.1007/s13167-022-00284-3

Hellis, E., and Mukaetova-Ladinska, E. B. (2023). Informal caregiving and Alzheimer's Disease: the psychological effect. *Medicina* 59:48. doi: 10.3390/MEDICINA59010048

Hentzen, N. B., Ferretti, M. T., Santuccione Chadha, A., Jaarsma, J., de Visser, M., Moro, E., et al. (2022). Mapping of European activities on the integration of sex and gender factors in neurology and neuroscience. *Eur. J. Neurol.* 29, 2572–2579. doi: 10.1111/ENE.15439

Herman, D., Andrea, V., Pablo, L., Simone, L., Andrea, B., Nicholas, A., et al. (2022). Menopause hormone therapy significantly alters pathophysiological biomarkers of Alzheimer's disease. *Alzheimers Dement.* [Online ahead of print]. doi: 10.1002/ALZ.12759

Holland, D., Desikan, R. S., Dale, A. M., and McEvoy, L. K. (2013). Higher rates of decline for women and apolipoprotein E epsilon4 carriers. *AJNR Am. J. Neuroradiol.* 34, 2287–2293. doi: 10.3174/ajnr.a3601

Huo, N., Vemuri, P., Graff-Radford, J., Syrjanen, J., Machulda, M., Knopman, D. S., et al. (2022). Sex differences in the association between midlife cardiovascular conditions or risk factors with midlife cognitive decline. *Neurology* 98:e623. doi: 10.1212/WNL.0000000000013174

Illán-Gala, I., Casaletto, K. B., Borrego-Écija, S., Arenaza-Urquijo, E. M., Wolf, A., Cobigo, Y., et al. (2021). Sex differences in the behavioral variant of frontotemporal dementia: a new window to executive and behavioral reserve. *Alzheimers Dement.* 17, 1329–1341. doi: 10.1002/ALZ.12299

Institute of Gender in Medicine (2023). *Gender-Sensitive Prevention Research in Medicine*. Available online at: https://gender.charite.de/en/research/ (accessed February 19, 2023).

Jack, C. R., Wiste, H. J., Weigand, S. D., Knopman, D. S., Vemuri, P., Mielke, M. M., et al. (2015). Age, sex, and APOE epsilon4 effects on memory, brain structure, and beta-amyloid across the adult life span. *JAMA Neurol.* 72, 511–519. doi: 10.1001/jamaneurol.2014.4821

Janusinfo. (2022). *Janusmed Sex and Gender*. Available online at: https://www.janusinfo.se/genus/in-english?letter=X (accessed February 20, 2023).

Jensen, A., Castro, A. W., Ferretti, M. T., Martinkova, J., Vasilevskaya, A., Chadha, A. S., et al. (2022). Sex and gender differences in the neurological and neuropsychiatric symptoms of long COVID: a narrative review. *Ital. J. Gender Spec. Med.* 8, 18–28. doi: 10.1723/3769.37563

Jensen, A., Stromme, M., Moyassari, S., Santuccione, A., Tartaglia, M. C., Szoeke, C., et al. (2021). *COVID-19 Vaccines: Open Questions towards Precision Medicine*. Available online at: https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/12963-Revision-of-the-EU-general-pharmaceuticals-legislation/F2242276_en (accessed November 6, 2022).

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., et al. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589. doi: 10.1038/S41586-021-03819-2

Karp, N. A., Mason, J., Beaudet, A. L., Benjamini, Y., Bower, L., Braun, R. E., et al. (2017). Prevalence of sexual dimorphism in mammalian phenotypic traits. *Nat. Commun.* 8, 1–12. doi: 10.1038/ncomms15475

Karp, N. A., and Reavey, N. (2019). Sex bias in preclinical research and an exploration of how to change the status quo. *Br. J. Pharmacol.* 176, 4107–4118. doi: 10.1111/BPH.14539

Kim, S., Kim, M. J., Kim, S., Kang, H. S., Lim, S. W., Myung, W., et al. (2015). Gender differences in risk factors for transition from mild cognitive impairment to Alzheimer's disease: a CREDOS study. *Compr. Psychiatry* 62, 114–122. doi: 10.1016/J.COMPPSYCH.2015.07.002

Liberale, L., Carbone, F., Montecucco, F., Gebhard, C., Lüscher, T. F., Wegener, S., et al. (2018). Ischemic stroke across sexes: What is the status quo? *Front. Neuroendocrinol.* 50:3–17. doi: 10.1016/j.yfrne.2018.05.001

LIBRA (2021). *Unifying Innovative Efforts of European Research Centres to Achieve Gender Equality in Academia*. Available online at: https://www.eu-libra.eu/ (accessed February 10, 2023).

Lin, K. A., Choudhury, K. R., Rathakrishnan, B. G., Marks, D. M., Petrella, J. R., and Doraiswamy, P. M. (2015). Marked gender differences in progression of mild cognitive impairment over 8 years. *Alzheimers Dement.* 1:103. doi: 10.1016/J.TRCI.2015.07.001

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., et al. (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet* 396:413. doi: 10.1016/S0140-6736(20)30367-6

Macgowan, S. H., Wilcock, G. K., and Scott, M. (1998). Effect of gender and apolipoprotein E genotype on response to anticholinesterase therapy in Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 13, 625–630.

Marseglia, A., Dahl Aslan, A. K., Fratiglioni, L., Santoni, G., Pedersen, N. L., and Xu, W. (2018). Cognitive trajectories of older adults with prediabetes and diabetes: a population-based cohort study. *J. Gerontol. A Biol. Sci. Med. Sci.* 73:400. doi: 10.1093/GERONA/GLX112

Martin Prince, A., Wimo, A., Guerchet, M., Gemma-Claire Ali, M., Wu, Y.-T., Prina, M., et al. (2015). *World Alzheimer Report 2015: The Global Impact of Dementia: An Analysis of Prevalence, Incidence, Cost and Trends*. Available online at: https://www.alzint.org/resource/world-alzheimer-report-2015/ (accessed November 19, 2022).

Martinkova, J., Quevenco, F. C., Karcher, H., Ferrari, A., Sandset, E. C., Szoeke, C., et al. (2021). Proportion of women and reporting of outcomes by sex in clinical trials for Alzheimer Disease: a systematic review and meta-analysis. *JAMA Netw. Open* 4:e2124124. doi: 10.1001/jamanetworkopen.2021.24124

Mielke, M. M. (2018). Sex and gender differences in Alzheimer's Disease dementia. *Psychiatr. Times* 35:14.

Mielke, M. M., Ferretti, M. T., Iulita, M. F., Hayden, K., and Khachaturian, A. S. (2018). Sex and gender in Alzheimer's disease – Does it matter? *Alzheimers Dement.* 14, 1101–1103. doi: 10.1016/J.JALZ.2018.08.003

Moguilner, S., Birba, A., Fittipaldi, S., Gonzalez-Campo, C., Tagliazucchi, E., Reyes, P., et al. (2022). Multi-feature computational framework for combined signatures of dementia in underrepresented settings. *J. Neural Eng.* 19:046048. doi: 10.1088/1741-2552/AC87D0

Moro, E., Arabia, G., Tartaglia, C., and Ferretti, M. T. (2022). *Sex and Gender Differences in Neurological Disease*. Cambridge, MA: Academic Press.

Mosconi, L., Rahman, A., Diaz, I., Wu, X., Scheyer, O., Hristov, H. W., et al. (2018). Increased Alzheimer's risk during the menopause transition: a 3-year longitudinal brain imaging study. *PLoS One* 13:e0207885. doi: 10.1371/JOURNAL.PONE.0207885

National Institutes of Health (2023). *Bench to Bedside: Integrating Sex and Gender to Improve Human Health Course*. Available online at: https://orwh.od.nih.gov/career-development-education/e-learning/bench-bedside (accessed February 11, 2023).

Neu, S. C., Pa, J., Kukull, W., Beekly, D., Kuzma, A., Gangadharan, P., et al. (2017). Apolipoprotein E genotype and sex risk factors for Alzheimer Disease. *JAMA Neurol* 74:1178. doi: 10.1001/jamaneurol.2017.2188

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., et al. (2015). A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 385, 2255–2263. doi: 10.1016/S0140-6736(15)60461-5

Nordström, A., and Nordström, P. (2018). Traumatic brain injury and the risk of dementia diagnosis: a nationwide cohort study. *PLoS Med.* 15:e1002496. doi: 10.1371/JOURNAL.PMED.1002496

Oertelt-Prigione, S., Gohlke, B.-O., Dunkel, M., Preissner, R., and Regitz-Zagrosek, V. (2014). GenderMedDB: an interactive database of sex and gender-specific medical literature. *Biol. Sex Differ.* 5:7. doi: 10.1186/2042-6410-5-7

Pallier, P. N., Ferrara, M., Romagnolo, F., Ferretti, M. T., Soreq, H., and Cerase, A. (2022). Chromosomal and environmental contributions to sex differences in the vulnerability to neurological and neuropsychiatric disorders: Implications for therapeutic interventions. *Prog. Neurobiol.* 219:102353. doi: 10.1016/J.PNEUROBIO.2022.102353

Palmqvist, S., Schöll, M., Strandberg, O., Mattsson, N., Stomrud, E., Zetterberg, H., et al. (2017). Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. *Nat. Commun.* 8:1214. doi: 10.1038/S41467-017-01150-X

Podcasy, J. L., and Epperson, C. N. (2016). Considering sex and gender in Alzheimer disease and other dementias. *Dial. Clin. Neurosci.* 18:437. doi: 10.31887/DCNS.2016.18.4/CEPPERSON

PORTIA (2020). *#ACTonGender*. Available online at: https://act-on-gender.eu/portia (accessed February 18, 2023).

Quevenco, F., Ferretti, M., Santuccione Chadha, A., and Schumacher Dimech, A. (2023). "Analysis of the Alzheimer's patient journey through a sex and gender lens: initial results from an international survey," in *Proceedings of the international Conference on Alzheimer's and Parkinson's Diseases (ADPD)*, Gothenburg.

Rahman, A., Jackson, H., Hristov, H., Isaacson, R. S., Saif, N., Shetty, T., et al. (2019). Sex and gender driven modifiers of Alzheimer's: the role for estrogenic control across age, race, medical, and lifestyle risks. *Front. Aging Neurosci.* 11:315. doi: 10.3389/FNAGI.2019.00315

Rahman, A., Schelbaum, E., Hoffman, K., Diaz, I., Hristov, H., Andrews, R., et al. (2020). Sex-driven modifiers of Alzheimer risk. *Neurology* 95, e166–e178. doi: 10.1212/WNL.0000000000009781

Rauen, K., Späni, C. B., Tartaglia, M. C., Ferretti, M. T., Reichelt, L., Probst, P., et al. (2021). Quality of life after traumatic brain injury: a cross-sectional analysis uncovers age- and sex-related differences over the adult life span. *Geroscience* 43, 263–278. doi: 10.1007/s11357-020-00273-2

Research on Women's Health (2023). *Putting Science to Work for the Health of Women*. Available online at: https://orwh.od.nih.gov/ (accessed January 19, 2023).

Richardson, P., Griffin, I., Tucker, C., Smith, D., Oechsle, O., Phelan, A., et al. (2020). Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet* 395, e30–e31. doi: 10.1016/S0140-6736(20)30304-4

Rich-Edwards, J. W., Kaiser, U. B., Chen, G. L., Manson, J. E., and Goldstein, J. M. (2018). Sex and gender differences research design for basic, clinical, and population studies: essentials for investigators. *Endocr. Rev.* 39, 424–439. doi: 10.1210/er.2017-00246

Rosende-Roca, M., Abdelnour, C., Esteban, E., Tartari, J. P., Alarcon, E., Martínez-Atienza, J., et al. (2021). The role of sex and gender in the selection of Alzheimer patients for clinical trial pre-screening. *Alzheimers Res. Ther.* 13:95. doi: 10.1186/S13195-021-00833-4

Sandset, E. C., and Ferretti, M. T. (2021). Sex and gender differences in stroke—The need for individualised follow-up. *Eur. J. Neurol.* 28, 365–366. doi: 10.1111/ene.14619

Santus, E., Marino, N., Cirillo, D., Chersoni, E., Montagud, A., Chadha, A. S., et al. (2021). Artificial intelligence-aided precision medicine for COVID-19: strategic areas of research and development. *J. Med. Internet Res.* 23:e22453. doi: 10.2196/22453

Senior, A. W., Evans, R., Jumper, J., Kirkpatrick, J., Sifre, L., Green, T., et al. (2020). Improved protein structure prediction using potentials from deep learning. *Nature* 577, 706–710. doi: 10.1038/S41586-019-1923-7

Shansky, R. M., and Anne, Z. (2021). *Biomedical Research Falls Short at Factoring in Sex and Gender*. Available online at: https://www.scientificamerican.com/article/biomedical-research-falls-short-at-factoring-in-sex-and-gender/ (accessed February 19, 2023).

Shoukr, E. M. M., Mohamed, A. A. E. R., El-Ashry, A. M., and Mohsen, H. A. (2022). Effect of psychological first aid program on stress level and psychological well-being among caregivers of older adults with Alzheimer's disease. *BMC Nurs.* 21:275. doi: 10.1186/S12912-022-01049-Z

Stanovsky, G., Smith, N. A., and Zettlemoyer, L. (2020). "Evaluating gender bias in machine translation," in *Proceedings of the Conference ACL 2019 - 57th Annual Meeting*

of the Association for Computational Linguistics, Vol. 117, (Florence), 1679–1684. doi: 10.18653/v1/p19-1164

Straw, I., and Wu, H. (2022). Investigating for bias in healthcare algorithms: a sex-stratified analysis of supervised machine learning models in liver disease prediction. *BMJ Health Care Inform.* 29:e100457. doi: 10.1136/BMJHCI-2021-100457

Sundermann, E. E., Barnes, L. L., Bondi, M. W., Bennett, D. A., Salmon, D. P., and Maki, P. M. (2021). Improving detection of amnestic mild cognitive impairment with sex-specific cognitive norms. *J. Alzheimers Dis.* 84, 1763–1770. doi: 10.3233/JAD-215260

Sundermann, E. E., Biegon, A., Rubin, L. H., Lipton, R. B., Mowrey, W., Landau, S., et al. (2016). Better verbal memory in women than men in MCI despite similar levels of hippocampal atrophy. *Neurology* 86:1368. doi: 10.1212/WNL.0000000000002570

Szoeke, C., Downie, S., Parker, A., and Phillips, S. (2020). Beyond sex differences: a spotlight on women's brain health. *Front. Neuroendocrinol.* 62:100927. doi: 10.1016/j.yfrne.2021.100927

Taslem Mourosi, J., Anwar, S., and Hosen, M. J. (2022). The sex and gender dimensions of COVID-19: a narrative review of the potential underlying factors. *Infect. Genet. Evolut.* 103:105338. doi: 10.1016/j.meegid.2022.105338

Tunyasuvunakool, K., Adler, J., Wu, Z., Green, T., Zielinski, M., Žídek, A., et al. (2021). Highly accurate protein structure prediction for the human proteome. *Nature* 596, 590–596. doi: 10.1038/S41586-021-03828-1

van Maurik, I. S., Vos, S. J., Bos, I., Bouwman, F. H., Teunissen, C. E., Scheltens, P., et al. (2019). Biomarker-based prognosis for people with mild cognitive impairment (ABIDE): a modelling study. *Lancet Neurol.* 18, 1034–1044. doi: 10.1016/S1474-4422(19)30283-2

Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G., et al. (2022). AlphaFold protein structure database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res.* 50, D439–D444. doi: 10.1093/NAR/GKAB1061

Winkler, A., Dlugaj, M., Weimar, C., Jöckel, K. H., Erbel, R., Dragano, N., et al. (2014). Association of diabetes mellitus and mild cognitive impairment in middle-aged men and women. *J. Alzheimers Dis.* 42, 1269–1277. doi: 10.3233/JAD-140696

World Health Organization [WHO] (2021). *Social Determinants of Health*. Available online at: https://www.who.int/publications/i/item/WHO-IER-CSDH-08.1 (accessed November 19, 2022).

World Health Organization [WHO] (2022a). *A Blueprint for Dementia Research*. Available online at: https://apps.who.int/iris/handle/10665/363341 (accessed November 19, 2022).

World Health Organization [WHO] (2022b). *Intersectoral Global Action Plan on Epilepsy and Other Neurological Disorders*. Available online at: https://apps.who.int/iris/handle/10665/325293 (accessed November 19, 2022).

World Health Organization [WHO] (2022c). *Optimizing Brain Health Across the Life Course*. Available online at: https://www.who.int/publications/i/item/9789240054561 (accessed November 19, 2022).

Zhang, X. X., Tian, Y., Wang, Z. T., Ma, Y. H., Tan, L., and Yu, J. T. (2021). The Epidemiology of Alzheimer's Disease Modifiable Risk Factors and Prevention. *J. Prev. Alzheimers Dis.* 8, 313–321. doi: 10.14283/jpad.2021.15

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Chronic consumption of a hypercaloric diet increases neuroinflammation and brain senescence, promoting cognitive decline in middle-aged female Wistar rats

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Being overweight and obesity are world health problems, with a higher prevalence in women, defined as abnormal or excessive fat accumulation that increases the risk of chronic diseases. Excess energy leads to adipose expansion, generating hypertrophic adipocytes that produce various pro-inflammatory molecules. These molecules cause chronic low-intensity inflammation, affecting the organism's functioning and the central nervous system (CNS), inducing neuroinflammation. The neuroinflammatory response during obesity occurs in different structures of the CNS involved in memory and learning, such as the cortex and the hippocampus. Here we analyzed how obesity-related peripheral inflammation can affect CNS physiology, generating neuroinflammation and promoting cellular senescence establishment. Since some studies have shown an increase in senescent cells during aging, obesity, and neurodegenerative diseases, we proposed that cellular senescence participation may contribute to the cognitive decline in an obesity model of middle-aged female Wistar rats. The inflammatory state of 6 and 13months-old female Wistar rats fed with a hypercaloric diet was measured in serum and CNS (cortex and hippocampus). Memory was evaluated using the novel object recognition (NOR) test; the presence of senescent markers was also determined. Our data suggest that the systemic inflammation generated by obesity induces a neuroinflammatory state in regions involved in learning and memory, with an increase in senescent markers, thus proposing senescence as a potential participant in the negative consequences of obesity in cognition.

KEYWORDS

obesity, female, neuroinflammation, senescence, cognitive deterioration

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that can harm health (World Health Organization, 2023), increasing the risk of developing different metabolic disorders and diseases (Awada et al., 2013), such as type 2 diabetes, cardiovascular diseases (Haslam and James, 2005), musculoskeletal disorders, and deterioration in cognitive function (Miller and Spencer, 2014). Interestingly, obesity is more common in women than men, independent of age, geographic region, or socioeconomic status (Kroll et al., 2020).

During obesity, the energy excess leads to adipose expansion generating hypertrophic adipocytes that produce a wide variety of proinflammatory molecules, such as monocyte chemoattractant protein-1 (MCP-1), TNF-α, IL-1β, and IL-6 that activate and attract the immune system cells (McArdle et al., 2013). The production of these molecules by adipocytes generates a chronic, low-intensity inflammation, affecting the organism's functioning; the central nervous system (CNS) may also be affected. Studies performed in rodent models show that obesity causes neuroinflammation (Miller and Spencer, 2014; Li et al., 2018; Zhou et al., 2020; Salas-Venegas et al., 2022). A hypercaloric diet induces the blood–brain barrier (BBB) loss of integrity, allowing proinflammatory cytokines to enter the CNS and promoting peripheral macrophage infiltration to the brain, which subsequently contributes, among other factors such as increased peripheral free fatty acid circulation, contributing to obesity-associated neuroinflammation (Samara et al., 2020). The inflammation in specific brain regions is related to memory impairment with significant cognitive decline (Palavra et al., 2016; Castro et al., 2017; Noble et al., 2017). Intriguingly, hippocampal neuroinflammation causes deficits in memory tasks in rodent models of obesity (Samara et al., 2020).

Inflammatory responses can be beneficial as acute, transient reactions to harmful conditions, promoting the defense, repair, and adaptation of host tissues. However, chronic and low-grade inflammation is detrimental to many tissues and likely to hamper normal functions (Calder et al., 2017). Chronic inflammation has become a hallmark of neurodegeneration in the brain, with persistent microglial activation, increased proinflammatory cytokines, and elevated levels of oxidative stress (Azam et al., 2021).

Additionally, some results indicate that the production of pro-inflammatory cytokines significantly reduces the expression of brain-derived neurotrophic factor (BDNF), the most expressed neurotrophin in the brain that plays a crucial role in learning and memory. The decreased BDNF expression has been associated with cognitive dysfunction and dementia (Budni et al., 2015). BDNF favors cognitive function by increasing neurogenesis, neuronal survival, axonal growth, dendritic growth, synaptic plasticity, neuronal development, and maintenance in the central nervous system neurons (Sandrini et al., 2018).

Moreover, clinical studies have shown that obesity increases the risk of developing mild cognitive impairment in short-term memory and executive function (Nguyen et al., 2014); however, the precise mechanisms are still unknown.

In addition, it has been reported that organisms fed with high-fat diets present an accumulation of senescent cells in tissues such as the prostate, heart, and adipose tissue with implications for the inflammatory state (Wang et al., 2009; Tikoo et al., 2017). Recently, Bussian et al. (2018) reported that the accumulation of senescent astrocytes and microglia increased neurodegeneration and cognitive decline. They observed that eliminating senescent cells prevented reactive gliosis and neurodegeneration in the cortex and hippocampus, thus preserving cognitive function.

Cellular senescence (CS) is a phenomenon in which cells stop proliferating in response to different stress stimuli, including oxidative stress, proteasome inhibition, autophagy malfunction, etc. Senescent cells are characterized by flattened and enlarged morphology and exhibit several molecular markers, including telomere-dysfunction-induced foci, senescence-associated heterochromatin foci (SAHF), lipofuscin granules, DNA scars, altered gene expression, absence of proliferative Ki-67 protein, the activity of senescence-associated β-galactosidase (SA-β-GAL), and the expression of tumor suppressors and cell cycle inhibitors (Watanabe et al., 2017; Yanagi et al., 2017; Dodig et al., 2019). They also show changes in gene expression, the absence of response to apoptotic and mitogenic stimuli, and metabolic reprogramming evidenced by the production of a secretome called "Senescence-Associated Secretory Phenotype" (SASP; Kuilman et al., 2010; Kong et al., 2011; Campisi, 2013; Salama et al., 2014). The SASP includes a variety of factors, such as interleukins, growth factors, chemokines, and metalloproteases. These factors can affect the surrounding cells by activating various cell surface receptors and signal transduction pathways (Coppé et al., 2010), creating a local tissue microenvironment that induces inflammation (Bianchi-Frias et al., 2010).

During obesity, the inflammation and oxidative stress generated in the brain increase the amount and accumulation of senescent cells, thus contributing to neuroinflammation due to the SASP secretion. The neuroinflammation induced by the senescent cells creates a vicious cycle that escalates inflammation and oxidative stress (Salas-Venegas et al., 2022). The components of SASP (proinflammatory and immunomodulatory cytokines) can interact with specific receptors, non-protein molecule factors, and exosomes (Gorgoulis et al., 2019). Thus, both soluble and insoluble interaction molecules are employed by senescent cells to influence the local microenvironment. However, with the accumulation of senescent cells during inflammation, its proinflammatory and potentially detrimental characteristics dominate and result in persistent inflammation and tissue damage (Nelke et al., 2022).

Two tumor suppressor pathways establish the CS, p53/p21, and p16INK4a/pRB, which can be used as proteins to validate the senescent state (McHugh and Gil, 2018). Animal models, mainly rodents, have been advantageous in studying obesity. Dietary manipulations through high-calorie intake are the most used for maintaining a more remarkable similarity with the establishment of obesity in humans (Von Diemen et al., 2006).

Notably, sexual differences have been reported concerning neurodegeneration and cognitive decline during aging (Santín-Márquez et al., 2021). Moreover, several neurological conditions are associated with sex differences in prevalence or outcome. For example, depression, multiple sclerosis, and Alzheimer's are common in women (Hanamsagar and Bilbo, 2016). So, it is crucial to study pathogenetic mechanisms, their progression, the age of onset, and possible treatment response in female models to develop the correct interventions for women. Ignoring these differences could alter the meaning of the obtained results (Piscopo et al., 2021).

The objective of this study was to determine the effect of systemic inflammation produced by the chronic consumption of a hypercaloric

diet on the establishment of senescence in the brain and its impact on cognitive deterioration in the obesity model of female Wistar rats.

Materials and methods

Chemicals

All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO). The reagents obtained from other sources are detailed throughout the text.

Animals

Sixty-four female Wistar rats (Rattus norvegicus) we used in this study. The animal was provided by the closed breeding colony at the Universidad Autónoma Metropolitana-Iztapalapa (UAM-I). They were housed four-per-cage in polycarbonate cages in a 12 h light–dark cycle and had free access to water and food. The animals' health status was constantly evaluated. A good state of health was considered when the animals did not have tumors, skin, or ear infections and when they ate and drank properly. Rats with tumors and those that went blind were discarded from the study. All animal procedures were strictly carried out according to Mexican Official Ethics Standard NOM-062-ZOO-1999 and the Standard for the disposal of biological waste (NOM-087-ECOL-1995).

Experimental groups

At 21 days of age, the 64 rats were randomly distributed into two groups: the Standard Diet (SD) group ($n = 32$) and the hypercaloric diet (HD) group ($n = 32$). The animals were euthanized at 13 months of age.

Animal diets

HD diet was prepared following the protocols previously reported (Bautista et al., 2017; Toledo-Pérez et al., 2021). The HD is based on an obesogenic diet with 23.5% protein, 20% animal lard (40% saturated fats), 5% corn oil, (60.7% polyunsaturated fats, 24.3% monounsaturated fats, 15% saturated fats, 0% cholesterol), 20.2% polysaccharides, 20.2% of simple sugars, 5% of fiber, 5% of the mineral mix, and 1% of vitamins (caloric intake, 4.9 kcal/g). The rats started consuming HD after weaning (at 21 days old) until they were euthanized at 13 months of age.

The SD groups were fed an Abene BDL-7100 diet containing 23% protein, 4.5% fat, and 46.5% carbohydrates (caloric intake, 3.2 kcal/g). The weekly food consumption was measured, and the average was plotted monthly. The results represent the consumption per box divided by the number of animals per box.

Morphometric and biochemical determinations

Animals were weighed and measured monthly to obtain the morphometric dimensions. The weight and naso-rectal length were used to obtain the Lee index to diagnose obesity in small animals, and those with an index greater than 0.30 were considered obese animals (Suárez Román et al., 2013). Rats were weighed with a digital scale 0SX40-SMART (Torrey, Mexico).

For the biochemical determinations, the blood (200 µl) was collected from the rats' tail veins after a fast of 8 h at 6 and 13 months of age. Serum was obtained by centrifugation at 3500 rpm for 10 min. Subsequently, glucose, creatinine, triglycerides, cholesterol, GOT, GPT, GGT, and HDL were determined using a biochemical blood analyzer (Spotchem EZ SP-4430; Arkray Inc., Kyoto, Japan) and reactive strips: Spotchem II Kenshin-2, glu, and cre2 (Arkray Inc., Kyoto, Japan). The atherogenic index was calculated as AI = (Total Cholesterol – High-Density Lipoprotein HDL)/HDL (Othman et al., 2019).

Sample preparation for the enzyme-linked immunosorbent assay (ELISA assay)

Treated and control animals were euthanized at 6 and 13 months of age. The blood samples were collected and centrifuged (Eppendorf, Hamburg, Germany) to obtain the serum (3,500 rpm at 4° C for 15 min). The whole brain was carefully extracted and washed with saline solution. Brain cortex (Cx) and hippocampus (Hc) were dissected and homogenized in 500 µl of lysis buffer (20 mM Tris, 0.25 M sucrose, 2 mM EDTA, 10 mM EGTA, 1% Triton X-100) containing protease inhibitor (11,836,153,001, Roche Diagnostics, Indianapolis, IN, United States). The samples were centrifugated at 13,500 rpm at 4°C for 15 min to collect the supernatant for the ELISA and the Western blots assays (Ugalde-Muñiz et al., 2020). At maximum speed, the tissue was homogenized using a polytron PTMR2100 7,549 (Omni International).

Cytokine and BDNF levels evaluation

TNFα, IL6, IL10, IL1β, MCP-1, and BDNF were measured in serum and brain tissue by sandwich ELISA assay following the provider's instructions. TNF-α DuoSet ELISA (DY510), IL-6 DuoSet ELISA (DY506), IL-10 DuoSet ELISA (DY522), IL-1β DuoSet ELISA (DY501), MCP-1 DuoSet ELISA (DY3144), BDNF DuoSet ELISA (DY248), and DuoSet ELISA Ancillary Reagent Kit (DY008), all purchased from R&D Systems (Minneapolis, MN, United States). All required solutions were prepared with deionized water from a Milli-RQ system (Millipore, MA). Serum and brain protein were incubated for 18 h at 4° C with PBS-Tween20 (0.05%)/0.5% BSA, washed three times, and incubated with the corresponding detection antibody for 2 h at room temperature. Bound detection antibodies were detected using system a-HRP (avidin-HRP/Streptavidin-HRP) using TMB/H2O2 as the substrate. Optical density readings were at 450 nm (Epoch BioTek, Winooski, VT, United States). All assays were performed by duplicate (Ugalde-Muñiz et al., 2020).

Novel object recognition test (NOR)

As reported previously, the NOR test was used to evaluate short-term and working memory in SD and HD groups at 6 and 13 months of age (Antunes and Biala, 2012; Santín-Márquez et al., 2021). The NOR test was performed using a 45 × 45 × 45 cm acrylic box. Each rat

was introduced into the box for 5 min daily over three consecutive days as a training period. On the fourth day, a pre-test was performed. Two random objects with different geometric shapes were placed in the box, and the animals explored them for 5 min. The exploration time for each object was recorded. The objects were changed and cleaned with 70% vol/vol ethanol before and between use. On the fifth day, the test was performed by placing one of the objects previously presented along with a novel one. The animals were then allowed to explore for 5 min, and the interaction time and the number of interactions with the old and new objects were recorded. The Novel preference index was calculated by dividing the time spent exploring the novel object by the total exploration time multiplied by 100 to obtain a percentage value.

Western blot analysis

Proteins (30 μg) from each fraction protein were separated on 12% SDS-PAGE, transferred to polyvinylidene difluoride membranes (Inmobilon-P, Millipore Billerica, MA), and incubated with specific primary antibodies against anti-βactin (sc-47,778), anti-γH2AX (sc-2,357), anti-p21 (sc-6,246), and anti-GLB (sc-65,670) dilution 1:1000. Membranes were washed three times with TBS-Tween and incubated with a horseradish peroxidase-conjugated secondary antibody dilution (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, United States) for 2 h. After three consecutive washes, the blots were developed using a commercial chemiluminescence reagent. The proportion of these proteins was quantified by densitometric analysis using Kodak Molecular Imaging Software (v.4.5.1).

Brain section

Rats were perfused transcardially with 4% paraformaldehyde in PBS and drop-fixed in 4% paraformaldehyde for 24 h. The brains were rapidly dissected and immersed in PBS/30% sucrose for 24 h. The brains were washed with PBS and embedded in tissue-tek (4,583 Sakura finetek, Torrance, CA, United States). Brain coronal sections (24 μm) from the frontal cortex were mounted serially.

SA-β-gal activity assay

The β-galactosidase activity was analyzed following the protocol described previously [26]. Brain sections were fixed with 4% paraformaldehyde, washed with PBS 1×, and stained with a solution containing 20 mg/ml of X-gal (V394A, Promega, Madison, WI, United States) in dimethylformamide, 0:2 M citric acid/sodium phosphate buffer pH = 6, 100 mM potassium ferrocyanide, 5 M sodium chloride, and 1 M magnesium chloride. Sections were incubated for 12 h at 37° C.

Statistical analysis

All data were analyzed and graphed with Prism 8 (GraphPad Software). Specific tests were performed according to each experimental design and are indicated in each figure.

Results

Morphometric parameters

Figure 1 shows the obesity establishment in HD-fed rats. From month 6 of age, a 15% increase in body weight was observed in the HD group compared to the SD group ($p < 0.034$). The increment continued until the last evaluation at 13 months, when the HD group presented a 51% higher body weight than the SD group ($p < 0.0001$). Table 1 illustrates the naso-rectal length in both animal groups at 6 and 13 months. As expected, the length increased in both groups over time, with 6% growth in the SD group and 9% in the HD group. However, no significant differences were found between them, indicating that the physical development of the animals was similar regardless of the diet.

The Lee index was determined using the data of body weight and naso-rectal length. Table 1 shows that the Lee index in the HD group increased by 8% at 6 months of age and by 13% at 13 months of age compared to the SD group, confirming that HD animals were obese from 6 months of age.

Food and water consumption

Food consumption was evaluated monthly (Supplementary Figure 1). In the third month, the SD group consumed 35% more food (measured in g) compared to the HD group, and from month 5 to month 10, there was an increase and then a decrease in food intake (32, 42, 36, 37, 35, and 25% respectively). However, as seen in Supplementary Figure 1B, the monthly kcal intake in the HD group was higher than in the SD group over time. The increase in calorie consumption in the HD group was 24% at month 4, and as time progressed, an increase of 22, 25, 29, and 26% was found at months 9, 10, 11, 12, and 13, respectively. Water consumption was also evaluated, but no differences were found between the groups (data not shown).

Biochemical parameters

The biochemical parameters, including the GOT/GPT ratio, triglycerides, creatinine, glucose, cholesterol, high-density lipoprotein (HDL), atherogenic index, and low-density lipoprotein (LDL), are shown in Table 2. Some parameters significantly increased in the HD group compared to the SD group. In particular, an 11-fold increase in triglyceride levels was observed in the HD group at 13 months of age (86 mg/dl) compared to levels in the same group at 6 months (7.8 mg/dl). Augmented glucose levels were observed in both groups at 13 months of age compared to the values obtained at 6 months. A 1.30-fold increase in glucose levels was observed in the SD group and a 1.68-fold increase in the HD group. Regarding cholesterol levels, the SD group showed increased levels compared to the HD group at 6 months; however, at 13 months, the HD group increased their cholesterol levels by 1.40 times compared to the values obtained at 6 months.

When evaluating the atherogenic index, a 2.4-fold increase was observed in the HD group compared to the SD group at 6 months. HDL concentrations were higher in the SD group compared to the HD group at 6 months of age. However, this parameter did not significantly change at 13 months of age.

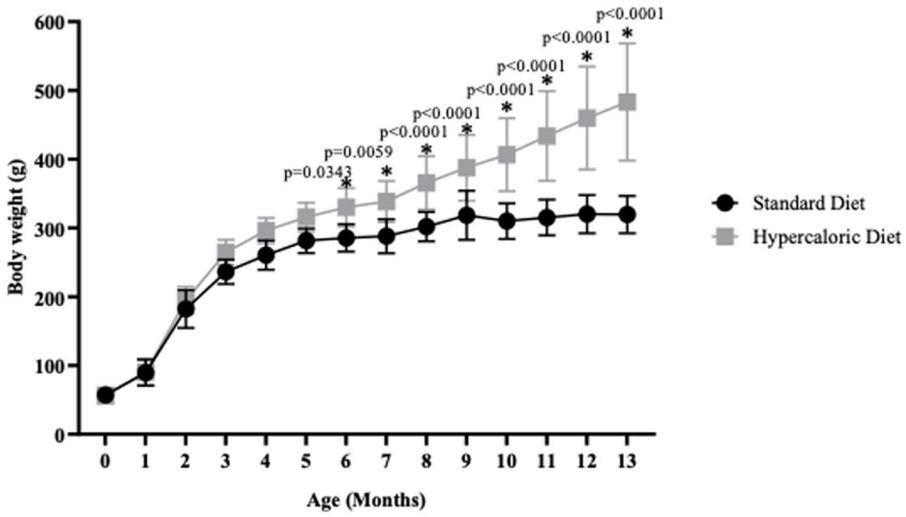

FIGURE 1
Body weight. Animal body weight was evaluated monthly (n=17 SD and n=15 HD). The data represent the mean±standard deviation (SD) and were analyzed by ANOVA and *post hoc* Holm-Sidak. The p-values are indicated in the graph<0.05. The significant differences between groups compared to the HD are marked with *.

TABLE 1 Lee's index.

Age	Group	Weight (g)	Lenght N-R (cm)	Lee's index
6 months	SD	280.5 ± 16.96	22.65 ± 0.4408	**0.2889** ± 0.0080
	HD	361 ± 16.39	22.7375 ± 0.4897	**0.3132** ± 0.010*
13 months	SD	329 ± 27.23	23.95 ± 0.04870	**0.2878** ± 0.0050
	HD	532.25 ± 81.65	24.8 ± 1.150	**0.3262** ± 0.0189*

The data were evaluated in rats fed with SD and HD at 6 and 13 months of age (n = 10 SD n = 10 HD). The Lee index was obtained as described in the materials and methods section. The data represent mean ± SD and were analyzed by two-way ANOVA, followed by a Tukey's *post hoc* test. The significant differences between groups compared to the SD are marked with *p < 0.05. The values in bold indicate the Lee´s index.

It is essential to highlight that, despite finding differences in some evaluated parameters, none of these values were outside the clinical parameter limits established for Wistar laboratory rats.

Inflammatory profile

Serum inflammatory profile

Once the obesity model was validated, the impact on the systemic inflammatory response was evaluated by quantifying the concentrations of the cytokines in the serum in both animal groups at 6 and 13 months of age. Figures 2A,B show that IL-1β and IL-6 levels in the HD group were significantly higher than in the SD group at 6 and 13 months of age; IL-1β levels were 2.07 times greater at 6 months and 3.23 times greater at 13 months in HD rats than in SD. While in the HD group, IL-6 levels increased 2.18 times at 6 months and 1.36 times at 13 months compared to the SD. Interestingly, in the SD group, an augment in this cytokine was also associated with age since it increased 1.58-fold at 13 months compared to 6 months.

The serum concentration of monocyte chemoattractant protein 1 (MCP-1) shown in Figure 2C presented the same behavior as IL-1β, both HD groups increased MCP-1 concentration compared to the SD groups (1.33 times at 6 months and 1.63 times at 13 months of age).

Figure 2D shows that TNF-α concentration in the HD group significantly increased by 2.36% at 6 months and 2.55% at 13 months of age compared to the SD group. However, both 13 moth-old groups decreased TNF-α concentration at month 13 of age. SD group significantly decreased by 2.06-fold and the HD group by 1.91-fold; the HF rats showed a higher TNF-α concentration.

IL-10 concentration was quantified to assess the anti-inflammatory response. Figure 2E shows that the HD group significantly increased by 1.33-fold at 6 months; however, at 13 months, IL-10 levels decreased in both groups, being more remarkable in the HD group (2.20 decrease fold) compared to the SD group (1.68-fold).

Cerebral cortex inflammatory profile

Figure 3 shows cytokine concentrations in the rat cerebral cortex. No differences were found in IL-1β and IL-10 concentrations (Figures 3A,E), but a significant increase in IL-6 was found at the two-time points measured in the HD group compared to the SD group (Figure 3B). At 6 months of age, IL-6 concentration in the HD group was 1.23 times higher than in the SD group, and at 13 months, it was 1.42 times higher than in the SD group. MCP-1 levels showed the same behavior. The HD groups had significantly higher levels compared to the SD group (1.78-fold increase at 6 months of age and 1.81-fold at 13 months), suggesting that the HD group had higher Cx

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TABLE 2 Biochemical parameters.

Parameter	6 months of age		13 months of age	
	SD	HD	SD	HD
GOT/GPT (IU/L)	2.669 ± 0.9436	7.651 ± 5.097	4.760 ± 0.9010	6.451 ± 1.515
TRIGLYCERIDES (mg/dl)	3.8 ± 5.495	7.8 ± 5.675	48.00 ± 17.65	86.00 ± 25.07 & $p = 0.0229$
CREATININE (mg/dl)	0.44 ± 0.2966	0.2 ± 0.07071	0.3400 ± 0.05477	0.3400 ± 0.05477
GLUCOSE (mg/dl)	88.2 ± 14.74	78.6 ± 10.69	115.4 ± 5.459 & $p = 0.0080$	132.8 ± 12.52 & $p < 0.0001$
CHOLESTEROL (mg/dl)	76.40 ± 9.317* $p = 0.0298$	56.60 ± 6.348	85.80 ± 12.50	79.60 ± 10.85 & $p = 0.0108$
HDL (mg/dl)	22.60 ± 6.107* $p = 0.0029$	8.800 ± 3.962	19.00 ± 6.519	14.40 ± 2.966
LDL (mg/dl)	53.04 ± 4.651	46.24 ± 3.648	57.20 ± 7.580	48.00 ± 7.331
ATHEROGENIC INDEX	2.569 ± 0.8980	6.270 ± 2.420* $p = 0.0321$	3.721 ± 0.7594	4.634 ± 0.8367

The table shows the values obtained of GOT/GPT, triglycerides, creatinine, glucose, cholesterol, HDL, LDL, and atherogenic index determined in rats fed with a standard diet (SD) and hypercaloric diet (HD) at 6 and 13 months of age ($n = 5$ SD and $n = 5$ HD). The data represent the mean ± SD and were analyzed by ANOVA. The significant differences between groups concerning the SD are marked with ∗, and differences at different ages are marked with & $p < 0.05$.

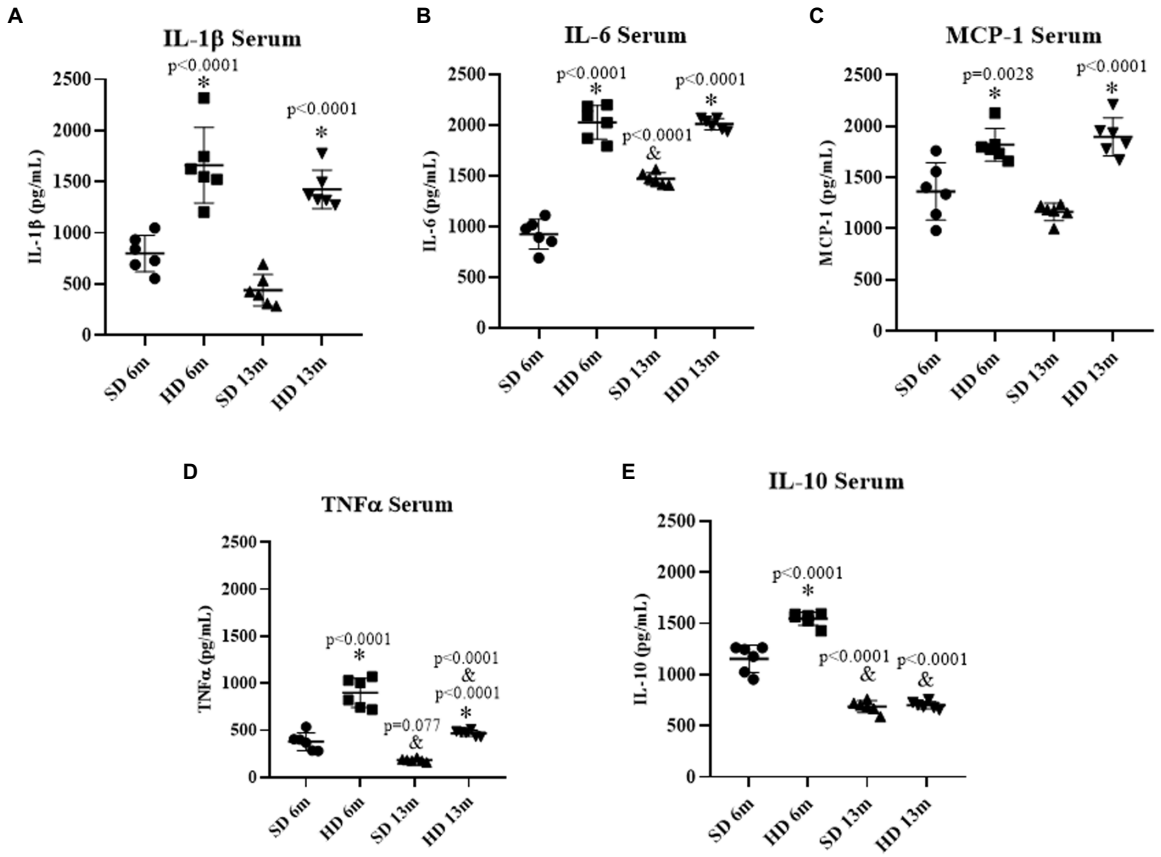

FIGURE 2
Serum inflammatory profile. The levels of IL1β **(A)**, IL6 **(B)**, MCP-1 **(C)**, TNFα **(D)**, and IL10 **(E)** were determined by ELISA (n=6 SD and n=6 HD). The data represent the mean±standard deviation and were analyzed by two-way ANOVA, followed by Tukey's *post hoc* test. The significant differences between groups with respect to the SD are marked with ∗, and differences at different ages are marked with & p<0.05.

inflammation (Figure 3C). TNF-α levels in the HD group were significantly higher compared to the SD group at 6 months of age (1.77-fold increase) and 13 months of age (3.02-fold increase).

Hippocampus inflammatory profile

Figure 4A shows a significant decrease in hippocampal IL-1β concentrations in both study groups (SD and HD) at 13 months of age, but no differences were observed regarding HD diet consumption. IL-6 concentration did not change at 6 months either (Figure 4B), but an increase was found at 13 months of age in the HD group (1.86-fold more than the same age SD group and the young groups). While MCP-1 concentrations in the HD group (Figure 4C) significantly increased at 6 months of age (1.37-fold) and 13 months of age (2.54-fold) compared to the SD

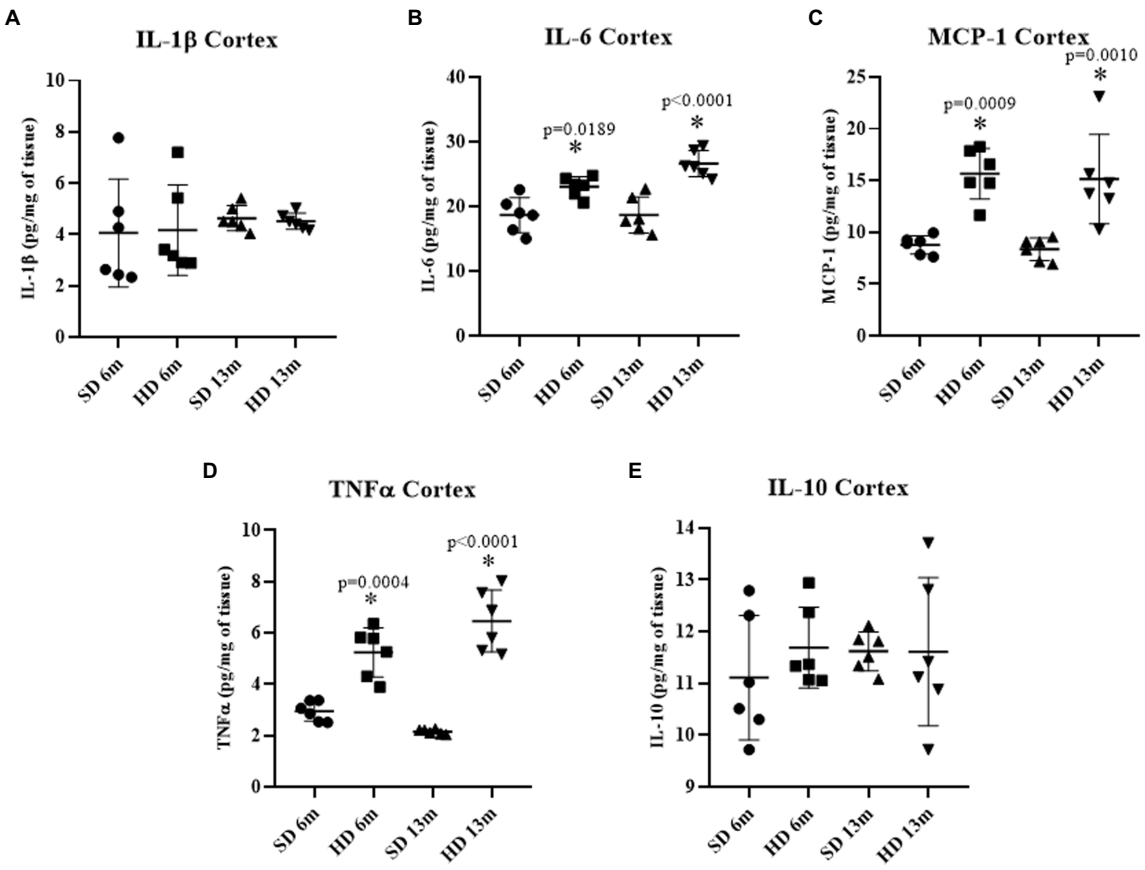

FIGURE 3
Cerebral cortex inflammatory profile. The levels of IL1β **(A)**, IL6 **(B)**, MCP-1 **(C)**, TNFα **(D)**, and IL10 **(E)** were analyzed by ELISA (n=6 SD and n=6 HD). The data represent the mean±SD and were analyzed by two-way ANOVA, followed by Tukey's *post hoc* test. The significant differences between groups compared to the SD are marked with ∗ p<0.05.

group. A decrease in the concentration of MCP-1 was observed in the SD group at 13 months of age compared to the younger group (1.72-fold). No significant differences were found in TNF-α and IL-10 concentrations (Figures 4D,E) between the two groups at 6 and 13 months. However, a 1.27-fold increase in IL-10 concentrations was observed in the SD group at 13 months compared to the 6 months group.

Brain-derived neurotrophic factor (BDNF)

Figure 5A shows that serum BDNF concentration decreased by 2.61 times in the HD group at 6 months of age compared to the SD group at the same age. A 3.28-fold decrease in BDNF concentration was also observed in the SD group at 13 months compared to the values obtained at 6 months in this same group. Concerning BDNF levels in the Cx (Figure 5B), a 4.1-fold decrease was observed in the HD group compared to the SD group at 6 months. However, at 13 months, BDNF increased in both groups (SD and HD) compared to the levels found at 6 months. In the Hc (Figure 5C), BDNF concentrations diminished in the HD group compared to the SD group at 6 and 13 months. In summary, BDNF increased in both groups (SD and HD) at 13 months, as opposed to 6 months.

Novel object recognition test (NOR)

The NOR test was performed to determine the possible detrimental effects of neuroinflammation on cognition; the NOR test was performed at 6 and 13 months of age following the scheme shown in Figure 6A. The interaction time with the novel object is shown in Figure 6B; the rats in the SD and HD groups spent more time exploring the novel object at 6 months than at 13. However, at 13 months, the SD group did not differentiate between the familiar and the novel object, equaling the exploration time between the objects.

When evaluating the number of interactions with each object, the SD group at 6 and 13 months interacted similarly with both objects (Figure 6C). With an evident decrease in the recognition index, the HD rats did not discriminate the novel object from the familiar object at 6 months, as seen in Figure 6D, being more pronounced in both groups at 13 months of age, especially in the HD group, which presented a more significant deficit in recognizing novel objects.

Senescence markers

To evaluate the participation of senescence in cognitive deterioration, GLB, p21, and γH2AX were determined in the Cx and

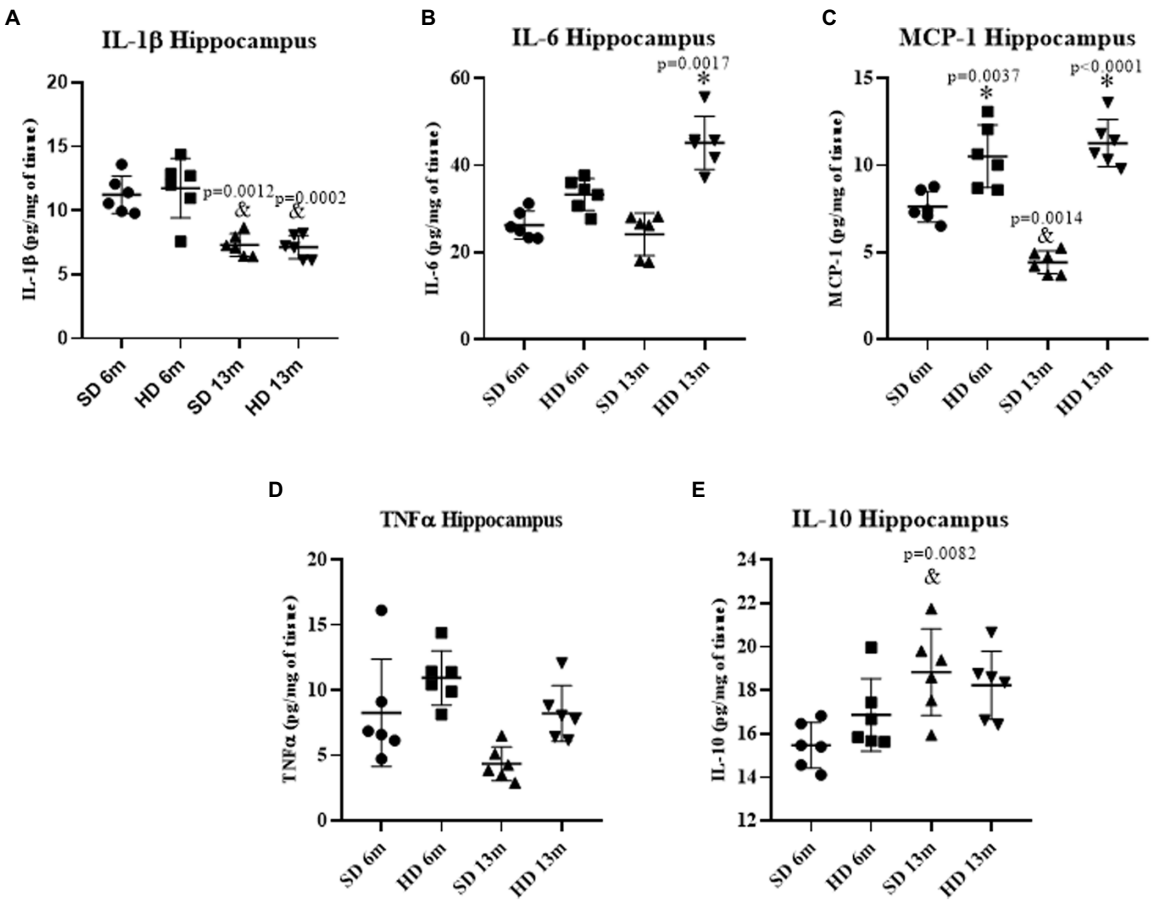

FIGURE 4
Hippocampus inflammatory profile. The levels of IL1β **(A)**, IL6 **(B)**, MCP-1 **(C)**, TNFα **(D)**, and IL10 **(E)** were analyzed by ELISA (n=6 SD and n=6 HD). The data represent the mean±SD and were analyzed by two-way ANOVA, followed by a Tukey's *post hoc* test. The significant differences between groups compared to the SD are marked with ∗, and differences at different ages are marked with & p<0.05.

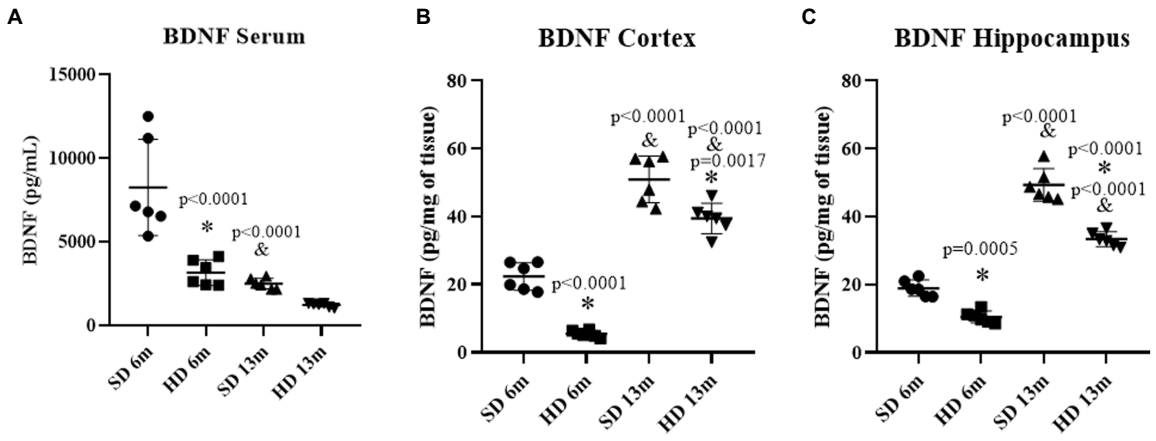

FIGURE 5
Brain Derived Neurotrophic Factor levels. The levels of BDNF in serum **(A)**, cortex **(B)**, and hippocampus **(C)** were determined by ELISA (n = 6 SD and n = 6 HD). The data represent the mean±SD and were analyzed by two-way ANOVA, followed by Tukey's *post hoc* test. The significant differences between groups compared to the SD are marked with ∗, and differences at different ages are marked with & p < 0.05.

Hc at 6 and 13 months of age. Figure 7A shows a significant increase of 1.12 times in GLB expression in the Cx of the 13-month-old HD group compared to the SD group at the same age. Unlike the SD group, where no changes in the expression of this enzyme were observed throughout life, changes were observed in the HD group at 13 months of age, with an increment of 2.12 times compared to

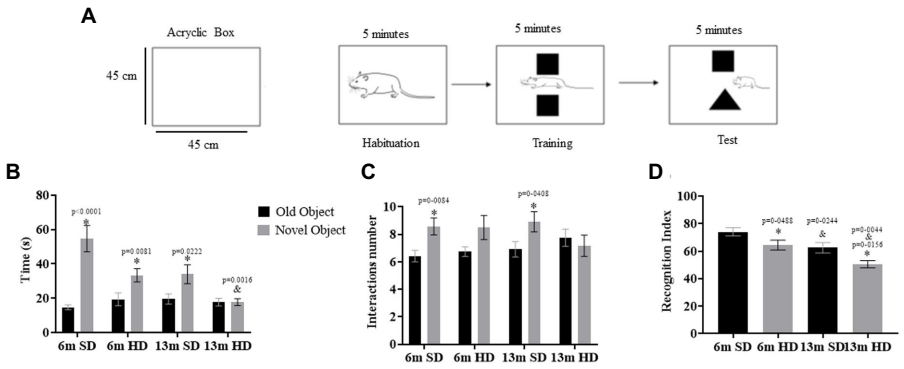

FIGURE 6
Novel object recognition test. **(A)** Schematic representation of protocol for a novel object recognition test. Familiar and novel object interaction time **(B)**, interactions number **(C)**, and recognition index **(D)** determined in rats fed with SD and HD at 6 and 13months of age (n=12 SD and n=12 HD). Each bar represents the mean±SD. The data were analyzed by two-way ANOVA and Tukey's *post hoc* test. The significant differences between groups with respect to the SD are marked with ∗, and differences at different ages are marked with & p<0.05.

6 months. No differences were observed in GLB expression in this region at 6 months old, regardless of the diet.

As observed in Figure 7B, GLB expression increased in the Hc at 13 months of age in rats in the HD group compared to the SD group. At 6 months, no differences in GLB expression were observed in this brain region between the SD and HD groups. Figure 7C indicates that the HD group had significantly higher levels of p21 in the Cx compared to the SD group at 6 months and 13 months, with an increase of 1.93 and 1.82-fold, respectively. The p21 levels in the SD group were low and stable, while in the HD group, they were high and constant. No differences were obtained in p21 expression in the Hc at 6 months of age (Figure 7D) between the SD and HD groups. However, at 13 months of age, a significant increase in p21 levels in the Hc was observed in the HD group compared to the SD group (1.38-fold). In addition, the same increase in p21 levels was found in the HD group at 13 months compared to the 6 months in the same group. Figure 7E shows that the expression of histone γH2AX in the Cx of HD rats increased 1.70 and 1.95 times compared to the SD group at 6 and 13 months, respectively; the levels of this histone increased in the HD group at 13 months compared to the 6 months old rats. When evaluating the expression levels of γH2AX in the Hc (Figure 7F), a significant increase of 1.82 times was found in the HD group at 13 months of age compared to the SD group. No changes in the expression of this histone were found at 6 months between both groups.

SA-β-gal assay

Senescent cells in the Cx and Hc were only evaluated at 13 months in SD and HD groups because we only found changes in all the senescence markers at this age.

Figure 8A is an anatomical diagram illustrating the specific regions of the brain in which the presence of senescent cells was studied. The CA2 region of the hypothalamus and the region of the somatosensory cortex are specifically mentioned. These regions are essential for memory and sensory perception, respectively. The lysosomal β-galactosidase assay was used to detect the presence of senescence in these brain areas. Figure 8B shows SA-β-gal positive cells in Hc (CA2 specifically) and Cx (somatosensory cortex) in rats with SD and HD at 13 months of age. The presence of senescent cells is more evident in the HD group in both areas analyzed in concordance with the increase of senescent markers previously shown.

Figures 8C,D show the quantification of the SA-β-gal positive cells in the Cx and Hc. In the HD group, there was an increase of 30% in SA-β-gal positive cells in the Cx and 14% in the Hc compared to the SD group. These results suggest a higher number of senescent cells in subjects in the HD group compared to those in the SD group.

Discussion

Obesity is a complex condition that affects individuals both physically and mentally. Physically, it increases the risk of chronic diseases such as diabetes, cardiovascular disease, cognitive decline, and certain cancers. At a psychological level, it can lead to low self-esteem, depression, and decreased quality of life. Our study focused on understanding the link between obesity and cognitive decline during long-term HD in middle-aged female rats since there is a higher rate of obesity in women, and they have a greater propensity to present dementia during advanced age (Albanese et al., 2015; Ghasemi Yngyknd et al., 2021).

The weight gain and the Lee index of the HD rats concord with previous studies (Malafaia et al., 2013; Suárez Román et al., 2013; Toledo-Pérez et al., 2021, p. 23), thus validating the obesity model. Moreover, we also found an increase in the atherogenic index, a risk marker for atherosclerosis and coronary heart disease (Niroumand et al., 2015), in the HD groups at 6 and 13 months of age. Interestingly, the SD group showed an increase in cholesterol and HDL levels, which could be due to the effect of reverse cholesterol transport, as described by Marques et al., 2018, as a mechanism for removing excess cholesterol from peripheral tissues and redistributing it or removing it from the body by the gallbladder.

At 13 months of age, both groups presented an increase in triglycerides, glucose, and cholesterol, which could be due to the transition from young adulthood to middle age, generating an accumulation of excess visceral fat, muscle mass, and strength loss, and an increase in adipocyte size in subcutaneous fat leading to

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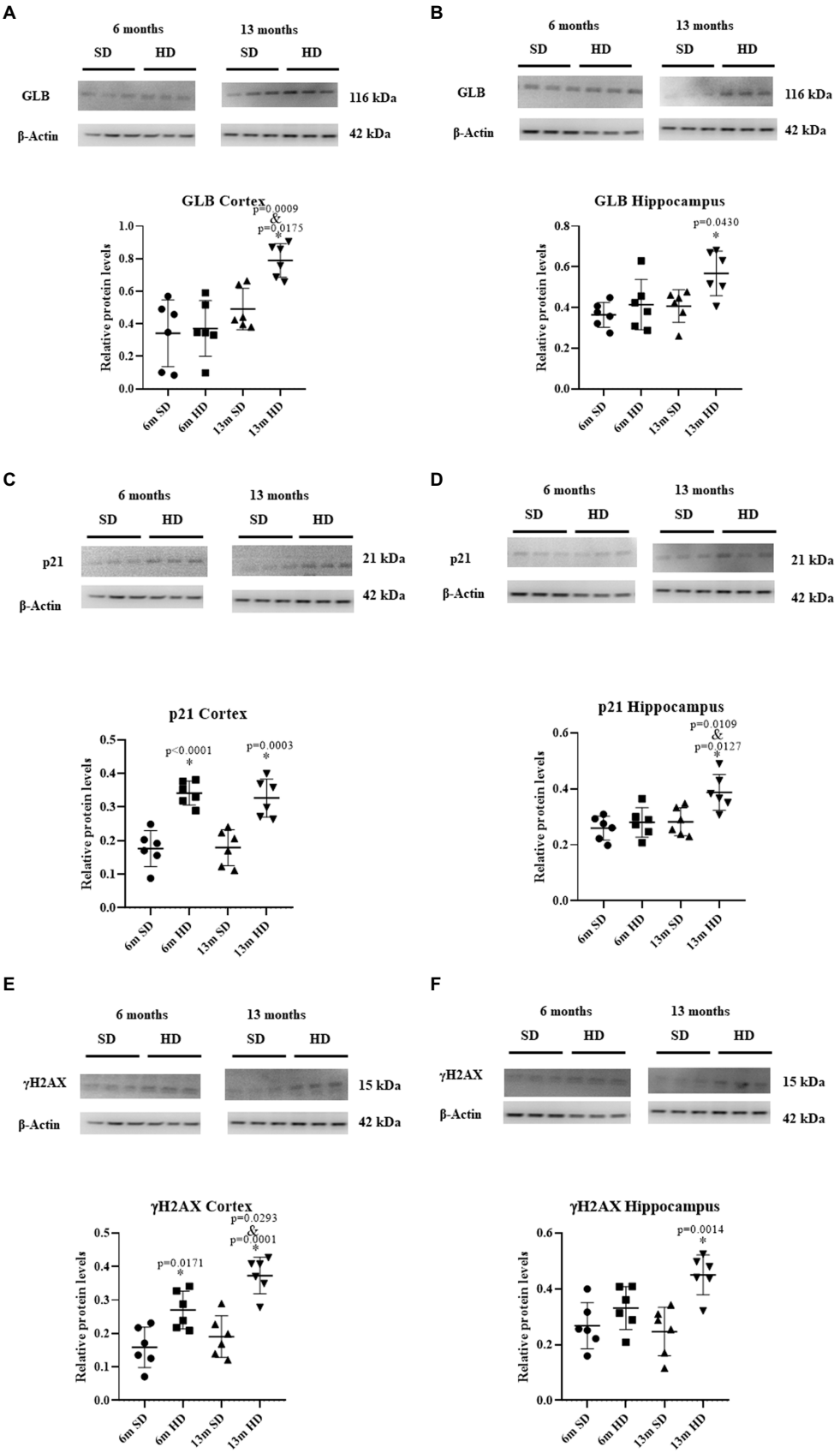

FIGURE 7
Expression of molecular markers of senescence in brain. Representative Western blot analysis of GLB in the Cx **(A)** and Hc **(B)**; p21 in the Cx **(C)** and Hc **(D)**; and γH2AX in the Cx **(E)** and Hc **(F)**. The graphs show the densitometric protein expression analysis from six animals per age group (each dot represents an animal; *n*=6 SD and *n*=6 HD). The data represent the mean±SD, the data were compared using the two-way ANOVA and Tukey's *post hoc* test. The significant differences between groups compared to the SD are marked with *, and differences at different ages are marked with & $p<0.05$.

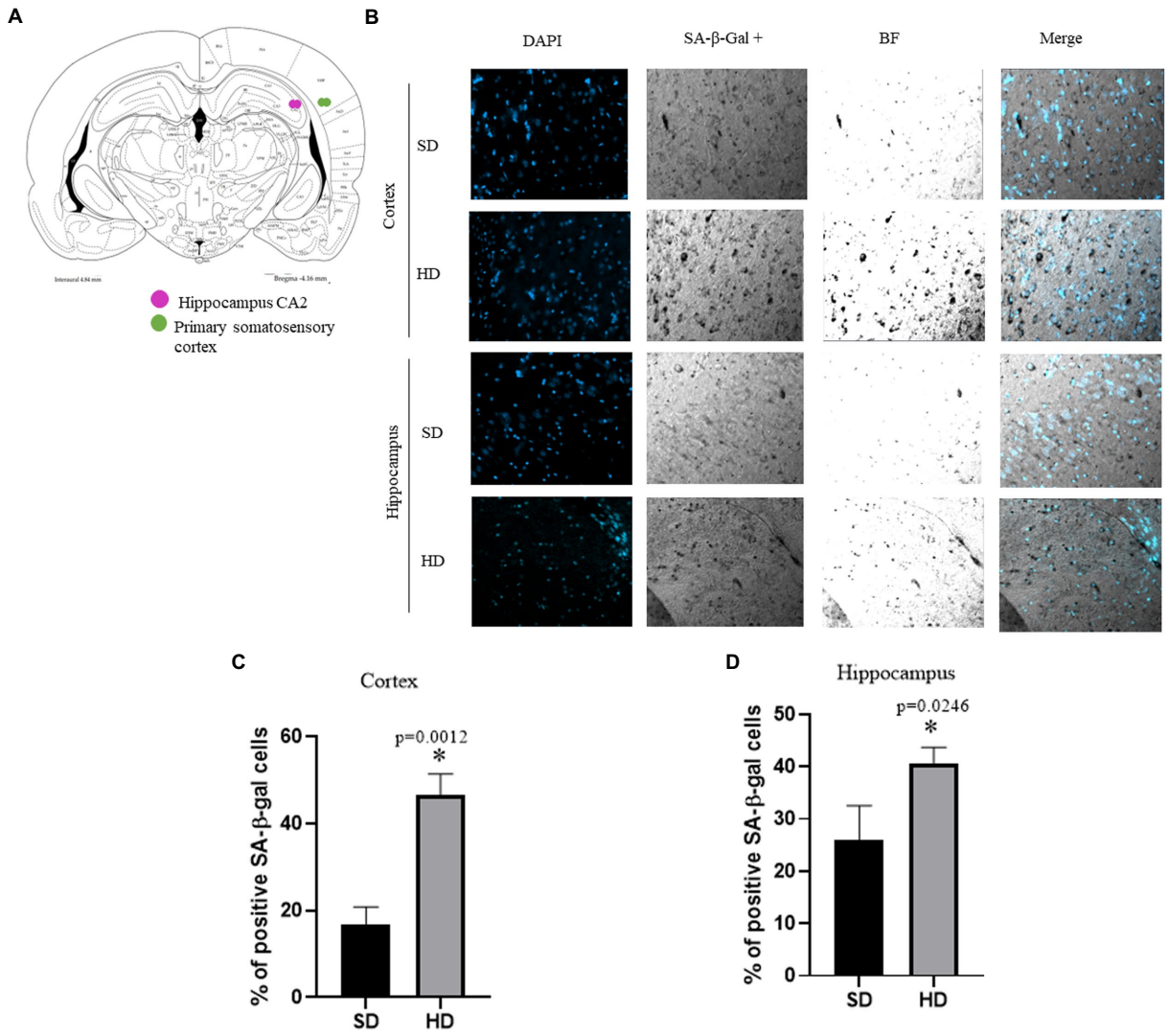

FIGURE 8
Evaluation of senescent cells in brain. **(A)** Anatomic diagram to locate the regions analyzed. **(B)** Representative images of different regions with cells with senescence features accumulate in the Cx and Hc in animals with HD at 13months (n=6 SD and n=6 HD). The percentage of SA-β-gal positive cells per area in each brain section in cortex **(C)** and hippocampus **(D)**. The average of three brains per group is graphed. The data represent the mean±SD and were analyzed by two-way ANOVA, followed by Tukey's *post hoc* test. The significant differences between groups compared to the SD are marked with ∗ and differences at different ages are marked with & p<0.05. Confocal fluorescence micrographs are 20× magnification. n=3.

increased release of free fatty acids (FFAs) associated with insulin resistance and other metabolic abnormalities, as reported by Palmer and Jensen, 2022. It is important to note that no significant differences were found when comparing the values of these parameters between the two groups at 6 and 13 months considering the Harlan manual (Giknis and Clifford, 2008).

Our results agree with previous findings, where obesity-induced chronic low-grade inflammation (Bing, 2015; Sindhu et al., 2015; Khanna et al., 2022) generates an increase in MCP-1, IL-1β, and IL-6 as observed in the HD group at 6 and 13 months. Serum levels of these cytokines are raised in obese individuals and those with chronic inflammatory conditions and abnormal serum lipid concentrations (Panee, 2012; Sindhu et al., 2015; El-Mikkawy et al., 2020), which aligns with the changes in the biochemical profile observed in our study (shown in Table 2). Additionally, IL-6 adversely impacts lipid metabolism by increasing triglyceride release and reducing lipoprotein lipase activity (Werida et al., 2021). Interestingly, we found an increase in the circulating levels of TNF-α in the HD groups at 6 and 13 months of age; however, those levels decreased at 13 months of age. This observation could be explained due to the individual immune system responsiveness and adaptability to a particular inflammatory condition regarding the degree of stimulation (severity) and persistence (chronicity; Italiani et al., 2020). The same behavior was observed for the anti-inflammatory cytokine IL-10, which increased in HD rats at 6 months and decreased in both groups at 13 months. This phenomenon is consistent with other studies that have reported that IL-10 decreases with age and obesity in animal and human models (Dagdeviren et al., 2017; Kondo et al., 2018), especially in obese women (Esposito et al., 2003).

Low-grade systemic inflammation induced by obesity also affects the central nervous system (CNS), generating neuroinflammation mainly in the Hc and Cx, impairing learning and memory (Henn

et al., 2022). Accordingly, we found elevated cytokine levels in those brain regions in the HD groups. IL-6, TNF-α, and MCP-1 levels increased in the Cx of HD-rats at 6 and 13 months of age (Figures 3B–D), while in the Hc, we found a rise in IL-6 in the HD group only at 13 months, and MCP-1 at 6 and 13 months. The cytokine increment indicates an ongoing inflammatory response in these brain regions, which may contribute to developing neurological disorders and learning deficits (Schmitt and Gaspar, 2023). Since the Cx has a higher number of neurons and fewer astrocytes and microglia than the Hc, these differences in cell composition could contribute to the observed differences in response to HD and obesity.

Moreover, obesity-induced inflammation changes the blood–brain barrier integrity, leading to increased permeability and the entrance of proinflammatory cytokines to the brain, which activates glial cells, such as microglia and astrocytes, leading to the activation of these cells (Salas-Venegas et al., 2022). This process can contribute to the harmful effects of obesity on the brain. Activated microglia secrete more proinflammatory cytokines, such as TNFα, IL-1β, and IL-6, which can perpetuate neuroinflammation and result in neuronal damage. Additionally, reactive astrocytes activated by proinflammatory signals have been linked to synaptic degeneration and glutamate dysregulation, contributing to neurodegeneration (Kwon and Koh, 2020). These mechanisms highlight the complex interplay between obesity-induced inflammation and brain function.

Brain-derived neurotrophic factor (BDNF) is a central molecule in neuronal outgrowth, differentiation, repair, and synaptic connections. Its levels are tightly regulated in response to different stimuli (Sandrini et al., 2018). Here a decrease in BDNF circulating levels in the HD group at 6 months of age was found, which further decreased with age. This observation is consistent with other studies that have found lower levels of BDNF in obese organisms. Reduced levels of BDNF can impact neural development and cognitive function by diminishing neuroplasticity, which is essential for learning, memory, and other cognitive functions. In rodents and humans, high levels of BDNF have been detected in various regions of a normal brain, including the hippocampus, amygdala, cerebellum, and cortex (Miranda et al., 2019).

Moreover, BDNF increases in the murine Hc during normal aging but not in pathological aging (Halbach, 2010). Here, lower BDNF levels in the Cx and Hc were determined in the HD group at 6 months. According to the literature, higher BDNF levels were determined at 13 months of age in the Cx and Hc of both animals groups when compared to the 6 months old rats; however, the levels observed in the HD group were significantly lower than the SD group in both brain regions, suggesting that BDNF decreased levels could contribute to the learning and memory deficits observed in obese individuals. Our results seem to align with previous studies that have reported a negative correlation between serum BDNF levels, obesity markers, and inflammation (Raharjo et al., 2021).

Research on animal models of obesity has suggested a link between obesity-induced inflammation and the development of cognitive alterations (Schmitt and Gaspar, 2023). Our results using the NOR test showed that rats in the HD group significantly decreased their ability to discriminate between the new and the familiar object at 6 months. That was more evident at 13 months of age. Interestingly, rats in the SD group also diminished their ability to discriminate the objects with age.

These results suggest that both diet-induced obesity and normal aging can impair the ability to discriminate a new object, which is an indicator of hippocampal-dependent learning and memory. The decline in performance could be due to the negative impact of obesity on the integrity of the Hc and other brain regions, such as the Cx, as well as the changes in synaptic plasticity and dendritic spine density associated with neuroinflammation and BDNF dysregulation. These findings highlight the importance of considering obesity and aging as risk factors for cognitive decline.

The onset of obesity is related to increased senescent cells within adipose tissue and other organs in response to various stressful stimuli (Chaib et al., 2021). Thus, the SASP is increasingly recognized as a critical driver of age-related pathologies, such as chronic inflammation. Of note, SASP components are effective proinflammatory cytokines capable of attracting and activating immune cells, thereby promoting an inflammatory tissue environment. Thus cells with a senescent phenotype resistant to apoptosis might provide a proinflammatory microenvironment sustaining the progression of neuroinflammatory diseases (Nelke et al., 2022).

Cellular senescence is a process in which cells change their characteristic phenotype in response to stress and enter a state of prolonged cell cycle arrest accompanied by a distinctive secretory phenotype which can alter the tissue microenvironment and contribute to inflammation, oxidative stress, and tissue dysfunction (Tchkonia et al., 2013; Rachmian and Krizhanovsky, 2022). It is known that senescent cells accumulate during obesity (Minamino et al., 2009; Ogrodnik et al., 2017; Schafer et al., 2017).

Senescent cells in the tissues contribute to chronic inflammation in two ways. First, additive or synergistic effects of paracrine senescence amplify the SASP and exacerbate inflammation. Mediators of paracrine senescence include reactive oxygen species (ROS) signaling through gap junctions, growth factors and chemokines, and small extracellular vesicles. Second, the impaired immune function and a reduced rate of senescent cell clearance. The resulting accumulation of senescent cells further reinforces the inflammation (Guerrero et al., 2021).

In a study realized by Ogrodnik and collaborators, they found that obesity results in the accumulation of senescent glial cells in proximity to the lateral ventricle, a region in which adult neurogenesis occurs, their elimination restored neurogenesis and alleviated anxiety-related behavior (Ogrodnik et al., 2017). We decided to evaluate the expression of senescence markers (GLB, p21, and γH2AX) in middle age rats (13 months old) during obesity. We found a significant rise in the three senescence markers at 13 months in the HD group in the Cx and Hc. In recent years, evidence emerged supporting the accumulation of senescent cells in the brain during several neurological disorders. This data correlated with an increase in the number of cells SA-β-gal positive cells in these brain regions. Some studies showed that the accumulation of senescent glial cells and neurons leads to structural and functional changes in the brain that result in cognitive impairment in the context of aging (Sikora et al., 2021). Hence, our study's contribution is that we indeed demonstrate that obesity-related cellular senescence can contribute to the development of age-related cognitive decline. The increased expression of senescence markers in the HD group reveals a higher burden of senescent cells in the Cx and Hc of obese rats, which may contribute to the observed decline in cognitive function. Their clearance has been shown to delay and alleviate those neurodegenerative symptoms (Kirkland and Tchkonia, 2017). Further research is needed to understand the exact mechanisms by which

senescent cells contribute to cognitive decline in obesity. Targeting senescence is a promising strategy to prevent obesity-related neuropsychiatric diseases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by COMISIÓN ACADÉMICA DE ETICA DE LA DIVISIÓN DE CIENCIAS BIOLÓGICAS Y DE LA SALUD. Dictamen: CECBS21-02.

Author contributions

VS-V was involved in the design of the study, generation, collection, and interpretation of the data, as well as in the manuscript writing. RS-M, RR-C, YR-C, and AC-M assisted in the generation, collection, and interpretation of the data. AL-L and AC were involved in the analysis and interpretation of the data. VS-V, MK, and NL-D were involved in the design of the study, analysis of the data, and revision of the manuscript. MK and NL-D also supervised the investigation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1162747/full#supplementary-material

References

Albanese, E., Davis, B., Jonsson, P. V., Chang, M., Aspelund, T., Garcia, M., et al. (2015). Overweight and obesity in midlife and brain structure and dementia 26 years later: the AGES-Reykjavik study. *Am. J. Epidemiol.* 181, 672–679. doi: 10.1093/aje/kwu331

Antunes, M., and Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process.* 13, 93–110. doi: 10.1007/s10339-011-0430-z

Awada, R., Parimisetty, A., and Lefebvre d'Hellencourt, C. (2013). *Influence of obesity on neurodegenerative diseases in neurodegenerative diseases*. ed. U. Kishore (InTech), 381–401.

Azam, S., Haque, M. E., Balakrishnan, R., Kim, I. S., and Choi, D. K. (2021). The ageing brain: molecular and cellular basis of neurodegeneration. *Front. Cell Dev. Biol.* 9:683459. doi: 10.3389/fcell.2021.683459

Bautista, C. J., Rodríguez-González, G. L., Morales, A., Lomas-Soria, C., Cruz-Pérez, F., Reyes-Castro, L. A., et al. (2017). Maternal obesity in the rat impairs male offspring aging of the testicular antioxidant defence system. *Reprod. Fertil. Dev.* 29, 1950–1957. doi: 10.1071/RD16277

Bianchi-Frias, D., Vakar-Lopez, F., Coleman, I. M., Plymate, S. R., Reed, M. J., and Nelson, P. S. (2010). The effects of aging on the molecular and cellular composition of the prostate microenvironment. *PLoS One* 5:e12501. doi: 10.1371/journal.pone.0012501

Bing, C. (2015). Is interleukin-1β a culprit in macrophage-adipocyte crosstalk in obesity? *Adipocytes* 4, 149–152. doi: 10.4161/21623945.2014.979661

Budni, J., Bellettini-Santos, T., Mina, F., Garcez, M. L., and Zugno, A. I. (2015). The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging Dis.* 6, 331–341. doi: 10.14336/AD.2015.0825

Bussian, T. J., Aziz, A., Meyer, C. F., Swenson, B. L., van Deursen, J. M., and Baker, D. J. (2018). Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. *Nature* 562, 578–582. doi: 10.1038/s41586-018-0543-y

Calder, P. C., Bosco, N., Bourdet-Sicard, R., Capuron, L., Delzenne, N., Doré, J., et al. (2017). Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition. *Ageing Res. Rev.* 40, 95–119. doi: 10.1016/j.arr.2017.09.001

Campisi, J. (2013). Aging, cellular senescence, and cancer. *Annu. Rev. Physiol.* 75, 685–705. doi: 10.1146/annurev-physiol-030212-183653

Castro, A. M., Macedo-de la Concha, L. E., and Pantoja-Meléndez, C. A. (2017). Low-grade inflammation and its relation to obesity and chronic degenerative diseases. *Rev. Medica del Hosp. Gen. de Mex.* 80, 101–105. doi: 10.1016/j.hgmx.2016.06.011

Chaib, S., Tchkonia, T., and Kirkland, J. L. (2021). "Obesity, senescence, and senolytics" in *From obesity to diabetes*. eds. J. Eckel and K. Clément (Cham: Springer International Publishing), 165–180.

Coppé, J. P., Desprez, P. Y., Krtolica, A., and Campisi, J. (2010). The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.: Mech. Dis.* 5, 99–118. doi: 10.1146/annurev-pathol-121808-102144

Dagdeviren, S., Jung, D. Y., Friedline, R. H., Noh, H. L., Kim, J. H., Patel, P. R., et al. (2017). IL-10 prevents aging-associated inflammation and insulin resistance in skeletal muscle. *FASEB J.* 31, 701–710. doi: 10.1096/fj.201600832R

Dodig, S., Čepelak, I., and Pavić, I. (2019). Hallmarks of senescence and aging. *Biochem. Med.* 29, 483–497. doi: 10.11613/BM.2019.030501

El-Mikkawy, D. M., EL-Sadek, M. A., EL-Badawy, M. A., and Samaha, D. (2020). Circulating level of interleukin-6 in relation to body mass indices and lipid profile in Egyptian adults with overweight and obesity. *Egypt. Rheumatol. Rehabil.* 47, 1–7. doi: 10.1186/s43166-020-00003-8

Esposito, K., Pontillo, A., Giugliano, F., Giugliano, G., Marfella, R., Nicoletti, G., et al. (2003). Association of low interleukin-10 levels with the metabolic syndrome in obese women. *J. Clin. Endocrinol. Metab.* 88, 1055–1058. doi: 10.1210/jc.2002-021437

Ghasemi Yngyknd, S., Asghari Jafarabadi, M., Ghanbari-Homayi, S., Laghousi, D., and Mirghafourvand, M. (2021). A systematic review of social determinants of healthy lifestyle among Iranian women. *Nurs. Open* 8, 2007–2017. doi: 10.1002/nop2.657

Giknis, M. L. A., and Clifford, C. (2008). "Clinical laboratory parameters for Crl: WI (Han)," Charles River Laboratories, vol. 14.

Gorgoulis, V., Adams, P. D., Alimonti, A., Bennett, D. C., Bischof, O., Bishop, C., et al. (2019). Cellular senescence: defining a path forward. *Cells* 179, 813–827. doi: 10.1016/j.cell.2019.10.005

Guerrero, A., De Strooper, B., and Arancibia-Cárcamo, I. L. (2021). Cellular senescence at the crossroads of inflammation and Alzheimer's disease. *Trends Neurosci.* 44, 714–727. doi: 10.1016/j.tins.2021.06.007

Halbach, O. V. B. U. (2010). Involvement of BDNF in age-dependent alterations in the hippocampus. *Front. Aging Neurosci.* 2:36. doi: 10.3389/fnagi.2010.00036

Hanamsagar, R., and Bilbo, S. D. (2016). Sex differences in neurodevelopmental and neurodegenerative disorders: focus on microglial function and neuroinflammation during development. *J. Steroid Biochem. Mol. Biol.* 160, 127–133. doi: 10.1016/j.jsbmb.2015.09.039

Haslam, D. W., and James, W. P. (2005). Obesity. *Lancet* 366, 1197–1209. doi: 10.1016/S0140-6736(05)67483-1

Henn, R. E., Elzinga, S. E., Glass, E., Parent, R., Guo, K., Allouch, A. A., et al. (2022). Obesity-induced neuroinflammation and cognitive impairment in young adult versus middle-aged mice. *Immun. Ageing* 19:67. doi: 10.1186/s12979-022-00323-7

Italiani, P., Mosca, E., Della Camera, G., Melillo, D., Migliorini, P., Milanesi, L., et al. (2020). Profiling the course of resolving vs. persistent inflammation in human monocytes: the role of IL-1 family molecules. Frontiers in. *Immunology* 11:1426. doi: 10.3389/fimmu.2020.01426

Khanna, D., Khanna, S., Khanna, P., Kahar, P., and Patel, B. M. (2022). Obesity: a chronic low-grade inflammation and its markers. *Cureus* 14:e22711. doi: 10.7759/cureus.22711

Kirkland, J. L., and Tchkonia, T. (2017). Cellular senescence: a translational perspective. *EBioMedicine* 21, 21–28. doi: 10.1016/j.ebiom.2017.04.013

Kondo, H., Abe, I., Gotoh, K., Fukui, A., Takanari, H., Ishii, Y., et al. (2018). Interleukin 10 treatment ameliorates high-fat diet–induced inflammatory atrial remodeling and fibrillation. *Circ. Arrhythm. Electrophysiol.* 11:e006040. doi: 10.1161/CIRCEP.117.006040

Kong, Y., Cui, H., Ramkumar, C., and Zhang, H. (2011). Regulation of senescence in cancer and aging. *J. Aging Res.* 2011, 1–15. doi: 10.4061/2011/963172

Kroll, D. S., Feldman, D. E., Biesecker, C. L., McPherson, K. L., Manza, P., Joseph, P. V., et al. (2020). Neuroimaging of sex/gender differences in obesity: a review of structure, function, and neurotransmission. *Nutrients* 12:1942. doi: 10.3390/nu12071942

Kuilman, T., Michaloglou, C., Mooi, W. J., and Peeper, D. S. (2010). The essence of senescence. *Genes Dev.* 24, 2463–2479. doi: 10.1101/gad.1971610

Kwon, H. S., and Koh, S. H. (2020). Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl. Neurodegener.* 9, 42–12. doi: 10.1186/s40035-020-00221-2

Li, J. W., Zong, Y., Cao, X. P., Tan, L., and Tan, L. (2018). Microglial priming in Alzheimer's disease. *Ann. Transl. Med.* 6:176. doi: 10.21037/atm.2018.04.22

Malafaia, A. B., Nassif, P. A. N., Ribas, C. A. P. M., Ariede, B. L., Sue, K. N., and Cruz, M. A. (2013). Obesity induction with high fat sucrose in rats. *ABCD: Arq. Bras. Cir. Dig.* 26, 17–21. doi: 10.1590/s0102-67202013000600005

Marques, L. R., Diniz, T. A., Antunes, B. M., Rossi, F. E., Caperuto, E. C., Lira, F. S., et al. (2018). Reverse cholesterol transport: molecular mechanisms and the non-medical approach to enhance HDL cholesterol. *Front. Physiol.* 9:526. doi: 10.3389/fphys.2018.00526

McArdle, M. A., Finucane, O. M., Connaughton, R. M., McMorrow, A. M., and Roche, H. M. (2013). Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. *Front. Endocrinol.* 4:52. doi: 10.3389/fendo.2013.00052

McHugh, D., and Gil, J. (2018). Senescence and aging: causes, consequences, and therapeutic avenues. *J. Cell Biol.* 217, 65–77. doi: 10.1083/jcb.201708092

Miller, A. A., and Spencer, S. J. (2014). Obesity and neuroinflammation: a pathway to cognitive impairment. *Brain Behav. Immun.* 42, 10–21. doi: 10.1016/j.bbi.2014.04.001

Minamino, T., Orimo, M., Shimizu, I., Kunieda, T., Yokoyama, M., Ito, T., et al. (2009). A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat. Med.* 15, 1082–1087. doi: 10.1038/nm.2014

Miranda, M., Morici, J. F., Zanoni, M. B., and Bekinschtein, P. (2019). Brain-derived neurotrophic factor: a key molecule for memory in the healthy and the pathological brain. *Front. Cell. Neurosci.* 13:363. doi: 10.3389/fncel.2019.00363

Nelke, C., Schroeter, C. B., Pawlitzki, M., Meuth, S. G., and Ruck, T. (2022). Cellular senescence in neuroinflammatory disease: new therapies for old cells? *Trends Mol. Med.* 28, 850–863. doi: 10.1016/j.molmed.2022.07.003

Nguyen, J. C., Killcross, A. S., and Jenkins, T. A. (2014). Obesity and cognitive decline: role of inflammation and vascular changes. *Front. Neurosci.* 8:375. doi: 10.3389/fnins.2014.00375

Niroumand, S., Khajedaluee, M., Khadem-Rezaiyan, M., Abrishami, M., Juya, M., Khodaee, G., et al. (2015). Atherogenic index of plasma (AIP): a marker of cardiovascular disease. *Med. J. Islam Repub. Iran* 29:240.

Noble, E. E., Hsu, T. M., and Kanoski, S. E. (2017). Gut to brain dysbiosis: mechanisms linking western diet consumption, the microbiome, and cognitive impairment. *Front. Behav. Neurosci.* 11:9. doi: 10.3389/fnbeh.2017.00009

Ogrodnik, M., Miwa, S., Tchkonia, T., Tiniakos, D., Wilson, C. L., Lahat, A., et al. (2017). Cellular senescence drives age-dependent hepatic steatosis. *Nat. Commun.* 8:15691. doi: 10.1038/ncomms15691

Othman, Z. A., Wan Ghazali, W. S., Noordin, L., Mohd Yusof, N. A., and Mohamed, M. (2019). Phenolic compounds and the anti-atherogenic effect of bee bread in high-fat diet-induced obese rats. *Antioxidants* 9:33. doi: 10.3390/antiox9010033

Palavra, F., Almeida, L., Ambrósio, A. F., and Reis, F. (2016). Obesity and brain inflammation: a focus on multiple sclerosis. *Obes. Rev.* 17, 211–224. doi: 10.1111/obr.12363

Palmer, A. K., and Jensen, M. D. (2022). Metabolic changes in aging humans: current evidence and therapeutic strategies. *J. Clin. Investig.* 132:e158451. doi: 10.1172/JCI158451

Panee, J. (2012). Monocyte chemoattractant protein 1 (MCP-1) in obesity and diabetes. *Cytokine* 60, 1–12. doi: 10.1016/j.cyto.2012.06.018

Piscopo, P., Bellenghi, M., Manzini, V., Crestini, A., Pontecorvi, G., Corbo, M., et al. (2021). A sex perspective in neurodegenerative diseases: microRNAs as possible peripheral biomarkers. *Int. J. Mol. Sci.* 22:4423. doi: 10.3390/ijms22094423

Rachmian, N., and Krizhanovsky, V. (2022). Senescent cells in the brain and where to find them. *FEBS J.* 290, 1256–1266. doi: 10.1111/febs.16649

Raharjo, S., Pranoto, A., Rejeki, P. S., Harisman, A. S. M., Pamungkas, Y. P., and Andiana, O. (2021). Negative correlation between serum brain-derived neurotrophic factor levels and obesity predictor markers and inflammation levels in females with obesity. *Open Access Maced. J. Med. Sci.* 9, 1021–1026. doi: 10.3889/oamjms.2021.6840

Salama, R., Sadaie, M., Hoare, M., and Narita, M. (2014). Cellular senescence and its effector programs. *Genes Dev.* 28, 99–114. doi: 10.1101/gad.235184.113

Salas-Venegas, V., Flores-Torres, R. P., Rodríguez-Cortés, Y. M., Rodríguez-Retana, D., Ramírez-Carreto, R. J., Concepción-Carrillo, L. E., et al. (2022). The obese brain: mechanisms of systemic and local inflammation, and interventions to reverse the cognitive deficit. *Front. Integr. Neurosci.* 16:798995. doi: 10.3389/fnint.2022.798995

Samara, A., Murphy, T., Strain, J., Rutlin, J., Sun, P., and Neyman, O. (2020). Neuroinflammation and white matter alterations in obesity assessed by diffusion basis spectrum imaging. *Front. Hum. Neurosci.* 13:464. doi: 10.3389/fnhum.2019.00464

Sandrini, L., Di Minno, A., Amadio, P., Ieraci, A., Tremoli, E., and Barbieri, S. S. (2018). Association between obesity and circulating brain-derived neurotrophic factor (BDNF) levels: systematic review of literature and meta-analysis. *Int. J. Mol. Sci.* 19:2281. doi: 10.3390/ijms19082281

Santín-Márquez, R., Ramírez-Cordero, B., Toledo-Pérez, R., Luna-López, A., López-Diazguerrero, N. E., Hernández-Arciga, U., et al. (2021). Sensory and memory processing in old female and male Wistar rat brain, and its relationship with the cortical and hippocampal redox state. *GeroScience* 43, 1899–1920. doi: 10.1007/s11357-021-00353-x

Schafer, M. J., White, T. A., Iijima, K., Haak, A. J., Ligresti, G., Atkinson, E. J., et al. (2017). Cellular senescence mediates fibrotic pulmonary disease. *Nat. Commun.* 8:14532. doi: 10.1038/ncomms14532

Schmitt, L. O., and Gaspar, J. M. (2023). Obesity-induced brain neuroinflammatory and mitochondrial changes. *Meta* 13:86. doi: 10.3390/metabo13010086

Sikora, E., Bielak-Zmijewska, A., Dudkowska, M., Krzystyniak, A., Mosieniak, G., Wesierska, M., et al. (2021). Cellular senescence in brain aging. *Front. Aging Neurosci.* 13:646924. doi: 10.3389/fnagi.2021.646924

Sindhu, S., Thomas, R., Shihab, P., Sriraman, D., Behbehani, K., and Ahmad, R. (2015). Obesity is a positive modulator of IL-6R and IL-6 expression in the subcutaneous adipose tissue: significance for metabolic inflammation. *PLoS One* 10:e0133494. doi: 10.1371/journal.pone.0133494

Suárez Román, G., Perera Calderín, A., Clapés Hernández, S., Fernández Romero, T., and Egaña Morales, E. (2013). Estandarización de un modelo para inducir obesidad en ratas. *Medisur* 11, 569–573.

Tchkonia, T., Zhu, Y., Van Deursen, J., Campisi, J., and Kirkland, J. L. (2013). Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J. Clin. Invest.* 123, 966–972. doi: 10.1172/JCI64098

Tikoo, K., Vikram, A., Shrivastava, S., Jena, G., Shah, H., and Chhabra, R. (2017). Parental high-fat diet promotes inflammatory and senescence-related changes in prostate. *Oxidative Med. Cell. Longev.* 2017, 1–13. doi: 10.1155/2017/4962950

Toledo-Pérez, R., Lopéz-Cervantes, S. P., Hernández-Álvarez, D., Mena-Montes, B., Pedraza-Vázquez, G., Sánchez-Garibay, C., et al. (2021). Metformin and tBHQ treatment combined with an exercise regime prevents osteosarcopenic obesity in middle-aged Wistar female rats. *Oxidative Med. Cell. Longev.* 2021, 1–18. doi: 10.1155/2021/5294266

Ugalde-Muñiz, P., Fetter-Pruneda, I., Navarro, L., García, E., and Chavarría, A. (2020). Chronic systemic inflammation exacerbates neurotoxicity in a Parkinson's disease model. *Oxidative Med. Cell. Longev.* 2020, 1–19. doi: 10.1155/2020/4807179

Von Diemen, V., Trindade, E. N., and Trindade, M. R. M. (2006). Experimental model to induce obesity in rats. *Acta Cir. Bras.* 21, 425–429. doi: 10.1590/s0102-86502006000600013

Wang, C., Jurk, D., Maddick, M., Nelson, G., Martin-Ruiz, C., and Von Zglinicki, T. (2009). DNA damage response and cellular senescence in tissues of aging mice. *Aging Cell* 8, 311–323. doi: 10.1111/j.1474-9726.2009.00481.x

Watanabe, S., Kawamoto, S., Ohtani, N., and Hara, E. (2017). Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases. *Cancer Sci.* 108, 563–569. doi: 10.1111/cas.13184

Werida, R. H., El-Gharbawy, N. M., and Mostafa, T. M. (2021). Circulating IL-6, clusterin and irisin in obese subjects with different grades of obesity: association with insulin resistance and sexual dimorphism. *Arch. Endocrinol. Metab.* 65, 126–136. doi: 10.20945/2359-3997000000336

World Health Organization (2023). Obesity. Available at: https://www.who.int/health-topics/obesity#tab=tab_1 (Accessed January 28, 2023).

Yanagi, S., Tsubouchi, H., Miura, A., Matsuo, A., Matsumoto, N., and Nakazato, M. (2017). The impacts of cellular senescence in elderly pneumonia and in age-related lung diseases that increase the risk of respiratory infections. *Int. J. Mol. Sci.* 18:503. doi: 10.3390/ijms18030503

Zhou, Q., Mareljic, N., Michaelsen, M., Parhizkar, S., Heindl, S., Nuscher, B., et al. (2020). Active poly-GA vaccination prevents microglia activation and motor deficits in a C9orf72 mouse model. *EMBO Mol. Med.* 12:e10919. doi: 10.15252/emmm.201910919

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The role of sex differences in depression in pathologically defined Alzheimer's disease

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Introduction: Sex differences in Alzheimer's disease (AD) may contribute to disease heterogeneity and affect prevalence, risk factors, disease trajectories and outcomes. Depression impacts a large number of patients with AD and has been reported to be more prevalent in women. We aimed to better understand the interaction between sex, depression and AD neuropathology, which could have implications for detection of symptoms, earlier diagnosis, therapeutic management, and enhanced quality of life.

Methods: We compared 338 cases with clinicopathologically confirmed AD (46% women) to 258 control cases (50% women), without dementia, parkinsonism or a significant pathological diagnosis. Depression was assessed both, using the Hamilton Depression Scale (HAM-D), and as being reported in their medical history combined with treatment with antidepressant medication.

Results: In the control group, women showed a higher depression severity, and a higher proportion of women were found to meet the cut-off score for depression on the HAM-D (32 vs. 16%) and having an history of depression (33 vs. 21%), while these sex differences were not observed in AD. Further, in both groups, female sex independently predicted the presence of depression, with covariates for age and cognitive status. AD subjects had higher mean HAM-D scores, were more likely to meet cutoff scores for depression (41 vs. 24%) and have a history of depression than controls (47 vs. 27%). When comparing the increase in frequency of depression in controls versus AD, the difference was significantly greater in men (AD men - control men: 24%) than in women (AD women - control women: 9%). Although subjects with depression were more likely to have higher levels of AD neuropathology, these differences were not observed when investigating the control or AD group separately.

Discussion: Control women had a higher likelihood and severity of depression than control men, but this sex difference was not noted when considering only those with pathologically defined AD, emphasizing the importance of considering sex in aging studies. AD was associated with higher rates of depression and men may be more likely to report or be diagnosed with depression once they develop AD indicating the importance of more frequent depression screenings in men.

KEYWORDS

gender, neuropsychaitric symptoms, behavioral and psychiatric symptoms of dementia, neuropathology, postmortem, sex differences, depression, Hamilton rating scale for depression

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease and cause of dementia. AD is associated with progressive decline in memory, executive, and other cognitive functions leading to functional decline and can manifest with diverse clinical presentations and heterogeneity (Murray et al., 2011; Atri, 2019; Knopman et al., 2021). Sex differences in AD have been shown to contribute to this heterogeneity and affect prevalence, risks factors, disease trajectories, and outcomes (Nebel et al., 2018; Katabathula et al., 2022). Depression is a frequent neuropsychiatric symptom that affects many patients with AD and has been shown to be more prevalent in women in the general population as well as in dementia (Lovheim et al., 2009; Chi et al., 2015). Therefore, a better understanding of the neuropathology of sex differences in depression among patients with AD may offer insights into strategies for prevention, diagnosis, therapy, and quality of life (Ferretti et al., 2018).

Sex differences in AD have been previously reported in terms of epidemiology, symptomatology, progression, risk factors, and biomarkers of AD (Zhu et al., 2021). The proportion of women with clinical AD is substantially higher than for men, and although sex differences in the risk of developing AD have yielded mixed findings, AD was found to be more prevalent in women at older ages (Mielke et al., 2014; Rocca, 2017; Ferretti et al., 2018; Zhu et al., 2021). Women were also shown to demonstrate a faster cognitive decline and greater clinical and pathological severity (Filon et al., 2016; Koran et al., 2017; Barnes et al., 2019; Zhu et al., 2021). Therefore, more studies are needed to better understand the effect of sex on disease heterogeneity and how they can be used in profiling disease phenotypes (Cahill, 2006; Filon et al., 2016; Liesinger et al., 2018; Nebel et al., 2018; Buckley et al., 2019).

Depression is one of the most common psychiatric disorders and impacts a large number of patients with AD, with studies reporting between 20 to 60% of patients affected with depression (Lyketsos and Olin, 2002; Chi et al., 2015; Moustafa et al., 2022). Depression considerably undermines the quality of life in patients and their caregivers, increases caregiver burden and aggravates psychological pain. In addition, depression itself has been suggested to be a risk factor of AD as well as a predictor of cognitive decline (Tsuno and Homma, 2009; Panza et al., 2010; Barnes et al., 2012; Diniz et al., 2013; Hudon et al., 2020; Anstey et al., 2021; Saiz-Vazquez et al., 2021). Sex differences have been identified in depression, with a higher prevalence and a greater illness severity observed in women in the general population (Parker and Brotchie, 2010; Salk et al., 2017; Labaka et al., 2018; Eid et al., 2019; Bangasser and Cuarenta, 2021). In AD dementia, women were also more likely to have depressive symptoms, however, studies have yielded inconsistent results (Lovheim et al., 2009; Lee et al., 2017; Tao et al., 2018; Eikelboom et al., 2022). Moreover, inconsistent findings have been reported regarding the influence of sex in the association of depression as a risk factor for AD (Underwood et al., 2019; Zhu et al., 2021). These discrepancies may be related to the use of different tests to diagnose depression and difficulties in diagnosing depression in AD due to the presence of several neuropsychiatric symptoms, overlapping symptoms between depression and dementia, the lack of consensus criteria to diagnose depression in AD and relying on the therapeutic metaphor with discrete psychiatric disorders (Tariot, 1999; Lyketsos et al., 2011; Novais and Starkstein, 2015; Burke et al., 2019; Moustafa et al., 2022). Further, all these studies lack pathological confirmation of an AD diagnosis which creates additional uncertainties as dementia in older individuals may be related to non-AD or mixed pathologies in the brain (Beach and Malek-Ahmadi, 2021).

Hence, this study aimed to investigate sex differences in depression, comparing both measures of depression from a validated scale scored test as well as depression reported in a subject's medical history combined with treatment with anti-depressant medication, and its link to neuropathology of AD in a well-characterized group of subjects with cognition ranging from unimpaired to dementia, derived from a longitudinal clinicopathological study.

2. Methods

2.1. Subjects

Subjects included in this study were volunteers enrolled in the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND) and Brain and Body Donation Program (BBDP; www.brainandbodydonationprogram.org), a longitudinal clinicopathological study at Banner Sun Health Research Institute (BISHRI) in metropolitan Phoenix, Arizona (Beach et al., 2015). All subjects signed informed consents, approved by BSHRI Institutional Review Boards, for both clinical assessment and brain donation for research purposes. Subjects are clinically characterized with annual standardized test batteries, consisting of general neurological, cognitive, and movement disorders components that are assessed by cognitive/behavioral neurologists, movement disorders neurologists and neuropsychologists (Beach et al., 2008, 2015).

Subjects of the current study were chosen by searching the BBDP database for cases that were assigned a final clinicopathological consensus diagnosis of AD dementia or control (defined as clinically lacking dementia or parkinsonism); these subjects could have mild cognitive impairment or incidental pathology but did not meet criteria for clinical or pathological diagnosis of a neurodegenerative disease (Beach et al., 2015). All subjects completed at least one clinical assessment of symptoms of depression using a scaled validated tool, before death. Data available included global cognition assessment using the Mini-Mental State Examination (MMSE) as well as the age of onset of dementia symptoms, which was used to calculate the duration of dementia. A total of 596 cases, between 2001 and 2021, including 338 (157 women/181 men) cases with AD and 258 (131 women/ 127 men) control cases were included in this study. The age at death of subjects ranged from 56 to 104 years with a mean age of death of 87 years (report to Table 1).

2.2. Depression assessments:

Different assessment scales for depression have been used over the years in BBDP including the clinician-administered Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1960), the Geriatric Depression Scale (GDS; Yesavage et al., 1982) and depressive symptom assessment as part of the Neuropsychiatric Inventory Questionnaire (NPI-Q; Cummings et al., 1994). Depression tests were administered by neuropsychologists and trained psychometrists. Of the 596 cases included, 590 (99%) had a least one assessment for the HAM-D and 409 (68.6%) had at least one assessment using the GDS while 444

TABLE 1 General, cognitive, and depression-related characteristics of all study subjects divided by group and sex.

	Controls (258)		AD (338)	
	Women (131)	Men (127)	Women (157)	Men (181)
Age at death	88.8 (6.9)	87.6 (6.4)	87.7 (7.7)	85.4 (7.2)* #
Dementia, age onset			81.8 (8.4)	79.2 (8.3)*
Dementia, duration			6.2 (4.1)	6.0 (4.3)
MMSE	27.7 (2.2)	27.4 (2.2)	16.2 (8.8)	15.8 (8.2)#
Education	14.8 (2.5)	15.1 (2.9)	14.0 (2.5)	15.3 (2.8)* #
HAM-D	6.2 (4.7)	4.7 (3.3)*	7.1 (4.7)	7.0 (4.4)#
GDS	4.5 (3.7)	3.9 (3.2)	4.2 (3.3)	3.9 (3.2)
NPI-Q	3.1 (3.6)	2.7 (3.8)	7.2 (5.1)	8.2 (5.6)#

Data are presented as means (SD). MMSE = last Mini Mental State Examination score; HAM-D = Hamilton Rating Scale for Depression; GDS = Geriatric Depression Scale (missing data for 203 subjects); NPI-Q = Neuropsychiatric Inventory Questionnaire (missing data for 152 subjects).
*$p < 0.05$ for sex comparisons within AD subjects or controls subjects.
#$p < 0.05$ for comparison of all 4 groups.

(74.5%) subjects received at least one NPIQ assessment. As the HAM-D was the most commonly used scale for depression in our program, we used the results from HAM-D (Total possible score = 52) for statistical analysis, but also reported mean results from other depression scales. If more than one test was performed before death, the worst score was used to capture any symptoms of depression. To assess the presence of clinical depression, previously recommended cut-off criteria for HAM-D were used as follows; a score between 0 to 7 indicated no depression; 8 to 17 mild depression, 18 to 24 moderate depression, and a score over 24 indicated severe depression (Cusin et al., 2009; Romera et al., 2011; Zimmerman et al., 2013). Moreover, any previous diagnosis of depression and use of antidepressants are recorded as part of a subject's medical history, using the medical history questionnaire performed at each BBDP visit or from the private medical records obtained. Therefore, in addition to scale scored depression, the combination of a medical history of depression and treatment with antidepressants was also used to define depression in a second set of analyses. Medication included selective serotonin reuptake inhibitors (SSRIs), Serotonin and norepinephrine reuptake inhibitors (SNRIs), nortriptyline, mirtazapine and trazodone.

2.3. Neuropathological evaluation

A complete neuropathological examination was performed after death, as previously described (Beach et al., 2015). Assignments for AD Braak neurofibrillary (NF) stages (Braak et al., 2006), CERAD neuritic plaque density score (Mirra et al., 1991), Thal amyloid phase for Aβ plaque brain distribution (Thal et al., 2002), and alpha-synuclein (aSyn) stage according to the Unified Staging System for Lewy Body Disorders (USSLBD; Beach et al., 2009). Data also included regional and summary cortical brain density measures for tau neurofibrillary (NF) tangle and plaque load (for both, there is a total possible score of 15 based on summary of 0–3 scores in each of 5 regions: frontal association cortex, parietal association cortex, temporal association cortex, hippocampus CA1, and entorhinal/transentorhinal areas). Neuropathological AD diagnoses were defined as having "intermediate" or "high" criteria according to the National Institute on Aging/Reagan Institute criteria combined with a history of clinical dementia (Nia and Group, R. I. W, 1997; Hyman et al., 2012; Montine et al., 2012). Some AD subjects (183 cases; 30.7% of which 55% were men and 45% women) had additional comorbid neuropathologically-diagnosed conditions, including Parkinson's disease (PD; $n = 41$), dementia with Lewy bodies (DLB; $n = 56$), vascular dementia (VaD; $n = 73$), progressive supranuclear palsy (PSP; $n = 31$), frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP; $n = 9$), and corticobasal degeneration (CBD; $n = 1$). These subjects were grouped as a "multiple diagnoses" group and were excluded in a second set of analyses to account for the influence of comorbid (non-AD) brain disease.

2.4. Statistical analyses

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 23.0). Non-parametric Mann–Whitney U-test, Kruskal Wallis ANOVA with Bonferroni *post hoc* comparisons, ANCOVA, as well as chi-square test or Fisher exact test were used, as appropriate, for group and sex comparisons. Non-parametric Spearman correlations were used to assess correlations between depression severity scores and clinical and neuropathologic characteristics. A series of logistic regressions, with depression as the dependent variable (binary: presence or not of depression), was then performed to assess if sex was a predictor of depression, with covariates for age and MMSE as well as neuropathologic characteristics.

3. Results

Refer to Table 1 for basic demographic and clinical characteristics and Table 2 for neuropathologic characteristics of the cases included for both women and men in each group. Of the included subjects, 48.3% were women and 51.7% were men while 54.5% of women and 58.8% of men had a final clinicopathological diagnosis of AD, these differences were not significant (NS). Age at death was significantly higher in control subjects than in AD subjects (88.2 ± 6.7 vs. 86.5 ± 7.5; U = 49.6; $p = 0.004$).

Age at death was significantly greater in women (88.2 ± 7.4 vs. 86.3 ± 6.3; U = 51.55; $p < 0.001$) and age at onset of dementia was also higher in women (81.8 vs. 79.2; U = 16.20; $p = 0.002$). Men had more education than women (U = 35.81; $p < 0.001$). No sex differences were found for duration of dementia, or last MMSE test score (U = 46.8 $p = 0.08$) prior to death. Apathy, as measured though the NPI-Q, was significantly more frequent and severe (U = 11808.0; $p = 0.001$) in AD than in controls (69% in AD vs. 27% in controls; $\chi^2 = 10.37$; $p = 0.001$; $n = 444$); no sex differences were found either in the control group (31.3% in women vs. 22.4% in men) nor in the AD group (69% in women vs. 69% in men).

When looking at neuropathologic characteristics, brain weight was lower in women ($p < 0.001$), even when corrected for age, while no other sex differences were found for Braak stage, plaque density, total brain NF or plaque load in the overall group. In the control group only, plaque density was higher in women than in men ($p = 0.038$), but was not significant after age correction ($p = 0.066$).

TABLE 2 Neuropathological characteristics of all study subjects divided by group and sex.

	Controls (258)		AD (338)	
	Women (131)	Men (127)	Women (157)	Men (181)
Brain weight	1112.7 (105.5)	1247.1 (114.4)*	1055.8 (109.6)	1188.7 (118.9)* #
Braak stage	3.5 (0.8)	3.1 (0.9)	4.8 (0.9)	4.7 (0.9)#
Plaque density	1.7 (1.2)	1.4 (1.2)	2.9 (0.2)	2.9 (0.3)#
Total brain NF load	5.9 (2.3)	5.5 (2.7)	10.6 (3.8)	10.3 (3.6)#
Total brain plaque load	6.8 (5.6)	5.6 (5.7)	12.7 (2.8)	13.0 (2.3)#

Data are presented as means (SD).
*$p < 0.05$ for sex comparisons within AD or controls subjects.
#$p < 0.05$ for comparison of all 4 groups.

3.1. Depression as measured by the HAM-D

In the whole group, 31% of subjects met criteria for depression on the HAM-D, of these 92.4% met criteria for mild depression, 7% met criteria for moderate depression and 0.5% met criteria for severe depression. Group differences were observed, as AD subjects had higher mean HAM-D scores (7.0 ± 4.5 vs. 5.5 ± 4.1; U = 33.04; $p < 0.001$) and were more likely to meet cutoff scores for depression than controls (40.7 vs. 24.1%; $\chi^2 = 17.7$; $p < 0.001$). The proportion of women meeting the cut-off score for depression (105/286 = 36.7%) was not significantly different than the proportion of men (93/304 = 30.6%; $\chi^2 = 2.48$; $p = 0.115$) and no significant sex differences were found in mean HAM-D scores (6.7 ± 4.7 vs. 6.0 ± 4.2; U = 46.05; $p = 0.211$) in all subjects for both groups. However, when looking separately in control and AD groups, sex differences in depression were observed in the control group, with higher mean HAM-D scores in women (6.2 ± 4.7 vs. 4.7 ± 3.3; U = 9360.0; $p = 0.018$) and a higher proportion of women with depression (31.5%), when compared to control men (16.3%; $\chi^2 = 8.06$; $p = 0.0045$). In the AD group, no sex differences, in either mean HAM-D score (U = 14.07; $p = 0.9$) or proportion of subjects with depression ($\chi^2 = 0.017$; $p = 0.89$), were observed between women (41.0%) and men (40.3%; Figures 1, 2A). A trend was observed but no significant difference was reported between control women and women with AD ($\chi^2 = 2.75$; $p = 0.09$) while a higher proportion of men with AD, when compared to control men, had depression ($\chi^2 = 19.98$; $p < 0.001$). When comparing the increase in frequency of depression in controls versus AD the difference was significantly greater in men (AD men – control men: 24.0%) than in women (AD women – control women: 9.5%; $\chi^2 = 6.95$; $p = 0.008$; Figure 3).

In the control group, 35% of cases (49.4% of women, 50.6% of men) were found to have MCI. Of these, 24.7% met criteria for depression on the HAM-D, no gender differences were found in the proportion of cases with MCI (20.4% in men vs. 28.9% in women; $\chi^2 = 0.85$; $p = 0.36$). Moreover, no differences in proportions of cases with depression were observed between control cases with MCI (24.7%) and cognitively normal controls (23.1%; $\chi^2 = 0.082$; $p = 0.77$) while depression was more frequent in AD cases than in control-MCI cases ($\chi^2 = 14.90$; $p = 0.0001$).

Mild univariate correlations were found between HAM-D score and age at death (Rho = −0.100; $p = 0.015$), MMSE (Rho = −0.190; $p < 0.001$), age of onset of dementia (Rho = −0.180; $p < 0.001$), brain weight (Rho = −0.143; $p < 0.001$), number of major neuropathological diagnoses (Rho = 0.190; $p < 0.001$), Braak NF stage (Rho = 0.099; $p = 0.016$), plaque density (Rho = 0.120; $p = 0.003$), total brain NF load (Rho = 0.104; $p < 0.012$) and total brain plaque load (Rho = 0.151; $p < 0.001$) but HAM-D score did not correlate with years of education or duration of dementia. For subjects with more than one depression scale available, the HAM-D was correlated to the GDS depression score (Rho = 0.430; $p < 0.001$; $n = 409$), the depression severity (Rho = 0.265; $p < 0.001$; $n = 439$) and apathy severity (Rho = 0.241; $p < 0.001$; $n = 439$) of the NPI-Q. In controls, HAM-D was only correlated with brain weight (Rho = −0.218; $p < 0.012$). In the AD group only, Ham-D correlated with age at death (Rho = −0.161; $p < 0.001$) and age of onset of dementia (Rho = −0.180; $p = 0.001$).

In the overall group, subjects with depression were younger ($p = 0.017$), and, when adjusted for age and sex, they were more likely to have a lower MMSE score ($p < 0.001$), a higher number of major neuropathological diagnoses ($p = 0.001$) and a lower brain weight ($p < 0,001$), as well as a higher Braak NF stage ($p = 0.025$), plaque density ($p < 0.0001$), total brain NF load ($p = 0.011$) and total brain plaque load ($p < 0.0001$). However, when investigating the controls, only brain weight ($p = 0.014$) was lower in cases with depression, but no significant differences survived age and sex correction. In the AD group, subjects with depression were younger but no other differences were observed. Further, when correcting for group (AD vs. control) none of the neuropathologic characteristic significantly predicted depression.

Further, logistic regression modeling found a lower MMSE score ($p = 0.005$) and female sex ($p = 0.05$), but not age or number of major neuropathologic diagnoses, to be significant predictors of depression [χ^2 (4) = 26.84; $p < 0.001$; $R^2 = 0.062$; for model]. In a model excluding all subjects with a second major neuropathologic diagnosis other than AD, i.e., including controls and subjects with AD diagnosis solely, both female sex ($p = 0.03$) and MMSE ($p < 0.001$) remained significant independent predictors of depression [χ^2 (3) = 23.12; $p < 0.001$; $R^2 = 0.078$; for model]. When adding Braak NF stage, plaque density, total NF tangle and plaque brain load the model was still significant [χ^2 (7) = 27.86; $p < 0.001$; R2 = 0.095], with sex ($p = 0.034$), MMSE ($p = 0.002$) and higher total plaque load ($p = 0.016$) being significant independent predictors of depression.

When investigating controls only, female sex was found to be the only independent predictor of depression, with covariates for age, MMSE, Braak stage and plaque density [χ^2 (5) = 11.63; $p = 0.04$; $R^2 = 0.068$]. A model including the AD group only significantly predicted depression [χ^2 (4) = 11.92; $p = 0.018$; $R^2 = 0.048$] with younger age being the only independent predictor of depression ($p = 0.009$).

Refer to Table 3A for significance, odd ratios (OR) and 95% confidence interval (CI) of each predictor for different models.

3.2. Depression reported in medical history combined with treatment with antidepressants

In the overall group, 38.4% of subjects had both a medical history of depression and received treatment with anti-depressants. A previous diagnosis of depression was significantly more frequent in AD subjects (47%) than in control subjects (27.1%; $\chi^2 = 24.51$;

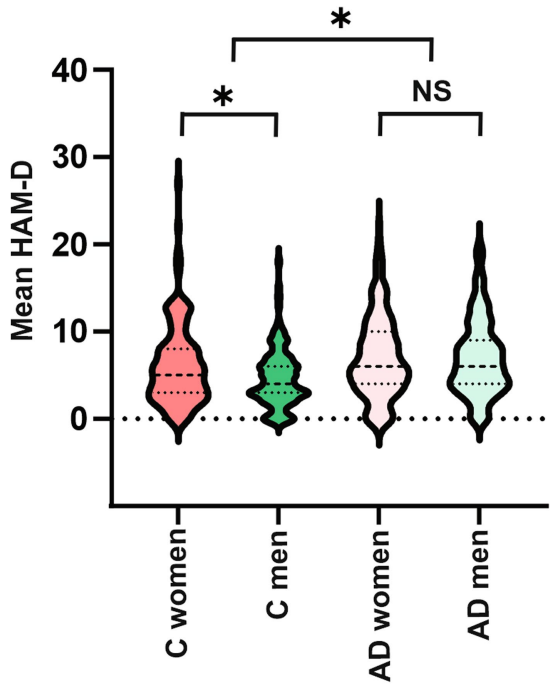

FIGURE 1
Violin plots of HAM-D depression scores between each group and sex. Sex difference is found only in controls, with a higher severity of depression in control women than men. No sex differences are found in AD. AD subjects have higher depression severity than controls. C=controls, AD=Alzheimer's disease, HAM-D=Hamilton Rating Scale for Depression.

$p < 0.001$) and depression was more frequent in AD women (49.7%) than in control women (32.8%; $\chi^2 = 8.33$; $p = 0.004$) as well as in AD men (44.8%) when compared to control men (21.3%; $\chi^2 = 17.35$; $p < 0.001$). In the overall group, no significant statistical difference was reported in the frequency of depression reported by women (41.7%) and men (35.4%; $\chi^2 = 2.48$; $p = 0.11$), when excluding subjects with a second neuropathologic diagnosis, this sex difference was significant ($\chi^2 = 4.37$; $p = 0.04$). Similar to the results yielded using the HAM-D, a sex difference was observed when investigating the control group only, in which a higher proportion of women (32.8%) had depression when compared to men (21.3%; $\chi^2 = 4.06$; $p = 0.044$) but no statistical differences were found between women (49.7%) and men (44.8%) with AD ($\chi^2 = 0.82$; $p = 0.37$). The increase in frequency of depression in controls versus AD was not significantly different in men (AD men – control men: 23.5%) than in women (AD women – control women: 16.9%; $\chi^2 = 1.50$; $p = 0.22$; Figure 2B).

Of control cases that had MCI, 30.3% had an history of depression, no significant gender differences were found in the proportion of cases with MCI (25.0% in men vs. 35.6% in women; $\chi^2 = 0.173$; $p = 0.28$). Moreover, no differences in proportions of cases with depression were observed between control cases with MCI (30.3%) and cognitively normal controls (25.4%; $\chi^2 = 0.701$; $p = 0.4$) while depression was more frequent in AD cases than in control-MCI cases ($\chi^2 = 7.99$; $p = 0.004$).

Subjects with depression were younger ($p < 0.001$), and, when adjusted for age and sex, they were more likely to have a lower MMSE score ($p < 0{,}001$), a worse HAM-D score ($p < 0{,}001$) and GDS ($p < 0{,}001$) depression score, a greater number of major neuropathological diagnoses ($p = 0.001$) as well as a higher Braak NF stage ($p = 0.005$), plaque density ($p = 0.002$), total brain NF load ($p = 0.04$) and total brain plaque load ($p = 0.014$). However, when investigating the control group only, no significant differences survived age and sex correction. In the AD group, subjects with depression were younger but no other group differences were observed. Further, when correcting for group (AD vs. control) none of the neuropathologic characteristic significantly predicted depression.

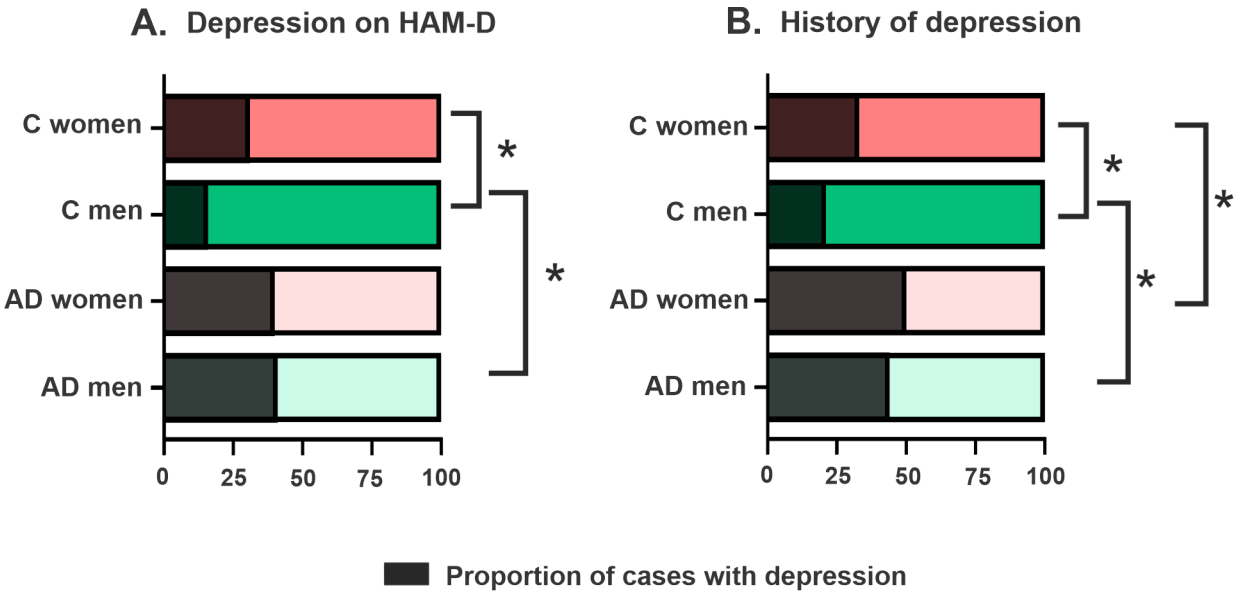

FIGURE 2
Proportions of subjects with depression in each group and sex. **(A)** When depression is defined by the HAM-D, proportion of cases with depression is higher in control women than control men and in control men than in AD men. **(B)** When depression is defined with history of depression and treatment with anti-depressant, depression is higher in control women than control men or AD women and in control men than in AD men.

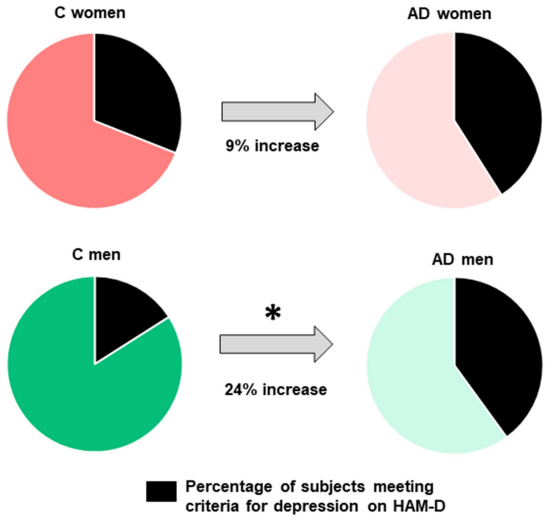

FIGURE 3
Increase between control and AD subjects in the proportion of subjects with depression. The increase in frequency of depression in controls versus AD is significantly greater in men (AD men - control men: 24%) than in women (AD women - control women: 9%).

Moreover, logistic regression modelling significantly predicted the presence of depression [χ^2 (4) = 38.97; $p < 0.001$; $R^2 = 0.087$], with female sex ($p = 0.02$), younger age ($p = 0.004$) and a higher number of major neuropathologic diagnoses ($p = 0.001$), but not MMSE, as significant independent predictors of depression. When excluding all subjects with a second major neuropathologic diagnosis, the model still predicted the presence of depression [χ^2 (3) = 15.14; $p = 0.002$; $R^2 = 0.051$], with sex ($p = 0.024$;), age ($p = 0.048$) and a lower MMSE ($p = 0.012$) being significant independent predictors. When adding Braak NF stage, plaque density, total NF tangle and plaque brain load, the model was still significant [χ^2 (7) = 15.93; $p < 0.026$; $R^2 = 0.054$] with the only independent predictors of depression remaining female sex ($p = 0.034$) and younger age ($p = 0.034$). In control only, the model was not significant [χ^2 (5) = 7.45; $p = 0.189$; $R^2 = 0.042$], while in the AD only group the model significantly predicted depression [χ^2 (4) = 10.42; $p = 0.034$; $R^2 = 0.041$] and younger age was the only significant independent predictor ($p = 0.006$; Table 3B).

4. Discussion

This clinicopathological study investigated sex differences in depression in pathologically well-defined subjects with AD dementia as well as non-demented controls. We confirm that AD dementia was associated with higher rates of depression than controls both when measured using a validated scale and by history of depression combined with anti-depressant treatment. Our results demonstrate that women had a higher likelihood of depression than men, but this sex difference was not observed when considering only those with AD dementia. Further, the increase in rates of depression, in AD subjects in comparison to controls, was greater in men than in women. These differences emphasize the importance of studying sex differences in AD and can have important implications for management of depression in AD, including more frequent depression screenings particularly in men who may be less likely to self-report depressive symptoms.

Depression has been shown to affect a large number of subjects with AD, we also report increased frequency of depression in AD affecting 40 to 47% of subjects (measured by HAM-D and history of depression respectively) when compared to 24 to 27% of subjects affected by depression in controls. A higher prevalence and severity of symptoms of depression have been repeatedly reported in women when compared to men (Parker and Brotchie, 2010; Eid et al., 2019). In our sample, the proportion of all women versus all men with depression did not reach statistical significance (36.7 to 41.7% in women and 30.6 to 35.4% in men) and we did not observe sex differences in severity of depression as measured by the HAM-D scores in the whole group. However, in logistic modelling, female sex was found to be a significant independent predictor of depression when controlling for age and cognitive status as measured by the MMSE. This effect remained when excluding subjects with a second major neuropathologic diagnosis, to account for the effect of multiple brain pathologies, and when controlling for AD neuropathology. When looking in both groups separately, we found that this sex difference observed was driven by the non-demented control group that showed a higher proportion of women with depression and more severe HAM-D scores in women than in men, while no sex differences were detected when considering only the AD group. It is possible that women have greater vulnerability to network disruptions or neurochemical imbalances that lead to mood symptoms in prodromal stages and reach a ceiling affect once they reach dementia stages. Future studies should correlate depression in women with biological markers of AD pathology such as serum amyloid beta, phosphor-tau, CSF and imaging markers.

While most studies report sex differences in depression, when investigating AD specifically, inconsistencies regarding sex differences in depression (Lovheim et al., 2009; Lee et al., 2017; Tao et al., 2018; Eikelboom et al., 2022) as well as the influence of sex in the association of depression as a risk factor for AD have been reported (Underwood et al., 2019; Zhu et al., 2021). These inconsistent results in AD may be related to the challenges of diagnosing depression in AD, as symptoms of depression and dementia overlap and there is no clear consensus to diagnose depression in AD (Burke et al., 2019). This highlights the need for more accurate tools to diagnose depression in AD. Moreover, these studies frequently lack pathological or biomarker confirmation of AD diagnosis which may also affect the results as distinct sex differences were reported in other frequent comorbid pathologies, such as Lewy body pathology, and sex differences in depression may present differently in other diseases affecting the brain (Perrin et al., 2017; Devanand et al., 2022; Chiu et al., 2023). Several hypotheses may be put forward as to why we do not find sex differences in AD in our study. Interestingly, when contrasting AD to non demented controls, we found that the AD-control increase in frequency of cases with depression (using the HAM-D score) was significantly greater in men (24%) than in women (9%). This result might suggest that men may be more likely to report or be diagnosed with depression when clinically affected with AD. One can speculate that these results could link to a gender bias in diagnosing mood disorders, in which men would be less likely to be diagnosed with a mood disorder in the absence of other medical conditions, such as dementia here (Norman, 2014; Salk et al., 2017). Several other

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TABLE 3 Multiple logistic regression modelling to determine predictors of depression.

Predictors	*p*-value	Odds ratios	95% CI
A: With depression (dependent variable) defined by a cut-off score on the HAM-D.			
All subjects			
Equation	**<0.001**		
Sex	**0.05**	0.70	0.49, 1.00
Age	0.07	0.98	0.95, 1.00
MMSE	**0.005**	0.97	0.95, 0.99
Nb nptx dx	0.3	1.13	0.92, 1.38
Excluding subjects with a second major nptx dx (including controls and subjects with AD solely)			
Equation	**<0.001**		
Sex	**0.03**	1.683	1.05, 2.54
Age	0.9	1.00	0.97, 1.03
MMSE	**< 0.001**	0.909	0.87, 0.94
Adding AD pathology			
Equation	**<0.001**		
Sex	**0.034**	1.63	1.05, 2.54
Age	0.63	1.00	0.97, 1.03
MMSE	**0.002**	0.95	0.92, 0.98
Braak NF stage	0.69	0.94	0.74, 1.18
Plaque density	0.12	0.73	0.88, 1.38
NF load	0.65	0.98	0.90,1.07
Plaque load	**0.016**	1.11	1.02, 1.21
In control subjects only			
Equation	**0.02**		
Sex	**0.003**	2.56	1.37, 4.79
Age	0.3	1.02	0.97, 1.06
MMSE	0.4	0.96	0.84, 1.09
Braak NF stage	0.2	0.77	0.54, 1.10
Plaque density	0.7	1.0	0.82, 1.35
In AD subjects only			
Equation	**0.02**		
Sex	0.72	1.09	0.69, 1.72
Age	**0.009**	0.96	0.93, 0.99
MMSE	0.19	0.98	0.96, 1.00
Nb nptx dx	0.60	0.92	0.67, 1.26
B: With depression (dependent variable) reported in a subject's medical history combined with a treatment with antidepressants.			
All subjects			
Equation	**<0.001**		
Sex	**0.02**	1.51	1.07, 2.15
Age	**0.004**	0.94	0.94, 1.00
MMSE	0.181	0.96	0.96, 1.00
Nb nptx dx	**0.001**	1.39	1.14, 1.70
Excluding subjects with a second major nptx dx (including controls and subjects with AD solely)			
Equation	**0.002**		
Sex	**0.024**	1.64	1.07, 2.51
Age	**0.048**	0.97	0.94, 1.00
MMSE	**0.012**	0.97	0.94, 0.99
Adding AD pathology			
Equation	**0.026**		
Sex	**0.034**	1.60	1.04, 2.46
Age	**0.034**	0.97	0.94, 0.99
MMSE	0.217	0.98	0.95, 1.00
Braak stage	0.870	0.92	0.83, 1.30
Plaque density	0.260	1.24	0.89, 1.80
NF tangle load	0.519	1.03	0.95, 1.11
Plaque load	0.404	0.96	0.89, 1,05
In control subjects only			
Equation	0.189		
Sex	**0.03**	1.92	1.07, 3.45
Age	0.25	0.97	0.93, 1.01
MMSE	0.14	0.92	0.81, 1.04
Braak stage	0.43	0.90	0.62, 1,22
Plaque density	0.92	1.0	0.80, 1.28
In AD subjects only			
Equation	**0.034**		
Sex	0.16	1.38	0.88, 2.16
Age	**0.006**	0.93	0.93, 0.99
MMSE	0.94	0.99	0.97, 1.03
Nb nptx dx	0.35	1.16	0.67, 1.26

MMSE = last Mini Mental State Examination score; Nb nptx dx: number or major neuropathologic diagnoses. Depression was used as the dependent variable, subjects with depression were coded as 1 and subjects without depression as 0. Female sex was coded as 1 while male sex was coded as 0. Significant *p* values are in bold.

neuropsychiatric symptoms such as apathy also frequently manifest in AD dementia and it may be difficult to separately assess depression from other symptoms (Eikelboom et al., 2022). While apathy measures were available only for a subset of subjects, no sex differences in apathy frequency or severity were found in either the AD or control group. Some of the neuropsychiatric manifestations, such as disinhibition in particular, to also play a role in this observed difference between men control and men with AD. It could be hypothesized that if men lose their inhibition when developing dementia this could lead to them, or their caregivers, reporting more symptoms of depression. Future studies should investigate the possible impact of disinhibition on the increase of depressive symptoms of depression in men. Alternatively, biological sex differences in depression have been reported and women have been shown to present with higher levels of inflammatory, neurotrophic, and serotonergic markers that were correlated to

severity of depressive symptoms (Labaka et al., 2018). Future studies could further investigate potential sex differences in depletion of serotonergic, dopaminergic, or noradrenergic cortical afferents between men and women with AD.

Depression has been suggested to be an early manifestation of AD; depressive symptoms have been associated with an increased risk of developing dementia and depression is a predictor of progression from normal cognition to MCI and to dementia (Panza et al., 2010; Barnes et al., 2012; Hudon et al., 2020; Saiz-Vazquez et al., 2021). Consequently, depression was suggested to be a manifestation of AD biological process, sometimes preceding cognitive decline. The concept of Mild Behavioral Impairment (MBI) describes this trajectory of depression and/or other debilitating neuropsychiatric symptoms preceding the onset of AD dementia symptoms (Ismail et al., 2016). In our group, only a low number of cases had MCI and we found no differences in depression in control cases that had MCI when compared to cognitively normal controls. Overall, we found subjects with depression to be younger and more likely to have a lower MMSE score, as well as a greater level of AD neuropathology both in terms of NF tangles and neuritic plaque burden, when compared to subjects without depression. Previous studies also reported a more severe NF pathology in depression with co-morbid AD (Rupp et al., 2008) and early neurofibrillary pathology was associated to increased odd for depression and other neuropsychiatric symptoms (Ehrenberg et al., 2018). Accordingly, NF tangles affect noradrenergic and serotonergic brainstem neurons early in AD (Braak et al., 2006; Grudzien et al., 2007). However, the literature has yielded mixed results and in a larger study, using data from the National Alzheimer's Coordinating Center, neuropathology was not associated with depression in subjects that had died with MCI and early AD dementia (McCutcheon et al., 2016). Our result might be linked to higher proportions of AD cases with depression as it is important to note that we did not find any differences in AD neuropathology when considering only the AD or only the control group and neither tau nor amyloid burden significantly predicted depression in controls. These results suggest that AD neuropathology markers do not explain the sex differences observed in the proportions of cases with depression found in controls and that depression in healthy aging is independent of NFT and Amyloid pathology, at least when measured using whole brain burden.

Several sex differences were reported in large pathologically well-defined cohort of AD suggesting a higher clinical and pathological severity in women including a higher neurofibrillary burden and a greater AD – Control brain weight loss (Filon et al., 2016; Liesinger et al., 2018; Barnes et al., 2019). We report an older age of onset of dementia and age of death in women as well as a lower brain weight while MMSE and other neuropathologic characteristic were similar between the sexes when controlled for age, but this is probably a result of having a smaller sample size in this study. A better understanding of sex differences in AD will lead to better recognition of risk factors of AD and better therapeutic management of patients.

We acknowledge some limitations. This study examined cross-sectional relationships between AD neuropathology and depression before death and causal inferences cannot be drawn. Depression had been reported to be recurrent and the use of medication might have improved the score on the HAM-D in well-controlled depression, thus, we used the worst HAM-D score when more than one test result was available, to capture any depression. Even though the HAM-D is a validated and widely used depression rating scale, it is designed as a semi-structured interview that requires a trained interviewer as well as a fair amount of judgement and interaction with the patient which makes this test likely to be an imperfect measure to use with demented patients (Cusin et al., 2009). Though, neuropsychologists and trained psychometrists routinely administers these tests in BBDP. Another limitation is that only one scale was used to assess depression, even though the GDS and NPIQ measures of depression were available for a subset of patients, it would significantly reduce the sample size and tests were not administered in the same time frame which would make it less likely to capture depression on two separate tests. Future studies using similar comparable measures would be more beneficial. Moreover, the use of medication is an important limitation of this study that should not be overlooked; a considerable number of subjects (38%) had received antidepressant medication that might have affected the test result. Information on depression treatment were limited, and subjects with dementia might have been prescribed less commonly used medications for depression such as mood stabilizers, while other types of treatment strategies such as cognitive behavioral therapy, might have not been captured in our dataset. To account for this, one strength of this study is that we used two complementary ways to define depression, both a validated clinician administered scale and history of depression in the subject's medical history combined with treatment with anti-depressants, which have yielded similar results. Another possible confounder that wasn't assess in this study is that the use of antidepressants may be associated with an increased risk of dementia (Wang et al., 2018; Kodesh et al., 2019).

In conclusion, in a large sample of subject with pathologically defined AD and controls, we found higher rates of depression in AD dementia. Female sex was a significant predictor of depression and women had a higher likelihood of depression than men in controls, while this difference was not noted when considering only AD subjects. This difference observed in controls was not explained by levels of AD neuropathology markers. The increase in rates of depression, in AD subjects in comparison to controls, was greater in men than in women which might suggest that men may be more likely to report or be diagnosed with depression when clinically affected with AD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Banner Sun Health Research Institute. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CT, PC, TB, and GS: conception and design. CT, PC, CB, IL, DG, TB, and GS: experimentations. CT and TB: statistical analysis, CT: writing—original draft preparation. CT, PC, CB, IL, DG, TB, and GS: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Anstey, K. J., Peters, R., Mortby, M. E., Kiely, K. M., Eramudugolla, R., Cherbuin, N., et al. (2021). Association of sex differences in dementia risk factors with sex differences in memory decline in a population-based cohort spanning 20-76 years. *Sci. Rep.* 11:7710. doi: 10.1038/s41598-021-86397-7

Atri, A. (2019). The Alzheimer's disease clinical Spectrum: diagnosis and management. *Med. Clin. North Am.* 103, 263–293. doi: 10.1016/j.mcna.2018.10.009

Bangasser, D. A., and Cuarenta, A. (2021). Sex differences in anxiety and depression: circuits and mechanisms. *Nat. Rev. Neurosci.* 22, 674–684. doi: 10.1038/s41583-021-00513-0

Barnes, L. L., Lamar, M., and Schneider, J. A. (2019). Sex differences in mixed Neuropathologies in community-dwelling older adults. *Brain Res.* 1719, 11–16. doi: 10.1016/j.brainres.2019.05.028

Barnes, D. E., Yaffe, K., Byers, A. L., Mccormick, M., Schaefer, C., and Whitmer, R. A. (2012). Midlife vs late-life depressive symptoms and risk of dementia: differential effects for Alzheimer disease and vascular dementia. *Arch. Gen. Psychiatry* 69, 493–498. doi: 10.1001/archgenpsychiatry.2011.1481

Beach, T. G., Adler, C. H., Lue, L., Sue, L. I., Bachalakuri, J., Henry-Watson, J., et al. (2009). Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathol.* 117, 613–634. doi: 10.1007/s00401-009-0538-8

Beach, T. G., Adler, C. H., Sue, L. I., Serrano, G., Shill, H. A., Walker, D. G., et al. (2015). Arizona study of aging and neurodegenerative disorders and brain and body donation program. *Neuropathology* 35, 354–389. doi: 10.1111/neup.12189

Beach, T. G., and Malek-Ahmadi, M. (2021). Alzheimer's disease neuropathological comorbidities are common in the younger-old. *J. Alzheimers Dis.* 79, 389–400. doi: 10.3233/JAD-201213

Beach, T. G., Sue, L. I., Walker, D. G., Roher, A. E., Lue, L., Vedders, L., et al. (2008). The Sun Health Research Institute brain donation program: description and experience, 1987-2007. *Cell Tissue Bank.* 9, 229–245. doi: 10.1007/s10561-008-9067-2

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., and Del Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 112, 389–404. doi: 10.1007/s00401-006-0127-z

Buckley, R. F., Waller, M., Masters, C. L., and Dobson, A. (2019). To what extent does age at death account for sex differences in rates of mortality from Alzheimer disease? *Am. J. Epidemiol.* 188, 1213–1223. doi: 10.1093/aje/kwz048

Burke, A. D., Goldfarb, D., Bollam, P., and Khokher, S. (2019). Diagnosing and treating depression in patients with Alzheimer's disease. *Neurol. Ther.* 8, 325–350. doi: 10.1007/s40120-019-00148-5

Cahill, L. (2006). Why sex matters for neuroscience. *Nat. Rev. Neurosci.* 7, 477–484. doi: 10.1038/nrn1909

Chi, S., Wang, C., Jiang, T., Zhu, X. C., Yu, J. T., and Tan, L. (2015). The prevalence of depression in Alzheimer's disease: a systematic review and Meta-analysis. *Curr. Alzheimer Res.* 12, 189–198. doi: 10.2174/1567205012666150204124310

Chiu, S. Y., Wyman-Chick, K. A., Ferman, T. J., Bayram, E., Holden, S. K., Choudhury, P., et al. (2023). Sex differences in dementia with Lewy bodies: focused review of available evidence and future directions. *Parkinsonism Relat. Disord.* 107:105285. doi: 10.1016/j.parkreldis.2023.105285

Cummings, J. L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D. A., and Gornbein, J. (1994). The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44, 2308–2314. doi: 10.1212/WNL.44.12.2308

Cusin, C., Yang, H., Yeung, A., and Fava, M. (2009). "Rating scales for depression" in *Handbook of clinical rating scales and assessment in psychiatry and mental health*. eds. L. Baer and M. A. Blais (Totowa, Nj: Humana Press)

Devanand, D. P., Lee, S., Huey, E. D., and Goldberg, T. E. (2022). Associations between neuropsychiatric symptoms and neuropathological diagnoses of Alzheimer disease and related dementias. *JAMA Psychiatry* 79, 359–367. doi: 10.1001/jamapsychiatry.2021.4363

Diniz, B. S., Butters, M. A., Albert, S. M., Dew, M. A., and Reynolds, C. F. 3rd (2013). Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and Meta-analysis of community-based cohort studies. *Br. J. Psychiatry* 202, 329–335. doi: 10.1192/bjp.bp.112.118307

Ehrenberg, A. J., Suemoto, C. K., Franca Resende, E. P., Petersen, C., Leite, R. E. P., Rodriguez, R. D., et al. (2018). Neuropathologic correlates of psychiatric symptoms in Alzheimer's disease. *J. Alzheimers Dis.* 66, 115–126. doi: 10.3233/JAD-180688

Eid, R. S., Gobinath, A. R., and Galea, L. A. M. (2019). Sex differences in depression: insights from clinical and preclinical studies. *Prog. Neurobiol.* 176, 86–102. doi: 10.1016/j.pneurobio.2019.01.006

Eikelboom, W. S., Pan, M., Ossenkoppele, R., Coesmans, M., Gatchel, J. R., Ismail, Z., et al. (2022). Sex differences in neuropsychiatric symptoms in Alzheimer's disease dementia: a meta-analysis. *Alzheimers Res. Ther.* 14:48. doi: 10.1186/s13195-022-00991-z

Ferretti, M. T., Iulita, M. F., Cavedo, E., Chiesa, P. A., Schumacher Dimech, A., Santuccione Chadha, A., et al. (2018). Sex differences in Alzheimer disease—the gateway to precision medicine. *Nat. Rev. Neurol.* 14, 457–469. doi: 10.1038/s41582-018-0032-9

Filon, J. R., Intorcia, A. J., Sue, L. I., Vazquez Arreola, E., Wilson, J., Davis, K. J., et al. (2016). Gender differences in Alzheimer disease: brain atrophy, histopathology burden, and cognition. *J. Neuropathol. Exp. Neurol.* 75, 748–754. doi: 10.1093/jnen/nlw047

Grudzien, A., Shaw, P., Weintraub, S., Bigio, E., Mash, D. C., and Mesulam, M. M. (2007). Locus coeruleus neurofibrillary degeneration in aging, mild cognitive

impairment and early Alzheimer's disease. *Neurobiol. Aging* 28, 327–335. doi: 10.1016/j.neurobiolaging.2006.02.007

Hamilton, M. (1960). A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* 23, 56–62. doi: 10.1136/jnnp.23.1.56

Hudon, C., Escudier, F., de Roy, J., Croteau, J., Cross, N., Dang-Vu, T. T., et al. (2020). Behavioral and psychological symptoms that predict cognitive decline or impairment in cognitively Normal middle-aged or older adults: a Meta-analysis. *Neuropsychol. Rev.* 30, 558–579. doi: 10.1007/s11065-020-09437-5

Hyman, B. T., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Carrillo, M. C., et al. (2012). National Institute on Aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement.* 8, 1–13. doi: 10.1016/j.jalz.2011.10.007

Ismail, Z., Smith, E. E., Geda, Y., Sultzer, D., Brodaty, H., Smith, G., et al. (2016). Neuropsychiatric symptoms as early manifestations of emergent dementia: provisional diagnostic criteria for mild behavioral impairment. *Alzheimers Dement.* 12, 195–202. doi: 10.1016/j.jalz.2015.05.017

Katabathula, S., Davis, P. B., and Xu, R.Alzheimer's Disease Neuroimaging, I (2022). Sex-specific heterogeneity of mild cognitive impairment identified based on multi-modal data analysis. *J. Alzheimers Dis.* 91, 233–243. doi: 10.3233/JAD-220600

Knopman, D. S., Amieva, H., Petersen, R. C., Chetelat, G., Holtzman, D. M., Hyman, B. T., et al. (2021). Alzheimer Disease. *Nat. Rev. Dis. Primers* 7:33. doi: 10.1038/s41572-021-00269-y

Kodesh, A., Sandin, S., Reichenberg, A., Rotstein, A., Pedersen, N. L., Ericsson, M., et al. (2019). Exposure to antidepressant medication and the risk of incident dementia. *Am. J. Geriatr. Psychiatry* 27, 1177–1188. doi: 10.1016/j.jagp.2019.05.019

Koran, M. E. I., Wagener, M., Hohman, T. J., and Alzheimer's Neuroimaging, I. (2017). Sex differences in the association between ad biomarkers and cognitive decline. *Brain Imaging Behav.* 11, 205–213. doi: 10.1007/s11682-016-9523-8

Labaka, A., Goñi-Balentziaga, O., Lebeña, A., and Pérez-Tejada, J. (2018). Biological sex differences in depression: a systematic review. *Biol. Res. Nurs.* 20, 383–392. doi: 10.1177/1099800418776082

Lee, J., Lee, K. J., and Kim, H. (2017). Gender differences in behavioral and psychological symptoms of patients with Alzheimer's disease. *Asian J. Psychiatr.* 26, 124–128. doi: 10.1016/j.ajp.2017.01.027

Liesinger, A. M., Graff-Radford, N. R., Duara, R., Carter, R. E., Hanna Al-Shaikh, F. S., Koga, S., et al. (2018). Sex and age interact to determine clinicopathologic differences in Alzheimer's disease. *Acta Neuropathol.* 136, 873–885. doi: 10.1007/s00401-018-1908-x

Lovheim, H., Sandman, P. O., Karlsson, S., and Gustafson, Y. (2009). Sex differences in the prevalence of behavioral and psychological symptoms of dementia. *Int. Psychogeriatr.* 21, 469–475. doi: 10.1017/S1041610209008497

Lyketsos, C. G., Carrillo, M. C., Ryan, J. M., Khachaturian, A. S., Trzepacz, P., Amatniek, J., et al. (2011). Neuropsychiatric symptoms in Alzheimer's disease. *Alzheimers Dement.* 7, 532–539. doi: 10.1016/j.jalz.2011.05.2410

Lyketsos, C. G., and Olin, J. (2002). Depression in Alzheimer's disease: overview and treatment. *Biol. Psychiatry* 52, 243–252. doi: 10.1016/S0006-3223(02)01348-3

McCutcheon, S. T., Han, D., Troncoso, J., Koliatsos, V. E., Albert, M., Lyketsos, C. G., et al (2016). Clinicopathological correlates of depression in early Alzheimer's disease in the NACC. *Int J Geriatr Psychiatry.* 31, 1301–1311. doi: 10.1002/gps.4435

Mielke, M. M., Vemuri, P., and Rocca, W. A. (2014). Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. *Clin. Epidemiol.* 6, 37–48. doi: 10.2147/CLEP.S37929

Mirra, S. S., Heyman, A., Mckeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., et al. (1991). The consortium to establish a registry for Alzheimer's disease (Cerad). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 41, 479–486. doi: 10.1212/WNL.41.4.479

Montine, T. J., Phelps, C. H., Beach, T. G., Bigio, E. H., Cairns, N. J., Dickson, D. W., et al. (2012). National Institute on aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol.* 123, 1–11. doi: 10.1007/s00401-011-0910-3

Moustafa, A. A., Jaroudi, W., Helal, A., Bilson, L., and El Haj, M. (2022). "Chapter 1 - measures of depression in Alzheimer's disease" in *Alzheimer's disease*. ed. A. A. Moustafa (Cambridge, Massachusetts: Academic Press). doi: 10.1016/B978-0-12-821334-6.00011-9

Murray, M. E., Graff-Radford, N. R., Ross, O. A., Petersen, R. C., Duara, R., and Dickson, D. W. (2011). Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. *Lancet Neurol.* 10, 785–796. doi: 10.1016/S1474-4422(11)70156-9

Nebel, R. A., Aggarwal, N. T., Barnes, L. L., Gallagher, A., Goldstein, J. M., Kantarci, K., et al. (2018). Understanding the impact of sex and gender in Alzheimer's disease: a call to action. *Alzheimers Dement.* 14, 1171–1183. doi: 10.1016/j.jalz.2018.04.008

Nia and Group, R. I. W (1997). Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan institute working group on diagnostic criteria for the neuropathological assessment of Alzheimer's disease. *Neurobiol. Aging* 18, S1–S2.

Norman, J. (2014). Gender Bias in the diagnosis and treatment of depression. *Int. J. Ment. Health* 33, 32–43). doi: 10.1080/00207411.2004.11043365

Novais, F., and Starkstein, S. (2015). Phenomenology of depression in Alzheimer's disease. *J. Alzheimers Dis.* 47, 845–855. doi: 10.3233/JAD-148004

Panza, F., Frisardi, V., Capurso, C., D'Introno, A., Colacicco, A. M., Imbimbo, B. P., et al. (2010). Late-life depression, mild cognitive impairment, and dementia: possible continuum? *Am. J. Geriatr. Psychiatry* 18, 98–116. doi: 10.1097/JGP.0b013e3181b0fa13

Parker, G., and Brotchie, H. (2010). Gender differences in depression. *Int. Rev. Psychiatry* 22, 429–436. doi: 10.3109/09540261.2010.492391

Perrin, A. J., Nosova, E., Co, K., Book, A., Iu, O., Silva, V., et al. (2017). Gender differences in Parkinson's disease depression. *Parkinsonism Relat. Disord.* 36, 93–97. doi: 10.1016/j.parkreldis.2016.12.026

Rocca, W. A. (2017). Time, sex, gender, history, and dementia. *Alzheimer Dis. Assoc. Disord.* 31, 76–79. doi: 10.1097/WAD.0000000000000187

Romera, I., Perez, V., Menchon, J. M., Polavieja, P., and Gilaberte, I. (2011). Optimal cutoff point of the Hamilton rating scale for depression according to normal levels of social and occupational functioning. *Psychiatry Res.* 186, 133–137. doi: 10.1016/j.psychres.2010.06.023

Rapp, M. A., Schnaider-Beeri, M., Purohit, D. P., Perl, D.P., Haroutunian, V., and Sanon, M. (2008). Increased neurofibrillary tangles in patients with Alzheimer disease with comorbid depression. *Am J Geriatr Psychiatry*, 16, 168–74. doi: 10.1097/JGP.0b013e31816029ec

Saiz-Vazquez, O., Gracia-Garcia, P., Ubillos-Landa, S., Puente-Martinez, A., Casado-Yusta, S., Olaya, B., et al. (2021). Depression as a risk factor for Alzheimer's disease: a systematic review of longitudinal meta-analyses. *J. Clin. Med.* 10: 1809. doi: 10.3390/jcm10091809

Salk, R. H., Hyde, J. S., and Abramson, L. Y. (2017). Gender differences in depression in representative National Samples: Meta-analyses of diagnoses and symptoms. *Psychol. Bull.* 143, 783–822. doi: 10.1037/bul0000102

Tao, Y., Peters, M. E., Drye, L. T., Devanand, D. P., Mintzer, J. E., Pollock, B. G., et al. (2018). Sex differences in the neuropsychiatric symptoms of patients with Alzheimer's disease. *Am. J. Alzheimers Dis. Other Dement.* 33, 450–457. doi: 10.1177/1533317518783278

Tariot, P. N. (1999). Treatment of agitation in dementia. *J. Clin. Psychiatry* 60, 11–20.

Thal, D. R., Rüb, U., Orantes, M., and Braak, H. (2002). Phases of Aβ-deposition in the human brain and its relevance for the development of ad. *Neurology* 58, 1791–1800. doi: 10.1212/WNL.58.12.1791

Tsuno, N., and Homma, A. (2009). What is the association between depression and Alzheimer's disease? *Expert. Rev. Neurother.* 9, 1667–1676. doi: 10.1586/ern.09.106

Underwood, E. A., Davidson, H. P., Azam, A. B., and Tierney, M. C. (2019). Sex differences in depression as a risk factor for Alzheimer's disease: a systematic review. *Innov. Aging* 3:Igz015. doi: 10.1093/geroni/igz015

Wang, Y. C., Tai, P. A., Poly, T. N., Islam, M. M., Yang, H. C., Wu, C. C., et al. (2018). Increased risk of dementia in patients with antidepressants: a Meta-analysis of observational studies. *Behav. Neurol.* 2018:5315098. doi: 10.1155/2018/5315098

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., et al. (1982). Development and validation of a geriatric depression screening scale: a preliminary report. *J. Psychiatr. Res.* 17, 37–49. doi: 10.1016/0022-3956(82)90033-4

Zhu, D., Montagne, A., and Zhao, Z. (2021). Alzheimer's pathogenic mechanisms and underlying sex difference. *Cell. Mol. Life Sci.* 78, 4907–4920. doi: 10.1007/s00018-021-03830-w

Zimmerman, M., Martinez, J. H., Young, D., Chelminski, I., and Dalrymple, K. (2013). Severity classification on the Hamilton depression rating scale. *J. Affect. Disord.* 150, 384–388. doi: 10.1016/j.jad.2013.04.028

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Decoding the genetic relationship between Alzheimer's disease and type 2 diabetes: potential risk variants and future direction for North Africa

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Introduction: Alzheimer's disease (AD) and Type 2 diabetes (T2D) are both age-associated diseases. Identification of shared genes could help develop early diagnosis and preventive strategies. Although genetic background plays a crucial role in these diseases, we noticed an underrepresentation tendency of North African populations in omics studies.

Materials and methods: First, we conducted a comprehensive review of genes and pathways shared between T2D and AD through PubMed. Then, the function of the identified genes and variants was investigated using annotation tools including PolyPhen2, RegulomeDB, and miRdSNP. Pathways enrichment analyses were performed with g:Profiler and EnrichmentMap. Next, we analyzed variant distributions in 16 worldwide populations using PLINK2, R, and STRUCTURE software. Finally, we performed an inter-ethnic comparison based on the minor allele frequency of T2D-AD common variants.

Results: A total of 59 eligible papers were included in our study. We found 231 variants and 363 genes shared between T2D and AD. Variant annotation revealed six single nucleotide polymorphisms (SNP) with a high pathogenic score, three SNPs with regulatory effects on the brain, and six SNPs with potential effects on miRNA-binding sites. The miRNAs affected were implicated in T2D, insulin signaling pathways, and AD. Moreover, replicated genes were significantly enriched in pathways related to plasma protein binding, positive regulation of amyloid fibril deposition, microglia activation, and cholesterol metabolism. Multidimensional screening performed based on the 363 shared genes showed that main North African populations are clustered together and are divergent from other worldwide populations. Interestingly, our results showed that 49 SNP associated with T2D and AD were present in North African populations. Among them, 11 variants located in *DNM3*, *CFH*, *PPARG*, *ROHA*, *AGER*, *CLU*, *BDNF1*, *CST9*, and *PLCG1* genes display significant differences in risk allele frequencies between North African and other populations.

Conclusion: Our study highlighted the complexity and the unique molecular architecture of North African populations regarding T2D-AD shared genes. In conclusion, we emphasize the importance of T2D-AD shared genes and ethnicity-specific investigation studies for a better understanding of the link

behind these diseases and to develop accurate diagnoses using personalized genetic biomarkers.

KEYWORDS

pathogenic variants, pathways, ethnicity, PRISMA, admixure, multidimensional scaling plot, miRNA

Introduction

The world is experiencing the oldest living population (United Nations Department of Economic and Social Affairs, Population Division, 2020). The increase in lifespan and unhealthy habits coincides with an increase in age-related diseases, such as dementia and type 2 diabetes (T2D) (Hayden, 2019). Globally, more than 57.4 million adults live with dementia, and this number is estimated to increase to 152.8 million by 2050 (Nichols et al., 2022). Similarly, 537 million T2D patients, and this figure is expected to increase to 780 million (Magliano et al., 2021).

Alzheimer's disease (AD) is the most common form of dementia worldwide, accounting for more than 70% of all cases (2022 Alzheimer's Disease Facts and Figures, n.d.). Currently, more than 6.2 million adults above the age of 65 years live with AD in the United States alone (2022 Alzheimer's Disease Facts and Figures, n.d.). The increasing prevalence of AD imposes a heavy socioeconomic burden on families and societies (Shu et al., 2022). AD is a complex disease, and the absence of modifying treatments adds another constraint. Therefore, a shift from a curative to a preventive approach is essential (Silva-Spínola et al., 2022). One approach is to work on the causes and risk factors of disease. For example, genetic investigation of AD risk factors could help shape our understanding of the disease and provide a promising tool for identifying presymptomatic AD (de Rojas et al., 2021). Furthermore, T2D is a major risk factor for AD development (Thomassen et al., 2020), and compelling evidence supports the interaction between these diseases (Barbagallo and Dominguez, 2014). T2D and AD share several molecular mechanisms including insulin resistance, oxidative stress (De Sousa et al., 2020), inflammation, and mitochondrial dysfunction (Silzer and Phillips, 2018). Thus, well-established genetic variants and pathways that are common between T2D and AD are of great significance for AD prevention and early diagnosis.

Advances in omics technologies, such as Genomics and Transcriptomics, have greatly enhanced our knowledge of the pathophysiology of T2D and AD at a detailed molecular level (Karczewski and Snyder, 2018). Several omics results have paved the way for new findings regarding the interactions between these diseases.

Although omics technologies represent great promise for science revolution and precision medicine implementation, a vast number of omics research cohorts are of European ancestry (Popejoy and Fullerton, 2016). This could lead to a serious research gap since European ancestry findings do not necessarily replicate across other populations (Martin et al., 2019). Genetic background is an important element when studying common diseases, such as AD and T2D (Huang et al., 2017). Consequently, there is an urgent need to integrate more underrepresented populations to maximize the potential of discovering genes and pathways that are common between T2D and AD (Popejoy and Fullerton, 2016) and to fulfill the promise of precision medicine.

North African populations have highly diverse and complex genetic structures. It is characterized by a rich genetic background due to the admixture between Berber (early settlers in North Africa) and Eurasiatic and Sub-Saharan components (Kefi et al., 2015). Like a mosaic, the North African genetic background represents a valuable and unique source for genetic investigations (Ben Halima et al., 2017; Jmel et al., 2018; Arauna et al., 2019; Romdhane et al., 2021; Dallali et al., 2022) and the implementation of precision medicine.

Abbreviations: ABC transporter, ATP Binding Cassette transporter; AD, Alzheimer's disease; ADORA2A, Adenosine A2a receptor; AGER, Advanced glycosylation end-product specific; AGPAT1, 1-acylglycerol-3-phosphate O-acyltransferase 1; AGT, Angiotensinogen; AMR, American; ANK1, Ankyrin 1; ApoB, Apolipoprotein B; APOE, Apolipoprotein E; ASW, African ancestry in the South Western USA; Aβ, Amyloid-beta; BBB, Blood–brain barrier; BDNF1, Brain derived neurotrophic factor; BMI, Body mass index; CEU, Northwestern and Western European ancestry populations of Utah from the CEPH collection; CFH, Complement factor H; CHB, Han Chinese in Beijing, China; CHD, Chinese population of metropolitan Denver, Colorado, USA; CLU, Clusterin; CNS, Central nervous system; CST3, Cystatin C; CST9, Cystatin C9; DMRT3, Doublesex and mab-3 related transcription factor 3; DNM3, Dynamin 3; EAS, East Asian; EFCAB5, EF-hand calcium binding domain 5; EIF2S2P3, Eukaryotic translation initiation factor 2 subunit 2 beta pseudogene 3; EPHX2, Epoxide hydrolase 2; EUR, European; GLUT4, Solute carrier family 2; H3K27ac, Histone 3 lysine 27 acetylation; H3K4me1, Monomethylation of lysine 4 on histone H3 protein subunit; HbA1c, Glycated hemoglobin; HHEX, Hematopoietically expressed homeobox; hIAPP, Human islet amyloid polypeptide; HWE, Hardy–Weinberg equilibrium; ICAM1, Intercellular adhesion molecule 1; JPT, Japanese in Tokyo, Japan; LD, Linkage disequilibrium; LDL, Low-density lipoprotein; LOAD, Late-onset Alzheimer's disease; LPL, Lipoprotein lipase; MADD, MAP kinase activating death domain; MAF, Minor allele frequency; MDS, Multidimensional scaling plot; MEX, Mexican ancestry living in Los Angeles, California, USA; miRNA, Micro ribonucleic acid; Morocco_N, North Morocco; Morocco_S, South Morocco; Myc, MYC proto-oncogene, bhlh transcription factor; NAF, North African populations; NECTIN2, Nectin cell adhesion molecule 2; NR2C2, Nuclear receptor subfamily 2 group C member 2; PLCG1, Phospholipase C gamma 1; PPARG, Peroxisome proliferator activated receptor gamma; ROHA, Ras homolog family member A; SNP, Single nucleotide polymorphism; Spain_BASC, Spain Basic populations; Spain_N, North Spain; Spain_NW, North West of Spain; Spain_S, South Spain; T2D, Type 2 diabetes; TC, Total cholesterol; TCF3, Transcription factor 3; TFB, Transcription factor binding motif; TN_Ber, Tunisia Douiret; TP53, Tumor protein p53; TSI, Toscani people of Italy; USF1, Upstream transcription factor 1; USF2, Upstream transcription factor 2, c-fos interacting; VEP, Variant effect predictor.

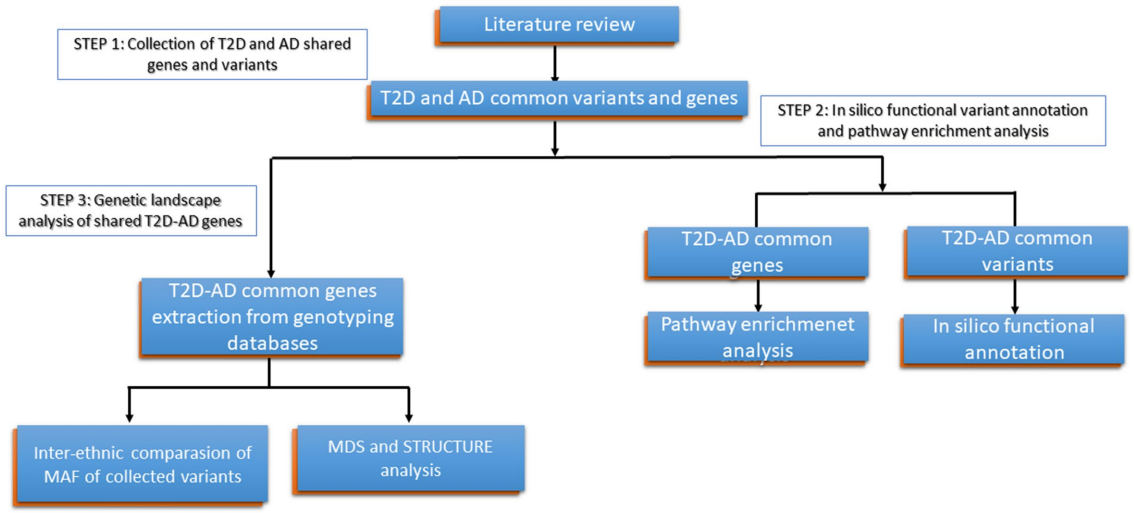

FIGURE 1
Detailed research work flow. Type 2 Diabetes (T2D); Alzheimer's disease (AD), Minor allele frequency (MAF).

In this study, we aimed to identify the most common variants and pathways shared between T2D and AD and to explore their genetic variability in North African populations compared to other populations worldwide.

Materials and methods

To attend our objectives, (1) we developed the present workflow (Figure 1), in which we conducted in the first step a general review of the literature to collect genes and variants previously identified in common between T2D and AD, (2) Then, in the second step, we performed *in silico* functional analysis and pathway enrichment analysis of the collected variants and genes shared betweenT2D and AD, and (3) In the third step, we conducted a multidimensional scaling plot (MDS) and Structure analysis of these variants on genotyping data available publicly in order to explore the genetic landscape of T2D-AD shared genes in North African populations and in comparison, to other populations.

Step 1: T2D-AD shared genes and variants collection

To collect T2D-AD shared genes and variants from the literature, we developed a study protocol using the PRISMA statement (Page et al., 2021). The public database PubMed[1] was searched from August 2001 to the 4th of September 2022. The search terms were limited to "Type 2 diabetes" AND "Alzheimer's disease" AND "gene" OR "biomarker" OR "Proteomic" OR "Methylation." Initially, we established a systematic screening for all articles published during that period according to their title and abstract relevance. Articles on animal models, *in vivo* studies, and mitochondrial DNA were excluded. The final selection criteria were as follows: (1) relevant articles, (2) available in English, (3) studies conducted on human samples, and (4) genetic, transcriptomic, proteomic, and methylation studies.

Step 2: *In silico* functional variant annotation and pathway enrichment analysis

Variant annotation and functional effect prediction

The collected single nucleotide polymorphism (SNPs) from the selected studies were annotated using the VEP (Variant Effect Predictor) tool from Ensembl (McLaren et al., 2016) and the SNPnexus web server (Oscanoa et al., 2020). Next, we used two databases to annotate the functionalities of the variants, depending on their locations. Variants in the coding region have been functionally annotated using Polyphen-2 (Adzhubei et al., 2010). Polyphen-2 is a web-based software that can predict the possible impact of amino acid substitutions on the structure and function of human proteins using physical and evolutionary comparative considerations (Adzhubei et al., 2010). The PolyPhen2 scores range between 0 and 1, with 1 being the most likely deleterious variant. Similarly, RegulomeDB[2] was used to prioritize non-coding and modifier variants. RegulomeDB is an open-access database that annotates variants in the intergenic region based on ENCODE releases, Gene Ontology, Chromatin States from the Roadmap Epigenome Consortium, and updates to DNase footprinting. The RegulomeDB probability score is ranging from 0 to 1, with 1 being the most likely to be a regulatory variant (Boyle et al., 2012). Top-ranked SNPs (RegulomeDB score = 1) were further

1 https://pubmed.ncbi.nlm.nih.gov/

2 https://regulomedb.org/regulome-search/

investigated using the GTEx portal (Stanfill and Cao, 2021) and FeatSNP to assess their association with epigenetic effects in the human brain (Ma et al., 2019). Additionally, SNPinfo, a web-based server, was used to detect SNPs with potential miRNA-binding sites (Xu and Taylor, 2009). Variants with predicted effects on miRNA-binding sites were explored using miRdSNP (Bruno et al., 2012). The list of miRNAs was then used to generate a heat map of pathways affected by this miRNA using miRPathDB 2.0 (Kehl et al., 2020).

Pathway enrichment analysis and visualization

Pathway enrichment analysis is an efficient method for gaining mechanistic insight into a specific gene list by identifying biological pathways enriched in that gene set (Reimand et al., 2019). We performed pathway enrichment analysis for the replicated genes among studies using the g:Profiler tool (Raudvere et al., 2019). It searches a collection of gene sets representing Gene Ontology (GO) terms and pathways (KEGG pathway, Reactome, and WikiPathway). The Bonferroni correction was applied as the significance threshold for all enrichment analyses. The user threshold was set to 0.05. However, pathway enrichment analysis often highlights several versions of the same pathway (Reimand et al., 2019). Visualization tools can help facilitate the interpretation of analysis results. Hence, we used EnrichmentMap (Merico et al., 2010) to visualize the non-redundant pathways.

Step 3: Genetic landscape analysis of T2D-AD shared genes

Genotyping data and quality control analysis

Genotyping data of 829 individuals from 16 populations were downloaded from the International 1,000 Genome Project phase III (1000 Genome, n.d)[3] and published data (Li et al., 2008; Henn et al., 2012). The studied populations included those of American: African ancestry in the South Western USA (ASW) and people of Mexican ancestry living in Los Angeles, California, USA (MEX); European ancestry: Northwestern and Western European ancestry populations of Utah from the CEPH collection (CEU), Toscani people of Italy (TSI), South Spain (Spain_S), North Spain (Spain_N), North West of Spain (Spain_NW) and Spain Basic populations (Spain_BASC); individuals from East Asian ancestry: Han Chinese in Beijing, China (CHB), the Chinese population of metropolitan Denver, Colorado, USA (CHD) and Japanese in Tokyo, Japan; (JPT), Individuals from North Africa: Algeria (Algeria), Egypt (Egypt), Libya (Libya), Tunisia Douiret (TN_Ber), South Morocco (Morocco_S), North Morocco (Morocco_N).

We used the PLINK v2 software (Chang et al., 2015) to extract variants of the selected common genes between T2D and AD, from the genotyping data.

First, to study the genetic landscape of all variants (common and rare), we excluded variants deviating from the Hardy–Weinberg equilibrium (HWE) (p-value $< 10^{-4}$) and those with a genotyping rate $\leq 95\%$ for each of the studied populations. Second, we retrained variants with minor allele frequency (MAF) $>10^{-2}$ to explore the genetic landscape of common variants given their importance in the development of complex diseases (de Rojas et al., 2021; Shoily et al., 2021).

Statistical analysis

Merged data were pruned based on the physical distances between adjacent markers and linkage disequilibrium (LD). High-density markers that did not provide additional information were excluded. Next, pruning data were used to create a multidimensional scaling plot (MDS) to study the landscape of the selected common T2D-AD gene regions. To this end, a symmetric matrix of identity-by-state (IBS) distances for all pairs of individuals was based on the proportion of shared common alleles. This analysis was performed using the Plink and R software (R: The R Project for Statistical Computing, n.d).[4]

After calculating the allele frequencies of the T2D-AD shared variants, the populations were clustered according to their geographic origins. Four groups were generated: North African (NAF), East Asian (EAS), American (AMR), and European (EUR). The Chi-square test was used to compare the risk allele frequencies of candidate variants between NAF populations and other populations. Bonferroni's adjustment was applied to the level of significance set at a value of p threshold of 5% divided by the number of studied variants. All analyses were conducted using the R software.

Analyses of population genetic structures

We used a Bayesian clustering algorithm, STRUCTURE Ver. 2.3.4 software (Pritchard et al., 2000; FALUSH et al., 2007) to explore the variability of the common T2D and AD variants in terms of population structure. The algorithm assigns samples within a hypothetical K number of ancestries. We set a range of possible numbers of clusters ranging from $K = 2$ to $K = 10$, and four trials were run for each K. The Markov Chain Monte Carlo iteration for each structure analysis was run for 10,000 after an initial burn-in period of 10,000 steps. To assess the most likely number of clusters, we calculated Delta K, as proposed by Evanno et al. (2005). The similarity of the runs at each K level was evaluated using CLUMPP software as implemented online (Jakobsson and Rosenberg, 2007). Distruct software was used to visualize the best alignment of subpopulations, inferring population substructure and individual assignment across the best runs at each K level.

Results

Step 1: T2D-AD shared genes and variants collection

The PubMed search based on our defined search terms yielded 226 results. Studies with irrelevant results and those conducted using animal models ($n = 163$) were excluded. Additionally, four relevant studies were excluded due to access issues. The PRISMA

3 www.internationalgenome.org

4 https://www.R-project.org/

flow diagram for the selected studies is represented in Figure 2. Finally, 59 publications were included in the present study (Supplementary file 1; Table 1).

The majority of the studies used data from American (Native American, Latino American, African American, and Mexican), Asian (Han Chinese, Japanese, and Korean), and European ancestry populations.

The literature search revealed 231 variants and 363 genes shared between T2D and AD identified by these studies. The 363 genes include those mapped to the 231 variants. A total of 46 genes were replicated in different studies (Supplementary file 1; Tables 2, 3).

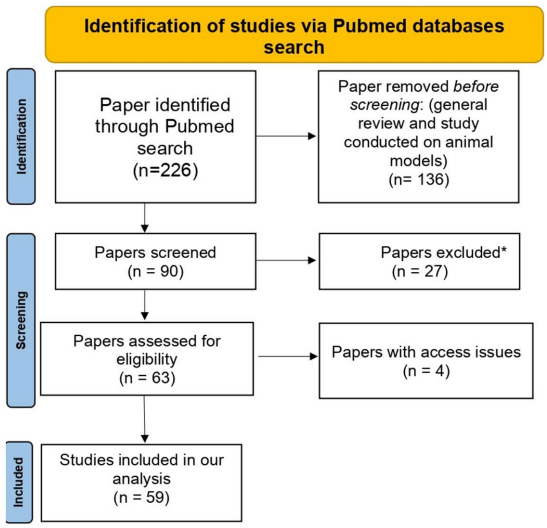

FIGURE 2
PRISMA flow diagram of selected studies. *Excluded papers are those with irrelevant results.

Step 2: *In silico* functional variant annotation and pathway enrichment analysis

Variant annotation and functional effect prediction

Variant annotation of the 231 common SNPs showed that chromosome 19 had the highest number of SNPs ($n = 32$), followed by chromosome 17 ($n = 24$ SNPs), and chromosome 11 ($n = 20$ SNPs). The results of the variant annotation are shown in Supplementary file 2; Tables 1, 2. The shared SNPs were mapped to 106 genes. The top 6 genes with the highest number of SNPs were MAP kinase activating death domain (*MADD*) (chromosome (CHR) 11, 9 SNPs); EF-hand calcium binding domain 5 (*EFCAB5*) (CHR 17, 9 SNPs); nectin cell adhesion molecule 2 (*NECTIN2*) (CHR 19, 6 SNPs); cystatin C (*CST3*) (CHR 20, 6 SNPs), apolipoprotein E (*APOE*) (CHR 19, 5 SNPs) and 1-acylglycerol-3-phosphate O-acyltransferase 1 (*AGPAT1*) (CHR 6, 5 SNPs). Variant annotation revealed the association of nine SNPs with drug response (Table 1) and 10 variants were annotated as clinically likely pathogenic/pathogenic (Table 2).

A total of 66 variants located in 52 genes were identified as missense or stop-gain mutations. Among these, 24 SNPs were predicted to be possibly damaging by PolyPhen2. We identified six SNPs (rs7412, rs2070600, rs4762, rs11540654, rs1799969, and rs751141) located in the *APOE*, advanced glycosylation end-product specific (*AGER*), angiotensinogen (*AGT*), tumor protein p53 (*TP53*), intercellular adhesion molecule 1 (*ICAM1*), and epoxide hydrolase 2 (*EPHX2*) gene, that had

TABLE 1 Summary of T2D-AD shared variants associated with drug response.

CHR	PB	Mapped gene	rs ID	Drug
1	11,796,321	*MTHFR*	rs1801133	l-methylfolate, Vitamin B-complex, Incl. Combinations, methotrexate, bevacizumab, carboplatin, cisplatin, cyanocobalamin, folic acid, pemetrexed capecitabine, fluorouracil, leucovorin, oxaliplatin, clozapine, olanzapine, nitrous oxide
15	78,590,583	*CHRNA5*	rs16969968	nicotine, cocaine, bupropion, Drugs used in nicotine dependence, varenicline, ethanol, Opium alkaloids and derivatives
19	44,905,579	*APOE*	rs405509	Selective serotonin reuptake inhibitor
19	44,908,684	*APOE*	rs429358	acenocoumarol, warfarin, hmg coa reductase inhibitors, Antivirals for treatment of HIV infections, combinations, ritonavir, simvastatin
19	44,908,822	*APOE*	rs7412	atorvastatin, warfarin, Antivirals for treatment of HIV infections, combinations, ritonavir, fenofibrate, fluvastatin, pravastatin
19	44,911,194	*APOE*	rs439401	Warfarin
22	19,963,748	*COMT*	rs4680	nicotine, naloxone, oxycodone, fentanyl, methadone, antipsychotics, opioids, entacapone, propranolol, modafinil, Analgesics, Antiinflammatory agents, non-steroids, Ergot alkaloids, sumatriptan, clozapine, venlafaxine, buprenorphine, fluvoxamine, remifentanil, risperidone
4	88,131,171	*ABCG2*	rs2231142	rosuvastatin, cyclophosphamide, doxorubicin, fluorouracil, imatinib, gemcitabine, dolutegravir, simvastatin, tenofovir, sunitinib, methotrexate, atorvastatin, apixaban, efavirenz, sulfasalazine, fluvastatin, lamotrigine, allopurinol, Opioid anesthetics, Other general anesthetics, volatile anesthetics, gefitinib, capecitabine, fluorouracil, leucovorin, oxaliplatin
6	31,575,254	*LTA-TNF*	rs1800629	etanercept, carbamazepine, sorafenib, carboplatin, gemcitabine, ethambutol, isoniazid, pyrazinamide, rifampin, cyclosporine, mycophenolate mofetil, Tumor necrosis factor alpha (TNF-alpha) inhibitors, atorvastatin

the highest pathogenicity score (PolyPhen2 score = 1). Polyphen2 results are provided in Table 3 of Supplementary file 2.

Most SNPs extracted from the included studies were located in the non-coding region and have a "modifier" impact. Please correct the following sentence Functional annotation using the RegulomeDB identified three variants (rs1544210, rs12679834, and rs515071) located in the hematopoietically expressed homeobox *(HHEX)*, lipoprotein lipase *(LPL)*, and ankyrin 1 *(ANK1)* gene which were the most likely regulatory SNPs (Table 3). The full list of RegulomeDB outputs is provided in Table 4 of Supplementary file 2.

To better assess the role of these variants in genetic cis-regulation in the brain, we explored the GTEx pathway. We found an association between the minor allele A rs1544210 and the under-expression of the *EIF2S2P3* pseudogene in the substantia nigra and hypothalamus. Similarly, the minor allele G of the variant rs515071 was associated with decreased *ANK1* expression in the cerebellum and the cerebellar hemisphere. The search for rs12679834 revealed no ci-regulation of this SNP in the brain tissues (Table 3).

To further explore the regulatory effects of these variants, the FeatSNP database was searched to evaluate the possible epigenetic effects in different brain regions. We identified four potential transcription factor (TF)-binding motifs associated with allele A of rs1544210: USF1, Myc, USF2, and DMRT3. However, histone modification signals associated with the selected SNP were not detected.

A search of the FeatSNP database showed one potential TF-binding motif (NR2C2) associated with the A allele of rs12679834. Our results showed a strong correlation between NR2C2 (nuclear receptor subfamily 2 group C member 2) expression and *LPL* expression in two different brain regions: putamen ($r = 0.736$) and caudate ($r = 0.651$). Furthermore, we found that the region tagged with SNP rs12679834 was enriched for strong active histone modification signals, including H3K4me1 and H3K27ac, in all three brain tissues.

Finally, the results for rs515071 showed that one TF-binding motif (TCF3) was associated with the G allele of this variant. The region tagged by this SNP was enriched for the active histone modification H3K27ac in the inferior temporal lobe, angular gyrus, and anterior caudate region.

The SNPinfo results showed that only seven variants were predicted to have a potential effect on miRNA-binding sites (Table 4). All the identified SNPs, except one (rs6997), affected miRNAs such as rs6859 that affect hsa-miR-378 (Supplementary file 2; Tables 5, 6). Our results showed that the majority of miRNAs were mapped to several pathways involved in different diseases such as cancers,T2D, AD, and insulin signaling pathways (Figure 3).

Pathway enrichment analysis and visualization

To gain a deeper understanding of T2D and AD common genes we conducted a pathway enrichment analysis using g:Profiler. In order to obtain accurate results in terms of the relationship between these diseases, the gene set was limited to genes replicated among the selected studies. The g:Profiler pathway enrichment analysis results are shown in Table 5 and Supplementary files 3 and 4. The obtained results were then visualized using EnrichmentMap (Figure 4). The main enriched pathways were: lipid subunit organization, positive regulation of protein binding, positive regulation of amyloid fibril formation, microglial cell activation, (value of $p = 0.01$). Furthermore, analysis of the KEGG pathway Reactome, and WikiPathway revealed enrichment of cholesterol metabolism (p-value $= 2.584 \times 10^{-3}$) plasma lipoprotein assembly (p-value $= 7.260 \times 10^{-3}$), and Statin inhibition of cholesterol production (p-value $= 1.041 \times 10^{-3}$).

Step 3: Genetic landscape analysis of shared T2D-AD genes

Statistical analysis

All variants located in in T2D-AD common genes were extracted from the genotyping data of 829 individuals from the studied populations (Supplementary file 5). A total of 212,688 variants were identified after merging of the genotyping data. Among them, we did

TABLE 2 Summary of T2D-AD common variants clinically likely pathogenic.

CHR	Position	Mapped gene	rs ID	Phenotype associated in ClinVar
1	11,796,321	*MTHFR*	rs1801133	Homocystinuria Due To Methylene Tetrahydrofolate Reductase Deficiency
17	7,676,040	*TP53*	rs11540654	Li-Fraumeni Syndrome, Hereditary Cancer-Predisposing Syndrome
19	44,908,684	*APOE*	rs429358	Familial Type 3 Hyperlipoproteinemia, APOE3 ISOFORM
19	44,908,822	*APOE*	rs7412	Familial Type 3 Hyperlipoproteinemia, Hyperlipoproteinemia Due To APOE1
20	23,637,790	*CST3*	rs1064039	Macular Degeneration, Age-Related
3	39,265,671	*CX3CR1*	rs3732378	Coronary Artery Disease, Resistance To, Human Immunodeficiency Virus Type 1, Rapid Progression To AIDS, Age Related Macular Degeneration 12
6	26,090,951	*HFE*	rs1799945	Hereditary Hemochromatosis, Alzheimer disease Type 1 Familial Porphyria Cutanea Tarda Variegate Porphyria Hemochromatosis Type 1 Microvascular Complications Of Diabetes, Transferrin Serum Level Quantitative Trait Locus 2, Cardiomyopathy, Abnormality Of Iron Homeostasis, Variegate Porphyria
6	26,092,913	*HFE*	rs1800562	Hereditary Hemochromatosis Type 1, Hereditary Cancer-Predisposing Syndrome, Abdominal Pain, Peripheral Neuropathy, Pain, Abnormal Peripheral Nervous System Morphology, Abnormality Of The Male Genitalia, Behavioral Abnormality, Abnormality Of The Nervous System, Cardiomyopathy, Hemochromatosis Type 2, HFE-Related Disorder, Hemochromatosis, Juvenile, Digenic, Alzheimer disease
7	150,999,023	*NOS3*	rs1799983	Susceptibility To Metabolic Syndrome

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TABLE 3 Regulatory T2D-AD shared SNPs.

Gene	rsID	RegulomeDB rank	GTEx e-QTL in brain tissues	TFB motifs	Histone modifications
HHEX	rs1544210	1b	*EIF2S2P3* in Substantia nigra (value of $p = 9.4e^{-7}$) and Hypothalamus (value of $p = 0.000025$)	USF1, Myc, USF2, DMRT3	
LPL	rs12679834	1b		ZNF354C, NR2C2, NKX2-8	Located in Substantia Nigra region marked by H3K4me1 and H3K27ac histone modifications
ANK1	rs515071	1b	*ANK1* in Cerebellum (value of $p = 1.9e^{-54}$) and in Cerebellum Hemisphere (value of $p = 7.5e^{-49}$)	Tcf3	Located in Inferior Temporal Lobe, Angular Gyrus and Anterior Caudate regions marked by H3K27ac histone modifications

Expression quantitative trait loci (e-QTL), Genotype Tissue Expression (GTEx), Transcription factor binding motif (TFB motifs).

TABLE 4 Summary of variants with potential effect on miRNAs binding sites.

Position	Mapped gene	SNP	miRNA	Disease
1: 109275684	*CELSR2*	rs629301	hsa-miR-338-3p, hsa-miR-224, hsa-miR-214, hsa-miR-186, hsa-miR-193b, hsa-miR-193a-3p, hsa-miR-193, hsa-miR-103, hsa-miR-107, hsa-miR-485-5p, hsa-miR-9, hsa-miR-125b, hsa-miR-125a-5p, hsa-miR-125a, hsa-miR-431, hsa-miR-17-5p, hsa-miR-106a, hsa-miR-20a, hsa-miR-106b, hsa-miR-93, hsa-miR-519d, hsa-miR-20b, hsa-miR-17, hsa-miR372, hsa-miR-20, hsa-miR-1,271, hsa-miR-96	
19: 44878777	*PVRL2*	rs6859	hsa-miR-378	Late onset Alzheimer's Disease
3: 49357401	*GPX1*	rs1050450	hsa-miR-1,233, hsa-miR-129-3p	Breast cancer, Lung cancer, Kashin-Beck disease
3: 9757089	*OGG1*	rs1052133	hsa-miR-1,256	Lung cancer, Colorectal cancer, Gallbladder cancer
6: 32180626	*RNF5*	rs8365	has-miR-196a, has-miR-196b, has-let-7b, has-let-7d, has-let-7i, has-let-7a, has-let-7f, has-let-7c, has-let-7e, has-let-7 g, has-miR-98	
7: 75986787	*POR*	rs17685	hsa-miR-603	

not find the rare variants of interest reported in the literature. Then, we generated a second set of common variants after excluding SNPs with MAF $< 10^{-2}$. In total, 123,115 common variants were retrained. MDS analysis describing the genetic landscape of these genetic variants was generated for the two sets of variants (set 1 with MAF $< 10^{-2} = 212{,}688$ variants, and set 2 without MAF $< 10^{-2} = 123{,}115$). There was no difference between the MDS plots generated by the two sets of variants. The MDS plot showed that the North African populations (Algeria, Egypt, Libya, Morocco-N, Morocco-S, Tunisia) were clustered within the European populations (CEU, Spain-S, Spain-Basic, Spain-NW, and TSI) and distinguished from the American (ASW, MEX) and Asian (CHB, CHD, JPT) populations (Figures 5A,B). Better individualization was observed in MDS performed across continents. In addition, there is great divergence among the North African (NAF), American (AMR), and East Asian (EAS) groups. However, slight proximity was found between the NAF and EUR clusters (Figure 5C).

Among the 231 variants of interest, only 49 risk alleles variants were identified in the studied populations. Interethnic comparison based on the selected MAF variants revealed significant differences at the level of 11 SNPs between North African, European, and East Asian populations. The 11 SNPs were located in *DNM3, CFH, PPARG, ROHA, RAGE, CLU, BDNF1, CST9*, and *PLCG1* genes (Supplementary file 6). No significant differences were found for the risk allele frequency of candidate genes between the North African and American populations (Supplementary file 7).

Analyses of population genetic structures

To determine the distribution of common T2D-AD variants between the studied populations, we adopted a Bayesian iterative algorithm using the STRUCTURE software. In accordance with Evanno's ΔK method for STRUCTURE, the hypothetical K number of ancestries was set at three (K = 3) to detect the most likely number of genetic clusters (Supplementary file 8). The Bar plot shows three components: Africa, Asia, and Europe. STRUCTURE analysis confirmed the ancestral diversity of the North African populations with evidence of the predominance of European components (Figure 6A). The Triangle of the structure shows that the NAF cluster is close to the European cluster and distinct from the EAS cluster (Figure 6B).

Discussion

In the present study, we collected T2D-AD common variants and genes from the literature. Then, we analyzed their functional predictions and pathways. Finally, we explored the genetic variability of the collected variants among North African populations in comparison with other populations worldwide.

TABLE 5 Molecular pathways enriched by shared genes between T2D and AD.

Category	Term ID	Description	Adjusted_*p*_value	Genes
KEGG	KEGG:04979	Cholesterol metabolism	0.0025837551982072883	*LPL, ABCA1, APOC1, APOE*
REAC	REAC:R-HSA-8963898	Plasma lipoprotein assembly	0.0072601471697531315	*ABCA1, APOC1, APOE*
WP	WP:WP430	Statin inhibition of cholesterol production	0.0010409155290130897	*LPL, ABCA1, APOC1, APOE*

Alzheimer's disease (AD), Kyoto Encyclopedia of Genes and Genomes (KEEG), Reactome (REAC), Type 2 Diabetes (T2D), Wikipathway (WP).

Common variants and genes between T2D and AD

Our literature search revealed 231 variants and 363 genes in common between T2D and AD. Annotation of the 231 shared SNPs showed that *MADD* and *EFCAB5* harbored the highest number of these variants (nine SNPs). *MADD* gene, also known as *IG20*, plays a critical role in the development of glucose intolerance (Dupuis et al., 2010; Hu et al., 2010; Strawbridge et al., 2011; Wagner et al., 2011) and AD (Del Villar and Miller, 2004; Hassan et al., 2021). In accordance with our findings, a large-scale genome-wide cross-trait study identified *MADD* as the only gene significantly associated with AD and fasting glucose exclusively in pituitary tissue; it is also the only shared gene found in both cross-trait Meta-analysis and Transcriptomic-wide association studies. Thus, the pituitary gland may link T2D and AD by regulating glucose metabolism and neuronal viability through *MADD* (Zhu et al., 2019).

Regarding the *EFCAB5* gene, it encodes the EF-hand calcium-binding domain 5. Our results are in accordance with those of Karki et al. (2020) highlighting the importance of this gene inT2D and AD development. Among the nine identified variants in this gene, two SNPs (rs9902453 and rs7221743) are associated with coffee consumption (Cheung et al., 2012; Cornelis et al., 2015). In this context functional studies showed the protective role of coffee consumption against AD (Kwok et al., 2016; Zhou et al., 2018) and T2D-associated memory impairment through adenosine A2 receptor (ADORA2A) blockage (Duarte et al., 2019). Thus, we suggest that variants in *EFCAB5* could affect memory impairment in T2D subjects.

We found six SNPs located in *NECTIN2*, which encodes the nectin cell adhesion molecule 2 protein involved in T-cell signaling (Zhu et al., 2016). These variants have been previously reported to be shared between T2D and AD (Wang et al., 2017). Indeed, these variants are associated with lipid metabolite measurements, emphasizing their critical role in the development of AD in T2D patients (Xiao et al., 2022).

CST3 encodes cystatin C inhibitors of cysteine proteases (Maniwa et al., 2020). It is one of the genes harboring the highest number of common SNPs, between T2D and AD (Karki et al., 2020). A recent study showed that CST3 protein aggregation abolishes its function and slightly increases amyloid-beta 1–40 (Aβ1-40) fibril formation, enhancing neurodegeneration (Sheikh et al., 2021). Furthermore, the exogenous Cystatin C induces impairment of insulin signaling in hippocampal neurons, which could promote cognitive decline and AD development (Luo et al., 2018). In contrast, other studies have suggested that Cystatin C exerts neuroprotective effects by inhibiting cysteine proteases, rescuing neurodegeneration, inhibiting Aβ oligomerization and amyloid fibril formation, inducing autophagy, and neurogenesis (Mathews and Levy, 2016). This discrepancy may be explained by Cystatin C conformation or its levels in the brain. Indeed, the shared T2D-AD variant (rs1064039) was previously associated with reduced Cystatin C levels owing to impaired signal peptide cleavage (Benussi et al., 2003). We hypothesized that *CST3* plays a crucial role in the development of T2D-induced AD pathology through the regulation of cerebral amyloid angiopathy and insulin signaling in a dose dependent manner. Further studies are needed to determine its exact function in T2D-inducing AD condition.

We found five shared SNPs between T2D and AD within the *APOE* gene. These findings are in line with the literature. *APOE* is a leading factor for AD development in T2D subjects (Zhen et al., 2018; Shinohara et al., 2020). Three variants among the five were associated with body mass index (Yengo et al., 2018). Our findings support the synergic effect of obesity and *APOE* genotype on the development of T2D and AD (Jones and Rebeck, 2018).

AGPAT1 encodes for 1-acylglycerol-3-phosphate O-acyltransferase 1. It harbors five SNPs among the 231 T2D-AD shared variants identified in this study. Deletion of this gene induces low glucose and lipid plasma levels, as well as neurological disturbances (Agarwal et al., 2017). These findings emphasize the importance of *AGPAT1* in the regulation of glucose homeostasis and neuron viability. Further studies are needed to investigate its role in T2D and AD.

The functional annotation of the coding variants revealed six probably damaging SNPs located in *APOE, AGER*, *AGT*, *TP53*, *ICAM1*, and *EPHX2* gene. All the variants have been identified by the Karki et al. (2020), study. Interestingly the two variants rs7412 and rs2070600 have been reported by other studies (Wang et al., 2016; Kim et al., 2022). Minor allele (T) carriers of the variant rs7412 are classified as APOEε2 carriers. It has been largely proven that *APOEε2* has a protective effect against AD (Shinohara et al., 2016). Controversially, Shinohara et al., (2020) showed that *APOEε2* accelerates cognitive decline in diabetic patients by 4 years. This could be explained by the synergic effect between diabetes (Hardigan et al., 2016; Peng et al., 2021) and the *APOEε2* genotype in enhancing neurovascular impairment and tauopathies (Kim et al., 2022).

The second SNP (rs2070600) located in *AGER* (advanced glycosylation end-product specific receptor), causes a conversion at position 82 from glycine to serine (G82S) responsible for a decrease of AGER proteolyze and increase of AGEs plasmatic levels (Serveaux-Dancer et al., 2019). Several studies shed the light on the role of AGEs in T2D and AD through oxidative stress and amyloid regulation mechanisms (Michailidis et al., 2022). As a result, we hypothesize that T2D patient carriers of these risk allele variants have a greater risk to develop AD.Likely, the functional annotation of non-coding variants revealed three top-ranked SNPs rs1544210, rs12679834, and rs515071 located, respectively, in *HHEX, LPL*, and *ANK1* gene. The minor allele A of the variant rs1544210 is linked to an under-expression of *EIF2S2P3* pseudogene in the substantia nigra and hypothalamus regions. *EIF2SS2P3* is a pseudogene located in chromosome 10. Transcriptomic-wide association studies revealed a significant enrichment of *EIF2S2P3* for depressive symptoms, T2D (Génin, 2020) and T2D patients skipping breakfast

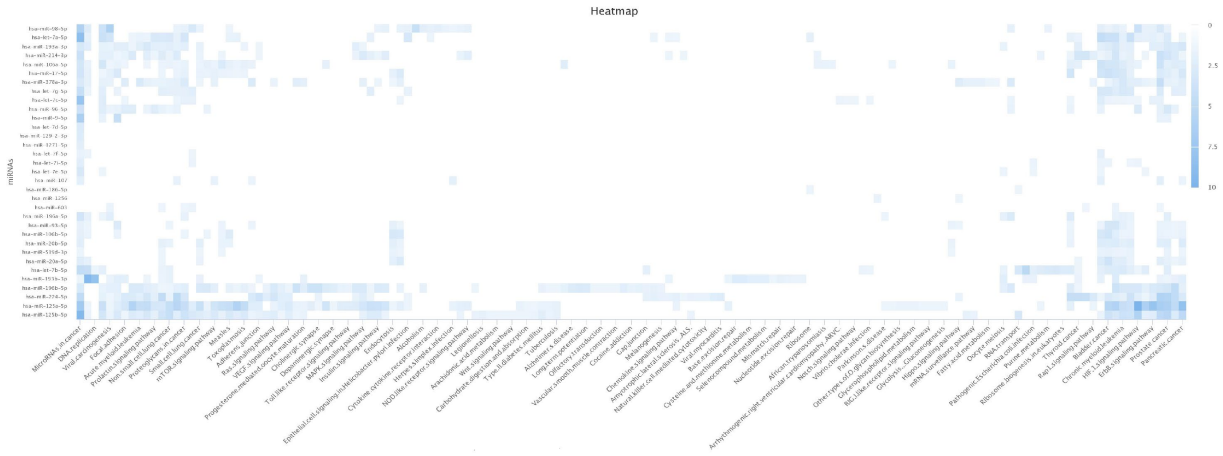

FIGURE 3
Heatmap of T2D_AD miRNAs enriched pathways. Our results showed that the majority of miRNAs were mapped to several pathways involved in different diseases such as cancer, T2D, AD and insulin signaling pathways. Type 2 Diabetes (T2D); Alzheimer's disease (AD),micro Ribonucleic Acid (miRNA).

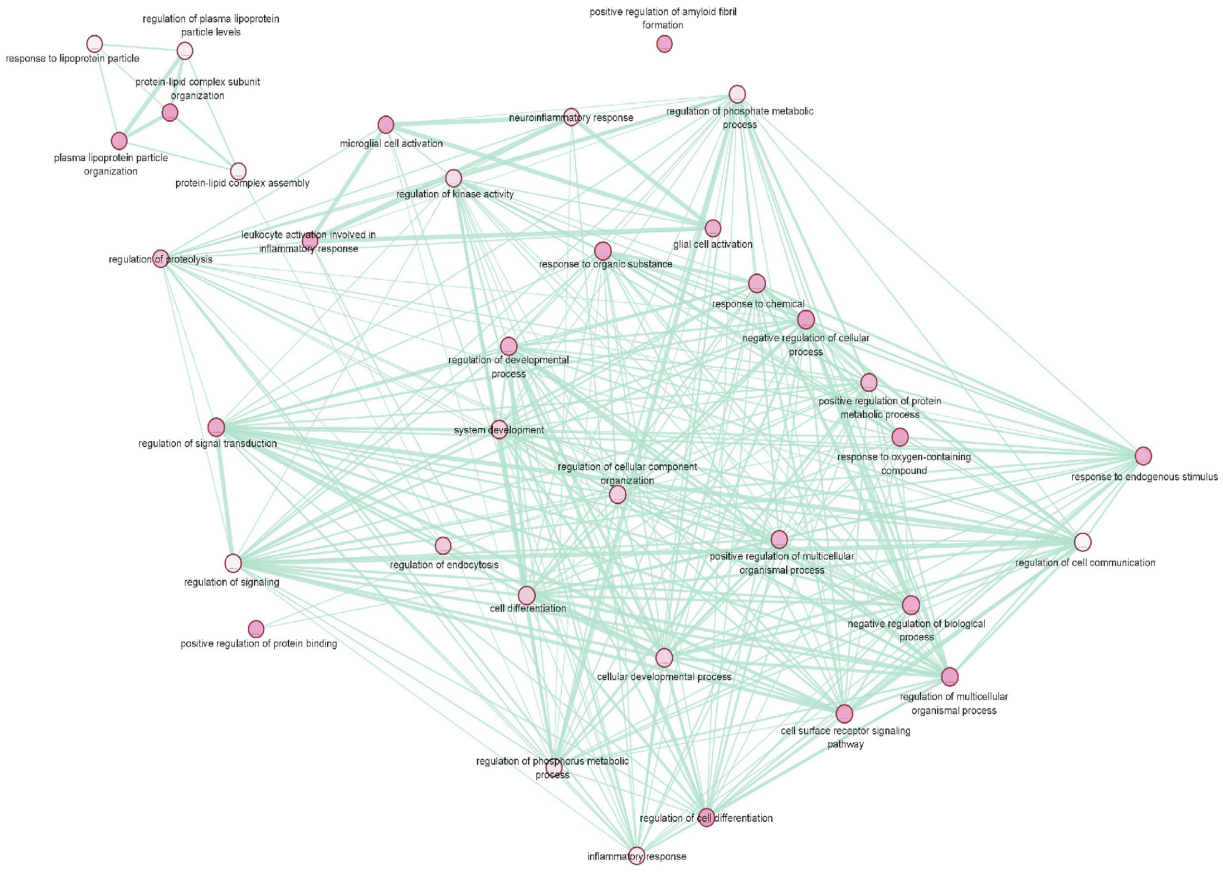

FIGURE 4
Pathway enrichment analysis of T2D and AD common genes. The main enriched pathways were: lipid subunit organization, positive regulation of protein binding, positive regulation of amyloid fibril formation, microglial cell activation (p-value=0.01).

(Chen et al., 2022), but its function remains unclear. Available evidence showed an increased risk for dementia development in T2D patients with depression (Katon et al., 2012). These findings highlights the role of *EIF2S2P3* pseudogene in the development of T2D-induced dementia through enhancing depression. Further studies should be conducted to assess the role of *EIF2S2P3* in T2D and AD development.

The same allele was associated with TFB motif "USF1," that regulates *APOE* gene a major linking factor between T2D and AD (Isotalo et al., 2012).

The second top-ranked SNP is rs12679834 located in the *LPL* gene. We found a TBF motif (NR2C2) with a high score associated with the G allele of this variant. NR2C2, also known as TR4, is an orphan nuclear receptor targeting many genes involved in metabolism including *APOE*

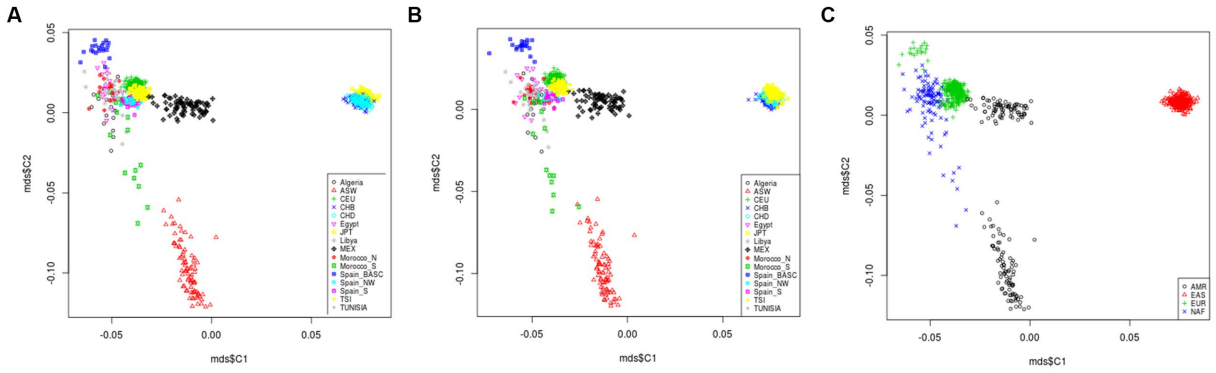

FIGURE 5
Multidimensional scaling plot of T2D and AD shared variants landscape in worldwide populations. The plot shows that North African populations (Algeria, Egypt, Libya, Morocco-N, Morocco-S, Tunisia) are clustered within the European populations (CEU, Sapin-S, Spain-Basic, Spain-NW, and TSI) and distinguished from the American (ASW, MEX) and Asian (CHB, CHD, JPT) populations **(A,B)**. Better individualization was observed in MDS performed across continents. In addition, there is a great divergence among the North African (NAF), American (AMR) and East Asian (EAS) groups. However, slight proximity was observed between the NAF and European EUR clusters **(C)**. *Rare variants are those with MAF<10^{-2}. **Comment variants are those with MAF>10^{-2}. Type 2 Diabetes (T2D); Alzheimer's disease (AD).

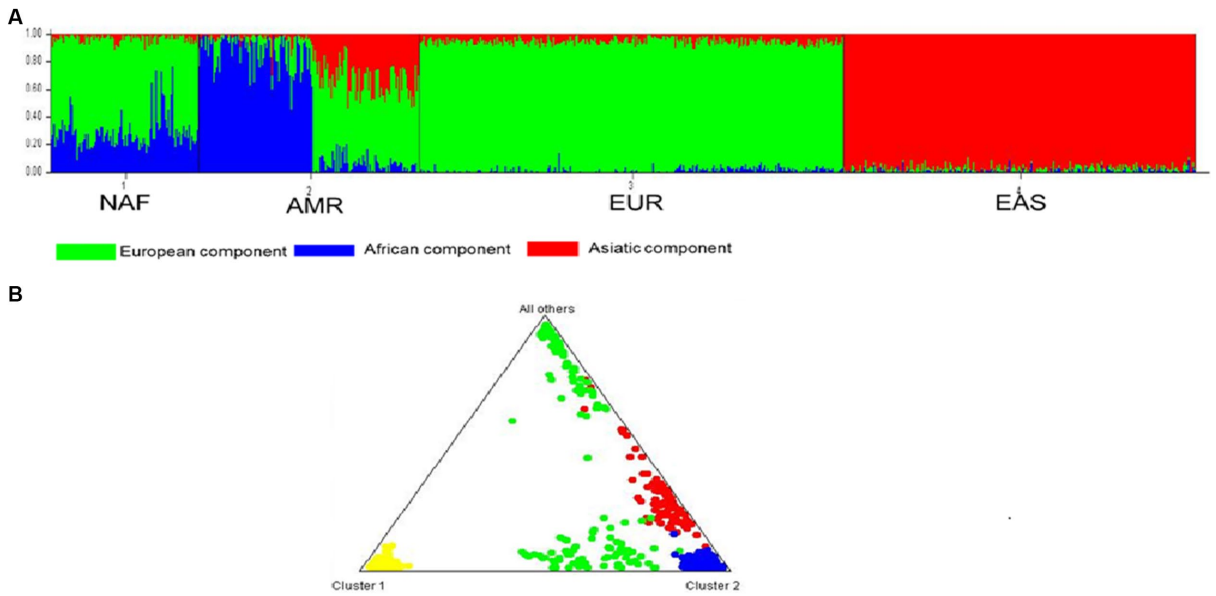

FIGURE 6
STRUCTURE analysis of the genetic relationship between the three group of populations. **(A)** The Bar plot shows three components: Africa, Asia and Europe. STRUCTURE analysis confirmed the ancestral diversity of the North African populations with evidence of the predominance of European component. **(B)** The Triangle of the structure shows that the NAF cluster is close to the EUR cluster and distinct from the EAS cluster. North African (NAF), European (EUR), and East Asian (EAS).

(Fogarty et al., 2013). This variant was also marked by H3K4me1 and H3K27ac histone modification in three brain regions. Furthermore, an increased expression of *LPL* in microglia appears to have a protective effect against AD (Keren-Shaul et al., 2017) and obesity (Gao et al., 2017). We suggest that rs12679834 possesses a protective effect against T2D and AD development via increasing lipid and lipoprotein uptake in the Central nervous system (CNS).

The third SNP was rs515071 located in the *ANK1* gene. The minor allele G of this variant was associated with decreased expression of *ANK1* gene in the cerebellum and cerebellar hemisphere regions. In agreement with our results, the GG genotype of the rs515071 variant is associated with a greater risk for T2D (Sun et al., 2017, p. 1) and AD (Chi et al., 2016). A reduced expression of *ANK1* gene could enhance T2D and AD development by affecting mediated metabolism, signal transduction (Sun et al., 2017), and inflammatory process (Morris et al., 2019).

Shared miRNA and pathways between T2D and AD

Six SNPs reported in our study were found to affect miRNA molecules implicated in LOAD, insulin signaling, and T2D

pathways. miR-1965-5p was enriched in the AD, T2D, and insulin signaling pathways. It has a positive effect on insulin biogenesis by enhancing insulin activity (Panda et al., 2014). miR-196b-5p down regulation has been implicated in innate immune response, apoptosis, and depression (Zhang et al., 2018). Inconsistent results have been found to be associated with the regulation trend in patients with AD (Pichler et al., 2017). We suggest that miR-196b-5p may play a protective role against T2D and AD development through insulin, immune response, and apoptosis regulation. However, further studies are required to elucidate their role in T2D and AD. Controversially, miR-378, previously associated with LOAD (Lusardi et al., 2017), was also enriched in the insulin-signaling pathway. Interestingly, miR-378 induces insulin resistance by targeting *P110a* and *SIRT7* (Deng and Guo, 2019). Furthermore, genetic depletion of miR-378a-3p ameliorates inflammatory stress and insulin resistance via protein kinase R inhibition (Wang et al., 2021). Likewise, upregulation of this miRNA has also been found in patients with AD (Dong et al., 2021; Li and Cai, 2021). This evidence emphasizes its role in T2D-induced AD through Central insulin signaling impairment (Gabbouj et al., 2019).

A previous study showed that miR-125a-5p and miR-125b-5p shared between T2D and the insulin pathway could ameliorate gluconeogenesis, glycogen synthesis (Xu et al., 2018), and insulin sensitivity (Yu et al., 2019). Interestingly, the same miRNA was found to be downregulated in the gray matter of patients with AD (Wang et al., 2011). We found that miR-98-5p was enriched in the insulin-signaling pathway. Decreased expression of this miRNA has been observed in T2D patients (Kokkinopoulou et al., 2019). The same study also reported a negative correlation between miR-98-5p and insulin levels in patients. Interestingly, Chen et al. (2019) found that in AD mice, miR-98 binds to HEY2 inducing a decrease of Aβ production, improve oxidative stress, and mitochondrial dysfunction through activating the Notch signaling pathway. We hypothesized that low levels of miR-98-5p could serve as a biomarker for insulin resistance and Aβ aggregation. A recent study found a significant downregulation of miR-214-3p blood levels in T2D patients (Avgeris et al., 2020). Similarly, miR-124-3p is downregulated in patients with AD and animal models (Kou et al., 2020), suggesting its potential role as a biomarker and therapeutic target for insulin resistance (Cheng et al., 2020) and cognitive defects (Zhang et al., 2018).

For a more in-depth understanding of the shared genes, we conducted a pathway enrichment analysis. The results revealed that the replicated genes were mainly enriched in lipid subunit organization, positive regulation of protein binding, positive regulation of amyloid fibril formation, microglial cell activation, cholesterol metabolism, plasma lipoprotein assembly, and Statin inhibition of cholesterol production pathways. Our findings are supported by those of previous studies. Plasma protein binding (PPB) has been implicated in several mechanisms, particularly drug binding and pharmacokinetics (Smith et al., 2010). A recent study identified an enrichment of positive regulation of PPB in 3 × Tg-AD mice fed rosmarinic acid, a preventive molecule against AD (Yamamoto et al., 2021). Rosmarinic acid is a potent suppressor of Aβ and an inhibitor of phosphorylated tau accumulation (Yamamoto et al., 2021). Interestingly, Rosmarinic acid possesses a therapeutic effect against T2D through the remodeling of amyloid aggregates (Wu et al., 2021). We identified, for the first time, the implication of positive regulation of PPB in T2D and AD. We hypothesized that the regulation of the PPB pathway could serve as a potential therapeutic target for these diseases.

The accumulation of amyloid fibrils is a hallmark of several degenerative diseases including T2D and AD. Insulin resistance promotes the oxidative stress generation and proinflammatory cytokines secretion in beta-cells inducing mitochondrial dysfunction and accumulation of protein aggregates, including human islet amyloid polypeptide (hIAPP) (Rocha et al., 2020). The latter can across the blood–brain barrier (BBB) inducing AD pathology (Lupaescu et al., 2022; Marrano et al., 2023).

Furthermore, amyloid deposition causes microglial and astrocyte activation leading to cytotoxic molecules release (Lupaescu et al., 2022). Recent study has demonstrated that hyperinsulinemia impaired GLUT4 translocation inducing mitochondrial fission, microglial M1 polarization, and neuroinflammation (Yang et al., 2022). Moreover, long-term high fat diet induces microglial M1 polarization which explains obesity/diabetes-associated cognitive impairment (Wu et al., 2020).

Cholesterol metabolism involves energy metabolism, cell membrane composition, and myelination. Dysregulation of these biological processes induces several pathologies, mainly T2D and AD. Reports suggested that a long-term high-fat diet could induce AD by enhancing Aβ and phosphorylated tau accumulation (Czuba et al., 2017). Downregulation of cholesterol biogenesis has been observed in diabetic (Suzuki et al., 2010) and AD (Varma et al., 2021) brains. Cholesterol is biosynthesized in astrocytes via the Bloch pathway and is transported to neurons by APOE via the ABC transporter. For utilization by neurons, APOE-containing cholesterol should be absorbed by LRP1/LDLR (Czuba et al., 2017). A previous study successfully demonstrated that insulin resistance suppresses *LRP1* expression, which may further compromise insulin signaling and cholesterol metabolism in neurons (Liu et al., 2015). Thus, our pathway enrichment analysis confirmed previous findings supporting the role of cholesterol metabolism and lipoprotein processes as linking factors between T2D and AD.

T2D-AD genetic landscape in North African populations

It is likely that genetic background plays an important role in the development of preventive strategies targeting modifier risk factors, such as T2D. Despite the high prevalence of T2D (Magliano et al., 2021) and AD (Nichols et al., 2022) in North African populations, we noticed an under or non-representation of these groups in the consortiums investigating these diseases (Martin et al., 2019). Taking all these evidences into consideration, it is important to dissect the genetic landscape of T2D-AD shared genes in North Africa in comparison with other well-studied populations. We determined the genetic landscape of T2D-AD shared genes in 829 individuals from 16 different populations whose genotyping data are publicly available [African ancestry in the South Western USA(ASW), Mexican ancestry living in Los Angeles, California, USA (MEX), Western European ancestry populations of Utah from the CEPH collection

(CEU), Toscani people of Italy (TSI),South Spain (Spain_S), North Spain (Spain_N), North West of Spain (Spain_NW), Spain Basic populations (Spain_BASC), Han Chinese in Beijing, China (CHB),Chinese population of metropolitan Denver, Colorado, USA (CHD), Japanese in Tokyo, Japan; (JPT), Individuals from North Africa: Algeria (Algeria), Egypt (Egypt), Libya (Libya), Tunisia Douiret (TN_Ber), South Morocco (Morocco_S), and North Morocco (Morocco_N),],. MDS analysis showed genetic similarity among North African populations (Algeria, Egypt, TN_Ber, Morocco_N, Morocco_Sand Libya), reflected by a consistent cluster. A better individualization of the North African populations was identified when the MDS analysis was conducted at the population group level. A slight similarity between North African and Southwestern European populations (CEU, Spain-S, Spain_NW, and TSI) was detected. However, a great divergence between North African and East Asian populations was observed in the two MDS representations. These results were further confirmed by STRUCTURE analysis conducted on four clusters of populations: North African, European, East Asian, and American. STRUCTURE representation shows a high admixture of the genetic structure of North African populations consisting mainly of European and African components, with minimum penetrance of East Asian components. Our findings are consistent with those of previous studies. Indeed, several genes/polymorphisms in T2D (Chande et al., 2020) and AD (Rubin et al., 2021) are highly variable among ethnic groups. Similar genetic positioning was observed among North African, European, and Asian populations regarding Metabolic Syndrome (MetS) pharmacogenes (Jmel et al., 2018). It is important to note that genes explored by Jmel et al. (2018) were also investigated in our study because MetS share several mechanisms with T2D and AD (Hayden, 2019). The genetic positioning of the North African cluster could be explained by the high ethnic heterogeneity of these populations. North Africans are multi-ethnic populations with several ancestral components: Middle Eastern, Sub-Saharan African, European, and autochthonous (Arauna et al., 2019). The high heterogeneity of the T2D-AD genetic background in North African populations reflects previous historical events such as invasion and migration (Botigué et al., 2013; Arauna et al., 2017; Fregel et al., 2018). Our results also support the conserved and ancient divergence between the North African and East Asian populations going back 550 centuries ago (Tateno et al., 2014). Thus, the non-replication of some genetic biomarkers of T2D (Baroudi et al., 2009; BaroudiOuederni et al., 2009; Ezzidi et al., 2009; Turki et al., 2012) and AD (Smach et al., 2011, p. 1; Rassas et al., 2013; Landoulsi et al., 2018) in the North African group could be due to its high heterogeneity and unicity. Indeed, among 231 risk variants studied, only 49 SNPs were present in the North African group. This could be the result of ethnic selection because some AD variants are also ethnicity-specific biomarkers (Huang et al., 2017) or due to the limited size of the North African populations investigated.

Furthermore, the inter-ethnic risk allele frequency comparison of the 49 variants between North African populations and other population groups revealed significant differences in allele frequency of 11 SNPs between North African, European, and East Asian populations located in *DNM3*, *CFH*, *PPARG*, *ROHA*, *AGER*, *CLU*, *BDNF1*, *CST9*, and *PLCG1* genes.

MAF of two variants, rs4504922 and rs7539972, located in the dynamin 3 (*DNM3*) gene, was significantly different between North African and European populations and between North African and East Asian populations. *DNM3* is enriched in the Fc gamma R-mediated phagocytosis pathway associated with AD and T2D (Hao et al., 2015; Caputo et al., 2020). These variants were previously identified as SNPs shared between T2D and AD (Caputo et al., 2020). We suggest that North African carriers of these risk allele variants may be at increased risk of T2D and AD.

The rs800292 G > A SNP, located in the complement factor H (*CFH*) gene, has been previously reported to be associated with a higher risk of age-related macular degeneration (Guindo-Martínez et al., 2021) and diabetic retinopathy (Wang et al., 2013).

Furthermore, the inter-ethnic comparison of risk alleles revealed a significant difference in the MAF of three variants (rs6809832, rs6997, and rs11715915) located in peroxisome proliferator activated receptor gamma (*PPARG*) and Ras homolog family member A (*RHOA*) genes between North African and East Asian populations. These variants have been associated with increased BMI and HbA1c levels (Merino et al., 2017; Yengo et al., 2018; Barton et al., 2021; Jurgens et al., 2023). We hypothesized that North African carriers of risk alleles of these variants may have an increased risk of developing obesity and insulin resistance pathologies, such as T2D and AD (Magliano et al., 2021; Nichols et al., 2022).

Moreover, we identified significant differences in the MAF of three SNPs (rs2070600, rs11136000, and rs6265) located in *AGER*, clusterin (*CLU*), and brain derived neurotrophic factor (*BDNF1*) between North African and East Asian populations. These variants have been previously associated with MCI/AD development in T2D subjects (Cai et al., 2016; Wang et al., 2016; Daily and Park, 2017; Stepler and Robinson, 2019; Bradley, 2020). The high MAF of these variants in North Africans could partly explain their greater risk of developing T2D-AD pathology, (Magliano et al., 2021; Alzheimer's Disease Facts and Figures, n.d.).

The variant rs3004145 C > G, located downstream of the Cystatin C9 (*CST9*) gene, presents a significant MAF difference between North African and East Asian populations. This variant has previously been associated with elevated cystatin C levels in the European population (Jurgens et al., 2023). High serum cystatin C levels have been previously associated with an increased risk of T2D (Yuan et al., 2022), T2D-related neuropathy (Hu et al., 2014), and AD (Straface et al., 2005). Thus, we suggest that North African carriers of the rs3004145 G allele may have an increased risk of T2D-induced AD.

Finally, our statistical analysis revealed a significant MAF difference in the exonic variant rs753381 T > C between North African and European populations. This variant is located in the phospholipase C gamma 1 (*PLCG1*) gene, recently identified as a potential therapeutic target for T2D (Ganekal et al., 2023). It has previously been associated with metabolic syndrome (Brown and Walker, 2016) and elevated serum cholesterol, LDL, and ApoB levels in individuals of African, East Asian, European, Hispanic, and South Asian ancestry (Graham et al., 2021). We suggest that the differences in rs753381 C allele frequency in North Africa explain the low plasma levels of TC, LDL-C, and ApoB compared to European populations (Najah et al., 2013). Further studies are required to elucidate the relationship between this variant and T2D-induced AD development in North Africa.

Study highlights and testable hypotheses

The present study generates several hypotheses: (1) *MADD* and *AGPAT1* genes regulate glucose homeostasis and neuronal viability. (2) *EFCAB5* could be a potential pharmacogene for ADORA2A agonist anti-AD therapies. (3) *NECTIN2* plays a critical role in T2D-induced AD through regulating lipid metabolism. (4) *CST3* regulates cerebral amyloid angiopathy and insulin signaling in a dose dependent manner. (5) Individuals carrier of rs7412, rs1800562, rs2070600 rs1544210, rs12679834, and rs515071 risk alleles are of great risk to develop T2D and AD. (6) miR-378, miR-125a-5p, miR-125b-5p, miR-196b-5p, miR-98-5p, and miR-214-3p are potential therapeutic target for T2D-induced AD. (7) Plasma protein binding pathway could serve as a potential therapeutic target for T2D-induced AD. (8) North African's carrier of minor alleles of variants located in, *DNM3*, *CFH*, *PPARG*, *ROHA*, *AGER*, *CLU*, *BDNF1*, *CST9*, and *PLCG1* genes are of great risk to develop T2D and AD.

Study limitations

Although our study's results give rise to several hypotheses consistent with the published literature, we also have some limitations. First, our search strategy has been limited to one database "PubMed" with one query for search builder. This strategy can lead to information leakage. Therefore, other datasets and search terms should be examined to consolidate our findings. Second, the restricted size of the studied populations, especially in North Africa, could lead to fewer genetic variations present in these populations. Finally, the predicted results should be supported by further experimental studies. Despite these limitations, our study findings are relevant and pave the way to further investigation because of their general consistency with previous results.

Conclusion

Our study contributes to efforts made to better understand the genetic variability and molecular mechanisms shared between T2D and AD. It is well established that the determination of the genetic component of these diseases could help develop new diagnostic and therapeutic strategies in the context of precision medicine. However, the promise of precision genomic medicine cannot be fulfilled without a broad representation of the global population. Here, we identified pathogenic variants and regulatory pathways shared between these diseases. Our study is the first to investigate the genetic landscape of shared T2D-AD genes in North African populations in comparison to other worldwide populations. Our results support the high heterogeneity and the unicity of North African populations regarding T2D and AD common genes. The inter-ethnic comparison between North African populations and worldwide populations revealed significant difference of eleven risk allele frequency variants. This finding might be one of the contributing factors to the higher prevalence of T2D and AD in North African populations. Furthermore, our results could pave the way for new target gene sequencing or functional follow-up of putative loci to investigate the exact role of these variants in North African populations. Finally, we emphasize the importance of further ethnicity-specific contributions in omics studies for a better understanding of the link between T2D and AD, and for developing an accurate diagnosis using personalized genetic biomarkers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

WB designed the study, collected and curated the original data, conducted analysis and validated results, and drafted the initial manuscript. HJ designed the study, collected and curated the original data, conducted and validated analysis, and wrote—reviewed and edited the manuscript. NK, IG, HD, and MH helped to revise the manuscript. RK conceived the idea, designed the study, supervised the analysis, validated results, wrote—reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1114810/full#supplementary-material

References

1000 Genome (n.d.). A deep catalog of human genetic variation. Available at: https://www.internationalgenome.org/ (Accessed November 15, 2022).

(2022). 2022 Alzheimer's disease facts and figures. *Alzheimers Dement* 18, 700–789. doi: 10.1002/alz.12638

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., et al. (2010). A method and server for predicting damaging missense mutations. *Nat. Methods* 7, 248–249. doi: 10.1038/nmeth0410-248

Agarwal, A. K., Tunison, K., Dalal, J. S., Nagamma, S. S., Hamra, F. K., Sankella, S., et al. (2017). Metabolic, reproductive, and neurologic abnormalities in Agpat1-null mice. *Endocrinology* 158, 3954–3973. doi: 10.1210/en.2017-00511

Arauna, L. R., Hellenthal, G., and Comas, D. (2019). Dissecting human North African gene-flow into its Western coastal surroundings. *Proc. R. Soc. B* 286:20190471. doi: 10.1098/rspb.2019.0471

Arauna, L. R., Mendoza-Revilla, J., Mas-Sandoval, A., Izaabel, H., Bekada, A., Benhamamouch, S., et al. (2017). Recent historical migrations have shaped the gene pool of Arabs and Berbers in North Africa. *Mol. Biol. Evol.* 34, 318–329. doi: 10.1093/molbev/msw218

Avgeris, M., Kokkinopoulou, I., Maratou, E., Mitrou, P., Boutati, E., Scorilas, A., et al. (2020). Blood-based analysis of 84 microRNAs identifies molecules deregulated in individuals with type-2 diabetes, risk factors for the disease or metabolic syndrome. *Diabetes Research and Clinical Practice* 164, 108187. doi: 10.1016/j.diabres.2020.108187

Barbagallo, M., and Dominguez, L. J. (2014). Type 2 diabetes mellitus and Alzheimer's disease. *World J. Diabetes* 5, 889–893. doi: 10.4239/wjd.v5.i6.889

Baroudi, T., Bouhaha, R., Moran-Moguel, C., Sanchez-Corona, J., Ben Maiz, H., Kammoun Abid, H., et al. (2009). Association of the insertion/deletion polymorphism of the angiotensin-converting enzyme gene with type 2 diabetes in two ethnic groups of Jerba Island in Tunisia. *J. Renin. Angiotensin. Aldosterone. Syst.* 10, 35–40. doi: 10.1177/1470320309102314

BaroudiOuederni, T., Sanchez-Corona, J., Martinez, S. E. F., Ben Maiz, H., AounallahSkhiri, H., Kammoun Abid, H., et al. (2009). The G1057D polymorphism of IRS-2 gene is not associated with type 2 diabetes and obese patients among ethnic groups in Tunisian population. *Clin. Biochem.* 42, 1169–1173. doi: 10.1016/j.clinbiochem.2009.03.018

Barton, A. R., Sherman, M. A., Mukamel, R. E., and Loh, P.-R. (2021). Whole-exome imputation within UK Biobank powers rare coding variant association and fine-mapping analyses. *Nat. Genet.* 53, 1260–1269. doi: 10.1038/s41588-021-00892-1

Ben Halima, Y., Kefi, R., Sazzini, M., Giuliani, C., De Fanti, S., Nouali, C., et al. (2017). Lactase persistence in Tunisia as a result of admixture with other Mediterranean populations. *Genes Nutr.* 12:20. doi: 10.1186/s12263-017-0573-3

Benussi, L., Ghidoni, R., Steinhoff, T., Alberici, A., Villa, A., Mazzoli, F., et al. (2003). Alzheimer disease-associated cystatin C variant undergoes impaired secretion. *Neurobiol. Dis.* 13, 15–21. doi: 10.1016/S0969-9961(03)00012-3

Botigué, L. R., Henn, B. M., Gravel, S., Maples, B. K., Gignoux, C. R., Corona, E., et al. (2013). Gene flow from North Africa contributes to differential human genetic diversity in Southern Europe. *Proc. Natl. Acad. Sci. U. S. A.* 110, 11791–11796. doi: 10.1073/pnas.1306223110

Boyle, A. P., Hong, E. L., Hariharan, M., Cheng, Y., Schaub, M. A., Kasowski, M., et al. (2012). Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 22, 1790–1797. doi: 10.1101/gr.137323.112

Bradley, D. (2020). Clusterin as a potential biomarker of obesity-related Alzheimer's disease risk. *Biomark. Insights* 15:1177271920964108. doi: 10.1177/1177271920964108

Brown, A. E., and Walker, M. (2016). Genetics of insulin resistance and the metabolic syndrome. *Curr. Cardiol. Rep.* 18:75. doi: 10.1007/s11886-016-0755-4

Bruno, A. E., Li, L., Kalabus, J. L., Pan, Y., Yu, A., and Hu, Z. (2012). miRdSNP: a database of disease-associated SNPs and microRNA target sites on 3'UTRs of human genes. *BMC Genomics* 13:44. doi: 10.1186/1471-2164-13-44

Cai, R., Han, J., Sun, J., Huang, R., Tian, S., Shen, Y., et al. (2016). Plasma clusterin and the CLU gene rs11136000 variant are associated with mild cognitive impairment in type 2 diabetic patients. *Front. Aging Neurosci.* 8:179. doi: 10.3389/fnagi.2016.00179

Caputo, V., Termine, A., Strafella, C., Giardina, E., and Cascella, R. (2020). Shared (epi)genomic background connecting neurodegenerative diseases and type 2 diabetes. *World J. Diabetes* 11, 155–164. doi: 10.4239/wjd.v11.i5.155

Chande, A. T., Rishishwar, L., Conley, A. B., Valderrama-Aguirre, A., Medina-Rivas, M. A., and Jordan, I. K. (2020). Ancestry effects on type 2 diabetes genetic risk inference in Hispanic/Latino populations. *BMC Med. Genet.* 21:132. doi: 10.1186/s12881-020-01068-0

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., and Lee, J. J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaSci* 4:7. doi: 10.1186/s13742-015-0047-8

Chen, D., Wu, H., Wang, X., Huang, T., and Jia, J. (2022). Shared genetic basis and causal relationship between television watching, breakfast skipping and type 2 diabetes: evidence from a comprehensive genetic analysis. *Front. Endocrinol.* 13:836023. doi: 10.3389/fendo.2022.836023

Chen, F.-Z., Zhao, Y., and Chen, H.-Z. (2019). MicroRNA-98 reduces amyloid β-protein production and improves oxidative stress and mitochondrial dysfunction through the Notch signaling pathway via HEY2 in Alzheimer's disease mice. *Int. J. Mol. Med.* 43, 91–102. doi: 10.3892/ijmm.2018.3957

Cheng, F., Yuan, G., He, J., Shao, Y., Zhang, J., Guo, X. (2020). Aberrant expression of miR-214 is associated with obesity-induced insulin resistance as a biomarker and therapeutic. *Diagn Pathol* 15, 18. doi: 10.1186/s13000-019-0914-1

Cheung, C.-L., Sham, P.-C., Xiao, S.-M., Bow, C. H., and Kung, A. W.-C. (2012). Meta-analysis of gene-based genome-wide association studies of bone mineral density in Chinese and European subjects. *Osteoporos. Int.* 23, 131–142. doi: 10.1007/s00198-011-1779-7

Chi, S., Song, J.-H., Tan, M.-S., Zhang, W., Wang, Z.-X., Jiang, T., et al. (2016). Association of Single-Nucleotide Polymorphism in ANK1 with Late-Onset Alzheimer's Disease in Han Chinese. *Mol. Neurobiol.* 53, 6476–6481. doi: 10.1007/s12035-015-9547-x

Cornelis, M. C., Byrne, E. M., Esko, T., Nalls, M. A., Ganna, A., Paynter, N., et al. (2015). Genome-wide Meta-analysis identifies six novel loci associated with habitual coffee consumption. *Mol. Psychiatry* 20, 647–656. doi: 10.1038/mp.2014.107

Czuba, E., Steliga, A., Lietzau, G., and Kowiański, P. (2017). Cholesterol as a modifying agent of the neurovascular unit structure and function under physiological and pathological conditions. *Metab. Brain Dis.* 32, 935–948. doi: 10.1007/s11011-017-0015-3

Daily, J. W., and Park, S. (2017). Interaction of BDNF rs6265 variants and energy and protein intake in the risk for glucose intolerance and type 2 diabetes in middle-aged adults. *Nutrition* 33, 187–194. doi: 10.1016/j.nut.2016.07.001

Dallali, H., Hechmi, M., Morjane, I., Elouej, S., Jmel, H., Ben Halima, Y., et al. (2022). Association of HNF1A gene variants and haplotypes with metabolic syndrome: a case–control study in the Tunisian population and a meta-analysis. *Diabetol. Metab. Syndr.* 14, 1–11. doi: 10.1186/s13098-022-00794-0

de Rojas, I., Moreno-Grau, S., Tesi, N., Grenier-Boley, B., Andrade, V., Jansen, I. E., et al. (2021). Common variants in Alzheimer's disease and risk stratification by polygenic risk scores. *Nat. Commun.* 12:3417. doi: 10.1038/s41467-021-22491-8

De Sousa, R. A. L., Harmer, A. R., Freitas, D. A., Mendonça, V. A., Lacerda, A. C. R., and Leite, H. R. (2020). An update on potential links between type 2 diabetes mellitus and Alzheimer's disease. *Mol. Biol. Rep.* 47, 6347–6356. doi: 10.1007/s11033-020-05693-z

Del Villar, K., and Miller, C. A. (2004). Down-regulation of DENN/MADD, a TNF receptor binding protein, correlates with neuronal cell death in Alzheimer's disease brain and hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4210–4215. doi: 10.1073/pnas.0307349101

Deng, J., and Guo, F. (2019). MicroRNAs and type 2 diabetes. *ExRNA* 1:36. doi: 10.1186/s41544-019-0038-5

Dong, Z., Gu, H., Guo, Q., Liang, S., Xue, J., Yao, F. (2021). Profiling of Serum Exosome MiRNA Reveals the Potential of a MiRNA Panel as Diagnostic Biomarker for Alzheimer's Disease. *Mol Neurobiol* 58, 3084–3094. doi: 10.1007/s12035-021-02323-y

Duarte, J. M. N., Skoug, C., Silva, H. B., Carvalho, R. A., Gruetter, R., and Cunha, R. A. (2019). Impact of caffeine consumption on type 2 diabetes-induced spatial memory impairment and neurochemical alterations in the hippocampus. *Front. Neurosci.* 12:1015. doi: 10.3389/fnins.2018.01015

Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A. U., et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat. Genet.* 42, 105–116. doi: 10.1038/ng.520

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14, 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Ezzidi, I., Mtiraoui, N., Cauchi, S., Vaillant, E., Dechaume, A., Chaieb, M., et al. (2009). Contribution of type 2 diabetes associated loci in the Arabic population from Tunisia: a case-control study. *BMC Med. Genet.* 10:33. doi: 10.1186/1471-2350-10-33

Falush, D., Stephens, M., and Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* 7, 574–578. doi: 10.1111/j.1471-8286.2007.01758.x

Fogarty, M. P., Panhuis, T. M., Vadlamudi, S., Buchkovich, M. L., and Mohlke, K. L. (2013). Allele-specific transcriptional activity at type 2 diabetes–associated single nucleotide polymorphisms in regions of pancreatic islet open chromatin at the JAZF1 locus. *Diabetes* 62, 1756–1762. doi: 10.2337/db12-0972

Fregel, R., Méndez, F. L., Bokbot, Y., Martín-Socas, D., Camalich-Massieu, M. D., Santana, J., et al. (2018). Ancient genomes from North Africa evidence prehistoric migrations to the Maghreb from both the Levant and Europe. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6774–6779. doi: 10.1073/pnas.1800851115

Gabbouj, S., Ryhänen, S., Marttinen, M., Wittrahm, R., Takalo, M., Kemppainen, S., et al. (2019). Altered insulin signaling in Alzheimer's disease brain—special emphasis on PI3K-Akt pathway. *Front. Neurosci.* 13:629. doi: 10.3389/fnins.2019.00629

Ganekal, P., Vastrad, B., Kavatagimath, S., Vastrad, C., and Kotrashetti, S. (2023). Bioinformatics and next-generation data analysis for identification of genes and

molecular pathways involved in subjects with diabetes and obesity. *Medicina* 59:309. doi: 10.3390/medicina59020309

Gao, Y., Vidal-Itriago, A., Kalsbeek, M. J., Layritz, C., García-Cáceres, C., Tom, R. Z., et al. (2017). Lipoprotein lipase maintains microglial innate immunity in obesity. *Cell Rep.* 20, 3034–3042. doi: 10.1016/j.celrep.2017.09.008

Génin, E. (2020). 49th European Mathematical Genetics Meeting (EMGM) 2021. *Hum. Hered.* 85, 69–100. doi: 10.1159/000516194

Graham, S. E., Clarke, S. L., Wu, K.-H. H., Kanoni, S., Zajac, G. J. M., Ramdas, S., et al. (2021). The power of genetic diversity in genome-wide association studies of lipids. *Nature* 600, 675–679. doi: 10.1038/s41586-021-04064-3

Guindo-Martínez, M., Amela, R., Bonàs-Guarch, S., Puiggròs, M., Salvoro, C., Miguel-Escalada, I., et al. (2021). The impact of non-additive genetic associations on age-related complex diseases. *Nat. Commun.* 12:2436. doi: 10.1038/s41467-021-21952-4

Hao, K., Di Narzo, A. F., Ho, L., Luo, W., Li, S., Chen, R., et al. (2015). Shared genetic etiology underlying Alzheimer's disease and type 2 diabetes. *Mol. Asp. Med.* 43–44, 66–76. doi: 10.1016/j.mam.2015.06.006

Hardigan, T., Ward, R., and Ergul, A. (2016). Cerebrovascular complications of diabetes: focus on cognitive dysfunction. *Clin. Sci.* 130, 1807–1822. doi: 10.1042/CS20160397

Hassan, M., Zahid, S., Alashwal, H., Kloczkowski, A., and Moustafa, A. A. (2021). Mechanistic insights into TNFR1/MADD death domains in Alzheimer's disease through conformational molecular dynamic analysis. *Sci. Rep.* 11:12256. doi: 10.1038/s41598-021-91606-4

Hayden, M. R. (2019). Type 2 Diabetes Mellitus Increases the risk of late-onset Alzheimer's disease: ultrastructural remodeling of the neurovascular unit and diabetic gliopathy. *Brain Sci.* 9:262. doi: 10.3390/brainsci9100262

Henn, B. M., Botigué, L. R., Gravel, S., Wang, W., Brisbin, A., Byrnes, J. K., et al. (2012). Genomic ancestry of North Africans supports back-to-Africa migrations. *PLoS Genet.* 8:e1002397. doi: 10.1371/journal.pgen.1002397

Hu, Y., Liu, F., Shen, J., Zeng, H., Li, L., Zhao, J., et al. (2014). Association between serum cystatin C and diabetic peripheral neuropathy: a cross-sectional study of a Chinese type 2 diabetic population. *Eur. J. Endocrinol.* 171, 641–648. doi: 10.1530/EJE-14-0381

Hu, C., Zhang, R., Wang, C., Wang, J., Ma, X., Hou, X., et al. (2010). Variants from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1, SLC30A8 and IGF1 are associated with glucose metabolism in the Chinese. *PLoS One* 5:e15542. doi: 10.1371/journal.pone.0015542

Huang, P., Hsieh, S.-W., Chang, Y.-H., Hour, A.-L., Chen, H.-Y., and Liu, C.-K. (2017). Differences in the frequency of Alzheimer's disease-associated genomic variations in populations of different races. *Geriatr. Gerontol. Int.* 17, 2184–2193. doi: 10.1111/ggi.13059

Isotalo, K., Kok, E. H., Luoto, T. M., Haikonen, S., Haapasalo, H., Lehtimäki, T., et al. (2012). Upstream Transcription Factor 1 (USF1) Polymorphisms associate with Alzheimer's disease-related neuropathological lesions: Tampere Autopsy Study. *Brain Pathol.* 22, 765–775. doi: 10.1111/j.1750-3639.2012.00586.x

Jakobsson, M., and Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23, 1801–1806. doi: 10.1093/bioinformatics/btm233

Jmel, H., Romdhane, L., Ben Halima, Y., Hechmi, M., Naouali, C., Dallali, H., et al. (2018). Pharmacogenetic landscape of Metabolic Syndrome components drug response in Tunisia and comparison with worldwide populations. *PLoS One* 13:e0194842. doi: 10.1371/journal.pone.0194842

Jurgens, S.J., Pirruccello, J. P., Choi, S. H., Morrill, V. N., Chaffin, M., Lubitz, S. A., et al. (2023). Adjusting for common variant polygenic scores improves yield in rare variant association analyses. *Nat Genet* 55:544–548. doi: 10.1038/s41588-023-01342-w

Jones, N. S., and Rebeck, G. W. (2018). The synergistic effects of APOE genotype and obesity on Alzheimer's disease risk. *Int. J. Mol. Sci.* 20:63. doi: 10.3390/ijms20010063

Karczewski, K. J., and Snyder, M. P. (2018). Integrative omics for health and disease. *Nat. Rev. Genet.* 19, 299–310. doi: 10.1038/nrg.2018.4

Karki, R., Madan, S., Gadiya, Y., Domingo-Fernández, D., Kodamullil, A. T., and Hofmann-Apitius, M. (2020). Data-driven modeling of knowledge assemblies in understanding comorbidity between type 2 diabetes mellitus and Alzheimer's disease. *J. Alzheimers Dis.* 78, 87–95. doi: 10.3233/JAD-200752

Katon, W., Lyles, C. R., Parker, M. M., Karter, A. J., Huang, E. S., and Whitmer, R. A. (2012). Depression increases risk of dementia in patients with type 2 diabetes: the Diabetes & Aging Study. *Arch. Gen. Psychiatry* 69, 410–417. doi: 10.1001/archgenpsychiatry.2011.154

Kefi, R., Hsouna, S., Ben Halim, N., Lasram, K., Romdhane, L., Messai, H., et al. (2015). Phylogeny and genetic structure of Tunisians and their position within Mediterranean populations. *Mitochondrial DNA* 26, 593–604. doi: 10.3109/19401736.2013.879649

Kehl, T., Kern, F., Backes, C., Fehlmann, T., Stöckel, D., Meese, E., et al. (2020). miRPathDB 2.0: a novel release of the miRNA pathway dictionary database. *Nucleic Acids Res.* 48, D142–D147. doi: 10.1093/nar/gkz1022

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T. K., et al. (2017). A unique microglia type associated with restricting development of Alzheimer's disease. *Cells* 169, 1276–1290.e17. doi: 10.1016/j.cell.2017.05.018

Kim, H., Devanand, D. P., Carlson, S., and Goldberg, T. E. (2022). Apolipoprotein E Genotype e2: Neuroprotection and Its Limits. *Front. Aging Neurosci.* 14:919712. doi: 10.3389/fnagi.2022.919712

Kokkinopoulou, I., Maratou, E., Mitrou, P., Boutati, E., Sideris, D. C., Fragoulis, E. G., et al. (2019). Decreased expression of microRNAs targeting type-2 diabetes susceptibility genes in peripheral blood of patients and predisposed individuals. *Endocrine* 66, 226–239. doi: 10.1007/s12020-019-02062-0

Kou, X., Chen, D., and Chen, N. (2020). The Regulation of microRNAs in Alzheimer's Disease. Frontiers in Neurology 11. Available at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00288 [Accessed November 29, 2022].

Kwok, M. K., Leung, G. M., and Schooling, C. M. (2016). Habitual coffee consumption and risk of type 2 diabetes, ischemic heart disease, depression and Alzheimer's disease: a Mendelian randomization study. *Sci. Rep.* 6:36500. doi: 10.1038/srep36500

Landoulsi, Z., Ben Djebara, M., Kacem, I., Sidhom, Y., Kefi, R., Abdelhak, S., et al. (2018). Genetic Analysis of TREM2 variants in Tunisian patients with Alzheimer's disease. *Med. Princ. Pract.* 27, 317–322. doi: 10.1159/000489779

Li, J. Z., Absher, D. M., Tang, H., Southwick, A. M., Casto, A. M., Ramachandran, S., et al. (2008). Worldwide human relationships inferred from genome-wide patterns of variation. *Science* 319, 1100–1104. doi: 10.1126/science.1153717

Li, Q.-S., and Cai, D. (2021). Integrated miRNA-Seq and mRNA-Seq Study to Identify miRNAs Associated With Alzheimer's Disease Using Post-mortem Brain Tissue Samples. *Front Neurosci* 15, 620899. doi: 10.3389/fnins.2021.620899

Liu, C.-C., Hu, J., Tsai, C.-W., Yue, M., Melrose, H. L., Kanekiyo, T., et al. (2015). Neuronal LRP1 regulates glucose metabolism and insulin signaling in the brain. *J. Neurosci.* 35, 5851–5859. doi: 10.1523/JNEUROSCI.5180-14.2015

Luo, L., Ma, J., Li, Y., Hu, Z., Jiang, C., Cai, H., et al. (2018). Cystatin C induces insulin resistance in hippocampal neurons and promotes cognitive dysfunction in rodents. *Neurosci. Bull.* 34, 543–545. doi: 10.1007/s12264-018-0226-6

Lupaescu, A.-V., Iavorschi, M., and Covasa, M. (2022). The use of bioactive compounds in hyperglycemia-and amyloid fibrils-induced toxicity in type 2 diabetes and Alzheimer's disease. *Pharmaceutics* 14:235. doi: 10.3390/pharmaceutics14020235

Lusardi, T. A., Phillips, J. I., Wiedrick, J. T., Harrington, C. A., Lind, B., Lapidus, A., et al (2017). MicroRNAs in Human Cerebrospinal Fluid as Biomarkers for Alzheimer's Disease. *J Alzheimers Dis* 55:1223–1233. doi: 10.3233/JAD-160835

Magliano, D. J., Boyko, E. J. (2021). IDF Diabetes Atlas 10th edition scientific committee (2021). IDF DIABETES ATLAS. 10th ed. Brussels: International Diabetes Federation Available at: http://www.ncbi.nlm.nih.gov/books/NBK581934/

Ma, C., Madden, P., Gontarz, P., Wang, T., and Zhang, B. (2019). FeatSNP: An Interactive Database for Brain-Specific Epigenetic Annotation of Human SNPs. *Front. Genet.* 10:262. doi: 10.3389/fgene.2019.00262

Maniwa, K., Yano, S., Sheikh, A. M., Onoda, K., Mitaki, S., Isomura, M., et al. (2020). Association between cystatin C gene polymorphism and the prevalence of white matter lesion in elderly healthy subjects. *Sci. Rep.* 10:4688. doi: 10.1038/s41598-020-61383-7

Marrano, N., Biondi, G., Borrelli, A., Rella, M., Zambetta, T., Di Gioia, L., et al. (2023). Type 2 diabetes and Alzheimer's disease: the emerging role of cellular lipotoxicity. *Biomol. Ther.* 13:183. doi: 10.3390/biom13010183

Martin, A. R., Kanai, M., Kamatani, Y., Okada, Y., Neale, B. M., and Daly, M. J. (2019). Clinical use of current polygenic risk scores may exacerbate health disparities. *Nat. Genet.* 51, 584–591. doi: 10.1038/s41588-019-0379-x

Mathews, P. M., and Levy, E. (2016). Cystatin C in aging and in Alzheimer's disease. *Ageing Res. Rev.* 32, 38–50. doi: 10.1016/j.arr.2016.06.003

McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R. S., Thormann, A., et al. (2016). The ensembl variant effect predictor. *Genome Biol.* 17:122. doi: 10.1186/s13059-016-0974-4

Merico, D., Isserlin, R., Stueker, O., Emili, A., and Bader, G. D. (2010). Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. *PLoS One* 5:e13984. doi: 10.1371/journal.pone.0013984

Merino, J., Leong, A., Posner, D. C., Porneala, B., Masana, L., Dupuis, J., et al. (2017). Genetically driven hyperglycemia increases risk of coronary artery disease separately from type 2 diabetes. *Diabetes Care* 40, 687–693. doi: 10.2337/dc16-2625

Michailidis, M., Moraitou, D., Tata, D. A., Kalinderi, K., Papamitsou, T., and Papaliagkas, V. (2022). Alzheimer's disease as type 3 diabetes: common pathophysiological mechanisms between Alzheimer's disease and type 2 diabetes. *Int. J. Mol. Sci.* 23:2687. doi: 10.3390/ijms23052687

Morris, G., Berk, M., Maes, M., and Puri, B. K. (2019). Could Alzheimer's disease originate in the periphery and if so how so? *Mol. Neurobiol.* 56, 406–434. doi: 10.1007/s12035-018-1092-y

Najah, M., Youssef, S. M., Yahia, H. M., Afef, S., Awatef, J., Saber, H., et al. (2013). Molecular characterization of Tunisian families with abetalipoproteinemia and identification of a novel mutation in MTTP gene. *Diagn. Pathol.* 8:54. doi: 10.1186/1746-1596-8-54

Nichols, E., Steinmetz, J. D., Vollset, S. E., Fukutaki, K., Chalek, J., Abd-Allah, F., et al. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050:

an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, 7:e105–e125. doi: 10.1016/S2468-2667(21)00249-8

Oscanoa, J., Sivapalan, L., Gadaleta, E., Dayem Ullah, A. Z., Lemoine, N. R., and Chelala, C. (2020). SNPnexus: a web server for functional annotation of human genome sequence variation (2020 update). *Nucleic Acids Res.* 48, W185–W192. doi: 10.1093/nar/gkaa420

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372:n71. doi: 10.1136/bmj.n71

Panda, A. C., Sahu, I., Kulkarni, S. D., Martindale, J. L., Abdelmohsen, K., Vindu, A., et al. (2014). miR-196b-Mediated Translation Regulation of Mouse Insulin2 via the 5′UTR. *PLoS One* 9:e101084. doi: 10.1371/journal.pone.0101084

Peng, L., Fang, X., Xu, F., Liu, S., Qian, Y., Gong, X., et al. (2021). Amelioration of hippocampal insulin resistance reduces tau hyperphosphorylation and cognitive decline induced by isoflurane in mice. *Front. Aging Neurosci.* 13:686506. doi: 10.3389/fnagi.2021.686506

Pichler, S., Gu, W., Hartl, D., Gasparoni, G., Leidinger, P., Keller, A., et al. (2017). The miRNome of Alzheimer's disease: consistent downregulation of the miR-132/212 cluster. *Neurobiol. Aging* 50, 167.e1–167.e10. doi: 10.1016/j.neurobiolaging.2016.09.019

Popejoy, A. B., and Fullerton, S. M. (2016). Genomics is failing on diversity. *Nature* 538, 161–164. doi: 10.1038/538161a

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. doi: 10.1093/genetics/155.2.945

R: The R Project for Statistical Computing (n.d.). Available at: https://www.r-project.org/ (Accessed November 15, 2022).

Rassas, A. A., Fredj, S. H., Khiari, H. M., Sahnoun, S., Bibi, A., Siala, H., et al. (2013). No association between an intronic polymorphism in the presenilin-1 gene and Alzheimer disease in a Tunisian population. *J. Neural Transm. (Vienna)* 120, 1355–1358. doi: 10.1007/s00702-013-0985-1

Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., et al. (2019). g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* 47, W191–W198. doi: 10.1093/nar/gkz369

Reimand, J., Isserlin, R., Voisin, V., Kucera, M., Tannus-Lopes, C., Rostamianfar, A., et al. (2019). Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. *Nat. Protoc.* 14, 482–517. doi: 10.1038/s41596-018-0103-9

Rocha, M., Apostolova, N., Diaz-Rua, R., Muntane, J., and Victor, V. M. (2020). Mitochondria and T2D: role of autophagy, ER stress, and inflammasome. *Trends Endocrinol. Metab.* 31, 725–741. doi: 10.1016/j.tem.2020.03.004

Romdhane, L., Mezzi, N., Dallali, H., Messaoud, O., Shan, J., Fakhro, K. A., et al. (2021). A map of copy number variations in the Tunisian population: a valuable tool for medical genomics in North Africa. *NPJ Genom. Med.* 6, 1–18. doi: 10.1038/s41525-020-00166-5

Rubin, L., Ingram, L. A., Resciniti, N. V., Ashford-Carroll, B., Leith, K. H., Rose, A., et al. (2021). Genetic Risk Factors for Alzheimer's Disease in Racial/Ethnic Minority Populations in the U.S.: a scoping review. *Front. Public Health* 9:784958. doi: 10.3389/fpubh.2021.784958

Serveaux-Dancer, M., Jabaudon, M., Creveaux, I., Belville, C., Blondonnet, R., Gross, C., et al. (2019). Pathological Implications of Receptor for Advanced Glycation End-Product (AGER) Gene Polymorphism. *Dis. Markers* 2019, 2067353–2067317. doi: 10.1155/2019/2067353

Sheikh, A. M., Wada, Y., Tabassum, S., Inagaki, S., Mitaki, S., Yano, S., et al. (2021). Aggregation of cystatin C changes its inhibitory functions on protease activities and amyloid β fibril formation. *Int. J. Mol. Sci.* 22:9682. doi: 10.3390/ijms22189682

Shinohara, M., Kanekiyo, T., Yang, L., Linthicum, D., Shinohara, M., Fu, Y., et al. (2016). APOE2 eases cognitive decline during Aging: Clinical and preclinical evaluations. *Ann. Neurol.* 79, 758–774. doi: 10.1002/ana.24628

Shinohara, M., Tashiro, Y., Suzuki, K., Fukumori, A., Bu, G., and Sato, N. (2020). Interaction between APOE genotype and diabetes in cognitive decline. *Alzheimers Dement (Amst).* 12:e12006. doi: 10.1002/dad2.12006

Shoily, S. S., Ahsan, T., Fatema, K., and Sajib, A. A. (2021). Common genetic variants and pathways in diabetes and associated complications and vulnerability of populations with different ethnic origins. *Sci Rep* 11:7504. doi: 10.1038/s41598-021-86801-2

Shu, J., Li, N., Wei, W., and Zhang, L. (2022). Detection of molecular signatures and pathways shared by Alzheimer's disease and type 2 diabetes. *Gene* 810:146070. doi: 10.1016/j.gene.2021.146070

Silva-Spínola, A., Baldeiras, I., Arrais, J. P., and Santana, I. (2022). The road to personalized medicine in Alzheimer's disease: the use of artificial intelligence. *Biomedicine* 10:315. doi: 10.3390/biomedicines10020315

Silzer, T. K., and Phillips, N. R. (2018). Etiology of type 2 diabetes and Alzheimer's disease: exploring the mitochondria. *Mitochondrion* 43, 16–24. doi: 10.1016/j.mito.2018.04.004

Smach, M. A., Edziri, H., Charfeddine, B., Ben Othman, L., Lammouchi, T., Ltaief, A., et al. (2011). Polymorphism in apoA1 influences high-density lipoprotein cholesterol levels but is not a major risk factor of Alzheimer's disease. *Dement. Geriatr. Cogn. Dis. Extra* 1, 249–257. doi: 10.1159/000329910

Smith, D. A., Di, L., and Kerns, E. H. (2010). The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. *Nat. Rev. Drug Discov.* 9, 929–939. doi: 10.1038/nrd3287

Stanfill, A. G., and Cao, X. (2021). Enhancing research through the use of the genotype-tissue expression (GTEx) database. *Biol. Res. Nurs.* 23, 533–540. doi: 10.1177/1099800421994186

Stepler, K. E., and Robinson, R. A. S. (2019). The potential of 'omics to link lipid metabolism and genetic and comorbidity risk factors of Alzheimer's disease in African Americans. *Adv. Exp. Med. Biol.* 1118, 1–28. doi:10.1007/978-3-030-05542-4_1.

Straface, E., Matarrese, P., Gambardella, L., Vona, R., Sgadari, A., Silveri, M. C., et al. (2005). Oxidative imbalance and cathepsin D changes as peripheral blood biomarkers of Alzheimer disease: a pilot study. *FEBS Lett.* 579, 2759–2766. doi: 10.1016/j.febslet.2005.03.094

Strawbridge, R. J., Dupuis, J., Prokopenko, I., Barker, A., Ahlqvist, E., Rybin, D., et al. (2011). Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. *Diabetes* 60, 2624–2634. doi: 10.2337/db11-0415

Sun, L., Zhang, X., Wang, T., Chen, M., and Qiao, H. (2017). Association of ANK1 variants with new-onset type 2 diabetes in a Han Chinese population from Northeast China. *Exp. Ther. Med.* 14, 3184–3190. doi: 10.3892/etm.2017.4866

Suzuki, R., Lee, K., Jing, E., Biddinger, S. B., McDonald, J. G., Montine, T. J., et al. (2010). Diabetes and insulin in regulation of brain cholesterol metabolism. *Cell Metab.* 12, 567–579. doi: 10.1016/j.cmet.2010.11.006

Tateno, Y., Komiyama, T., Katoh, T., Munkhbat, B., Oka, A., Haida, Y., et al. (2014). Divergence of East Asians and Europeans estimated using male-and female-specific genetic markers. *Genome Biol. Evol.* 6, 466–473. doi: 10.1093/gbe/evu027

Thomassen, J. Q., Tolstrup, J. S., Benn, M., and Frikke-Schmidt, R. (2020). Type-2 diabetes and risk of dementia: observational and Mendelian randomisation studies in 1 million individuals. *Epidemiol. Psychiatr. Sci.* 29:e118. doi: 10.1017/S2045796020000347

Turki, A., Mtiraoui, N., Al-Busaidi, A. S., Khirallah, M., Mahjoub, T., and Almawi, W. Y. (2012). Lack of association between genetic polymorphisms within KCNQ1 locus and type 2 diabetes in Tunisian Arabs. *Diabetes Res. Clin. Pract.* 98, 452–458. doi: 10.1016/j.diabres.2012.10.006

United Nations Department of Economic and Social Affairs, Population Division (2020). *World population ageing 2020 highlights: living arrangements of older persons*. New York: United Nations.

Varma, V. R., BüşraLüleci, H., Oommen, A. M., Varma, S., Blackshear, C. T., Griswold, M. E., et al. (2021). Abnormal brain cholesterol homeostasis in Alzheimer's disease—a targeted metabolomic and transcriptomic study. *NPJ Aging Mech. Dis.* 7, 11–14. doi: 10.1038/s41514-021-00064-9

Wagner, R., Dudziak, K., Herzberg-Schäfer, S. A., Machicao, F., Stefan, N., Staiger, H., et al. (2011). Glucose-raising genetic variants in MADD and ADCY5 impair conversion of proinsulin to insulin. *PLoS One* 6:e23639. doi: 10.1371/journal.pone.0023639

Wang, H., Song, Y., Wu, Y., Kumar, V., Mahato, R. I., Su, Q. (2021). Activation of dsRNA-Dependent Protein Kinase R by miR-378 Sustains Metabolic Inflammation in Hepatic Insulin Resistance. *Diabetes*, 70, 710–719. doi: 10.2337/db20-0181

Wang, W.-X., Huang, Q., Hu, Y., Stromberg, A. J., and Nelson, P. T. (2011). Patterns of microRNA expression in normal and early Alzheimer's disease human temporal cortex: white matter versus gray matter. *Acta Neuropathol.* 121, 193–205. doi: 10.1007/s00401-010-0756-0

Wang, P., Huang, R., Lu, S., Xia, W., Cai, R., Sun, H., et al. (2016). RAGE and AGEs in mild cognitive impairment of diabetic patients: a cross-sectional study. *PLoS One* 11:e0145521. doi: 10.1371/journal.pone.0145521

Wang, X.-F., Lin, X., Li, D.-Y., Zhou, R., Greenbaum, J., Chen, Y.-C., et al. (2017). Linking Alzheimer's disease and type 2 diabetes: Novel shared susceptibility genes detected by cFDR approach. *J. Neurol. Sci.* 380, 262–272. doi: 10.1016/j.jns.2017.07.044

Wang, J., Yang, M. M., Li, Y. B., Liu, G. D., Teng, Y., and Liu, X. M. (2013). Association of CFH and CFB gene polymorphisms with retinopathy in type 2 diabetic patients. *Mediat. Inflamm.* 2013:e748435. doi: 10.1155/2013/748435

Wu, M., Liao, M., Huang, R., Chen, C., Tian, T., Wang, H., et al. (2020). Hippocampal overexpression of TREM2 ameliorates high fat diet induced cognitive impairment and modulates phenotypic polarization of the microglia. *Genes Dis* 9, 401–414. doi: 10.1016/j.gendis.2020.05.005

Wu, L., Velander, P., Brown, A. M., Wang, Y., Liu, D., Bevan, D. R., et al. (2021). Rosmarinic acid potently detoxifies amylin amyloid and ameliorates diabetic pathology in a transgenic rat model of type 2 diabetes. *ACS Pharmacol. Transl. Sci.* 4, 1322–1337. doi: 10.1021/acsptsci.1c00028

Xiao, Q., Xi, J., Wang, R., Zhao, Q., Liang, X., Wu, W., et al. (2022). The relationship between low-density lipoprotein cholesterol and progression of mild cognitive impairment: the influence of rs6859 in PVRL2. *Front. Genet.* 13:823406. doi: 10.3389/fgene.2022.823406

Xu, L., Li, Y., Yin, L., Qi, Y., Sun, H., Sun, P., et al. (2018). miR-125a-5p ameliorates hepatic glycolipid metabolism disorder in type 2 diabetes mellitus through targeting of STAT3. *Theranostics* 8, 5593–5609. doi: 10.7150/thno.27425

Xu, Z., and Taylor, J. A. (2009). SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res.* 37, W600–W605. doi: 10.1093/nar/gkp290

Yamamoto, S., Kayama, T., Noguchi-Shinohara, M., Hamaguchi, T., Yamada, M., Abe, K., et al. (2021). Rosmarinic acid suppresses tau phosphorylation and cognitive decline by downregulating the JNK signaling pathway. *NPJ Sci. Food* 5:1. doi: 10.1038/s41538-021-00084-5

Yang, X., Xu, Y., Gao, W., Wang, L., Zhao, X., Liu, G., et al. (2022). Hyperinsulinemia-induced microglial mitochondrial dynamic and metabolic alterations lead to neuroinflammation in vivo and in vitro. *Front. Neurosci.* 16:1036872. doi: 10.3389/fnins.2022.1036872

Yengo, L., Sidorenko, J., Kemper, K. E., Zheng, Z., Wood, A. R., Weedon, M. N., et al. (2018). Meta-analysis of genome-wide association studies for height and body mass index in ∼700000 individuals of European ancestry. *Hum. Mol. Genet.* 27, 3641–3649. doi: 10.1093/hmg/ddy271

Yu, C.-Y., Yang, C.-Y., and Rui, Z.-L. (2019). MicroRNA-125b-5p improves pancreatic β-cell function through inhibiting JNK signaling pathway by targeting DACT1 in mice with type 2 diabetes mellitus. *Life Sci.* 224, 67–75. doi: 10.1016/j.lfs.2019.01.031

Yuan, H., Miao, J., Xu, J., Zhu, S., Xu, F., Wang, X., et al. (2022). Increased serum cystatin C levels and responses of pancreatic α-and β-cells in type 2 diabetes. *Endocr. Connect.* 11:e210597. doi: 10.1530/EC-21-0597

Zhang, Y., Zhao, Y., Tian, C., Wang, J., Li, W., and Zhong, C. (2018). Differential exosomal microRNA profile in the serum of a patient with depression. *Eur. J. Psychiatr.* 32, 105–112. doi: 10.1016/j.ejpsy.2017.10.002

Zhen, J., Lin, T., Huang, X., Zhang, H., Dong, S., Wu, Y., et al. (2018). Association of ApoE genetic polymorphism and type 2 diabetes with cognition in non-demented aging Chinese adults: a community Based cross-sectional study. *Aging Dis.* 9, 346–357. doi: 10.14336/AD.2017.0715

Zhou, A., Taylor, A. E., Karhunen, V., Zhan, Y., Rovio, S. P., Lahti, J., et al. (2018). Habitual coffee consumption and cognitive function: a Mendelian randomization Meta-analysis in up to 415,530 participants. *Sci. Rep.* 8:7526. doi: 10.1038/s41598-018-25919-2

Zhu, Z., Lin, Y., Li, X., Driver, J.A., and Liang, L.. (2019). Shared genetic architecture between metabolic traits and Alzheimer's disease: A large scale genome-wide cross-trait analysis. *Hum Genet* 138:271–285. doi: 10.1007/s00439-019-01988-9

Zhu, Y., Paniccia, A., Schulick, A. C., Chen, W., Koenig, M. R., Byers, J. T., et al. (2016). Identification of CD112R as a novel checkpoint for human T cells. *J. Exp. Med.* 213, 167–176. doi: 10.1084/jem.20150785

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Latin American women in dementia research: outstanding contributions, barriers, and opportunities from Argentinian, Chilean, and Colombian colleagues

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Women's contributions to science have been consistently underrepresented throughout history. Despite many efforts and some progresses being made to reduce gender inequity in science, pursuing an academic career across disciplines, including Alzheimer's disease (AD) and other dementias, remains challenging for women. Idiosyncratic difficulties of Latin American countries likely accentuate the gender gap. In this Perspective, we celebrate outstanding contributions from Argentinian, Chilean, and Colombian colleagues in dementia research and discuss barriers and opportunities identified by them. We aim to acknowledge Latin American women's work and bring visibility to the challenges they face throughout their careers in order to inform potential solutions. Also, we highlight the need to perform a systematic assessment of the gender gap in the Latin American dementia community of researchers.

KEYWORDS

women, gender, inequity, diversity, dementia, Alzheimer's disease, Latin America

Introduction

Throughout the history of science, women's contributions have been consistently underrepresented, creating a misleading and unfair mindset: women are just not interested in scientific research (Saini, 2017). Such a simplistic point of view overlooked the many cultural, religious, and social hurdles that women had to overcome to produce new knowledge, including the lack of access to higher education and gender stereotypes that cause them to be perceived as less talented for science (Leslie et al., 2015) and more inclined toward care-taking roles (Ellemers, 2018). Despite many efforts and some progresses being made to reduce gender inequity in science, pursuing an academic career remains challenging for women.

Across disciplines, the women-to-men ratio is known to decrease from early to advanced career stages (Llorens et al., 2021). Several reasons have been proposed to explain such a trend. Women are less likely to occupy key (first and last) authorship positions in high profile journals

(West et al., 2013) and are cited less than men (Dworkin et al., 2020). These biases can lead to lower productivity metrics and hinder opportunities for funding acquisition and promotion to higher-level roles. Even in the case of equal outcomes, women often receive poorer evaluations of their performance (Moss-Racusin et al., 2012), fewer grants (Burns et al., 2019), and fewer prestigious awards (Lincoln et al., 2012) compared to men, indicating a systemic undervaluing of their contributions. Importantly, childcare and household responsibilities are mostly taken on by women, limiting opportunities for mobility, work presentations at scientific conferences, and networking. Taken together, these disadvantages hamper academic growth, recognition, and impact of the female scientific workforce.

This landscape is mirrored in Alzheimer's disease (AD) and other dementias' research. Women comprise 42.1% of all authorships in this field and only 32.8% of the last authorships (Menzel et al., 2019). Also, women have lower citation rates compared to men, publish fewer articles, and less often have prestigious authorships in collaborative papers with multiple authors (Menzel et al., 2019). Data from Alzheimer's Research UK revealed that women hold only 33% of major grants, contrasting with 64% of successful early career grants (Andreou et al., 2022), suggesting a loss of female researchers in the junior-to-senior transition as reported for other disciplines.

The outlook for Latin American countries is even more intricate. These countries face idiosyncratic difficulties related to the scarce availability of government funds for scientific research, the political instability, and patriarchal sociocultural norms that likely accentuate the gender gap (García-Holgado et al., 2019; Silva et al., 2022). While specific data on female researchers in the dementia field across Latin America is wanting, insights from related neuroscience field reveal that less than 20% of women attain the highest position (full professor), in comparison with 30% of men, despite female neuroscientists being overrepresented at the graduate level (Silva et al., 2022). Also, Latin American neuroscientists women report higher levels of career dissatisfaction (44%) than men (28%) (Silva et al., 2021).

Beyond the inequity concerns intrinsic to these numbers, gender diversity in teams is now recognized as a key driver of creativity, innovation, and scientific discovery (Nielsen et al., 2018). Female perspectives become particularly relevant in dementia research partially because dementia affects more women than men both in terms of higher prevalence (GBD 2019 Dementia Forecasting Collaborators, 2022) and care burden (Erol et al., 2016) worldwide. Latin America is not the exception; dementia is more prevalent in women (8.97%) than men (7.26%) (Ribeiro et al., 2022), and patterns of female-to-male ratio are projected to continue (GBD 2019 Dementia Forecasting Collaborators, 2022). In Latin American countries caregiving is also disproportionally delivered by women [with numbers as high as 80% in Chile (Slachevsky et al., 2013)] who mostly experience the emotional, social, and financial costs in contexts of poor governmental support (Ibanez et al., 2021). Achieving gender equity in dementia research, specifically in highly impacted regions such as Latin America where prevalence is expected to increase 200% by 2050 (GBD 2019 Dementia Forecasting Collaborators, 2022), is necessary to amplify the voices of key actors in this global health issue.

One of the first steps toward achieving this goal is to acknowledge women's work and bring visibility to the challenges they face throughout their careers in order to inform potential solutions. In this Perspective, we aim to celebrate outstanding contributions from Latin American women in dementia research and discuss barriers and opportunities identified by them. We surveyed 14 national Argentinian, Chilean, and Colombian female researchers from our local networks who (a) are currently working in the region, and (b) have significantly advanced our understanding of AD and other dementias with a local impact to mitigate the burden this condition (Figure 1). We included early-, mid-career, and senior researchers focused on both basic and translational science. Their trajectories are described below, followed by a discussion of barriers, opportunities, and future directions.

Argentina

Agustina Legaz, BS

Ph.D. student, Cognitive Neuroscience Center (CNC), Universidad de San Andres, Buenos Aires.

The research program of Ms. Legaz involves the study of socio-contextual modulations on memory and emotions in Latin American samples of AD, behavioral-variant frontotemporal dementia (bvFTD), and Parkinson's disease. Her work combines novel experimental paradigms with electrophysiological and structural and functional MRI techniques. In a recent work (Legaz et al., 2022), which has been highlighted among new developments in frontotemporal dementia by the editor-in-chief of Brain journal (Husain, 2022), Legaz and colleagues showed differential multimodal deficits in the ability to learn from social feedback in AD, bvFTD, and Parkinson's disease. In another work (Salamone et al., 2021), they showed how interoception (the sensing of visceral signals) primes the processing of emotions, and how this process is compromised in different neurodegenerative diseases. Her contributions allow a better characterization of dementia subtypes in underrepresented Latin American populations, with relevant theoretical and clinical implications in the realms of diagnosis and treatment.

Diana Bruno, PhD

Director, Instituto de Investigaciones en Psicología Básica y Aplicada (IIPBA), Facultad de Filosofía y Humanidades, Universidad Católica de Cuyo, San Juan.

Dr. Bruno is a neuropsychologist and researcher interested in the detection of and intervention in cognitive impairment and dementia. She has validated the Addenbrooke's Cognitive Examination III (ACE-III), a widely used cognitive screening, for the Argentinian and Chilean populations (Bruno et al., 2020), bringing local professional communities a sensitive instrument for the detection of dementia. A review on the psychometric properties and usability of the ACE-III was critical to identify difficulties associated with its use in the region (Bruno and Schurmann Vignaga, 2019). Dr. Bruno has also validated the Cognitive Complaints Questionnaire (CCQ) aimed to assess multidomain cognitive complaints (Nuñez and Bruno, 2021). Crucially, Dr. Bruno has published "Saber Acompañar," a toolkit to support caregivers and families of persons with dementia and other brain diseases (Torralva et al., 2021). The work of Dr. Bruno is relevant for professionals and the community. This is the result of her conviction that science must transcend academic boundaries to solve societal problems, improve quality of life, and guarantee an equative access to new developments.

FIGURE 1
Latin American women featured in this Perspective. Female scientists who are currently developing high-impact research on Alzheimer's Disease and other dementias in Argentina (blue), Chile (yellow), and Colombia (pink).

Lucía Crivelli, PhD

Head of Neuropsychology, Department of Cognitive Neurology, Neuropsychology, and Neuropsychiatry, FLENI Neurological Institute, Buenos Aires.

Dr. Crivelli is committed with the prevention and early detection of cognitive decline and dementia in developing countries. She serves as Principal Investigator on LatAm-FINGERS, the first multi-domain lifestyle intervention in the region (Crivelli, 2020). Her role has been crucial for designing the project and reuniting the most prestigious centers and leaders from Latin America. In addition, during the last 2 years, she has studied the consequences of COVID-19 on cognitive functions in adults with no prior history of cognitive impairment, revealing that even asymptomatic cases show cognitive sequelae (Crivelli et al., 2022a). The pandemic has also driven her work on the field of tele-neuropsychology, where she published the first recommendations for Latin America, identifying barriers and challenges unique to the region and potential solutions to overcome them (Crivelli et al., 2022b).

Silvia Kochen, PhD

Researcher, Hospital El Cruce and National Scientific and Technical Research Council (CONICET), Buenos Aires.

Dr. Kochen's work is focused on the study of cognition in older adults and the development of effective interventions for dementia in vulnerable populations from suburbs of Buenos Aires province. She has run a prospective longitudinal study in the memory clinic of the Neurosciences and Complex Systems Unit (EnyS, Hospital El Cruce, Florencio Varela, Buenos Aires) which revealed critical needs of users in terms of cognitive assessment and treatment (Kochen et al., 2018). Her research has also contributed to a better understanding of memory-enhancing strategies in healthy older adults (Tassone et al., 2020). Overall, Dr. Kochen's work has implications for the development of public policies around dementia prevention, diagnosis, treatment, care, and support in populations in need from Argentina. Dr. Kochen describes herself as a "feminist scientist" committed to the integration of a gender perspective in her activities.

Teresa Torralva, PhD

Neuropsychologist and researcher, Institute of Cognitive and Translational Neuroscience (INCYT) and INECO Foundation, Buenos Aires.

Dr. Torralva has devoted her career to clinical neuropsychology and research, with a focus on the study of the frontal lobes and executive functions in frontotemporal dementia. In one of her most impactful publications (Torralva et al., 2009a), she showed that an Executive and Social Cognition Battery was more sensitive than classical cognitive measures to detect specific deficits in early bvFTD. This work was pioneering in revealing the relevance of ecological tests to assess early bvFTD, which can sometimes be very challenging. Dr. Torralva and her team have also developed the INECO Frontal Screening (IFS), a brief, sensitive, and specific tool to detect frontal executive impairment in neurodegeneration (Torralva et al., 2009b), which has been translated and adapted for multiple countries. Dr. Torralva has also made significant contributions in the field of social cognition in dementia, particularly showing patterns of alterations of theory of mind in bvFTD (Torralva et al., 2015). Dr. Torralva has published more than 80 papers in peer-reviewed journals, including Brain and Neuropsychologia, book chapters, and books on these topics, including "Saber Acompañar" (Torralva et al., 2021), and "Upgrade Cerebral" (Torralva, 2022).

Chile

Andrea Slachevsky, PhD

Associate Professor, Faculty of Medicine, Universidad de Chile, Santiago.

Dr. Slachevsky is a neurologist specialized in cognition and dementia. She is the clinical director of the Geroscience Center for Brain Health and Metabolism (GERO) which runs the largest longitudinal cohort of aging in Latin America (Slachevsky et al., 2020). Together with health professionals and caregivers, she founded the Professional Cooperation for Alzheimer's and Other Dementias (COPRAD), a non-profit, non-governmental organization dedicated to promoting public policies to address the needs of people living with dementia and face the challenges associated with the rapid increase of dementia prevalence. She has also participated in the preparation of the National Dementia Plan in Chile and in the creation of one of the three memory units that were implemented in the context of said Plan. Her research line focuses on neuropsychology and functionality in dementia and healthy aging. Dr. Slachevsky has developed and validated a new version of the Activities of Daily Living Questionnaire (ADLQ) that incorporates a subscale to assess the use of information and communication technologies, contributing to the evaluation of functionality in cognitive disorders (Muñoz-Neira et al., 2012). She is now working on the development of new methods to assess executive functions using serious games. Dr. Slachevsky has also participated in studies aimed to estimate the cost associated with dementia (Hojman et al., 2017) and the burden of care (Slachevsky et al., 2013) in Chile. Taken together, Dr. Slachevsky's work is relevant for the evaluation of dementia in Latin America and the estimation of its economic impact, and has policy implications.

Brigitte van Zundert, PhD

Full Professor, Universidad Andrés Bello, Santiago.

Dr. van Zundert is specialized in the molecular and cellular basis of learning and memory in health and neurodegenerative diseases. The laboratory of Dr. van Zundert analyzed the PSD95 epigenetic landscape in developing hippocampus and designed a PSD95 epigenetic-targeting strategy for AD. They established that epigenetic editing of the synaptic protein PSD95 prevented and even recovered hippocampal dysfunction and memory deficits in mouse models of AD, thus validating PSD95 as a key player in memory (Bustos et al., 2017). Dr. van Zundert and colleagues have also studied the effect of urban air pollution in epigenetic gene regulation and AD hallmarks in the brain of humans and mice. They found that young healthy humans chronically exposed to urban air pollution exhibit reduced repressive epigenetic marks, increased DNA damage, and presence of AD hallmarks. In addition, mice exposed for 7 months to intermediate/high air pollution (Santiago, Chile) displayed similar brain impacts (Calderon-Garciduenas et al., 2020). These findings established the role of epigenetic mechanisms in learning and memory in neurodegenerative diseases. Dr. van Zundert has published >50 scientific papers in prestigious journals such as Brain and Neuron.

Maria Isabel Behrens, PhD

Full Professor, Faculty of Medicine, Universidad de Chile, Santiago.

Dr. Behrens studies the molecular mechanisms of dementia, with a focus on the inverse relationship between AD and cancer. Remarkable, using a cohort of 600 patients from the Memory and Aging Program, Dr. Behrens and his co-worker, Dr. Catherine Roe, found that AD has a protective role against the future development of cancer. Moreover, patients with cancer are apparently protected from the development of AD. This finding was replicated in the Cardiovascular Health Study–Cognition Substudy cohort with 3,000 patients and in many other publications (Roe et al., 2005). Additionally, Dr. Behrens studied the genetic cause of Kufor-Rakeb syndrome (KRS), a rare juvenile hereditary disease. KRS patients develop the typical signs of Parkinson's disease, but also show symptoms of dementia. Dr. Behrens associated the origin of this syndrome with the loss-of-function mutation of the neuronal ATPase gene ATP13A2 (Ramirez et al., 2006). Overall, Dr. Behrens has contributed to understanding the mechanism of neurodegenerative diseases, paving the way to the development of effective therapies.

Natalia Salvadores, PhD

Assistant Professor, Universidad Mayor, Temuco.

The main interest of Dr. Salvadores is contributing to the understanding of neurological diseases. Her studies are focused on the molecular and cellular mechanisms underlying AD pathogenesis. Dr. Salvadores' work involves adapting and validating protein misfolding cyclic amplification (PMCA) technique as a new potential diagnosis tool for neurodegenerative diseases. In a recent study, Dr. Salvadores was able to distinguish AD patients from control individuals with high sensitivity and specificity using cerebrospinal fluid samples (Salvadores et al., 2014). Dr. Salvadores also studied the participation of necroptosis (a programmed form of cell death) in the pathomechanism of AD. She found that oligomeric Aβ aggregates (Aβo) correlate with necroptosis activation in human AD brains. She also showed that genetic and pharmacological inhibition of necroptosis ameliorates neurodegeneration and memory loss in an AD model based on intracerebral administration of Aβo (Salvadores et al., 2022). Dr.

Salvadores demonstrated for the first time the involvement of Aβ pathology in the activation of this death pathway.

Rommy von Bernhardi, PhD

Full Professor, Faculty of Medicine and Science, Universidad San Sebastián, Santiago.

Dr. von Bernhardi investigates the participation of glial cells in the development of neurodegenerative diseases. She is currently focused on the glial functional changes of aging that promote cytotoxic activation and neurodegeneration. She demonstrated that inflammatory stimuli induce the production of reactive oxygen species (ROS) in older mice, favoring oxidative damage during aging. The increase in ROS also induces neuroinflammation, generating a vicious circle, resulting in functional deficiencies such as memory and behavior alterations (von Bernhardi et al., 2015). She also studied the role of glial cells receptor Scavenger Receptor class A (SR-A) in AD. Dr. von Bernhardi reported the downregulation of SR-A expression in the hippocampus of aged animals and the APP/PS1 AD animal model. Additionally, Microglia and astrocytes lacking SR-A displayed impaired oxidative response and nitric oxide production (Cornejo et al., 2018). Taken together, Dr. von Bernhardi contribution allows understanding the correlation between microglial response and neurodegeneration.

Colombia

Claudia Ramos, M.D.

Psychiatrist, Neuroscience Group of University of Antioquia, Medellín.

Dr. Ramos serves as psychiatrist and researcher at the mental health program for patients with neurodegenerative diseases of the Neuroscience Group of University of Antioquia. She participated in a study assessing mental disorders in young adults from families with the presenilin-1 gene mutation e280a in the preclinical stage of AD (Villalba et al., 2019). Also, she conducted a review of different genetic variants that cause AD or frontotemporal dementia in Latin America (Ramos et al., 2020). In her more recent work (Ramos et al., 2022), she investigated the association between substance use and cognitive decline in carriers of the PSEN1-E280A genetic variant showing that cigarette and alcohol were associated with an improvement of some cognitive assessments, possibly by a survival bias. Taken together, Dr. Ramos' work helps to characterize mental health disorders in patients with dementia and develop prevention strategies.

Diana Matallana, PhD

Full Professor, Faculty of Medicine, Pontificia Universidad Javeriana, Bogotá.

As neuropsychologist and researcher, Dr. Matallana's work has focused on clinical studies aimed to improve the diagnosis and treatment of neurodegenerative diseases. She also uses neuropsychological measures to study the cognitive impairments associated with psychiatric disorders and mild traumatic brain injury. One of her recent studies (Cruz-Sanabria et al., 2021) compared the neuropsychological profiles, brain morphometry, and structural connectivity patterns between patients diagnosed with bvFTD and older-age bipolar disorder patients. She has also participated in genetic studies in neurodegenerative diseases identifying 21 pathogenic variants in AD-FTLD related genes in the Colombian population (Acosta-Uribe et al., 2022). Dr. Matallana is member of the Multi-partner consortium to expand dementia research in Latin America (ReDLat), a multi-site network aimed to understand the genetic and environmental factors influencing dementia in Latin America. In a recent work of this consortium, authors reported a survey to explore the ongoing work, needs, interests, potential barriers, and opportunities for future studies related to biomarkers in the region (Parra et al., 2022). Dr. Matallana has remarkably contributed to the characterization of neuropsychological profiles and clinical and genetic assessments of Colombian patients with neurodegenerative diseases.

Natalia Acosta Baena, BS

Ph.D. student, Neuroscience Group of University of Antioquia, Medellín.

Ms. Acosta Baena is clinical epidemiologist, and her work is focused on the genetics of neurodegenerative diseases, mainly AD. She also investigates the relationship between neurodevelopment and dementia. She has carried out one of the largest studies assessing descendants of individuals with a mutation in presenilin 1 (PSEN1) that causes familial AD, with the aim of identifying distinct stages of clinical progression to AD dementia (Acosta-Baena et al., 2011). She participated in the Alzheimer's Prevention Initiative Colombia Trial (Rios-Romenets et al., 2018), in which authors reported a participant retention of 94%, highlighting that this adherence plan plays a crucial role in maintaining treatment compliance and may offer guideposts for other prevention trials. Overall, Ms. Acosta Baena's work aims to improve the quality of life of patients with dementia, clarifying the diagnosis, informing about the characteristics of these diseases, and discovering options for possible future therapies.

Natalia Trujillo, PhD

Full professor, University of Antioquia, Medellín.

Dr. Trujillo studies cognitive and social processing and its intervention in neurological patients and populations characterized by disruptive behavior. In ReDLat multisite project, she evaluates the role of social determinants of health on the progression of AD and is involved in the transcultural validation of functional scales. She employs neuropsychological and electrophysiological methods for assessing patients with neurodegenerative diseases. She contributed to relevant works showing early action-verb production and action semantics in patients with Parkinson's disease (Bocanegra et al., 2015), and proposing an automated analysis of spontaneous discourse for the classification of Parkinson's disease patients (Garcia et al., 2016). Also, she participated in a study supporting the validity of the visual short-term memory binding test and their neural correlates in the early detection of AD (Pietto et al., 2016). Her work has implications for early detection and monitoring of neurodegenerative conditions.

Empowering Latin American women in dementia research: barriers and opportunities

In this Perspective, we celebrated the work of Latin American women in the field of dementia research. Using animal models, neuropsychological tools, and neuroimaging techniques, among

TABLE 1 Potential solutions and resources to address gender-related barriers and opportunities identified in the present work.

Barriers	Potential solutions
Struggles with work-life balance and childcare duties	• Institutions should consider maintaining a flexible and family-friendly work schedule and subsume childcare expenditures during work-related travel. • Institutions and conference organizers could provide free or affordable childcare options/lactation rooms. • Funding agencies and reviewers should take into account parenthood and care-related delays in the academic career when assessing candidates for a job, a grant, or an award (e.g., "stop the clock" policies).
Expectations of traditional female roles, prejudice, and discrimination	• All individuals should educate themselves regarding biases, stereotypes and prejudices, evaluate their own behavior, and be an active bystander against discrimination and harassment. • Institutions should consider offering regular (un)conscious bias training workshops for employers and employees. • Conference organizers should guarantee a balanced speaker's gender ratio. • Institutions and conference organizers should adopt zero-tolerance policies for discrimination, harassment, and abuse, and a code of conduct with clear consequences for such behaviors.
Difficulty in securing funding	• Authors and scientific journals should balance gender in references' lists to allow for more equitative academic success metrics. • Funding agencies and institutions should guarantee gender balance among reviewers, editorial panels, and awardees as well as double-blind review processes. • Institutions could actively outreach, suggest, and support applications for grants or promotions from female scientists.
Opportunities	**Resources**
Positive female role models and networking	• Repositories such as Women in science, Anne's list, and 500 Women Scientists feature female neuroscientists, allowing them to gain visibility. • Networking initiatives such as the Alliance of Women Alzheimer's Researchers (AWARE) hosted by the Alzheimer's Association connect female researchers.
Funding/awards specifically tailored to women from middle-and low-income countries	• The International Brain Research Organization (IBRO), the Society for Neuroscience (SfN), and the ALBA network offer tailored funding programs.
Visibility of the gender gap	• Initiatives such as BiasWatchNeuro (a website that tracks gender diversity in neuroscience conferences), public reports of grant success rates by gender, and the use of "Inclusion and diversity statement" in manuscripts allow to oversight gender (im)balance in science.

others, they have made remarkable contributions to improve the characterization, assessment, diagnosis, and treatment of neurodegenerative conditions, including AD, bvFTD, and Parkinson's disease. They have contributed to a better understanding of memory and other cognitive and sociocognitive domains in healthy adults and persons with dementia. In addition, they have characterized different genetic variants associated with dementia in Latin America, created and validated neuropsychological tests with local utility, led prevention initiatives, and developed recommendations for assessment and intervention in dementia targeting professionals, caregivers, and policy makers. Their work proves critical for the development of strategies to reduce the burden of dementia with local impact in a global context.

The barriers faced by women included in this article along their career are not essentially different from those reported in the literature. Struggles with work-life balance, childcare duties, expectations of traditional female roles, prejudice, discrimination, and difficulty in securing funding were recognized as disproportionally affecting them compared to men, as previously acknowledged by Latin American neuroscientists (Silva et al., 2021, 2022). Taken together, these barriers represent a "glass ceiling" that unequally makes harder for women to advance their careers (Cotter et al., 2001).

While much has been written about barriers of women in science, less is known about opportunities from the perspective of the persons involved. When asked to identify opportunities during their careers that empowered and energized them to stay on track, women surveyed for this article mainly mentioned: (1) working with female scientists that inspired them, (2) belonging to supportive networks that allowed them to overcome economic resources' limitations, (3) securing funding/awards specifically tailored to women from middle-and low-income countries, and (4) being aware of increased visibility of the gender gap and actions toward reducing it at the national and international sphere. Thus, despite barriers, positive female role models, networking, and the development of policies and programs aimed at increasing women in science, in particular from underrepresented backgrounds, are identified as opportunities to change.

Regardless of the discipline, promoting the engagement and retention of women in science requires systemic changes at different levels, including individual (e.g., education, bias awareness), institutional (e.g., fair promotion and family leave policies), and cultural/societal (e.g., reduction of explicit and implicit prejudice, legislations) dimensions. While a comprehensive proposal of solutions is beyond the scope of this work and can be found elsewhere (Schrouff et al., 2019; Llorens et al., 2021; Sibener et al., 2022; Silva et al., 2022). Table 1 summarizes some potential solutions and resources to address the main barriers and opportunities that were identified by women surveyed here.

We specifically highlight the need to perform a systematic assessment of the gender gap in the Latin American dementia community of researchers. Recent reports from the Interamerican Development Bank (López-Bassols et al., 2018) and the Latin America Regional Committee of International Brain Research Organization

(IBRO LARC) and the Economic Commission for Latin America and the Caribbean (ECLAC) (Tomassini et al., 2020) offer an analysis of the gender imbalance in (neuro)science, technology, and innovation across the region. However, women's situation specifically in the field of dementia research remains unexplored. Thus, additional work should be performed to systematically explore the participation, barriers, and opportunities of Latin American women researchers on dementia. Quantitative gender measures are needed to develop interventions aimed at reducing the gap and monitoring their impact.

The list of women featured in the present Perspective by no means intends to be exhaustive and we acknowledge that it is not representative of the entire Latin American research landscape on dementia. It consists of a selective sample from the authors' networks in Argentina, Chile, and Colombia as a recognition of all colleagues from Latin America who are devoting their careers to advance dementia research in the region. We also acknowledge that additional disparities might contribute to women's challenges in science, such as sexual orientation, ethnicity, religion, and socioeconomic status, among others, which need to be further examined in the Latin American context. We hope this work will inspire others and serve as a starting point to address these critical issues. Gender diversity and equity in research are critical to the global and local effort to tackle dementia.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SF, SB, and CD-A conceived this work and designed and performed the survey. SF wrote the manuscript in collaboration with SB, CGS, and CD-A. All authors discussed the content and substantively contributed to the revision of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Acosta-Baena, N., Sepulveda-Falla, D., Lopera-Gomez, C. M., Jaramillo-Elorza, M. C., Moreno, S., Aguirre-Acevedo, D. C., et al. (2011). Pre-dementia clinical stages in presenilin 1 E280A familial early-onset Alzheimer's disease: a retrospective cohort study. *Lancet Neurol.* 10, 213–220. doi: 10.1016/S1474-4422(10)70323-9

Acosta-Uribe, J., Aguillon, D., Cochran, J. N., Giraldo, M., Madrigal, L., Killingsworth, B. W., et al. (2022). A neurodegenerative disease landscape of rare mutations in Colombia due to founder effects. *Genome Med.* 14:27. doi: 10.1186/s13073-022-01035-9

Andreou, M., Choi, N., Magenti, J. G., Kohlhaas, S., and Sancho, R. (2022). The dementia research career pipeline: gender disparities in publication authorships and grant funding outcomes at different career stages. *Health Open Res.* 4:18. doi: 10.12688/amrcopenres.13072.1

Bocanegra, Y., Garcia, A. M., Pineda, D., Buritica, O., Villegas, A., Lopera, F., et al. (2015). Syntax, action verbs, action semantics, and object semantics in Parkinson's disease: dissociability, progression, and executive influences. *Cortex* 69, 237–254. doi: 10.1016/j.cortex.2015.05.022

Bruno, D., and Schurmann Vignaga, S. (2019). Addenbrooke's cognitive examination iii in the diagnosis of dementia: a critical review. *Neuropsychiatr. Dis. Treat.* 15, 441–447. doi: 10.2147/NDT.S151253

Bruno, D., Slachevsky, A., Fiorentino, N., Rueda, D. S., Bruno, G., Tagle, A. R., et al. (2020). Argentinian/Chilean validation of the Spanish-language version of Addenbrooke's cognitive examination iii for diagnosing dementia. *Neurologia (Engl Ed)* 35, 82–88. doi: 10.1016/j.nrl.2017.06.004

Burns, K. E. A., Straus, S. E., Liu, K., Rizvi, L., and Guyatt, G. (2019). Gender differences in grant and personnel award funding rates at the Canadian Institutes of Health Research based on research content area: a retrospective analysis. *PLoS Med.* 16:e1002935. doi: 10.1371/journal.pmed.1002935

Bustos, F. J., Ampuero, E., Jury, N., Aguilar, R., Falahi, F., Toledo, J., et al. (2017). Epigenetic editing of the Dlg4/Psd95 gene improves cognition in aged and Alzheimer's disease mice. *Brain* 140, 3252–3268. doi: 10.1093/brain/awx272

Calderon-Garciduenas, L., Herrera-Soto, A., Jury, N., Maher, B. A., Gonzalez-Maciel, A., et al. (2020). Reduced repressive epigenetic marks, increased Dna damage and Alzheimer's disease hallmarks in the brain of humans and mice exposed to particulate urban air pollution. *Environ. Res.* 183:109226. doi: 10.1016/j.envres.2020.109226

Cornejo, F., Vruwink, M., Metz, C., Munoz, P., Salgado, N., Poblete, J., et al. (2018). Scavenger receptor-a deficiency impairs immune response of microglia and astrocytes potentiating Alzheimer's disease pathophysiology. *Brain Behav. Immun.* 69, 336–350. doi: 10.1016/j.bbi.2017.12.007

Cotter, D. A., Hermsen, J. M., Ovadia, S., and Vanneman, R. (2001). The glass ceiling effect. *Social Forces* 80, 655–681. doi: 10.1353/sof.2001.0091

Crivelli, L. (2020). LatAm-Fingers (Latin America) world-wide Fingers network: the first global network of multidomain dementia prevention trials. *Alzheimers Dement.* 16:e046953

Crivelli, L., Palmer, K., Calandri, I., Guekht, A., Beghi, E., Carroll, W., et al. (2022a). Changes in cognitive functioning after Covid-19: a systematic review and meta-analysis. *Alzheimers Dement.* 18, 1047–1066. doi: 10.1002/alz.12644

Crivelli, L., Quiroz, Y. T., Calandri, I. L., Martin, M. E., Velilla, L. M., Cusicanqui, M. I., et al. (2022b). Working group recommendations for the practice of Teleneuropsychology in Latin America. *Arch. Clin. Neuropsychol.* 37, 553–567. doi: 10.1093/arclin/acab080

Cruz-Sanabria, F., Reyes, P. A., Trivino-Martinez, C., Garcia-Garcia, M., Carmassi, C., Pardo, R., et al. (2021). Exploring signatures of Neurodegeneration in early-onset older-age bipolar disorder and behavioral variant Frontotemporal dementia. *Front. Neurol.* 12:713388

Dworkin, J. D., Linn, K. A., Teich, E. G., Zurn, P., Shinohara, R. T., and Bassett, D. S. (2020). The extent and drivers of gender imbalance in neuroscience reference lists. *Nat. Neurosci.* 23, 918–926. doi: 10.1038/s41593-020-0658-y

Ellemers, N. (2018). Gender stereotypes. *Annu. Rev. Psychol.* 69, 275–298. doi: 10.1146/annurev-psych-122216-011719

Erol, R., Brooker, D., and Peel, E. (2016). The impact of dementia on women internationally: an integrative review. *Health Care Women Int.* 37, 1320–1341. doi: 10.1080/07399332.2016.1219357

Garcia, A. M., Carrillo, F., Orozco-Arroyave, J. R., Trujillo, N., Vargas Bonilla, J. F., Fittipaldi, S., et al. (2016). How language flows when movements don't: an automated analysis of spontaneous discourse in Parkinson's disease. *Brain Lang.* 162, 19–28. doi: 10.1016/j.bandl.2016.07.008

García-Holgado, A., Díaz, A. C., and García-Peñalvo, F. J. Engaging women into stem in Latin America: W-stem project. Proceedings of the Seventh International Conference on Technological Ecosystems for Enhancing Multiculturality, (2019). 232–239.

GBD 2019 Dementia Forecasting Collaborators. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the global burden of disease study 2019. *Lancet Public Health* 7, e105–e125.

Hojman, D. A., Duarte, F., Ruiz-Tagle, J., Budnich, M., Delgado, C., and Slachevsky, A. (2017). The cost of dementia in an unequal country: the case of Chile. *PLoS One* 12:e0172204. doi: 10.1371/journal.pone.0172204

Husain, M. (2022). New developments in frontotemporal dementia. *Brain* 145, 799–800. doi: 10.1093/brain/awac058

Ibanez, A., Pina-Escudero, S. D., Possin, K. L., Quiroz, Y. T., Peres, F. A., Slachevsky, A., et al. (2021). Dementia caregiving across Latin America and the Caribbean and brain health diplomacy. *Lancet Healthy Longev* 2, e222–e231.

Kochen, S., Sevillano, Z., Lisso, J., Nancy, M., and Solis, P. (2018). Screening of cognitive impairment: undiagnosed or over diagnosis in a vulnerable population. *J. Neurosci. Cogn. Stud.* 2:1009.

Legaz, A., Abrevaya, S., Dottori, M., Gonzalez Campo, C., Birba, A., Martorell Caro, M., et al. (2022). Multimodal mechanisms of human socially reinforced learning across neurodegenerative diseases. *Brain* 145, 1052–1068. doi: 10.1093/brain/awab345

Leslie, S. J., Cimpian, A., Meyer, M., and Freeland, E. (2015). Expectations of brilliance underlie gender distributions across academic disciplines. *Science* 347, 262–265. doi: 10.1126/science.1261375

Lincoln, A. E., Pincus, S., Koster, J. B., and Leboy, P. S. (2012). The matilda effect in science: awards and prizes in the us, 1990s and 2000s. *Soc. Stud. Sci.* 42, 307–320. doi: 10.1177/0306312711435830

Llorens, A., Tzovara, A., Bellier, L., Bhaya-Grossman, I., Bidet-Caulet, A., Chang, W. K., et al. (2021). Gender bias in academia: a lifetime problem that needs solutions. *Neuron* 109, 2047–2074. doi: 10.1016/j.neuron.2021.06.002

López-Bassols, V., Grazzi, M., Guillard, C., and Salazar, M. (2018). *Las brechas de género en ciencia, tecnología e innovación en América Latina y el Caribe: resultados de una recolección piloto y propuesta metodológica para la medición*, New York: Interamerican Development Bank.

Menzel, L. C., Kramer, P. W., Groneberg, D. A., and Bendels, M. H. K. (2019). Gender disparities in authorships of Alzheimer's disease and dementia research articles. *J. Alzheimers Dis.* 70, 1143–1152. doi: 10.3233/JAD-190216

Moss-Racusin, C. A., Dovidio, J. F., Brescoll, V. L., Graham, M. J., and Handelsman, J. (2012). Science faculty's subtle gender biases favor male students. *Proc. Natl. Acad. Sci. U. S. A.* 109, 16474–16479. doi: 10.1073/pnas.1211286109

Muñoz-Neira, C., López, O. L., Riveros, R., Núñez-Huasaf, J., Flores, P., and Slachevsky, A. (2012). The technology–activities of daily living questionnaire: a version with a technology-related subscale. *Dement. Geriatr. Cogn. Disord.* 33, 361–371. doi: 10.1159/000338606

Nielsen, M. W., Bloch, C. W., and Schiebinger, L. (2018). Making gender diversity work for scientific discovery and innovation. *Nat. Hum. Behav.* 2, 726–734. doi: 10.1038/s41562-018-0433-1

Nuñez, S. L., and Bruno, D. (2021). Validación del cuestionario de quejas cognitivas. *Neurol. Argent.* 13, 137–144. doi: 10.1016/j.neuarg.2020.09.004

Parra, M. A., Orellana, P., Leon, T., Victoria, C. G., Henriquez, F., Gomez, R., et al. (2022). Biomarkers for dementia in Latin American countries: gaps and opportunities. *Alzheim. Dem.* 19, 721–735. doi: 10.1002/alz.12757

Pietto, M., Parra, M. A., Trujillo, N., Flores, F., Garcia, A. M., Bustin, J., et al. (2016). Behavioral and electrophysiological correlates of memory binding deficits in patients at different risk levels for Alzheimer's disease. *J. Alzheimers Dis.* 53, 1325–1340. doi: 10.3233/JAD-160056

Ramirez, A., Heimbach, A., Gründemann, J., Stiller, B., Hampshire, D., Cid, L. P., et al. (2006). Hereditary parkinsonism with dementia is caused by mutations in Atp13A2, encoding a lysosomal type 5 P-type Atpase. *Nat. Genet.* 38, 1184–1191. doi: 10.1038/ng1884

Ramos, C., Aguillon, D., Cordano, C., and Lopera, F. (2020). Genetics of dementia: insights from Latin America. *Dem. Neuropsychol.* 14, 223–236. doi: 10.1590/1980-57642020dn14-030004

Ramos, C., Villalba, C., Garcia, J., Lanata, S., Lopez, H., Aguillon, D., et al. (2022). Substance use-related cognitive decline in families with autosomal dominant Alzheimer's disease: a cohort study. *J. Alzheimers Dis.* 85, 1423–1439. doi: 10.3233/JAD-215169

Ribeiro, F., Teixeira-Santos, A. C., Caramelli, P., and Leist, A. K. (2022). Prevalence of dementia in Latin America and Caribbean countries: systematic review and meta-analyses exploring age, sex, rurality, and education as possible determinants. *Ageing Res. Rev.* 81:101703. doi: 10.1016/j.arr.2022.101703

Rios-Romenets, S., Acosta-Baena, N., Lopez, L., Madrigal-Zapata, L., Street, H., Jakimovich, L., et al. (2018). Adherence/retention Alzheimer's prevention initiative Colombia plan. *Alzheim. Dement* 4, 283–287. doi: 10.1016/j.trci.2018.06.010

Roe, C. M., Behrens, M. I., Xiong, C., Miller, J. P., and Morris, J. C. (2005). Alzheimer disease and cancer. *Neurology* 64, 895–898. doi: 10.1212/01.WNL.0000152889.94785.51

Saini, A. (2017). *Inferior: How science got women wrong—and the new research that's rewriting the story*, Boston: Beacon Press.

Salamone, P. C., Legaz, A., Sedeno, L., Moguilner, S., Fraile-Vazquez, M., Campo, C. G., et al. (2021). Interoception primes emotional processing: multimodal evidence from Neurodegeneration. *J. Neurosci.* 41, 4276–4292. doi: 10.1523/JNEUROSCI.2578-20.2021

Salvadores, N., Moreno-Gonzalez, I., Gamez, N., Quiroz, G., Vegas-Gomez, L., Escandon, M., et al. (2022). Abeta oligomers trigger necroptosis-mediated neurodegeneration via microglia activation in Alzheimer's disease. *Acta Neuropathol. Commun.* 10:31. doi: 10.1186/s40478-022-01332-9

Salvadores, N., Shahnawaz, M., Scarpini, E., Tagliavini, F., and Soto, C. (2014). Detection of misfolded Abeta oligomers for sensitive biochemical diagnosis of Alzheimer's disease. *Cell Rep.* 7, 261–268. doi: 10.1016/j.celrep.2014.02.031

Schrouff, J., Pischedda, D., Genon, S., Fryns, G., Pinho, A. L., Vassena, E., et al. (2019). Gender bias in (neuro)science: facts, consequences, and solutions. *Eur. J. Neurosci.* 50, 3094–3100. doi: 10.1111/ejn.14397

Sibener, L. J., Kirchgessner, M. A., Steiner, S., Santiago, C., Cassataro, D., Rossa, M., et al. (2022). Lessons from the stories of women in neuroscience. *J. Neurosci.* 42, 4769–4773. doi: 10.1523/JNEUROSCI.0536-22.2022

Silva, A., Iyer, K., Cirulli, F., Del-Bel, E., Duenas, Z., Matta-Camacho, E., et al. (2022). Addressing the opportunity gap in the Latin American neuroscience community. *Nat. Neurosci.* 25, 1115–1118. doi: 10.1038/s41593-022-01154-x

Silva, A., Tomassini, C., Zurbrigg, J., Palacios, A. G., Amarante, V., and Bouzat, C. (2021). Gender inequality in Latin American neuroscience community. *Ibro Neurosci. Rep.* 10, 104–108. doi: 10.1016/j.ibneur.2021.01.006

Slachevsky, A., Budinich, M., Miranda-Castillo, C., Nunez-Huasaf, J., Silva, J. R., Munoz-Neira, C., et al. (2013). The Cuideme study: determinants of burden in Chilean primary caregivers of patients with dementia. *J. Alzheimers Dis.* 35, 297–306. doi: 10.3233/JAD-122086

Slachevsky, A., Zitko, P., Martinez-Pernia, D., Forno, G., Court, F. A., Lillo, P., et al. (2020). Gero cohort protocol, Chile, 2017-2022: community-based cohort of functional decline in subjective cognitive complaint elderly. *BMC Geriatr.* 20:505. doi: 10.1186/s12877-020-01866-4

Tassone, L. M., Urreta Benítez, F. A., Rochon, D., Martínez, P. B., Bonilla, M., Leon, C. S., et al. (2020). Memory reconsolidation as a tool to endure encoding deficits in elderly. *PLoS One* 15:e0237361. doi: 10.1371/journal.pone.0237361

Tomassini, C., Zurbrigg, J., Amarante, V., Silva, A., Palacios, A., and Bouzat, C. (2020). *Evaluation of gender inequities in Latin American neuroscience community*, Paris: Ibro Latin America Regional Committee/Eclac.

Torralva, T. (2022). *Upgrade cerebral: Cómo potenciar tus habilidades ejecutivas en la vida cotidiana*, Buenos Aires: Editorial El Ateneo.

Torralva, T., Bruno, D., Ciampa, M. A., and Bustin, J. (2021). *Saber acompañar*, Buenos Aires: Editorial El Ateneo.

Torralva, T., Gleichgerrcht, E., Ardila, M. J. T., Roca, M., and Manes, F. F. (2015). Differential cognitive and affective theory of mind abilities at mild and moderate stages of behavioral variant frontotemporal dementia. *Cogn. Behav. Neurol.* 28, 63–70. doi: 10.1097/WNN.0000000000000053

Torralva, T., Roca, M., Gleichgerrcht, E., Bekinschtein, T., and Manes, F. (2009a). A neuropsychological battery to detect specific executive and social cognitive

impairments in early frontotemporal dementia. *Brain* 132, 1299–1309. doi: 10.1093/brain/awp041

Torralva, T., Roca, M., Gleichgerrcht, E., Lopez, P., and Manes, F. (2009b). Ineco frontal screening (ifs): a brief, sensitive, and specific tool to assess executive functions in dementia. *J. Int. Neuropsychol. Soc.* 15, 777–786. doi: 10.1017/S1355617709990415

Villalba, A. C., Garcia, J., Ramos, C., Cuastumal, A. R., Aguillon, D., Aguirre-Acevedo, D. C., et al. (2019). Mental disorders in young adults from families with the Presenilin-1 gene mutation E280A in the preclinical stage of Alzheimer's disease. *J Alzheim. Dis. Rep.* 3, 241–250. doi: 10.3233/ADR-190139

Von Bernhardi, R., Eugenin-Von Bernhardi, L., and Eugenin, J. (2015). Microglial cell dysregulation in brain aging and neurodegeneration. *Front. Aging Neurosci.* 7:124.

West, J. D., Jacquet, J., King, M. M., Correll, S. J., and Bergstrom, C. T. (2013). The role of gender in scholarly authorship. *PLoS One* 8:e66212. doi: 10.1371/journal.pone.0066212

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Steroid hormones: risk and resilience in women's Alzheimer disease

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More women have Alzheimer disease (AD) than men, but the reasons for this phenomenon are still unknown. Including women in clinical research and studying their biology is key to understand not just their increased risk but also their resilience against the disease. In this sense, women are more affected by AD than men, but their reserve or resilience mechanisms might delay symptom onset. The aim of this review was to explore what is known about mechanisms underlying women's risk and resilience in AD and identify emerging themes in this area that merit further research. We conducted a review of studies analyzing molecular mechanisms that may induce neuroplasticity in women, as well as cognitive and brain reserve. We also analyzed how the loss of steroid hormones in aging may be linked to AD. We included empirical studies with human and animal models, literature reviews as well as meta-analyses. Our search identified the importance of 17-b-estradiol (E2) as a mechanism driving cognitive and brain reserve in women. More broadly, our analysis revealed the following emerging perspectives: (1) the importance of steroid hormones and their effects on both neurons and glia for the study of risk and resilience in AD, (2) E2's crucial role in women's brain reserve, (3) women's verbal memory advantage as a cognitive reserve factor, and (4) E2's potential role in linguistic experiences such as multilingualism and hearing loss. Future directions for research include analyzing the reserve mechanisms of steroid hormones on neuronal and glial plasticity, as well as identifying the links between steroid hormone loss in aging and risk for AD.

KEYWORDS

steroid hormones, resilience, Alzheimer's disease, macroglia, microglia, BDNF, plasticity, verbal memory

1. Introduction

Although abundant research has documented sex disparities in Alzheimer's disease (AD; World Health Organization, 2021), the sources of these sex differences remain disputed. Women live longer than men and this has been suggested as one of the reasons. Indeed, old age is the most common risk factor for AD, but age alone cannot explain the fact that two-thirds of the AD global cases are women (Snyder et al., 2016). There are likely other factors involved such as sex chromosomes, steroid hormones, brain structure differences, and varying life experiences. Moreover, there are several forms of AD, and they may affect women and men differently (Snyder et al., 2016). AD can be classified into early (≤65 years) or late, familial-sporadic (>65 years) onset. Early onset AD is rare (only 5% of all AD cases) and has a genetic background. Late onset AD represents most AD cases and has a less clear background (Bekris et al., 2010).

Consider the first case of AD identified which we would now call early onset – that of a 51-year-old woman named Auguste Deter. In 1906, Dr. Alois Alzheimer noticed that Auguste had abnormal behaviors such as short-term amnestic problems, disorientation, and dysphasia. He diagnosed the case as 'presenile dementia' and later brain biopsy showed senile plaques, neurofibrillary tangles, and atherosclerotic alteration. Since then, numerous studies have confirmed this constellation of signs as AD and the disease has become one of the most intractable, worldwide problems in medicine. AD is complex, there are different subgroups throughout the lifespan (Lima et al., 2022), and its trajectory and phenotypes are typically influenced by multiple pathological processes such as α-synucleinopathologies, vascular pathology, non-AD-tauopathies, etc. (Dubois et al., 2021). Despite its complexity, it is crucial to understand sex differences because biological sex may have a distinctive role in the cause and trajectory of AD.

In this respect, postmortem studies have shown that women have more severe neurodegeneration than men (Filon et al., 2016); and transgenic animal models of AD-like brain amyloidosis have found that female mice have increased plaque load burden and higher levels of soluble and insoluble Aβ40/Aβ42 than age-matched males (Callahan et al., 2001). In humans, it was initially thought that apolipoprotein ε4 (*APOE ε4*) may place women at higher risk than it does men (Altmann et al., 2014). However, a meta-analysis with data on nearly 60.000 participants revealed that only women with *APOE ε4* who are younger than 55 are at increased risk of developing AD (Neu et al., 2017). Recent evidence suggests that sex differences in AD may be more strongly related to regional tau pathology (Buckley et al., 2020) with a longitudinal PET study revealing that women have an increased accumulation rate of tau pathology, even when adjusting for baseline tau load (Smith et al., 2020). These findings demonstrate that clinical research needs to explore sex differences in order to discover specific pathways to AD (e.g., AD tauopathies vs. non-tauopathies, early vs. late onset, etc.).

Indeed, these studies have focused on only some of the theories that explain the cause of AD such as beta amyloid and tau, but female sex has a crucial role in most theories about AD. For instance, the mitochondrial cascade hypothesis posits that mitochondrial DNA (mtDNA) affects a person's risk of AD (Swerdlow et al., 2014), in this case mothers would have a bigger contribution to risk in their offspring, as mtDNA is maternally inherited. Moreover, estradiol loss due to menopause leads to a reduced mitochondrial function (Scheyer et al., 2018); which in turn may lead to cognitive impairment (van der Windt et al., 2012). Alternatively, AD may be the result of mixed disease pathology given multiple co-pathologies are present in AD patients. A typical AD co-pathology is vascular (Pasquier et al., 1998) as neurovascular dysfunction contributes to cognitive decline (Tarantini et al., 2017). Importantly, neurovascular function is influenced by gonadal hormones (Honarpisheh and McCullough, 2019); in particular, E2 improves blood flow (Collins et al., 1995). In this sense, the decrease in E2 production at menopause is a known risk factor for women's cardiovascular disease (Vogel et al., 2021). Moreover, the presynaptic protein α-synuclein (αSyn), mainly associated with synucleinopathies may be involved in the pathophysiology of AD. For instance, dementia with Lewy bodies (DLB) is one of the disorders referred to as a-synucleinopathies and, while the disease is more prevalent in men (Kane et al., 2018), once manifested, it has a more aggressive course in women (Van de Beek et al., 2020). Post-mortem examinations reveal that women generally present mixed pathology (DLB + AD) while men show 'pure' DLB pathology (Barnes et al., 2019; Van de Beek et al., 2020).

If there are possible sex differences in risk, cause, and trajectory of AD, it is intuitive that there might also be sex differences in resilience–a general term used to refer to several mechanisms that increase reserve and maintain function in the face of AD pathology (Stern et al., 2020). There is both cognitive and brain reserve. Individuals with high cognitive reserve show better than expected cognitive performance given the degree of pathology (Stern, 2002). Brain reserve is the neurobiological state of the brain, such as number of neurons. It can be measured by the connectivity, response, and structural integrity of key brain areas like the hippocampus. Brain maintenance, another type of resilience, refers to reduced age-related brain changes over time based on biological factors or lifestyle; factors that lead to brain maintenance can add to brain reserve (Stern et al., 2020). Since women live longer than men, it is important to consider these resilience mechanisms that are also involved in women's aging. Specifically, there might be sex differences in resilience related to steroid hormones and sex chromosomes. For instance, a second X chromosome may offer resilience to women (Davis et al., 2020) and steroid hormones might also influence plasticity (Jacobs and Goldstein, 2018) shaping women's cognitive and brain reserve.

The first report of resilience, while it did not analyze data by sex, was a study with a cohort that was 81% women. The authors examined post-mortem brains of 137 older adults and found extensive AD pathology in some of these patients, but also greater number of neurons, and higher brain weight when compared to age matched controls (Katzman et al., 1988). When considering this study from the perspective of brain reserve, one can imagine that these patients might have had some resilience mechanism that helped them to avoid neuron loss. An example of cognitive reserve comes from one of the most famous longitudinal studies of aging, a cohort of all women (Snowdon, 1997). The authors studied the written autobiographies of 678 novitiate nuns from the Notre Dame congregation, using linguistic density (ability to produce complex human communication) to predict resilience. The results showed that early life texts lacking linguistic density were correlated with increased late-life AD. This was one of the first studies using verbal processing to measure resilience. Nowadays, a verbal advantage is suggested as a form of cognitive reserve in women (Sundermann et al., 2016), but the underlying mechanisms are still not clear.

The purpose of the present review is to explore the possible mechanisms underlying risk and resilience in women especially as they might be mediated by steroid hormones. Note that we limited our searches to studies considering biological sex. The Canadian Institute of health research (CIHR) defines sex as female or male but recognizing variation in the biological aspects that comprise sex and how those aspects are expressed. Gender, on the other hand, refers to the socially constructed roles, behaviors, and expressions of gender diverse people. In this manuscript, we focus on sex instead of gender because we review articles that have investigated biological aspects (e.g., sex hormones, chromosomes) related to risk and resilience. First, we review the role of steroid hormones loss in aging as risk factor for AD. Then, we review the evidence that steroid hormones play a role in the plasticity of neuronal as well as nonneuronal brain cells considering plasticity's contribution to resilience. We also focus on other possible factors that may further confer cognitive and brain

reserve against AD in women. Finally, we highlight existing gaps in the literature, and new avenues for future research.

2. Literature search

We undertook a wide search of the literature in order to understand the interconnectedness of steroid hormones, risk for AD, plasticity, and resilience. To do this, we used a combination of all the following key words or phrases for the search: aging, AD, brain plasticity, steroid hormones, sex differences, glial cells, estrogens, estradiol, estrogen replacement therapy, cognitive reserve, brain reserve, verbal memory in women, and linguistic experience. Main searches were conducted using PubMed, and PsychINFO databases. A manual search of the reference lists of included studies was also used to identify relevant articles–Google Scholar was used for these specific searches. Articles published between 1990 and April 2023 were included. We included articles starting in 1990 because the National Institutes of Health (NIH) Revitalization Act that required the inclusion of women in NIH-funded clinical research was done in 1993.We conducted a synthetic review of the literature and followed PRISMA guidelines for the selection of articles. We then organized articles into themes and subthemes to make results clearer. This combined approach allows for the integration of many different types of articles, the creation of new theoretical perspectives, the identification of gaps in the literature and the implementation of rigorous methodology for the selection of articles. Thus, we included empirical studies with human and animal models, literature reviews as well as meta-analyses to enhance our knowledge of the literature and be able to identify new emerging perspectives. Inclusion criteria were: 1990 and later, articles that were written in English. Exclusion criteria were: articles primarily focused on neurodegenerative diseases other than AD (e.g., Huntington), neuronal or nonneuronal associations with dementias other than AD, and studies focusing on gender instead of biological sex. We also excluded gray literature such as theses or conference proceedings. Initially, a total of 4,808 articles were identified as potentially relevant, but after screening only 158 full articles were selected (see Figure 1). Note that we also drew on foundational papers that were not part of the search – such as Hebb (1949)–but that formed the conceptual foundation of our questions. Where these are used, they are marked with $^{+}$ to designate that they were not part of the search. Moreover, our search led to the referencing of other disorders – such as fronto-temporal dementia (FTD); strictly speaking these are not part of the results but we do address them in the discussion.

We extracted data for all 158 studies. We then entered these data into an Excel file with the following headers: data source, study title, methods and main findings. Based on the full-article texts, the articles were divided into 2 categories: (1) Steroid hormone effects on neuronal/glial cells and risk for AD, and (2) Resilience in women. These categories were not mutually exclusive–meaning that articles addressing risk could also address resilience. We further evaluated articles in each category and identified main themes. To do this, we looked for ideas discussed across and in most articles. Thus, each article was assigned to at least one of the following main themes: (a) the positive or negative effect of steroid hormones on neurons, (b) the positive or negative effect of steroid hormones on glia, (c) neuroplasticity, steroid hormones and brain reserve, (d) verbal cognitive reserve, (e) sex differences and linguistic experience (Figure 2). A narrative synthesis method was used to describe the results.

3. Results

3.1. Steroid hormone effects on neuronal/glial cells and risk for AD

The average adult brain contains approximately 100 billion cells including neurons and glia cells (nonneuronal). Both neurons and glia are influenced by progesterone, estrogens, and androgens (testosterone) (Chowen and Garcia-Segura, 2021). These steroid hormones modulate neural activity, synaptic plasticity, growth factor expression, neuronal survival (Pike et al., 2009), and cognition (Sherwin, 1997; Boyle et al., 2021). Steroid hormones also interact with certain genes located on the X and the Y chromosomes (Raznahan and Disteche, 2021), and they affect glial cells (Mellon and Vaudry, 2001; Garcia-Ovejero et al., 2005). Recent evidence indicates that steroid hormones may protect the brain against AD pathology (Pike et al., 2009; Vegeto et al., 2020); and thus may explain the sex differences in AD (Einstein, 1999; Nebel et al., 2018; Vegeto et al., 2020; Guo et al., 2022). However, most of the evidence comes from studies analyzing steroid action on neurons, showing sex specific patterns of disease manifestation and differences in the rates of cognitive decline and pathology (for a review see Ferretti et al., 2018). The question of how glia cells contribute to sex differences in AD is less clear. The focus of this section is to review what is known about the impact of steroid hormones at the cellular level in both neurons and glia to better understand how they might contribute to sex differences in risk for AD.

3.1.1. The positive or negative effect of steroids hormones on neurons

Estrogens (estradiol, estriol and estrone), pregnanes (progesterone and allopregnanolone) and androgens (testosterone and dihydrotestosterone) are steroidal hormones produced by the gonads, adrenal glands and other tissues; perhaps most importantly, the brain (Ruiz-Cortés, 2012). All steroid hormones are present in both biological sexes, but at different levels (Miller et al., 2017). There are multiple and different receptors for estrogens (ER, subtypes α and β), progesterone (PRs, subtypes A and B), and androgens (AR). These receptor's interaction with steroid hormones can affect the nervous system directly or indirectly(Ruiz-Cortés, 2012).

For the most part, research exploring the mechanisms underlying sex differences in AD have focused on the effects of E2 on neurons. For instance, in an animal study, Miranda et al. (1999) showed sex differences in estrogen deprivation on aged rodent dentate granule cells. The authors deprived and restored estrogens to young and old gonadectomized female and male rats and explored whether the secretion of gonadal steroids led to morphological changes to neurons in the hippocampus–an important brain region for memory and learning and affected early in AD (Mielke et al., 2012). Results showed that dentate granule cells of estradiol-deprived female rats had lower spine density than that of testosterone-deprived males or young females with short term estrogen deprivation. Similar effects were reported in female macaques after surgical menopause, showing spine

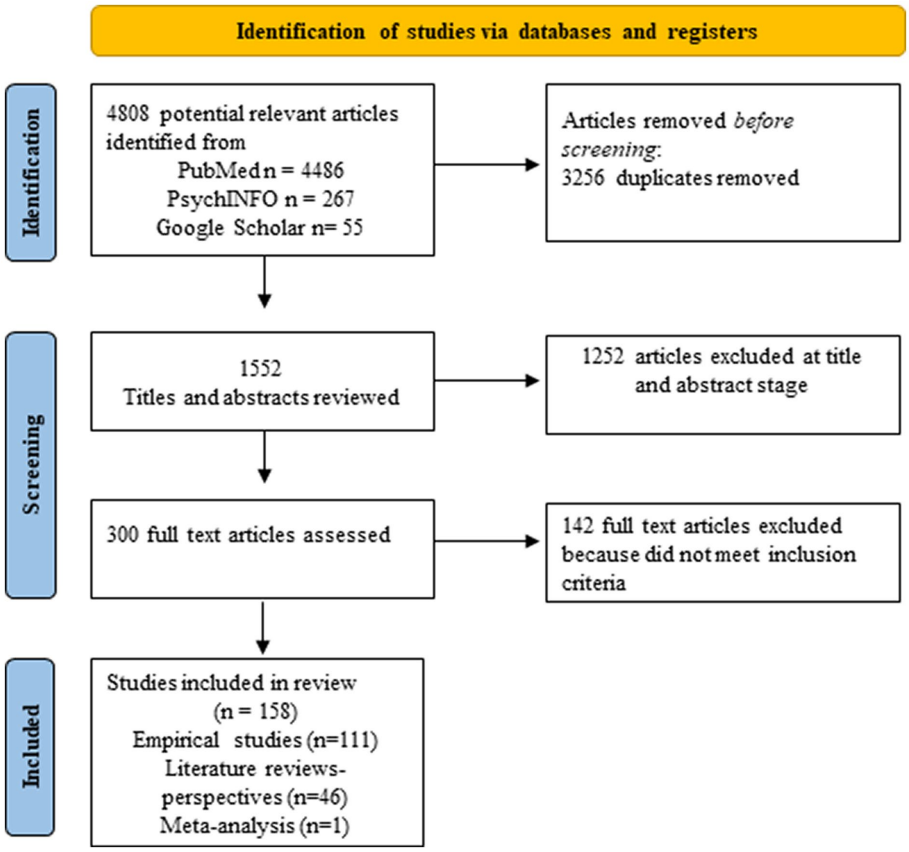

FIGURE 1
PRISMA flow diagram of the study selection process. The diagram describes all articles included in section 3.

density loss in multiple cortical regions including hippocampal CA1 neurons (Dumitriu et al., 2010). In humans, steroid patterns were reported for postmenopausal women in a study exploring the effects of estrogen in AD. In this case, the authors studied estrogen use in a cohort of 1,124 elderly women in a longitudinal study of aging. E2 use was associated with a delay in the onset as well as a lower risk for AD (Tang et al., 1996). However, the question of hormone replacement therapy (HRT) for older women and in AD is a controversial one. The hypothesis behind this question is that the use of exogenous hormones (e.g., E2) may ameliorate cognitive decline in menopause. The question has critical implications for clinical treatment but human research has reported mixed findings.

Several studies replicated the findings by Tang et al. (1996) using prospective approaches (Baldereschi et al., 1998; Jacobs et al., 1998; Zandi et al., 2002; Shao et al., 2012), cross-sectional (Steffens et al., 1999; Lau et al., 2010), and case control studies (Henderson et al., 1994, 2005). Indeed, a recent meta-analysis including studies using different approaches (prospective, retrospective, etc.) confirmed the benefits of postmenopausal use of E2 and concluded that it significantly decreased the risk of onset and/or development of AD (Song et al., 2020). This meta-analysis; however, did not include the largest randomized clinical trial linking HRT to AD which found the opposite effect. The Women's Health Initiative (WHI; Shumaker et al., 2003) was a complex study exploring strategies for the prevention of heart diseases in postmenopausal women. In brief, the results showed that HRT did not benefit and might even be detrimental with respect to heart disease, stroke, blood clots, breast cancer and cognitive decline. Notably, the Women's Health Initiative Memory Study (WHIMS; Shumaker et al., 1998, 2004) replicated these findings–specifically for cognition. Taken together, these studies led to ceasing recommending conjugated equine estrogens (CEE) plus medroxyprogesterone acetate (MPA) to postmenopausal women. Debate about the methodology and cohort of the WHI and its ancillary studies (WHIMS) suggested that these studies were not assessing prevention as most of the women included in the cohort were many years past menopause (Lobo, 2013). The question of the benefits of estrogen replacement therapy (ERT as opposed to CEE) still needs answering because E2 loss after menopause has been correlated with cognitive decline and hence, risk for AD. Also, the question of when hormone replacement begins is important. A recent study found that earlier age at menopause and late initiation of HRT were significantly associated with increased regional tau in the context of high β-amyloid (Coughlan et al., 2023). If women began HRT exactly at the onset of menopause, they did not have higher tau proteins in the brain. However, AD markers were elevated if they delayed the treatment to more than 5 years after menopause.

The possible positive effects of estrogens can also be studied in women who have had bilateral salpingo-oophorectomy (BSO) which leads to a rapid and deep decrease level of E2; they show increased risk

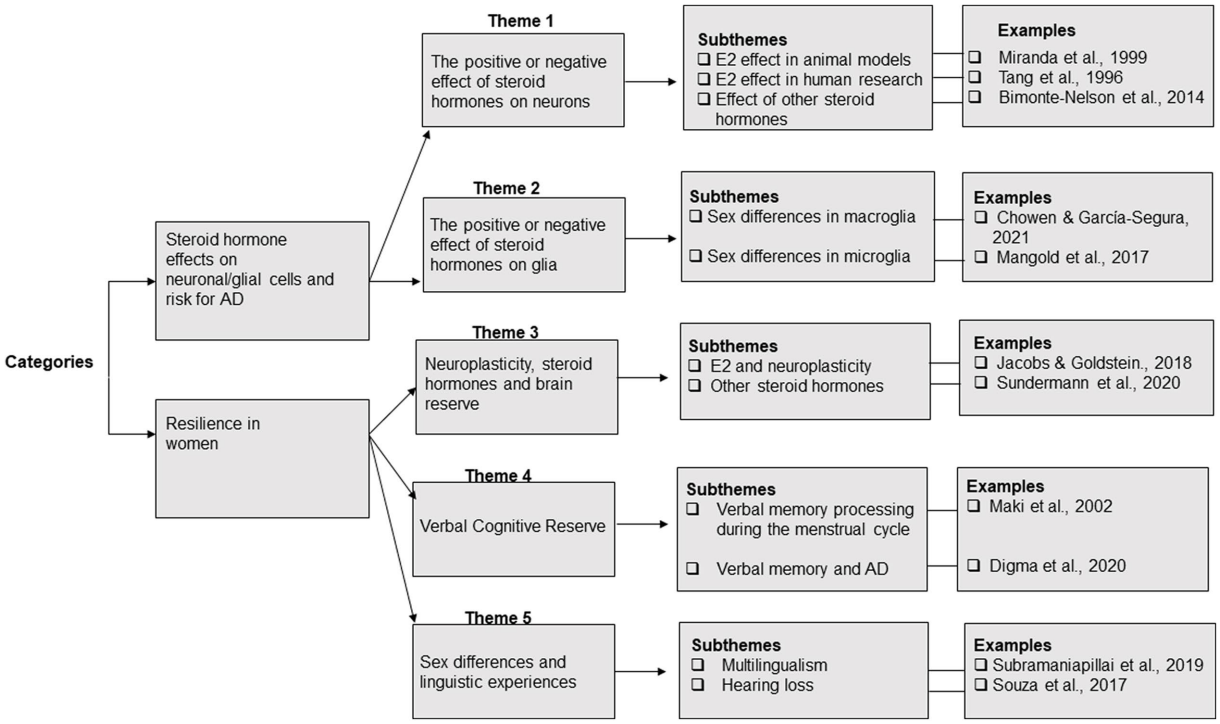

FIGURE 2
Overview of the analytic process and the categories, themes and subthemes that emerged from the analysis.

of cognitive impairment in late life (Rocca et al., 2007) as well as up to 5 years post-BSO (Gervais et al., 2020), sleep disturbances (Kaunitz et al., 2021), and increased inflammation (Au et al., 2016). Women who underwent BSO before 50 years old have shown smaller amygdala volumes, thinner parahippocampal-entorhinal cortices, and lower entorhinal white matter fractional anisotropy values than women who kept their ovaries (Zeydan et al., 2019). Women with BSO before spontaneous menopause also have an increased risk of MCI and decreased performance on global cognition, attention, executive functions, and short Mental status test (Rocca et al., 2021). Taken together, these findings show that early life BSO is a risk factor for AD which highlights the importance of understanding the unique effects of each menopause and steroid hormones loss (Edwards et al., 2019). There is a more generally acknowledged importance of E2 production in staving off cognitive decline and eventual AD (Pike, 1999; Koski et al., 2004; Quinn et al., 2022) with proposed mechanisms including inducing and maintaining hippocampal spine density (Woolley and McEwen, 1994; Miranda et al., 1999; Leranth et al., 2002; Wallace et al., 2006; Hajszan and Leranth, 2010; Bowman et al., 2015) as well as by controlling β-amyloid accumulation (Gandy and Petanceska, 2000; Qin et al., 2020).

Progesterone as well as estrogens regulate brain-derived neurotrophic factor (BDNF) which influences survival, growth, and maintenance of neurons (Chan and Ye, 2017), and thus the effects of progesterone should also be studied. In animal studies, progesterone has been linked to the survival rate of newborn hippocampal neurons in rats (Deniselle et al., 2007). Progesterone also has a neuroprotective effect, but it seems to be E2-dependent as it reduces E2-induced BDNF (Bimonte-Nelson et al., 2004). More specifically, long-term progesterone treatment in aged ovariectomized female rats has been found to block the beneficial effects of E2 on neurotrophic factor, nerve growth factor, and neurotrophin 3 (Bimonte-Nelson et al., 2004). Thus, the effects of progesterone on neurons should be carefully analyzed considering its interaction with estrogens. Indeed, the few studies that have been conducted in non-human animals indicate that progesterone alone may have some benefits on neuronal activity, but it may also have an antagonistic effect when combined with estrogens (Pike et al., 2009). Understanding the interaction between estrogens and progesterone is crucial because it may lead to a more accurate interpretation of research and clinical trials. For instance, the WHI trial mentioned above reported that CEE therapy leads to an increased risk of AD, but current empirical work suggests that interactions with progesterone may have obscured estrogen's effects on AD in that study (Pike et al., 2009).

Testosterone is important to consider as it, too, may regulate BDNF (Gao et al., 2019). In non-human animals, androgens reduce neurite outgrowth (Zelleroth et al., 2021), but they may also promote neuron viability (Creta et al., 2010), and offer protection against apoptosis in hippocampal neurons (Nguyen et al., 2010). Humans with prostate cancer treated by androgen deprivation have an increased risk for AD (Seruga and Tannock, 2008). Thus, androgens may also play a contributing role in supporting neurons against AD degeneration. In both women and men, low testosterone may increase the risk of AD. However, only a few studies have explored testosterone as an AD therapy; unsurprisingly, most of these studies are in men (Pike et al., 2009). To our knowledge, no study has explored the effects of testosterone therapy or its interaction with E2 therapy for AD in women.

3.1.2. The positive or negative effect of steroid hormones on glia

Glial cells include macroglia (oligodendrocytes and astrocytes) and microglia; together these non-neuronal cells transport nutrients and carry out other metabolic processes, influence synaptic communication, protect neurons from oxidative stress, and modulate neuronal activity (Heneka et al., 2010; Chowen and Garcia-Segura, 2021). In particular, oligodendrocytes provide insulation through the production of myelin, allowing for high-speed transmission of signals. They support and maintain the integrity of axons (Larson, 2018). Oligodendrocyte function depends on both astrocytes and microglia. Indeed, oligodendrocytes connect intracellularly with astrocytes to aid in the removal of myelin debris (Raff et al., 1988). Astrocytes contribute to brain homeostasis, regulate synaptic plasticity, neurotransmission (Ferrer, 2017), and have long been considered crucial in sleep, and local blood flow (Ramon y Cajal, 1895; Howarth, 2014). Microglia are considered "surveillance cells" and are in charge of maintaining brain homeostasis, protecting against disease and infection (Graeber and Streit, 2010). They regulate CNS processing both via their connections with astrocytes and by interacting with synapses (Wake et al., 2009). Microglia can be protective by clearing cellular debris or deleterious by driving inflammation depending on their state of activation and the influence of steroid hormones (Larson, 2018).

Glia cells are also linked with steroid hormones. Oligodendrocytes secrete progesterone, and astrocytes secrete progesterone, testosterone, and estradiol-among other steroids (Gago et al., 2001; Garcia-Ovejero et al., 2005). Microglia have receptors for some of these steroids and are thus steroid-sensitive (Garcia-Ovejero et al., 2005). Recent evidence indicates that there might be more pronounced age-related changes in cell state, gene expression, and localization of glia cells than in neurons (Allen et al., 2023). Thus, the effects of steroid hormones on nonneuronal cells are of utmost importance to understand sex differences in aging. Sex differences in glia may affect the etiology of pathological conditions, and thus may also contribute to the possible sex differences in risk.

There are sex differences in macroglia. For instance, female rodents' oligodendrocytes have a shorter lifespan than those in males but increased proliferation (Cerghet et al., 2006), and better migratory ability (Yasuda et al., 2020). With respect to neurodegenerative disease, an analysis of 80,660 single-nucleus transcriptomes from the postmortem prefrontal cortex of 48 individuals with AD revealed that higher AD pathology correlated with more global transcriptional activation in male oligodendrocytes than in female. Indeed, female oligodendrocyte precursors exhibited a down-regulation shift in response to pathology, a pattern that was not found in male cells (Mathys et al., 2019). These results offer new avenues to study myelination-related processes in AD pathogenesis. Given that E2 promotes oligodendrocytes myelin protein survival (Takao et al., 2004), these findings suggest that E2 play an important role in maintaining women's white matter. Sex differences have also been shown for astrocyte function, number, and morphology (Sofroniew and Vinters, 2010; Chowen and Garcia-Segura, 2021). In non-human animal models, astrocyte proliferation is higher in females than in males (Llorente et al., 2009). Notably, estrogens acting on astrogliosis may lead to neural regeneration (Le Prince et al., 1993), and neuroprotection against AD (Liang et al., 2002). Progesterone may also provide neuroprotection against Aβ-induced neuroinflammation in astrocytes (Hong et al., 2016). A recent study has shown astrocytic dysfunction in AD (Serrano-Pozo et al., 2022), but the question of steroid hormones and sex differences related to astrocytes during AD awaits further research.

Microglial sex differences have been reported in number (Mouton et al., 2002), gene expression (Villa et al., 2019), and morphology (Lenz et al., 2013). Aging impacts microglia in that it affects gene expression and their reaction to stimulation (Chowen and Garcia-Segura, 2021). Critically, microglia have been suggested as the predominant cell to target AD pathology (Hu et al., 2023) and female microglia may age faster (Olmedillas del Moral et al., 2020). Greater expression of inflammation-related transcripts of microglia has been found in aged females than in males (Mangold et al., 2017). Moreover, microglia may contribute to the higher AD risk associated with *APOE ε4* in females (Stephen et al., 2019). In this respect, a recent study has shown an effect of *APOEε4* on medial temporal microglial activation leading to AD progression, this effect was independent of Aβ plaques and tau tangles (Ferrari-Souza et al., 2023). The study controlled for sex, but did not look at sex differences. This is important because their cohort of cognitively impaired individuals was 78% women, while the MCI individuals were almost 70% men. Finally, it has also been suggested that microglia are vulnerable to death by ferroptosis-a type of cell death that involves iron-dependent lipid peroxide accumulation (Dixon et al., 2012). Ryan et al. (2023) hypothesized that ferroptosis in microglia may drive neurodegeneration. Using a novel tri-culture system to create neurons, astrocytes and microglia, the authors found that microglia were the most responsive to iron and that ferroptosis in response to iron overload had a unique transcriptional signature found in neurodegeneration. These results are important because they may explain crucial mechanisms underlying neurodegenerative diseases. Indeed, E2 depletion in women after menopause may result in significant transcriptomic changes related to inflammation (Sárvári et al., 2012), and thus steroid action on microglia may explain the possible sex differences in AD.

3.2. Resilience in women

The concept of resilience is used to explain inter-individual variability in the trajectories of cognitive decline and AD (Stern, 2002). However, this term represents multiple concepts: cognitive reserve, brain reserve, compensation; and each of these concepts have different definitions. Here, we follow the current consensus framework and refer to resilience as a general concept that includes any term related to the ability of the brain to maintain cognition and function against normal or pathological aging (Stern et al., 2020). Following this approach, we focus on cognitive reserve as the flexibility of cognitive processes in the face of normal aging or disease, and brain reserve as the neurobiological state of the brain at any point in time: number of neurons, synapses, white matter integrity, etc. (Stern et al., 2020).

Indeed, much attention has been devoted in the last decades to studying resilience against AD and arrive at a consensus definition, but little attention has been devoted to elucidating the resilience proxies for women (Subramaniapillai et al., 2021) which might be different to those found in men. It is hypothesized that women may have had less access than men to some reserve-building factors such as education or work opportunities, and this, might in turn, lead to

less cognitive reserve (Subramaniapillai et al., 2021). However, steroid hormones like estrogens and progesterone may affect brain plasticity and, in this way, contribute to brain reserve. A verbal advantage is also generally reported in women compared to males–even in MCI (Sundermann et al., 2016); thus, women's verbal advantage can be considered a cognitive reserve proxy only among women. Crucially, the relationship between loss of estrogens and cognitive decline is a well-established phenomenon (Bove et al., 2014), and it may be the link needed to understand plasticity and resilience in older women.

3.2.1. Neuroplasticity, steroid hormones, and brain reserve

Neuroplasticity is the result of functional and molecular changes within the CNS due to either experience/learning, injury, disease, or cell death (Hebb, 1949; Fuchs and Flügge, 2014). Steroid hormones play an essential role in neuroplasticity because they evoke changes in neuronal excitability that may induce synaptic plasticity (Baudry et al., 2013). Moreover, major hormonal shifts throughout the lifespan–puberty, pregnancy, menopause, and aging–typically trigger neuroplastic processes (Been et al., 2022). Aging is characterized by gonadal functional loss and is accompanied by subtle cognitive decline and frailty. These fluctuations of steroid hormones due to aging occur in both men and women, but the fluctuations in men are a gradual process and occur slowly with advancing age (Morley, 2003). In women, changes are more sudden, profound, drastic, and variable. For instance, motherhood is a period marked by deep neuroplastic changes (Pawluski et al., 2022; Orchard et al., 2023). Similarly, menopause may induce plasticity and neural reorganization (Jacobs and Goldstein, 2018); but there are several types of menopauses with the ovarian cycle ceasing for varied reasons – aging, ovarian surgery, cancer treatment–and at different ages (Edwards et al., 2019). Thus, it is important to also consider the reasons for menopause to determine the extent to which it induces neural plasticity. Despite this variability, neural plasticity – including regions that mediate memory–seems to be a constant in the lifespan of women (Sheppard et al., 2019). In humans, E2 shapes memory circuits in women by promoting hippocampal plasticity (Jacobs and Goldstein, 2018); in non-human animals it is been shown to control hippocampal and prefrontal cortical (PFC) dendritic spine proliferation (Hara et al., 2015). Indeed, the different type of estrogens may affect neuroplasticity and cognition in different ways; they may modulate hippocampal plasticity via cell proliferation and this effect is dependent on type, time and dose (Barha and Galea, 2010). Low levels of E2 may lead to better performance on spatial working memory and contextual fear conditioning, while high levels may impair spatial working memory, spatial reference memory, and contextual fear conditioning. Estrone also seems to impair contextual fear conditioning (Barha et al., 2010).

E2 has also been related to women's PFC neural efficiency. For instance, in healthy young women, optimal cortical dopamine (DA) activity was linked to overall reduced PFC activity. However, PFC activity enhanced with greater demands for cognitive control in accordance with the neural efficiency hypothesis (Jacobs and D'Esposito, 2011). Thus, E2 may enhance cortical dopamine activity and alter PFC-dependent cognitive functions such as spatial memory and executive function. Moreover, E2 may be linked to hippocampal neurogenesis (Sager et al., 2018), essential to preserve cognition (Klempin and Kempermann, 2007). Importantly, in female rodents E2 seems to modulate neurogenesis while in male rodents it is androgens (Yagi and Galea, 2019). In addition, repeated administration of E2 to female rodents was related to increased cell proliferation and decreased cell death in the dentate gyrus (Barker and Galea, 2008). A transgenic AD mouse model showed that perturbed neurogenesis may result in new neurons having hyperphosphorylated tau, reduced hippocampal circuitry, and cognitive deficits like those seen in AD (Hollands et al., 2017).

Microglia cells show the highest level of CNS plasticity (Augusto-Oliveira et al., 2022). Of note, females have higher microglia density in the hippocampus during early development than males (Yanguas-Casás, 2020). In menopause, E2 depletion has a significant impact on microglia which results in an increased inflammatory phenotype (Yanguas-Casás, 2020). Interestingly, a recent study has shown that resilience is highly heritable in both sexes, but there might be sex specific genetic drivers of resilience. Genetic predisposition toward resilience may be linked to less genetic risk for autoimmune conditions among females (Eissman et al., 2022). In this sense, there is a dysregulation in immune response in AD pathogenesis, inflammatory process may play a primary role (Sardi et al., 2011), and E2 action in microglia may help elucidate the mechanisms underlying inflammation, AD and resilience in women.

The effects of progesterone on plasticity are not fully understood. Only a few studies have researched this question and the evidence shows contradictory results. One study showed that in CA1 slices from 4-week-old rats progesterone had no effect in long term potentiation (Ito et al., 1999), while others have shown an effect of progesterone on hippocampal synaptic transmission, likely mediated by $GABA_A$ receptors (Foy et al., 2008). Progesterone may influence synaptic plasticity (Baudry et al., 2013), but its effects in resilience against AD for women are yet to be explored. As noted previously, interpretation about the effects of progesterone should carefully consider E2 activity. With mitochondria as a new perspective on women and AD (Nilsen and Brinton, 2004), a mouse model exploring the effects of progesterone and E2 on mitochondrial function is important. One study showed progesterone and E2 in opposition; both steroids improve mitochondrial function when administered separately but attenuate it when replaced in combination (Irwin et al., 2008).

Similarly, testosterone has been related to synaptic plasticity (Jia et al., 2016) but sex differences in resilience related to testosterone have not been fully explored. Benefits for men have been reported at the cognitive level. High testosterone may result in better long-term memory in men than in women (Barrett-Connor et al., 1999) and its decrease in men has been linked to poor performance in visual and verbal memory as well as visuospatial processing (Moffat et al., 2002). Thus, testosterone may also induce lifelong plasticity. However, the role of testosterone in cognitive decline for old adult men is not clear. In elderly men with low-normal gonadal status, a comprehensive neuropsychological evaluation showed no effect of low-normal testosterone status on cognition (Haren et al., 2005). A resilience effect of testosterone in women has been reported when analyzed in conjunction with genetic profile. A study of the 172 participants (113 men and 59 women) aged 55–90 years in the ADNI database showed that the effects of low testosterone are particularly detrimental to cognition in women who are APOE ε4carriers (Sundermann et al., 2020). The cohort included 15% cognitively normal participants, 56% with MCI and 23% with AD. In this case, the authors studied separate and interactive effects of testosterone levels and APOE ε4 on

cerebrospinal fluid p-tau level. Sex difference in p-tau level was also considered before and after adjusting for testosterone. The results showed that women had higher p–tau levels than men among *APOE ε4* carriers, and this difference was eliminated when adjusting for testosterone. Thus, testosterone may induce plasticity in both men and women. Moreover, it may be protective against p-tau among women who are *APOE ε4* carriers. Importantly, women have typically less testosterone than men during the lifespan, and thus may be more at risk for AD due to higher levels of pathological Tau.

3.2.2. Verbal cognitive reserve

Women have a verbal memory advantage as they age and as noted, this lasts into the pre-AD stage of MCI (Sundermann et al., 2016). The reasons for this are still unknown, but verbal memory in women is highly dependent on steroid hormone levels. Women show variations in verbal processing during pregnancy (Glynn, 2010), postpartum (de Groot et al., 2006), and menopause (Schaafsma et al., 2010). Fine motor coordination and verbal fluency depend on stage of the ovarian cycle with women at the early follicular (low estrogen and progesterone) performing worse than those at midluteal (high estrogen and progesterone; Maki et al., 2002). Mental rotation in women also depends on the menstrual cycle phase: mental rotation skills decreased during the midluteal phase but are on a par with men's at early follicular (Hampson et al., 2014; Peragine et al., 2020). On the other hand, verbal fluency improves at high estradiol phases (Maki et al., 2002).

Verbal memory also improves in some postmenopausal women after taking estradiol hormone therapy (ET). Women taking estrogens had better verbal memory performance than those without (Kampen and Sherwin, 1994). This finding has been replicated in numerous studies (Sherwin, 1988; Jacobs et al., 1998; Wolf et al., 1999; Zec and Trivedi, 2002). Deficits in verbal memory are also reported in women after acute loss of ovarian function (Ryan et al., 2014; Soni and Hogervorst, 2014), and these effects can also be reversed with ET (Phillips and Sherwin, 1992; Newton et al., 1996; Sherwin and Tulandi, 1996; Craig et al., 2008). Similarly, verbal memory deficits have been reported in subjective cognitive decline which may lead to an increased risk for AD (Reuben et al., 2021). Attention and memory complaints of young women also vary across the menopausal transition. Data from 120 women who were either premenopausal, perimenopausal or postmenopausal showed attention complaints during perimenopause, and verbal memory problems directly related to SCD and menopause (Schaafsma et al., 2010). A study of SCD in a cohort of cognitively unimpaired individuals with autosomal-dominant AD who will develop dementia revealed that the women reported more SCD than men, and among female mutation carriers SCD was associated with worse verbal memory (Martinez et al., 2022).

The complaints in verbal memory among older women may be related to the loss of estradiol during menopause. However, once pathological cognitive decline begins, verbal memory may act as a form of reserve in some women. In an analysis of 742 participants with normal cognitive aging (NC), and early mild cognitive impairment (eMCI), the relationship between biological sex, verbal memory, hippocampal volume (HV) and florbetapir PET (an agent used to bind amyloid plaques for the potential detection of AD) showed that women with NC had a robust verbal advantage even in the face of amyloid positivity. On the other hand, women in the eMCI group, only showed decreased verbal memory in the face of amyloid positivity. The authors interpreted this finding as a verbal memory advantage for women with earlier stages of AD, but not in a stage of prodromal AD (Caldwell et al., 2017). Similarly, women with low to moderate Aβ burden (but not high Aβ burden) had increased verbal memory while men did not. Interestingly, this effect was specific to MCI (Sundermann et al., 2018). The relationship between biological sex, tau and verbal memory was explored in two different databases (National Alzheimer's Coordinating Center, and the Alzheimer's Disease Neuroimaging initiative) which revealed that in both, women had higher verbal reserve and were able to maintain this verbal reserve in the face of greater tau pathology than men (Digma et al., 2020). More recently, an analysis of sex differences in verbal memory and the distribution of tau pathology in MCI and AD patients showed a strong association between left hemisphere tau and verbal memory mainly in women with MCI (Banks et al., 2021).

The left hippocampus has been related to verbal memory, while the right hippocampus to spatial memory (de Toledo-Morrell et al., 2000). Both sides play a central role in the consolidation of memories and both suffer AD pathology. A study analyzing verbal memory in old adults found that the left hippocampal head may be the region directly associated with verbal memory (Hackert et al., 2002). E2 also modulates the metabolism of this as well as other brain regions (frontal, parietal, and temporal regions), and a few studies have shown that acute ovarian loss function may be linked to a decreased activation in the left inferior frontal gyrus (LIFG) during verbal memory encoding (Craig et al., 2009). Taken together, it's important to consider that the maintenance of skills associated with the hippocampus and PFC such as verbal memory may be related to resilience in women.

3.2.3. Sex differences and linguistic experiences

Given that there might be women's verbal advantage linked to estradiol, leading to cognitive reserve, it is essential to analyze different linguistic experiences and how they may provide resilience to cognitive aging. For instance, multilingualism has also been suggested as a cognitive reserve proxy (Bialystok et al., 2007). Multilinguals with dementia can tolerate greater brain atrophy than monolinguals with similar clinical dementia levels (Schweizer et al., 2012). This pattern has been confirmed worldwide (Klein et al., 2016), and in different stages of neurodegeneration such as MCI (Duncan et al., 2018), conversion from MCI to AD (Calvo et al., 2023), and AD (Chertkow et al., 2010). A study of sex differences in bilingual and monolingual healthy old adults using the Wisconsin card sort test (WCST) revealed that women had more cognitive decline than men, but their performance improved with bilingualism (Subramaniapillai et al., 2019). Although the authors did not relate their finding to reserve, these results suggest a relationship between biological sex and multilingualism that may affect resilience mechanisms. These results should be taken with caution as the authors did not measure steroid hormones which is crucial for an analysis of this relationship.

Hearing loss is another linguistic experience worth considering to understand sex differences in risk of AD and plasticity (Bruce et al., 2019). Hearing impairment leads to problems in understanding spoken language and cognitive processing (Pichora-Fuller, 2003). Men are more affected by hearing loss than women in old age (Curhan et al., 2019) and recent studies indicate that estradiol may support the

auditory system (Reavis et al., 2023). Indeed, a study investigating hearing threshold during the menstrual cycle found that the highest threshold in women is when estradiol levels are at its highest level (Da Da Souza et al., 2017). Moreover, premenopausal women have shown better extended high frequency hearing than postmenopausal women (Zhang et al., 2018), which emphasizes the need to consider estradiol effects in hearing loss or deafness in the elderly.

4. Discussion

The first person diagnosed with AD was a woman and also some of the most significant reports of resilience were done in cohorts including mostly women (Katzman et al., 1988; Snowdon, 1997). However, the higher risk of AD in women has only gradually come to recognition (Einstein, 1999). Historically, women have been excluded from clinical research because their biology was considered more complicated than that of men. This applied to both human and non-human animal research (Beery and Zucker, 2011; Shansky, 2019). In non-human animal models, female rats would typically be removed from experimentation because they were considered more "variable" and more expensive than males since they had to be tested at each stage of their estrous cycle. This assumption has been tested and refuted empirically (Prendergast et al., 2014). Nonetheless, most animal models of AD continue excluding females from their research (Beery and Zucker, 2011). In humans, policies have been implemented to ensure that women are included in clinical research. This has led to an expansion of research about women's health and sex differences in health and disease. Importantly, more women are now enrolled in randomized clinical trials for AD than males which makes the analysis of sex differences in AD more feasible. However, only 12.5% of published articles have reported sex-stratified results (Martinkova et al., 2021). Thus, the full possibilities of sex differences in etiology, progression, treatment response, risk, and resilience in AD remain unexplored. Addressing sex differences in risk and resilience for AD is crucial for advancing successful interventions for the majority of the sufferers. By including empirical studies, reviews and meta-analyses looking at the effects of steroid hormones on aspects of AD and resilience, the current review is one step in that direction.

4.1. Neuronal effects

Our literature review indicated that steroid hormones could affect the etiology and progression of AD. Animal studies showed that estrogen deprivation results in spine density loss in crucial regions such as the hippocampus. In humans, it has been suggested that use of estrogens may decrease cognitive decline in postmenopausal women. The issue of HRT in AD is a critical one that has suffered from a scientific paradigm shift. Current evidence shows that the question of *when* HRT begins is crucial. Future research should also explore the benefits of ERT instead of CEE as most of the current evidence indicates that E2 loss after menopause may lead to AD. Importantly, the effects of E2 in women who have had BSO is a unique window to understand risk and resilience mechanisms in midlife.

Moreover, estradiol's effect on hippocampal plasticity and neurogenesis (Jacobs and Goldstein, 2018; Yagi and Galea, 2019) may shape cognitive reserve in functions such as verbal memory (Sundermann et al., 2016; Digma et al., 2020). Progesterone may also have a beneficial effect but these positive effects may be lost when combined with estrogens (Pike et al., 2009). Testosterone may play a role in neurodegeneration in males; however, it may have a crucial role in guarding against p-tau in women who are *APOE ε4* carriers (Sundermann et al., 2018).

4.2. Non-neuronal effects

At the non-neuronal level, oligodendrocytes may aid resilience by protecting women's white matter but the relationship with sex steroids has not yet been clarified. Astrocytes may depend on estradiol and progesterone for function and its presence may be related to resilience in AD (Liang et al., 2002; Serrano-Pozo et al., 2022), but no study has analyzed sex differences related to astrocytic function in AD. Microglia are more affected by aging in women than in men (Olmedillas del Moral et al., 2020). Microglia may also affect hippocampal neurogenesis (De Lucia et al., 2016). A few studies suggest that microglia may be associated with *APOE ε4* and higher risk for AD in women (Stephen et al., 2019). However, there is a dearth of studies that would illuminate the interaction of steroid hormones and microglia and its impact on microglial function in the etiology and progression of AD. This may be an important direction to pursue as inflammation is associated with AD, inflammation increases in menopausal women, and microglia play a role in brain inflammation. Thus, the effect of E2 decreases in microglia for postmenopausal women may help elucidate their role and the risks associated with inflammation in AD.

4.3. Factors leading to resilience

With regards to resilience, steroid hormones may impact both cognitive and brain reserve. For instance, verbal memory performance is one proxy of cognitive reserve for women. Brain regions crucial to verbal memory are the hippocampus, the PFC and the LIFG and they are all sensitive to E2. Indeed, E2 may be related to the neural efficiency of verbal memory in women (Jacobs and D'Esposito, 2011). Conversely, E2 loss has been associated with decreased activation in the LIFG during verbal memory encoding (Craig et al., 2009) as well as decrements in executive function and spatial memory performance, mediated by the PFC. Understanding the role of verbal memory as a reserve factor in women is crucial because most of the neuropsychological tests used to diagnose AD involve verbal processing. If verbal memory is, indeed, a cognitive reserve factor in women, and women have preserved verbal function compared to men, more sensitive tools may be needed to diagnose AD in women. Moreover, multilingualism should be considered as this has also been suggested as a proxy for cognitive reserve. Verbal memory and multilingualism may have an additive effect on cognitive reserve for women, and thus multilingual women may, in fact, be diagnosed so long into the disease that neural degeneration has progressed long past rescue. Further research is needed to understand the complex relationship between verbal memory and bilingualism in resilience for women. Importantly, studies assessing this question should focus on E2 effects in multilingual women to truly address reserve mechanisms

in this cohort. Hearing loss is another linguistic experience that should be carefully considered. It seems to be more prevalent in men than in women (Curhan et al., 2019). Importantly, more men work in professions that affect hearing (e.g., armed forces; Thompson et al., 2016) and there is some thought that E2 is beneficial for maintaining the auditory system (Reavis et al., 2023). However, only a few studies are starting to explore these associations.

The analysis of estradiol on different linguistic experiences leads to reflection about other disorders in which language is affected. For instance, some neurodegenerative diseases are marked by language disturbances and thus the effects of estradiol in linguistic processing are crucial to understand the cause and course of these diseases. Consider the language variant of frontotemporal dementia (FTD) or primary progressive aphasia (PPAs). PPAs are typically sub-classified according to different linguistic criteria (Gorno-Tempini et al., 2011): a non-fluent/agrammatic variant (naPPA), a semantic variant (svPPA), and a logopenic variant (lvPPA). Few recent studies indicate sex differences in FTD in that women may be more affected by PPAs than men (Pengo et al., 2022). Indeed, women may have greater reserve to face FTD (Illán-Gala et al., 2021). However, to the best of our knowledge, no study has investigated estradiol effects in frontotemporal dementia. This is a critical new avenue to pursue as it may lead to the discovery of biomarkers that may help in the diagnosis and treatment of FTD as well as AD. It may also lead to the discovery of resilience mechanisms that are specific to FTD but not AD, and *vice-versa*.

4.4. Strengths and weaknesses

The strength of the current review is that it is synthetic, highlighting themes arising from the AD literature on the role of steroid hormones in AD mechanism as well as in risk and resilience. There are, however, some limitations. All women age and some experience severe drops in steroid hormone but only some of them get AD. The reasons for this are still unknown. It might be that AD pathology is dependent on levels of E2, years of E2 exposure or that E2 levels interacting with other biologically related variables – such as contraceptives, past hormone therapy, parity: affect AD pathology differently. Our review did not address this question, and future studies should include these variables to fully understand the role of E2 behind risk and resilience. Moreover, life circumstances such as access to education, complex occupation, high socio-economic standing, and misogyny are social factors embedded in gender which may also affect biology and hence, the risk of AD. We limited our question to biological sex and specifically pituitary-gonadal steroid hormone levels and thus, did not include studies analyzing the role of gender in AD. However, as noted, gender is crucial for the advancement of health equity. Another weakness is that we reviewed both human and non-human animal models and the ovarian cycle as well as menopause are different. Typically, women have a 30-day menstrual cycle and rodents have a 4–5-day estrous cycle. As well, rodents do not go into 'menopause'; their aging ovarian state is either constant diestrous or constant estrous. Although the studies reviewed here suggest that E2 has a beneficial role in both humans and non-human animal modes, these differences in the estrous cycle and ovarian cessation should be considered carefully as they might have different impacts on cognitive decline.

5. Conclusion

Overall, the current review followed a synthetic approach to create a new theoretical perspective by rigorously analyzing patterns in the results of previous studies. We first explored new associations – steroid hormones, glia, AD–to identify how the loss of steroid hormones in aging may be related to risk in AD. We then investigated factors leading to AD resilience in women and identified new avenues for future research. Although more targeted studies are needed to understand these associations, the emerging themes summarized in this review highlight the importance of incorporating the analysis of steroid hormones in AD pathology. Specifically, the findings suggest that E2 loss after menopause may be one of the reasons for the increased risk of AD in women. Conversely, adequate levels of E2 are related to the brain and cognitive reserve mechanisms that may both lead to women's unique pathways toward resilience against AD. One of the features of AD is cell death and E2 prevents neuronal death. It also shapes hippocampal plasticity, modulates neurogenesis and promotes neural efficiency in the PFC. Studying the effects of E2 on multiple brain systems can help to elucidate the complex nature of women's risk and resilience and allow for the study of different types of reserve–brain and cognitive–in healthy or pathological aging. It may also help to identify new biomarkers with which to elucidate the risk, cause, trajectory, and resilience of women to many neurodegenerative diseases.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Allen, W. E., Blosser, T. R., Sullivan, Z. A., Dulac, C., and Zhuang, X. (2023). Molecular and spatial signatures of mouse brain aging at single-cell resolution. *Cell*. 186, 194–208. doi: 10.1016/j.cell.2022.12.010

Altmann, A., Tian, L., Henderson, V. W., Greicius, M. D., and Investigators, A. D. N. I. (2014). Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann. Neurol.* 75, 563–573. doi: 10.1002/ana.24135

Au, A., Feher, A., McPhee, L., Jessa, A., Oh, S., and Einstein, G. (2016). Estrogens, inflammation and cognition. *Front. Neuroendocrinol.* 40, 87–100. doi: 10.1016/j.yfrne.2016.01.002

Augusto-Oliveira, M., Arrifano, G. P., Delage, C. I., Tremblay, M.-È., Crespo-Lopez, M. E., and Verkhratsky, A. (2022). Plasticity of microglia. *Biol. Rev.* 97, 217–250. doi: 10.1111/brv.12797

Baldereschi, M., Di Carlo, A., Lepore, V., Bracco, L., Maggi, S., Grigoletto, F., et al. (1998). Estrogen-replacement therapy and Alzheimer's disease in the Italian longitudinal study on aging. *Neurology* 50, 996–1002. doi: 10.1212/WNL.50.4.996

Banks, S. J., Andrews, M. J., Digma, L., Madsen, J., Reas, E. T., Caldwell, J. Z., et al. (2021). Sex differences in Alzheimer's disease: do differences in tau explain the verbal memory gap? *Neurobiol. Aging* 107, 70–77. doi: 10.1016/j.neurobiolaging.2021.05.013

Barha, C. K., Dalton, G. L., and Galea, L. A. (2010). Low doses of 17α-estradiol and 17β-estradiol facilitate, whereas higher doses of estrone and 17α-and 17β-estradiol impair, contextual fear conditioning in adult female rats. *Neuropsychopharmacology* 35, 547–559. doi: 10.1038/npp.2009.161

Barha, C. K., and Galea, L. A. (2010). Influence of different estrogens on neuroplasticity and cognition in the hippocampus. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1800, 1056–1067. doi: 10.1016/j.bbagen.2010.01.006

Barker, J. M., and Galea, L. A. (2008). Repeated estradiol administration alters different aspects of neurogenesis and cell death in the hippocampus of female, but not male, rats. *Neuroscience* 152, 888–902. doi: 10.1016/j.neuroscience.2007.10.071

Barnes, L. L., Lamar, M., and Schneider, J. A. (2019). Sex differences in mixed neuropathologies in community-dwelling older adults. *Brain Res.* 1719, 11–16. doi: 10.1016/j.brainres.2019.05.028

Barrett-Connor, E., Goodman-Gruen, D., and Patay, B. (1999). Endogenous sex hormones and cognitive function in older men*. *J. Clin. Endocrinol. Metabol.* 84, 3681–3685. doi: 10.1210/jcem.84.10.6086

Baudry, M., Bi, X., and Aguirre, C. (2013). Progesterone–estrogen interactions in synaptic plasticity and neuroprotection. *Neuroscience* 239, 280–294. doi: 10.1016/j.neuroscience.2012.10.051

Been, L. E., Sheppard, P. A., Galea, L. A., and Glasper, E. R. (2022). Hormones and neuroplasticity: a lifetime of adaptive responses. *Neurosci. Biobehav. Rev.* 132, 679–690. doi: 10.1016/j.neubiorev.2021.11.029

Beery, A. K., and Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neurosci. Biobehav. Rev.* 35, 565–572. doi: 10.1016/j.neubiorev.2010.07.002

Bekris, L. M., Yu, C.-E., Bird, T. D., and Tsuang, D. W. (2010). Genetics of Alzheimer disease. *J. Geriatr. Psychiatry Neurol.* 23, 213–227. doi: 10.1177/0891988710383571

Bialystok, E., Craik, F. I., and Freedman, M. (2007). Bilingualism as a protection against the onset of symptoms of dementia. *Neuropsychologia* 45, 459–464. doi: 10.1016/j.neuropsychologia.2006.10.009

Bimonte-Nelson, H. A., Nelson, M. E., and Granholm, A.-C. E. (2004). Progesterone counteracts estrogen-induced increases in neurotrophins in the aged female rat brain. *Neuroreport* 15, 2659–2663. doi: 10.1097/00001756-200412030-00021

Bove, R., Secor, E., Chibnik, L. B., Barnes, L. L., Schneider, J. A., Bennett, D. A., et al. (2014). Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. *Neurology* 82, 222–229. doi: 10.1212/WNL.0000000000000033

Bowman, R. E., Luine, V., Weinstein, S. D., Khandaker, H., DeWolf, S., and Frankfurt, M. (2015). Bisphenol-a exposure during adolescence leads to enduring alterations in cognition and dendritic spine density in adult male and female rats. *Horm. Behav.* 69, 89–97. doi: 10.1016/j.yhbeh.2014.12.007

Boyle, C. P., Raji, C. A., Erickson, K. I., Lopez, O. L., Becker, J. T., Gach, H. M., et al. (2021). Estrogen, brain structure, and cognition in p ostmenopausal women. *Hum. Brain Mapp.* 42, 24–35. doi: 10.1002/hbm.25200

Bruce, H., Aponte, D., St-Onge, N., Phillips, N., Gagné, J.-P., and Li, K. Z. (2019). The effects of age and hearing loss on dual-task balance and listening. *J. Gerontol.* 74, 275–283. doi: 10.1093/geronb/gbx047

Buckley, R. F., Scott, M. R., Jacobs, H. I., Schultz, A. P., Properzi, M. J., Amariglio, R. E., et al. (2020). Sex mediates relationships between regional tau pathology and cognitive decline. *Ann. Neurol.* 88, 921–932. doi: 10.1002/ana.25878

Caldwell, J. Z. K., Berg, J.-L., Cummings, J. L., and Banks, S. J.for the Alzheimer's Disease Neuroimaging Initiative (2017). Moderating effects of sex on the impact of diagnosis and amyloid positivity on verbal memory and hippocampal volume. *Alzheimers Res. Ther.* 9:1. doi: 10.1186/s13195-017-0300-8

Callahan, M. J., Lipinski, W. J., Bian, F., Durham, R. A., Pack, A., and Walker, L. C. (2001). Augmented senile plaque load in aged female β-amyloid precursor protein-transgenic mice. *Am. J. Pathol.* 158, 1173–1177. doi: 10.1016/S0002-9440(10)64064-3

Calvo, N., Anderson, J. A., Berkes, M., Freedman, M., Craik, F. I., and Bialystok, E. (2023). Gray matter volume as evidence for cognitive Reserve in Bilinguals with Mild Cognitive Impairment. *Alzheimer Dis. Assoc. Disord.* 37, 7–12. doi: 10.1097/WAD.0000000000000549

Cerghet, M., Skoff, R. P., Bessert, D., Zhang, Z., Mullins, C., and Ghandour, M. S. (2006). Proliferation and death of oligodendrocytes and myelin proteins are differentially regulated in male and female rodents. *J. Neurosci.* 26, 1439–1447. doi: 10.1523/JNEUROSCI.2219-05.2006

Chan, C. B., and Ye, K. (2017). Sex differences in brain-derived neurotrophic factor signaling and functions. *J. Neurosci. Res.* 95, 328–335. doi: 10.1002/jnr.23863

Chertkow, H., Whitehead, V., Phillips, N., Wolfson, C., Atherton, J., and Bergman, H. (2010). Multilingualism (but not always bilingualism) delays the onset of Alzheimer disease: evidence from a bilingual community. *Alzheimer Dis. Assoc. Disord.* 24, 118–125. doi: 10.1097/WAD.0b013e3181ca1221

Chowen, J. A., and Garcia-Segura, L. M. (2021). Role of glial cells in the generation of sex differences in neurodegenerative diseases and brain aging. *Mech. Ageing Dev.* 196:111473. doi: 10.1016/j.mad.2021.111473

Collins, P., Rosano, G. M., Sarrel, P. M., Ulrich, L., Adamopoulos, S., Beale, C. M., et al. (1995). 17β-estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation* 92, 24–30. doi: 10.1161/01.CIR.92.1.24

Coughlan, G. T., Betthauser, T. J., Boyle, R., Koscik, R. L., Klinger, H. M., Chibnik, L. B., et al. (2023). Association of age at menopause and hormone therapy use with tau and β-amyloid positron emission tomography. *JAMA Neurol.* 80, 462–473. doi: 10.1001/jamaneurol.2023.0455

Craig, M. C., Fletcher, P. C., Daly, E. M., Rymer, J., Brammer, M., Giampietro, V., et al. (2008). Reversibility of the effects of acute ovarian hormone suppression on verbal memory and prefrontal function in pre-menopausal women. *Psychoneuroendocrinology* 33, 1426–1431. doi: 10.1016/j.psyneuen.2008.08.006

Craig, M. C., Fletcher, P. C., Daly, E. M., Rymer, J., Brammer, M., Giampietro, V., et al. (2009). The interactive effect of the cholinergic system and acute ovarian suppression on the brain: An fMRI study. *Horm. Behav.* 55, 41–49. doi: 10.1016/j.yhbeh.2008.08.008

Creta, M., Riccio, R., Chiancone, F., and Fusco, F. (2010). Androgens exert direct neuroprotective effects on the brain: a review of pre-clinical evidences. *J. Androl. Sci.* 17, 49–55.

Curhan, S. G., Willett, W. C., Grodstein, F., and Curhan, G. C. (2019). Longitudinal study of hearing loss and subjective cognitive function decline in men. *Alzheimers Dement.* 15, 525–533. doi: 10.1016/j.jalz.2018.11.004

Da Souza, D., Luckwu, B., Andrade, W. T. L., Pessoa, L. S. F., Nascimento, J. A. D., and Rosa, M. R. D. D. (2017). Variation in the hearing threshold in women during the menstrual cycle. *Int. Arch. Otorhinolaryngol.* 21, 323–328. doi: 10.1055/s-0037-1598601

Davis, E. J., Broestl, L., Abdulai-Saiku, S., Worden, K., Bonham, L. W., Miñones-Moyano, E., et al. (2020). A second X chromosome contributes to resilience in a mouse model of Alzheimer's disease. *Sci. Transl. Med.* 12:eaaz5677. doi: 10.1126/scitranslmed.aaz5677

de Groot, R. H. M., Vuurman, E. F. P. M., Hornstra, G., and Jolles, J. (2006). Differences in cognitive performance during pregnancy and early motherhood. *Psychol. Med.* 36, 1023–1032. doi: 10.1017/S0033291706007380

De Lucia, C., Rinchon, A., Olmos-Alonso, A., Riecken, K., Fehse, B., Boche, D., et al. (2016). Microglia regulate hippocampal neurogenesis during chronic neurodegeneration. *Brain Behav. Immun.* 55, 179–190. doi: 10.1016/j.bbi.2015.11.001

de Toledo-Morrell, L., Dickerson, B., Sullivan, M., Spanovic, C., Wilson, R., and Bennett, D. A. (2000). Hemispheric differences in hippocampal volume predict verbal and spatial memory performance in patients with Alzheimer's disease. *Hippocampus* 10, 136–142. doi: 10.1002/(SICI)1098-1063(2000)10:2<136::AID-HIPO2>3.0.CO;2-J

Deniselle, M. C. G., Garay, L., Gonzalez, S., Saravia, F., Labombarda, F., Guennoun, R., et al. (2007). Progesterone modulates brain-derived neurotrophic factor and choline acetyltransferase in degenerating wobbler motoneurons. *Exp. Neurol.* 203, 406–414. doi: 10.1016/j.expneurol.2006.08.019

Digma, L. A., Madsen, J. R., Rissman, R. A., Jacobs, D. M., Brewer, J. B., and Banks, S. J. (2020). Women can bear a bigger burden: ante-and post-mortem evidence for reserve in the face of tau. Brain. *Communications* 2:fcaa025. doi: 10.1093/braincomms/fcaa025

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cells* 149, 1060–1072. doi: 10.1016/j.cell.2012.03.042

Dubois, B., Villain, N., Frisoni, G. B., Rabinovici, G. D., Sabbagh, M., Cappa, S., et al. (2021). Clinical diagnosis of Alzheimer's disease: recommendations of the international working group. *Lancet Neurol.* 20, 484–496. doi: 10.1016/S1474-4422(21)00066-1

Dumitriu, D., Rapp, P. R., McEwen, B. S., and Morrison, J. H. (2010). Estrogen and the aging brain: An elixir for the weary cortical network. *Ann. N. Y. Acad. Sci.* 1204, 104–112. doi: 10.1111/j.1749-6632.2010.05529.x

Duncan, H. D., Nikelski, J., Pilon, R., Steffener, J., Chertkow, H., and Phillips, N. A. (2018). Structural brain differences between monolingual and multilingual patients with mild cognitive impairment and Alzheimer disease: Evidence for cognitive reserve. *Neuropsychologia* 109, 270–282. doi: 10.1016/j.neuropsychologia.2017.12.036

Edwards, H., Duchesne, A., Au, A. S., and Einstein, G. (2019). The many menopauses: searching the cognitive research literature for menopause types. *Menopause (New York, NY)* 26:45. doi: 10.1097/GME.0000000000001171

Einstein, G. (1999). To each her own: sexual dimorphisms in Alzheimer's disease. *Neurobiol. Aging* 20, 439–440. doi: 10.1016/S0197-4580(99)00079-2

Eissman, J. M., Dumitrescu, L., Mahoney, E. R., Smith, A. N., Mukherjee, S., Lee, M. L., et al. (2022). Sex differences in the genetic architecture of cognitive resilience to Alzheimer's disease. Brain: a. *J. Neurol.* 145, 2541–2554. doi: 10.1093/brain/awac177

Ferrari-Souza, J. P., Lussier, F. Z., Leffa, D. T., Therriault, J., Tissot, C., Bellaver, B., et al. (2023). APOE ε4 associates with microglial activation independently of Aβ plaques and tau tangles. Science. *Advances* 9:eade1474. doi: 10.1126/sciadv.ade1474

Ferrer, I. (2017). Diversity of astroglial responses across human neurodegenerative disorders and brain aging. *Brain Pathol.* 27, 645–674. doi: 10.1111/bpa.12538

Ferretti, M. T., Iulita, M. F., Cavedo, E., Chiesa, P. A., Schumacher Dimech, A., Santuccione Chadha, A., et al. (2018). Sex differences in Alzheimer disease—the gateway to precision medicine. *Nat. Rev. Neurol.* 14, 457–469. doi: 10.1038/s41582-018-0032-9

Filon, J. R., Intorcia, A. J., Sue, L. I., Vazquez Arreola, E., Wilson, J., Davis, K. J., et al. (2016). Gender differences in Alzheimer disease: brain atrophy, histopathology burden, and cognition. *J. Neuropathol. Exp. Neurol.* 75, 748–754. doi: 10.1093/jnen/nlw047

Foy, M. R., Akopian, G., and Thompson, R. F. (2008). Progesterone regulation of synaptic transmission and plasticity in rodent hippocampus. *Learn. Mem.* 15, 820–822. doi: 10.1101/lm.1124708

Fuchs, E., and Flügge, G. (2014). Adult neuroplasticity: more than 40 years of research. *Neural Plast.* 2014. doi: 10.1155/2014/541870

Gago, N., Akwa, Y., Sananès, N., Guennoun, R., Baulieu, E. E., El-Etr, M., et al. (2001). Progesterone and the oligodendroglial lineage: stage-dependent biosynthesis and metabolism. *Glia* 36, 295–308. doi: 10.1002/glia.1117

Gandy, S., and Petanceska, S. (2000). Regulation of Alzheimer β-amyloid precursor trafficking and metabolism. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1502, 44–52. doi: 10.1016/S0925-4439(00)00031-4

Gao, S., Chen, S., Chen, L., Zhao, Y., Sun, L., Cao, M., et al. (2019). Brain-derived neurotrophic factor: a steroidogenic regulator of Leydig cells. *J. Cell. Physiol.* 234, 14058–14067. doi: 10.1002/jcp.28095

Garcia-Ovejero, D., Azcoitia, I., DonCarlos, L. L., Melcangi, R. C., and Garcia-Segura, L. M. (2005). Glia-neuron crosstalk in the neuroprotective mechanisms of sex steroid hormones. *Brain Res. Rev.* 48, 273–286. doi: 10.1016/j.brainresrev.2004.12.018

Gervais, N. J., Au, A., Almey, A., Duchesne, A., Gravelsins, L., Brown, A., et al. (2020). Cognitive markers of dementia risk in middle-aged women with bilateral salpingo-oophorectomy prior to menopause. *Neurobiol. Aging* 94, 1–6. doi: 10.1016/j.neurobiolaging.2020.04.019

Glynn, L. M. (2010). Giving birth to a new brain: hormone exposures of pregnancy influence human memory. *Psychoneuroendocrinology* 35, 1148–1155. doi: 10.1016/j.psyneuen.2010.01.015

Gorno-Tempini, M. L., Hillis, A. E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S. F., et al. (2011). Classification of primary progressive aphasia and its variants. *Neurology* 76, 1006–1014. doi: 10.1212/WNL.0b013e31821103e6

Graeber, M. B., and Streit, W. J. (2010). Microglia: biology and pathology. *Acta Neuropathol.* 119, 89–105. doi: 10.1007/s00401-009-0622-0

Guo, L., Zhong, M. B., Zhang, L., Zhang, B., and Cai, D. (2022). Sex differences in Alzheimer's disease: insights from the multiomics landscape. *Biol. Psychiatry* 91, 61–71. doi: 10.1016/j.biopsych.2021.02.968

Hackert, V. H., den Heijer, T., Oudkerk, M., Koudstaal, P. J., Hofman, A., and Breteler, M. M. B. (2002). Hippocampal head size associated with verbal memory performance in nondemented elderly. *NeuroImage* 17, 1365–1372. doi: 10.1006/nimg.2002.1248

Hajszan, T., and Leranth, C. (2010). Bisphenol a interferes with synaptic remodeling. *Front. Neuroendocrinol.* 31, 519–530. doi: 10.1016/j.yfrne.2010.06.004

Hampson, E., Levy-Cooperman, N., and Korman, J. M. (2014). Estradiol and mental rotation: relation to dimensionality, difficulty, or angular disparity? *Horm. Behav.* 65, 238–248. doi: 10.1016/j.yhbeh.2013.12.016

Hara, Y., Waters, E. M., McEwen, B. S., and Morrison, J. H. (2015). Estrogen effects on cognitive and synaptic health over the Lifecourse. *Physiol. Rev.* 95, 785–807. doi: 10.1152/physrev.00036.2014

Haren, M. T., Wittert, G. A., Chapman, I. M., Coates, P., and Morley, J. E. (2005). Effect of oral testosterone undecanoate on visuospatial cognition, mood and quality of life in elderly men with low-normal gonadal status. *Maturitas* 50, 124–133. doi: 10.1016/j.maturitas.2004.05.002

Hebb, D. O. (1949). Organization of behavior. New York: Wiley. *J. Clin. Psychol.* 6, 335–307.

Henderson, V. W., Benke, K. S., Green, R. C., Cupples, L. A., and Farrer, L. A. (2005). Postmenopausal hormone therapy and Alzheimer's disease risk: interaction with age. *J. Neurol. Neurosurg. Psychiatry* 76, 103–105. doi: 10.1136/jnnp.2003.024927

Henderson, V. W., Paganini-Hill, A., Emanuel, C. K., Dunn, M. E., and Buckwalter, J. G. (1994). Estrogen replacement therapy in older women: comparisons between Alzheimer's disease cases and nondemented control subjects. *Arch. Neurol.* 51, 896–900. doi: 10.1001/archneur.1994.00540210068014

Heneka, M. T., Rodríguez, J. J., and Verkhratsky, A. (2010). Neuroglia in neurodegeneration. *Brain Res. Rev.* 63, 189–211. doi: 10.1016/j.brainresrev.2009.11.004

Hollands, C., Tobin, M. K., Hsu, M., Musaraca, K., Yu, T.-S., Mishra, R., et al. (2017). Depletion of adult neurogenesis exacerbates cognitive deficits in Alzheimer's disease by compromising hippocampal inhibition. *Mol. Neurodegener.* 12:64. doi: 10.1186/s13024-017-0207-7

Honarpisheh, P., and McCullough, L. D. (2019). Sex as a biological variable in the pathology and pharmacology of neurodegenerative and neurovascular diseases. *Br. J. Pharmacol.* 176, 4173–4192. doi: 10.1111/bph.14675

Hong, Y., Wang, X., Sun, S., Xue, G., Li, J., and Hou, Y. (2016). Progesterone exerts neuroprotective effects against Aβ-induced neuroinflammation by attenuating ER stress in astrocytes. *Int. Immunopharmacol.* 33, 83–89. doi: 10.1016/j.intimp.2016.02.002

Howarth, C. (2014). The contribution of astrocytes to the regulation of cerebral blood flow. *Front. Neurosci.* 8:103. doi: 10.3389/fnins.2014.00103

Hu, J., Chen, Q., Zhu, H., Hou, L., Liu, W., Yang, Q., et al. (2023). Microglial Piezo1 senses Aβ fibril stiffness to restrict Alzheimer's disease. *Neuron* 111, 15–29. doi: 10.1016/j.neuron.2022.10.021

Illán-Gala, I., Casaletto, K. B., Borrego-Écija, S., Arenaza-Urquijo, E. M., Wolf, A., Cobigo, Y., et al. (2021). Sex differences in the behavioral variant of frontotemporal dementia: a new window to executive and behavioral reserve. *Alzheimers Dement.* 17, 1329–1341. doi: 10.1002/alz.12299

Irwin, R. W., Yao, J., Hamilton, R. T., Cadenas, E., Brinton, R. D., and Nilsen, J. (2008). Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. *Endocrinology* 149, 3167–3175. doi: 10.1210/en.2007-1227

Ito, K.-I., Skinkle, K. L., and Hicks, T. P. (1999). Age-dependent, steroid-specific effects of oestrogen on long-term potentiation in rat hippocampal slices. *J. Physiol.* 515, 209–220. doi: 10.1111/j.1469-7793.1999.209ad.x

Jacobs, E., and D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J. Neurosci.* 31, 5286–5293. doi: 10.1523/JNEUROSCI.6394-10.2011

Jacobs, E. G., and Goldstein, J. M. (2018). The middle-aged brain: biological sex and sex hormones shape memory circuitry. *Curr. Opin. Behav. Sci.* 23, 84–91. doi: 10.1016/j.cobeha.2018.03.009

Jacobs, D. M., Tang, M.-X., Stern, Y., Sano, M., Marder, K., Bell, K. L., et al. (1998). Cognitive function in nondemented older women who took estrogen after menopause. *Neurology* 50, 368–373. doi: 10.1212/WNL.50.2.368

Jia, J., Cui, C., Yan, X., Zhang, B., Song, W., Huo, D., et al. (2016). Effects of testosterone on synaptic plasticity mediated by androgen receptors in male SAMP8 mice. *J. Toxic. Environ. Health A* 79, 849–855. doi: 10.1080/15287394.2016.1193113

Kampen, D. L., and Sherwin, B. B. (1994). Estrogen use and verbal memory in healthy postmenopausal women. *Obstet. Gynecol.* 83:6

Kane, J. P., Surendranathan, A., Bentley, A., Barker, S. A., Taylor, J.-P., Thomas, A. J., et al. (2018). Clinical prevalence of Lewy body dementia. *Alzheimers Res. Ther.* 10, 1–8. doi: 10.1186/s13195-018-0350-6

Katzman, R., Terry, R., DeTeresa, R., Brown, T., Davies, P., Fuld, P., et al. (1988). Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann. Neurol.* 23, 138–144. doi: 10.1002/ana.410230206

Kaunitz, A. M., Kapoor, E., and Faubion, S. (2021). Treatment of women after bilateral salpingo-oophorectomy performed prior to natural menopause. *JAMA* 326, 1429–1430. doi: 10.1001/jama.2021.3305

Klein, R. M., Christie, J., and Parkvall, M. (2016). Does multilingualism affect the incidence of Alzheimer's disease?: a worldwide analysis by country. *SSM-Popul. Health* 2, 463–467. doi: 10.1016/j.ssmph.2016.06.002

Klempin, F., and Kempermann, G. (2007). Adult hippocampal neurogenesis and aging. *Eur. Arch. Psychiatry Clin. Neurosci.* 257, 271–280. doi: 10.1007/s00406-007-0731-5

Koski, C. L., Hila, S., and Hoffman, G. E. (2004). Regulation of cytokine-induced neuron death by ovarian hormones: involvement of antiapoptotic protein expression and c-JUN N-terminal kinase-mediated proapoptotic signaling. *Endocrinology* 145, 95–103. doi: 10.1210/en.2003-0803

Larson, T. A. (2018). Sex steroids, adult neurogenesis, and inflammation in CNS homeostasis, degeneration, and repair. *Front. Endocrinol.* 9:205. doi: 10.3389/fendo.2018.00205

Lau, D. T., Mercaldo, N. D., Harris, A. T., Trittschuh, E., Shega, J., and Weintraub, S. (2010). Polypharmacy and potentially inappropriate medication use among community-dwelling elders with dementia. *Alzheimer Dis. Assoc. Disord.* 24:56. doi: 10.1097/WAD.0b013e31819d6ec9

Le Prince, G., Delaere, P., Fages, C., Duyckaerts, C., Hauw, J.-J., and Tardy, M. (1993). Alterations of glial fibrillary acidic protein mRNA level in the aging brain and in senile dementia of the Alzheimer type. *Neurosci. Lett.* 151:1

Lenz, K. M., Nugent, B. M., Haliyur, R., and McCarthy, M. M. (2013). Microglia are essential to masculinization of brain and behavior. *J. Neurosci.* 33, 2761–2772. doi: 10.1523/JNEUROSCI.1268-12.2013

Leranth, C., Shanabrough, M., and Redmond, D. E. Jr. (2002). Gonadal hormones are responsible for maintaining the integrity of spine synapses in the CA1 hippocampal subfield of female nonhuman primates. *J. Comp. Neurol.* 447, 34–42. doi: 10.1002/cne.10230

Liang, Z., Valla, J., Sefidvash-Hockley, S., Rogers, J., and Li, R. (2002). Effects of estrogen treatment on glutamate uptake in cultured human astrocytes derived from cortex of Alzheimer's disease patients. *J. Neurochem.* 80, 807–814. doi: 10.1046/j.0022-3042.2002.00779.x

Lima, B. S., Freedman, M., Binns, M., Tang-Wai, D. F., Black, S. E., Leach, L., et al. (2022). Identifying subtypes of Alzheimer's Disease: an analysis of possible cognitive subgroups through the life span. *Alzheimers Dement.* 18:e066207. doi: 10.1002/alz.066207

Llorente, R., Gallardo, M. L., Berzal, A. L., Prada, C., Garcia-Segura, L. M., and Viveros, M.-P. (2009). Early maternal deprivation in rats induces gender-dependent effects on developing hippocampal and cerebellar cells. *Int. J. Dev. Neurosci.* 27, 233–241. doi: 10.1016/j.ijdevneu.2009.01.002

Lobo, R. A. (2013). Where are we 10 years after the Women's health Initiative? *J. Clin. Endocrinol. Metabol.* 98, 1771–1780. doi: 10.1210/jc.2012-4070

Maki, P. M., Rich, J. B., and Shayna Rosenbaum, R. (2002). Implicit memory varies across the menstrual cycle: estrogen effects in young women. *Neuropsychologia* 40, 518–529. doi: 10.1016/S0028-3932(01)00126-9

Mangold, C. A., Wronowski, B., Du, M., Masser, D. R., Hadad, N., Bixler, G. V., et al. (2017). Sexually divergent induction of microglial-associated neuroinflammation with hippocampal aging. *J. Neuroinflammation* 14, 1–19. doi: 10.1186/s12974-017-0920-8

Martinez, J. E., Pardilla-Delgado, E., Guzmán-Vélez, E., Vila-Castelar, C., Amariglio, R., Gatchel, J., et al. (2022). Subjective cognitive decline and its relation to verbal memory and sex in cognitively unimpaired individuals from a Colombian cohort with autosomal-dominant Alzheimer's Disease. *J. Int. Neuropsychol. Soc.* 28:6. doi: 10.1017/S1355617721000801

Martinkova, J., Quevenco, F.-C., Karcher, H., Ferrari, A., Sandset, E. C., Szoeke, C., et al. (2021). Proportion of women and reporting of outcomes by sex in clinical trials for Alzheimer disease: a systematic review and meta-analysis. *JAMA Netw. Open* 4:e2124124. doi: 10.1001/jamanetworkopen.2021.24124

Mathys, H., Davila-Velderrain, J., Peng, Z., Gao, F., Mohammadi, S., Young, J. Z., et al. (2019). Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* 570, 332–337. doi: 10.1038/s41586-019-1195-2

Mellon, S. H., and Vaudry, H. (2001). Biosynthesis of neurosteroids and regulation of their sysnthesis. *Int. Rev. Neurobiol.* 46, 33–78. doi: 10.1016/S0074-7742(01)46058-2

Mielke, M. M., Okonkwo, O. C., Oishi, K., Mori, S., Tighe, S., Miller, M. I., et al. (2012). Fornix integrity and hippocampal volume predict memory decline and progression to Alzheimer's disease. *Alzheimers Dement.* 8, 105–113. doi: 10.1016/j.jalz.2011.05.2416

Miller, L. R., Marks, C., Becker, J. B., Hurn, P. D., Chen, W.-J., Woodruff, T., et al. (2017). Considering sex as a biological variable in preclinical research. *FASEB J.* 31:29. doi: 10.1096/fj.201600781R

Miranda, P., Williams, C. L., and Einstein, G. (1999). Granule cells in aging rats are sexually dimorphic in their response to estradiol. *J. Neurosci.* 19, 3316–3325. doi: 10.1523/JNEUROSCI.19-09-03316.1999

Moffat, S. D., Zonderman, A. B., Metter, E. J., Blackman, M. R., Harman, S. M., and Resnick, S. M. (2002). Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. *J. Clin. Endocrinol. Metabol.* 87, 5001–5007. doi: 10.1210/jc.2002-020419

Morley, J. E. (2003). Hormones and the aging process. *J. Am. Geriatr. Soc.* 51, S333–S337. doi: 10.1046/j.1365-2389.2003.51344.x

Mouton, P. R., Long, J. M., Lei, D.-L., Howard, V., Jucker, M., Calhoun, M. E., et al. (2002). Age and gender effects on microglia and astrocyte numbers in brains of mice. *Brain Res.* 956, 30–35. doi: 10.1016/S0006-8993(02)03475-3

Nebel, R. A., Aggarwal, N. T., Barnes, L. L., Gallagher, A., Goldstein, J. M., Kantarci, K., et al. (2018). Understanding the impact of sex and gender in Alzheimer's disease: a call to action. *Alzheimers Dement.* 14, 1171–1183. doi: 10.1016/j.jalz.2018.04.008

Neu, S. C., Pa, J., Kukull, W., Beekly, D., Kuzma, A., Gangadharan, P., et al. (2017). Apolipoprotein E genotype and sex risk factors for Alzheimer disease: a meta-analysis. *JAMA Neurol.* 74, 1178–1189. doi: 10.1001/jamaneurol.2017.2188

Newton, C., Slota, D., Yuzpe, A. A., and Tummon, I. S. (1996). Memory complaints associated with the use of gonadotropin-releasing hormone agonists: a preliminary study. *Fertil. Steril.* 65, 1253–1255. doi: 10.1016/S0015-0282(16)58351-4

Nguyen, T. V., Jayaraman, A., Quaglino, A., and Pike, C. J. (2010). Androgens selectively protect against apoptosis in hippocampal neurones. *J. Neuroendocrinol.* 22, 1013–1022. doi: 10.1111/j.1365-2826.2010.02044.x

Nilsen, J., and Brinton, R. D. (2004). Mitochondria as therapeutic targets of estrogen action in the central nervous system. *Curr. Drug Targ. Neurol. Disord.* 3, 297–313. doi: 10.2174/1568007043337193

Olmedillas del Moral, M., Fröhlich, N., Figarella, K., Mojtahedi, N., and Garaschuk, O. (2020). Effect of caloric restriction on the in vivo functional properties of aging microglia. *Front. Immunol.* 11:750. doi: 10.3389/fimmu.2020.00750

Orchard, E. R., Rutherford, H. J. V., Holmes, A. J., and Jamadar, S. D. (2023). Matrescence: lifetime impact of motherhood on cognition and the brain. *Trends Cogn. Sci.* 27, 302–316. doi: 10.1016/j.tics.2022.12.002

Pasquier, F., Leys, D., and Scheltens, P. (1998). The influence of coincidental vascular pathology on symptomatology and course of Alzheimer's disease. *J. Neural Transm. Suppl.* 54, 117–127. doi: 10.1007/978-3-7091-7508-8_11

Pawluski, J. L., Hoekzema, E., Leuner, B., and Lonstein, J. S. (2022). Less can be more: fine tuning the maternal brain. *Neurosci. Biobehav. Rev.* 133:104475. doi: 10.1016/j.neubiorev.2021.11.045

Pengo, M., Alberici, A., Libri, I., Benussi, A., Gadola, Y., Ashton, N. J., et al. (2022). Sex influences clinical phenotype in frontotemporal dementia. *Neurol. Sci.* 43, 5281–5287. doi: 10.1007/s10072-022-06185-7

Peragine, D., Simeon-Spezzaferro, C., Brown, A., Gervais, N. J., Hampson, E., and Einstein, G. (2020). Sex difference or hormonal difference in mental rotation? The influence of ovarian milieu. *Psychoneuroendocrinology* 115:104488. doi: 10.1016/j.psyneuen.2019.104488

Phillips, S. M., and Sherwin, B. B. (1992). Effects of estrogen on memory function in surgically menopausal women. *Psychoneuroendocrinology* 17, 485–495. doi: 10.1016/0306-4530(92)90007-T

Pichora-Fuller, M. K. (2003). Cognitive aging and auditory information processing. *Int. J. Audiol.* 42, 26–32. doi: 10.3109/1499202030907464

Pike, C. J. (1999). Estrogen modulates neuronal Bcl-xl expression and β-amyloid-induced apoptosis: relevance to Alzheimer's Disease. *J. Neurochem.* 72, 1552–1563. doi: 10.1046/j.1471-4159.1999.721552.x

Pike, C. J., Carroll, J. C., Rosario, E. R., and Barron, A. M. (2009). Protective actions of sex steroid hormones in Alzheimer's disease. *Front. Neuroendocrinol.* 30, 239–258. doi: 10.1016/j.yfrne.2009.04.015

Prendergast, B. J., Onishi, K. G., and Zucker, I. (2014). Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci. Biobehav. Rev.* 40, 1–5. doi: 10.1016/j.neubiorev.2014.01.001

Qin, Y., An, D., Xu, W., Qi, X., Wang, X., Chen, L., et al. (2020). Estradiol replacement at the critical period protects hippocampal neural stem cells to improve cognition in APP/PS1 mice. *Front. Aging Neurosci.* 12:240. doi: 10.3389/fnagi.2020.00240

Quinn, J. F., Kelly, M. J., Harris, C. J., Hack, W., Gray, N. E., Kulik, V., et al. (2022). The novel estrogen receptor modulator STX attenuates amyloid-β neurotoxicity in the 5XFAD mouse model of Alzheimer's disease. *Neurobiol. Dis.* 174:105888. doi: 10.1016/j.nbd.2022.105888

Raff, M. C., Lillien, L. E., Richardson, W. D., Burne, J. F., and Noble, M. D. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature* 333, 562–565. doi: 10.1038/333562a0

Ramon y Cajal, S. (1895). Algunas conjecturas sobre el mecanismo anatomico de la ideacion. *Revista Medica y Cirugia Practicas* 36, 479–508.

Raznahan, A., and Disteche, C. M. (2021). X-chromosome regulation and sex differences in brain anatomy. *Neurosci. Biobehav. Rev.* 120, 28–47. doi: 10.1016/j.neubiorev.2020.10.024

Reavis, K. M., Bisgaard, N., Canlon, B., Dubno, J. R., Frisina, R. D., Hertzano, R., et al. (2023). Sex-linked biology and gender-related research is essential to advancing hearing health. *Ear Hear.* 44, 10–27. doi: 10.1097/AUD.0000000000001291

Reuben, R., Karkaby, L., McNamee, C., Phillips, N. A., and Einstein, G. (2021). Menopause and cognitive complaints: are ovarian hormones linked with subjective cognitive decline? *Climacteric* 24:4. doi: 10.1080/13697137.2021.1892627

Rocca, W. A., Bower, J. H., Maraganore, D. M., Ahlskog, J. E., Grossardt, B. R., De Andrade, M., et al. (2007). Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 69, 1074–1083. doi: 10.1212/01.wnl.0000276984.19542.e6

Rocca, W. A., Lohse, C. M., Smith, C. Y., Fields, J. A., Machulda, M. M., and Mielke, M. M. (2021). Association of premenopausal bilateral oophorectomy with cognitive performance and risk of mild cognitive impairment. *JAMA Netw. Open* 4:e2131448. doi: 10.1001/jamanetworkopen.2021.31448

Ruiz-Cortés, Z. T. (2012). Gonadal sex steroids: production, action and interactions in mammals. *Steroids – from physiology to clinical medicine*. ed. S. M. Ostojic (London: IntechOpen), 3–44.

Ryan, J., Scali, J., Carriere, I., Amieva, H., Rouaud, O., Berr, C., et al. (2014). Impact of a premature menopause on cognitive function in later life. *BJOG Int. J. Obstet. Gynaecol.* 121, 1729–1739. doi: 10.1111/1471-0528.12828

Ryan, S. K., Zelic, M., Han, Y., Teeple, E., Chen, L., Sadeghi, M., et al. (2023). Microglia ferroptosis is regulated by SEC24B and contributes to neurodegeneration. *Nat. Neurosci.* 26, 12–26. doi: 10.1038/s41593-022-01221-3

Sager, T., Kashon, M. L., and Krajnak, K. (2018). Estrogen and environmental enrichment differentially affect neurogenesis, dendritic spine Immunolabeling and synaptogenesis in the Hippocampus of Young and reproductively senescent female rats. *Neuroendocrinology* 106, 252–263. doi: 10.1159/000479699

Sardi, F., Fassina, L., Venturini, L., Inguscio, M., Guerriero, F., Rolfo, E., et al. (2011). Alzheimer's disease, autoimmunity and inflammation. The good, the bad and the ugly. *Autoimmun. Rev.* 11, 149–153. doi: 10.1016/j.autrev.2011.09.005

Sárvári, M., Hrabovszky, E., Kalló, I., Solymosi, N., Likó, I., Berchtold, N., et al. (2012). Menopause leads to elevated expression of macrophage-associated genes in the aging frontal cortex: rat and human studies identify strikingly similar changes. *J. Neuroinflammation* 9, 1–13. doi: 10.1186/1742-2094-9-264

Schaafsma, M., Homewood, J., and Taylor, A. (2010). Subjective cognitive complaints at menopause associated with declines in performance of verbal memory and attentional processes. *Climacteric* 13, 84–98. doi: 10.3109/13697130903009187

Scheyer, O., Rahman, A., Hristov, H., Berkowitz, C., Isaacson, R. S., Diaz Brinton, R., et al. (2018). Female sex and Alzheimer's risk: the menopause connection. *J. Prev Alzheimers Dis.* 5, 225–230. doi: 10.14283/jpad.2018.34

Schweizer, T. A., Ware, J., Fischer, C. E., Craik, F. I., and Bialystok, E. (2012). Bilingualism as a contributor to cognitive reserve: evidence from brain atrophy in Alzheimer's disease. *Cortex* 48, 991–996. doi: 10.1016/j.cortex.2011.04.009

Serrano-Pozo, A., Li, Z., Woodbury, M. E., Munoz-Castro, C., Wachter, A., Jayakumar, R., et al. (2022). Astrocyte transcriptomic changes along the spatiotemporal progression of Alzheimer's disease. *BioRxiv*. 2022-12. doi: 10.1101/2022.12.03.518999

Seruga, B., and Tannock, I. F. (2008). Intermittent androgen blockade should be regarded as standard therapy in prostate cancer. *Nat. Clin. Pract. Oncol.* 5, 574–576. doi: 10.1038/ncponc1180

Shansky, R. M. (2019). Are hormones a "female problem" for animal research? *Science* 364, 825–826. doi: 10.1126/science.aaw7570

Shao, H., Breitner, J. C., Whitmer, R. A., Wang, J., Hayden, K., Wengreen, H., et al. (2012). Hormone therapy and Alzheimer disease dementia: new findings from the Cache County study. *Neurology* 79, 1846–1852. doi: 10.1212/WNL.0b013e318271f823

Sheppard, P. A., Choleris, E., and Galea, L. A. (2019). Structural plasticity of the hippocampus in response to estrogens in female rodents. *Mol. Brain* 12, 1–17. doi: 10.1186/s13041-019-0442-7

Sherwin, B. B. (1988). Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology* 13:4.

Sherwin, B. B. (1997). Estrogen effects on cognition in menopausal women. *Neurology* 48, 21S–26S.

Sherwin, B. B., and Tulandi, T. (1996). "Add-back" estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. *J. Clin. Endocrinol. Metabol.* 81, 2545–2549.

Shumaker, S. A., Legault, C., Kuller, L., Rapp, S. R., Thal, L., Lane, D. S., et al. (2004). Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's health Initiative memory study. *JAMA* 291, 2947–2958. doi: 10.1001/jama.291.24.2947

Shumaker, S. A., Legault, C., Rapp, S. R., Thal, L., Wallace, R. B., Ockene, J. K., et al. (2003). Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's health Initiative memory study: a randomized controlled trial. *JAMA* 289, 2651–2662. doi: 10.1001/jama.289.20.2651

Shumaker, S. A., Reboussin, B. A., Espeland, M. A., Rapp, S. R., McBee, W. L., Dailey, M., et al. (1998). The Women's health Initiative memory study (WHIMS): a trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. *Control. Clin. Trials* 19, 604–621. doi: 10.1016/S0197-2456(98)00038-5

Smith, R., Strandberg, O., Mattsson-Carlgren, N., Leuzy, A., Palmqvist, S., Pontecorvo, M. J., et al. (2020). The accumulation rate of tau aggregates is higher in females and younger amyloid-positive subjects. *Brain* 143, 3805–3815. doi: 10.1093/brain/awaa327

Snowdon, D. A. (1997). Aging and Alzheimer's disease: lessons from the Nun study. *The Gerontologist* 37, 150–156. doi: 10.1093/geront/37.2.150

Snyder, H. M., Asthana, S., Bain, L., Brinton, R., Craft, S., Dubal, D. B., et al. (2016). Sex biology contributions to vulnerability to Alzheimer's disease: a think tank convened by the Women's Alzheimer's research Initiative. *Alzheimers Dement.* 12, 1186–1196. doi: 10.1016/j.jalz.2016.08.004

Sofroniew, M. V., and Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol.* 119, 7–35. doi: 10.1007/s00401-009-0619-8

Song, Y., Li, S., Li, X., Chen, X., Wei, Z., Liu, Q., et al. (2020). The effect of estrogen replacement therapy on Alzheimer's disease and Parkinson's disease in postmenopausal women: a meta-analysis. *Front. Neurosci.* 14:157. doi: 10.3389/fnins.2020.00157

Soni, M., and Hogervorst, E. (2014). Premature ovarian insufficiency and neurological function. *Minerva Endocrinol.* 39:3

Steffens, D. C., Norton, M. C., Plassman, B. L., Tschanz, J. A. T., Wyse, B. W., Welsh-Bohmer, K. A., et al. (1999). Enhanced cognitive performance with estrogen use in nondemented community-dwelling older women. *J. Am. Geriatr. Soc.* 47, 1171–1175. doi: 10.1111/j.1532-5415.1999.tb05195.x

Stephen, T. L., Cacciottolo, M., Balu, D., Morgan, T. E., LaDu, M. J., Finch, C. E., et al. (2019). APOE genotype and sex affect microglial interactions with plaques in Alzheimer's disease mice. *Acta Neuropathol. Commun.* 7, 1–11. doi: 10.1186/s40478-019-0729-z

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *J. Int. Neuropsychol. Soc.* 8, 448–460. doi: 10.1017/S1355617702813248

Stern, Y., Arenaza-Urquijo, E. M., Bartrés-Faz, D., Belleville, S., Cantilon, M., Chetelat, G., et al. (2020). Whitepaper: defining and investigating cognitive reserve, brain reserve, and brain maintenance. *Alzheimers Dement.* 16, 1305–1311. doi: 10.1016/j.jalz.2018.07.219

Subramaniapillai, S., Almey, A., Rajah, M. N., and Einstein, G. (2021). Sex and gender differences in cognitive and brain reserve: implications for Alzheimer's disease in women. *Front. Neuroendocrinol.* 60:100879. doi: 10.1016/j.yfrne.2020.100879

Subramaniapillai, S., Rajah, M. N., Pasvanis, S., and Titone, D. (2019). Bilingual experience and executive control over the adult lifespan: the role of biological sex. *Biling. Lang. Congn.* 22, 733–751. doi: 10.1017/S1366728918000317

Sundermann, E. E., Biegon, A., Rubin, L. H., Lipton, R. B., Mowrey, W., Landau, S., et al. (2016). Better verbal memory in women than men in MCI despite similar levels of hippocampal atrophy. *Neurology* 86, 1368–1376. doi: 10.1212/WNL.0000000000002570

Sundermann, E. E., Panizzon, M. S., Chen, X., Andrews, M., Galasko, D., Banks, S. J., et al. (2020). Sex differences in Alzheimer's-related tau biomarkers and a mediating effect of testosterone. *Biol. Sex Differ.* 11:33. doi: 10.1186/s13293-020-00310-x

Sundermann, E. E., Tran, M., Maki, P. M., and Bondi, M. W. (2018). Sex differences in the association between apolipoprotein E ε4 allele and Alzheimer's disease markers Alzheimer's & Dementia. *Diagn. Assess. Dis. Monit.* 10, 438–447. doi: 10.1016/j.dadm.2018.06.004

Swerdlow, R. H., Burns, J. M., and Khan, S. M. (2014). The Alzheimer's disease mitochondrial cascade hypothesis: Progress and perspectives. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1842, 1219–1231. doi: 10.1016/j.bbadis.2013.09.010

Takao, T., Flint, N., Lee, L., Ying, X., Merrill, J., and Chandross, K. J. (2004). 17beta-estradiol protects oligodendrocytes from cytotoxicity induced cell death. *J. Neurochem.* 89, 660–673. doi: 10.1111/j.1471-4159.2004.02370.x

Tang, M.-X., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B., et al. (1996). Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* 348, 429–432. doi: 10.1016/S0140-6736(96)03356-9

Tarantini, S., Tran, C. H. T., Gordon, G. R., Ungvari, Z., and Csiszar, A. (2017). Impaired neurovascular coupling in aging and Alzheimer's disease: contribution of astrocyte dysfunction and endothelial impairment to cognitive decline. *Exp. Gerontol.* 94, 52–58. doi: 10.1016/j.exger.2016.11.004

Thompson, J. M., VanTil, L., Feder, K., Sweet, J., Boswall, M., Courchesne, C., et al. (2016). Prevalence of hearing problems among Canadian Armed Forces veterans: life after service studies. *J. Milit. Vet. Family Health* 2, 62–72. doi: 10.3138/jmvfh.4030

Van de Beek, M., Babapour Mofrad, R., Van Steenoven, I., Vanderstichele, H., Scheltens, P., Teunissen, C. E., et al. (2020). Sex-specific associations with cerebrospinal fluid biomarkers in dementia with Lewy bodies. *Alzheimers Res. Ther.* 12, 1–8. doi: 10.1186/s13195-020-00610-9

van der Windt, G. J., Everts, B., Chang, C.-H., Curtis, J. D., Freitas, T. C., Amiel, E., et al. (2012). Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* 36, 68–78. doi: 10.1016/j.immuni.2011.12.007

Vegeto, E., Villa, A., Della Torre, S., Crippa, V., Rusmini, P., Cristofani, R., et al. (2020). The role of sex and sex hormones in neurodegenerative diseases. *Endocr. Rev.* 41, 273–319. doi: 10.1210/endrev/bnz005

Villa, A., Della Torre, S., and Maggi, A. (2019). Sexual differentiation of microglia. *Front. Neuroendocrinol.* 52, 156–164. doi: 10.1016/j.yfrne.2018.11.003

Vogel, B., Acevedo, M., Appelman, Y., Merz, C. N. B., Chieffo, A., Figtree, G. A., et al. (2021). The lancet women and cardiovascular disease commission: reducing the global burden by 2030. *Lancet* 397, 2385–2438. doi: 10.1016/S0140-6736(21)00684-X

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980. doi: 10.1523/JNEUROSCI.4363-08.2009

Wallace, M., Luine, V., Arellanos, A., and Frankfurt, M. (2006). Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. *Brain Res.* 1126, 176–182. doi: 10.1016/j.brainres.2006.07.064

Wolf, O. T., Kudielka, B. M., Hellhammer, D. H., Törber, S., McEwen, B. S., and Kirschbaum, C. (1999). Two weeks of transdermal estradiol treatment in postmenopausal elderly women and its effect on memory and mood: verbal memory changes are associated with the treatment induced estradiol levels. *Psychoneuroendocrinology* 24:7. doi: 10.1016/S0306-4530(99)00025-6

Woolley, C. S., and McEwen, B. S. (1994). Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. *J. Neurosci.* 14, 7680–7687. doi: 10.1523/JNEUROSCI.14-12-07680.1994

World Health Organization. (2021). *Global status report on the public health response to dementia* Geneva: World Health Organization.

Yagi, S., and Galea, L. A. (2019). Sex differences in hippocampal cognition and neurogenesis. *Neuropsychopharmacology* 44, 200–213. doi: 10.1038/s41386-018-0208-4

Yanguas-Casás, N. (2020). Physiological sex differences in microglia and their relevance in neurological disorders. *Neuroimmunol. Neuroinflam.* 7, 13–22. doi: 10.20517/2347-8659.2019.31

Yasuda, K., Maki, T., Kinoshita, H., Kaji, S., Toyokawa, M., Nishigori, R., et al. (2020). Sex-specific differences in transcriptomic profiles and cellular characteristics of oligodendrocyte precursor cells. *Stem Cell Res.* 46:101866. doi: 10.1016/j.scr.2020.101866

Zandi, P. P., Carlson, M. C., Plassman, B. L., Welsh-Bohmer, K. A., Mayer, L. S., Steffens, D. C., et al. (2002). Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County study. *JAMA* 288, 2123–2129. doi: 10.1001/jama.288.17.2123

Zec, R. F., and Trivedi, M. A. (2002). The effects of estrogen replacement therapy on neuropsychological functioning in postmenopausal women with and without dementia: a critical and theoretical review. *Neuropsychol. Rev.* 12, 65–109. doi: 10.1023/A:1016880127635

Zelleroth, S., Nylander, E., Örtenblad, A., Stam, F., Nyberg, F., Grönbladh, A., et al. (2021). Structurally different anabolic androgenic steroids reduce neurite outgrowth and neuronal viability in primary rat cortical cell cultures. *J. Steroid Biochem. Mol. Biol.* 210:105863. doi: 10.1016/j.jsbmb.2021.105863

Zeydan, B., Tosakulwong, N., Schwarz, C. G., Senjem, M. L., Gunter, J. L., Reid, R. I., et al. (2019). Association of bilateral salpingo-oophorectomy before menopause onset with medial temporal lobe neurodegeneration. *JAMA Neurol.* 76, 95–100. doi: 10.1001/jamaneurol.2018.3057

Zhang, J., Zhang, T., Yu, L., Ruan, Q., Yin, L., Liu, D., et al. (2018). Effects of ovarian reserve and hormone therapy on hearing in premenopausal and postmenopausal women: a cross-sectional study. *Maturitas* 111, 77–81. doi: 10.1016/j.maturitas.2018.01.019

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Menopausal hormone therapy and risk of dementia: health insurance database in South Korea-based retrospective cohort study

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Introduction: Menopausal hormone therapy (MHT) is used to alleviate the symptoms associated with menopause, despite the lack of recommendations for MHT in preventing dementia. Recent nationwide studies have explored the association between MHT and dementia risk, but the findings remain limited. This study aims to investigate the association between MHT and the incidence of Alzheimer's disease (AD) and non-AD dementia using national population data from Korea.

Methods: We conducted a retrospective study using data from the National Health Insurance Service in Korea between January 1, 2002, and December 31, 2019. Women over 40 years were eligible for this study and classified into the MHT or non-MHT groups. The MHT group consisted of women who used Tibolone (TIB), combined estrogen plus progestin by the manufacturer (CEPM), estrogen, combined estrogen plus progestin by a physician (CEPP), and transdermal estrogen during menopause. We compared the risk of dementia between the MHT and non-MHT groups.

Results: The study included 1,399,256 patients, of whom 387,477 were in the MHT group, and 1,011,779 were in the non-MHT group. The median duration of MHT was 23 months (range: 10–55 months). After adjusting for available confounders, we found that different types of MHT had varying effects on the occurrence of dementia. TIB (HR 1.041, 95% confidence interval (CI) 1.01–1.072) and oral estrogen alone (HR 1.081, 95% CI 1.03–1.134) were associated with a higher risk of AD dementia. In contrast, there was no difference in the risk of AD dementia by CEPM (HR 0.975, 95% CI 0.93–1.019), CEPP (HR 1.131, 95% CI 0.997–1.283), and transdermal estrogen (HR 0.989, 95% CI 0.757–1.292) use. The use of TIB, CEPM, and oral estrogen alone increased the risk of non-AD dementia (HR 1.335, 95% CI 1.303–1.368; HR 1.25, 95% CI 1.21–1.292; and HR 1.128, 95% CI 1.079–1.179; respectively), but there was no risk of non-AD dementia in the other MHT groups (CEPP and topical estrogen).

Conclusion: Our findings indicate that MHT has varying effects on the incidence of AD and non-AD dementia. Specifically, TIB, CEPM, and oral estrogen alone increase the risk of non-AD dementia, while transdermal estrogen is not associated with dementia risk. It is essential to consider the type of MHT used when assessing the risk of dementia in women.

KEYWORDS

dementia, women, menopausal hormone therapy, menopause, tibolone

1. Introduction

Dementia is characterized by a decline in cognition involving one or more of the following cognitive domains: memory and learning, language, executive function, complex attention, or social cognition (McKhann et al., 2011). The number of people with dementia worldwide was estimated at 47.47 million in 2015 and is expected to reach 135.46 million in 2050. Due to the increasing number of older adults, dementia and age-related cognitive disorders present a significant challenge for health services and an increasing global problem (Ferri et al., 2005; Livingston et al., 2020; Tam et al., 2021).

This trend seems more rapid in South Korea (hereafter Korea). Based on the government-led data, it is estimated that the number of people aged ≥65 years will reach 8.53 million in 2021 and is expected to exceed 12.98 million by 2030 and 19 million (accounting for 39.8% of the older adult population) by 2050 (Jang et al., 2021; Kim J.H. et al., 2022). Additionally, the burden of dementia in Korea is an important issue and is expected to increase considerably, and the annual management cost per patient was approximately 20.72 million won in 2019 (Kong and Park, 2022). The cost of managing dementia at the national level is estimated to be 16.5 trillion won (approximately 0.86% of GDP), which is expected to increase to 63 trillion won by 2040 (Park et al., 2013; Kong and Park, 2022).

Most studies have shown that women have a higher prevalence of dementia than men. This trend, which is generally explained by differences in sex hormones, education, and life expectancy, becomes more evident as age increases, because the prevalence of Alzheimer's disease (AD) is higher in women aged >80 years than in men (Ferri et al., 2005; McKhann et al., 2011). These demographic characteristics suggest an anticipated increase in the burden of dementia in women. Identifying women at a higher risk of dementia and correcting modifiable risk factors are essential (Derreberry and Holroyd, 2019).

Menopausal hormone therapy (MHT), which is prescribed for postmenopausal women with moderate to severe menopausal vasomotor symptoms, is not recommended to prevent dementia (Lee et al., 2020; Pinkerton, 2020). Although some epidemiological and observational studies on MHT in aging women support the positive effects of estrogen on cognitive function (Imtiaz et al., 2017), data from the Women's Health Initiative and extensive research have shown that estrogen replacement does not protect against dementia and may increase its risk (Shumaker et al., 2004; McCarrey and Resnick, 2015; Pinkerton, 2020). Tibolone (TIB) treatment, as an alternative to conventional hormone replacement therapy, has estrogenic, progestogenic, and androgenic effects (Huang et al., 2010). Although there are many protective actions for the nervous system, TIB is contraindicated in women with a history of breast cancer and may increase the risk of stroke (Modelska and Cummings, 2002; Formoso et al., 2016). Although the number of participants was small, a recent nationwide case–control study showed that TIB treatment increased the risk of AD in women older than 60 years (Savolainen-Peltonen et al., 2019). Another nationwide study using Korean claims data suggested that TIB treatment did not significantly affect dementia risk aged in patients aged 50–80 years (Han et al., 2021). However, this study did not include possible confounders, such as the duration and initiation age of MHT. Furthermore, it did not adjust for various essential risk factors for dementia and gynecological factors, such as menstrual history, excessive alcohol intake, and physical activity.

Although recent nationwide studies have been conducted on the relationship between MHT and the risk of dementia, findings on this issue are limited. This study aimed to explore the risk of dementia and MHT using the Health Insurance Database of South Korea.

2. Materials and methods

2.1. Database

We conducted a retrospective study based on the national population by investigating data from the National Health Insurance Service (NHIS) in Korea from January 1, 2002, to December 31, 2019. Korea's health insurance system was integrated into a single system operated by the National Health Insurance Corporation in 2000 (Seong et al., 2017; Noh et al., 2019). Since the National Health Insurance covers most people living in Korea (about 51 million people), most disease health insurance information (age, sex, diagnostic name, surgical name, and prescription) can be checked, except for procedures not covered by insurance, such as plastic surgery (Kim et al., 2014; Noh et al., 2019). In addition, the NHIS recommends that employees and insured people aged ≥40 years undergo free cardiovascular health checkups every other year and that physical labor workers should be examined every year (Seong et al., 2017). Therefore, the NHIS provides additional health examination data for these people. Similarly, since the National Cancer Screening Program was introduced in 1999, all individuals at a certain age are given free gastric, liver, colon, breast, and cervical cancer screening every two years for all women aged >30 years (Yoo, 2008; Yun et al., 2020; Bolormaa et al., 2022). The NHIS provides additional self-survey data on history and cancer examination results. The International Classification of Diseases, 10th revision (ICD-10) was used to record the diagnosis name, and surgery and procedures were confirmed using the Korean Health Insurance Medical Care Expenses (2012, 2016, and 2019 versions) (Seong et al., 2017).

2.2. Selection of participants

Using a database of clinical records from 2002 to 2011, only women aged >40 years were eligible for this study, and participants were recruited into the MHT and non-MHT groups. The MHT group included patients prescribed at least one MHT between 2002 and 2011, while the non-MHT group were those who underwent a national health examination at least once between January 1, 2002, and December 31, 2011, excluding those who received MHT between 2002 and 2019. Participants with previous diagnostic codes for cancer (any Cxx), dementia (F00–03, G30–31), or Parkinson's disease (G20) before the 180th day from the start date of the study were excluded. Women diagnosed with menopause in 2002 and those aged <40 years were also excluded (Figure 1).

2.3. Outcome

The term 'AD dementia' used in this study is a clinical diagnostic term of AD dementia based on the ICD-10 code. AD dementia was defined based on diagnostic ICD-10 codes (F00 or G30) and non-AD

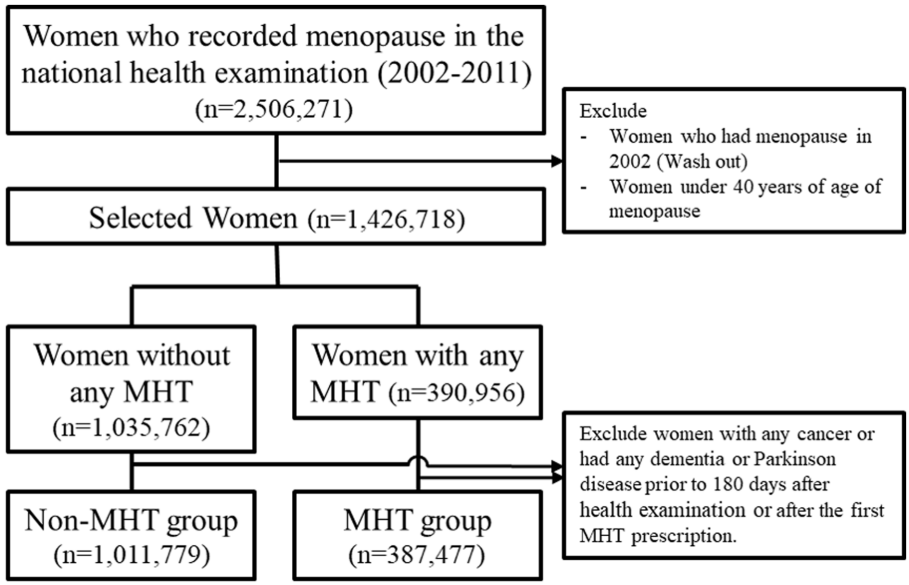

FIGURE 1
Flowchart for the selection of case–control according to MHT in Korea National Health Insurance Data, 2002–2019. MHT, menopausal hormone therapy.

dementia based on the codes (F01, F02, F03, G231, or G31), among patients who had visited three or more medical institutions. Total dementia (TD) was defined when the above two conditions are satisfied.

2.4. Variables

In this study, MHT during menopause refers to the use of TIB, combined estrogen plus progestin by the manufacturer (CEPM), estrogen, combined estrogen plus progestin by a physician (CEPP), and transdermal estrogen. A detailed list of the MHTs is provided in Supplementary Table S1. If it was confirmed that two or more MHTs were sequentially used, the last MHT used for more than 6 months was defined as the woman's treatment. The reference date for collection of data such as age, parity, age at menarche, age at menopause, body mass index (BMI), socioeconomic status (SES), region of the clinic, Charlson Comorbidity Index (CCI), period from menopause to inclusion date, smoking status, alcohol consumption status, and physical exercise was the date of participation in the study. MHT initiation date was defined as the date on which the first MHT was prescribed. On the other hand, the examination date in the institutions was defined as the treatment start date for the non-MHT group. If the start date of treatment is not confirmed and only the year of examination is recorded, June 30 of the year of examination is defined as the initiation date. The date of death (or December 31, 2019) was defined as the last day without other events. BMI in this study was evaluated based on the criteria presented from an Asia-Pacific perspective (WHO expert consultation, 2004). Patients on Medicaid for medical insurance were categorized as having an SES. An urban area is defined as an area in which the administrative district is a large city (WHO expert consultation, 2004). We calculated CCI using the diagnosis codes evaluated at the medical institutions visited from 1 year before the date of participation to the date of participation in the study (Quan et al., 2011). Current smokers were defined as those who smoked during our investigation. Never smokers and past smokers were defined as those who reported having smoked in their lifetime and those who reported having smoked but did not smoke during the study. Alcohol history was classified according to the weekly drinking frequency (None, ~2/week, 3 ~ 6/week, and daily). Physical exercise was classified according to the number of exercises performed for 30 min or more per week.

2.5. Statistical analyses

A two-sided test was performed for all statistics, and a value of $p < 0.05$ was considered statistically significant. All continuous data are presented as median [25th and 75th percentile], and categorical data are expressed as total number (%). Cox proportional hazard regression analysis, with or without adjustment for confounders, was used to analyze the associations between the variables and dementia. The pairwise deletion method was used when values were missing. All statistical analyses were carried out using SAS Enterprise Guide 6.1 (SAS Institute Inc.). To confirm the sensitivity analysis for evaluating the robustness of these results, we analyzed only participants prescribed medications by an obstetrics and gynecology specialist.

2.6. Ethics statement

This study was approved by the Institutional Review Board of the Sanggye Paik Hospital (approval number: SGPAIK-2020-08-002). Following the NHIS's information protection policy, this study removed individual-identifying variables that could only be analyzed using virtual servers within the NHIS. Therefore, this study was

conducted retrospectively using data obtained for clinical purposes, and the researcher could secure only the results. Furthermore, because it is impossible to specify any individual in the data, no possible harm can be caused. Moreover, this study did not require informed consent from the participants according to the Bioethics and Safety Act of South Korea.

3. Results

3.1. Baseline characteristics

Of the 2,506,271 women who recorded menopause in the national health examination during the study period (2002–2011), we excluded those who had menopause in 2002 (washout period) and those under 40 years old. Women with any cancer type, previous dementia, or Parkinsonian syndrome within 180 days after the health examination or after the first MHT prescription were also excluded. Finally, data from 1,399,256 patients were included in this study. Among them, 387,477 were classified into the MHT group and 1,011,779 into the non-MHT group (Figure 1).

The baseline characteristics of the women according to MHT exposure status are summarized in Table 1. The most frequently prescribed MHT was TIB (195,476; median age: 52 years), followed by CEPM (130,246; median age: 53 years), oral estrogen alone (53,143; median age: 54 years), CEPP (6,531; median age: 54 years), and transdermal estrogen (2,081; median age: 53 years). The number of patients with TD was 96,853 (9.6%) in the non-MHT group, 13,372 (6.8%) in the TIB group, 6,183 (4.7%) in the CEPM group, 3,696 (7%) in the oral estrogen alone group, 494 (7.6%) in the CEPP group, and 115 (5.5%) in the transdermal estrogen group.

3.2. Women receiving menopausal hormone therapy

Overall, the median duration of MHT was 23 months (range: 10–55 months). Only 23,145 (6%) women used hormone therapy for more than 10 years, whereas a shorter duration of MHT (shorter than 5 years) was observed in 297,801 (76.9%) women. According to the gynecology and non-gynecology specialties, physicians prescribed hormone therapy to 149,498 (38.6%) and 237,979 (61.4%) women, respectively. The detailed characteristics of the women receiving MHT in this study are shown in Table 2.

3.3. Association of reproductive factors with dementia risk in women receiving MHT

The hazard ratio for the risk of dementia according to reproductive factors are shown in Supplementary Table S2. In a dose-dependent manner, increase in the incidence of TD was significantly associated with later age at menarche, later age at inclusion, low SES, rural area, high CCI, parity, late menopausal age, smoking, and a long period from menopause to inclusion (years). Compared with short duration (5–9 years) from menopause to inclusion (hazard ratio (HR) 1.413, 95% confidence interval (CI) 1.379–1.448), long duration (10 years) was associated with a higher risk of TD (HR 2.165, 95% CI 2.103–2.228). Similar patterns were observed for both patients with AD and non-AD dementia (Supplementary Table S2). Supplementary Table S3 shows case/person-years of dementia according to the reproductive factors. Regardless of hormone type, there is an increased incidence of dementia with advanced age (60 ~ 69 years). Our findings also showed an increased risk of dementia with longer period from menopause to inclusion after menopause (<5 years versus >10 years).

3.4. Association MHT drug type and risk of dementia

In Table 3, an unadjusted analysis of dementia incidence showed that the incidence of TD was higher in the non-MHT group (9.6%) than in the MHT group (8.6%). After adjusting for available confounders, such as age group, age at menarche, age at menopause, BMI, SES, region, CCI, parity, period from menopause to inclusion, smoking status, alcohol consumption status, and physical exercise, we found that different MHTs had varying effects on dementia occurrence. Figure 2 shows the risk of each dementia phenotype based on the MHT formulation. TIB (HR 1.041, 95% confidence interval (CI) 1.01–1.072) and oral estrogen alone (HR 1.081, 95% CI 1.03–1.134) were associated with a higher risk of AD dementia. In contrast, there was no difference in the risk of AD dementia by CEPM (HR 0.975, 95% CI 0.93–1.019), CEPP (HR 1.131, 95% CI 0.997–1.283), and transdermal estrogen (HR 0.989, 95% CI 0.757–1.292) use. The use of TIB, CEPM, and oral estrogen alone increased the risk of non-AD dementia (HR 1.335, 95% CI 1.303–1.368; HR 1.25, 95% CI 1.21–1.292; and HR 1.128, 95% CI 1.079–1.179, respectively), but there was no risk of non-AD dementia in the other MHT groups (CEPP and topical estrogen). Similarly, TD risk increased in users of TIB (HR 1.212, 95% CI 1.188–1.236), CEPM (HR 1.137, 95% CI 1.11–1.169), and oral estrogen alone (HR 1.092, 95% CI 1.054–1.13), but not in the other groups.

4. Discussion

We conducted a large cohort study of menopausal women undergoing MHT to determine the association between MHT use and the risk of dementia. Our study revealed that TIB and oral estrogen alone were associated with an increased risk of AD dementia. In contrast, CEPM, CEPP, and topical estrogen were not associated with an increased risk of AD dementia. In this analysis, the use of TIB, CEPM, and oral estrogen alone was found to be related to an increased risk of non-AD dementia and TD. However, topical estrogen was not associated with an increased risk of AD or non-AD dementia.

A few studies have reported neither the benefit nor the risk of MHT concerning AD, which begins later than 65 or more than 5 years after menopause. A recent study that assessed the risks of developing TD and AD in women exposed to different types of MHT for different durations also reported no increased risks of developing dementia overall (Vinogradova et al., 2021). Furthermore, a retrospective study using a 10-year claims dataset found that MHT was associated with a reduced risk of all neurodegenerative diseases, including TD and AD (Kim et al., 2021). However, most studies have suggested the potential and overall risks of MHT later in life concerning dementia, including AD. The most

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TABLE 1 Baseline characteristics of women according to menopausal hormone exposure status at recruitment, Korea National Health Insurance Data, 2002–2019.

	Non-MHT	TIB	CEPM	Oral Estrogen	CEPP	Transdermal estrogen	Total
Number of women	1,011,779	195,476	130,246	53,143	6,531	2,081	1,399,256
Median age (years)	53 [50–57]	52 [50–56]	53 [49–57]	54 [51–59]	54 [51–59]	53 [50–58]	58 [52–64]
Median follow-up period (years)	11.5 [9.5–13.5]	12.8 [10.5–14.8]	11.9 [10.1–14.3]	13.9 [11.4–15.7]	13.8 [11.1–15.9]	14.2 [12.2–15.7]	11.5 [9.5–13.5]
Age at inclusion (years)							
40 ~ 49	85,683 (8.5)	34,331 (17.6)	29,826 (22.9)	13,831 (26)	1,143 (17.5)	439 (21.1)	165,253 (11.8)
50 ~ 59	493,744 (48.8)	128,026 (65.5)	86,702 (66.6)	29,760 (56)	3,850 (58.9)	1,271 (61.1)	743,353 (53.1)
60 ~ 69	304,315 (34.4)	29,443 (15.4)	12,679 (9.8)	7,873 (15.3)	1,345 (21.2)	336 (16.4)	355,991 (28.2)
70~	128,037 (12.7)	3,676 (1.9)	1,039 (0.8)	1,679 (3.2)	193 (3)	35 (1.7)	134,659 (9.6)
Median BMI (kg/m^2)	24 [22.1–26.1]	23.5 [21.8–25.4]	23.1 [21.5–25]	23.7 [22–25.7]	23.3 [21.6–25.2]	23.7 [22–25.6]	23.8 [21.9–25.9]
BMI (kg/m2)							
<18.5	18,757 (1.9)	3,347 (1.7)	2,608 (2)	775 (1.5)	134 (2.1)	45 (2.2)	25,666 (1.9)
18.5–22.9	340,070 (34.3)	78,531 (40.6)	58,556 (45.3)	19,491 (37)	2,696 (41.6)	768 (37.2)	500,112 (36.3)
23–24.9	262,812 (26.5)	53,719 (27.7)	34,763 (26.9)	14,746 (28)	1,815 (28)	552 (26.7)	368,407 (26.8)
25–29.9	328,197 (33.1)	53,116 (27.4)	30,852 (23.9)	15,912 (30.2)	1,689 (26.1)	641 (31.1)	430,407 (31.3)
≥30	43,002 (4.3)	4,909 (2.5)	2,557 (2)	1,786 (3.4)	142 (2.2)	58 (2.8)	52,454 (3.8)
SES							
Mid ~ high SES	970,198 (95.9)	188,565 (96.5)	126,981 (97.5)	51,674 (97.2)	6,365 (97.5)	2,021 (97.1)	1,345,804 (96.2)
Low SES	41,581 (4.1)	6,911 (3.5)	3,265 (2.5)	1,469 (2.8)	166 (2.5)	60 (2.9)	53,452 (3.8)
Region							
Urban area	298,338 (29.5)	60,734 (31.1)	43,925 (33.7)	16,682 (31.4)	3,297 (50.5)	942 (45.3)	423,918 (30.3)
Rural area	713,441 (70.5)	134,742 (68.9)	86,321 (66.3)	36,461 (68.6)	3,234 (49.5)	1,139 (54.7)	975,338 (69.7)
CCI							
0	664,370 (65.7)	132,453 (67.8)	91,893 (70.6)	36,856 (69.4)	4,498 (68.9)	1,351 (64.9)	931,421 (66.6)
1	201,286 (19.9)	38,429 (19.7)	23,834 (18.3)	9,849 (18.5)	1,234 (18.9)	393 (18.9)	275,025 (19.7)
≥2	146,123 (14.4)	24,594 (12.6)	14,519 (11.1)	6,438 (12.1)	799 (12.2)	337 (16.2)	192,810 (13.8)
Parity (years)							
0 or not respond	165,454 (16.4)	30,289 (15.5)	16,242 (12.5)	11,153 (21)	1,285 (19.7)	455 (21.9)	224,878 (16.1)
1	60,098 (5.9)	17,054 (8.7)	13,578 (10.4)	4,091 (7.7)	487 (7.5)	167 (8)	95,475 (6.8)
2	664,541 (74.7)	130,455 (73.4)	90,873 (75.3)	32,657 (68.2)	4,091 (69.8)	1,251 (66.8)	923,868 (74.3)
≥3	121,686 (12)	17,678 (9)	9,553 (7.3)	5,242 (9.9)	668 (10.2)	208 (10)	155,035 (11.1)
Age at menarche (years)							
<13	157,857 (15.7)	28,371 (14.6)	18,522 (14.3)	9,752 (18.6)	1,212 (18.7)	387 (18.8)	216,101 (15.5)
≥13	849,189 (84.3)	165,588 (85.4)	110,982 (85.7)	42,709 (81.4)	5,264 (81.3)	1,669 (81.2)	1,175,401 (84.5)
Age at menopause (years)							
40–44	120,943 (12)	23,411 (12)	14,623 (11.2)	11,459 (21.6)	834 (12.8)	412 (19.8)	171,682 (12.3)
45–49	291,724 (28.8)	63,608 (32.5)	43,607 (33.5)	18,852 (35.5)	2,095 (32.1)	751 (36.1)	420,637 (30.1)
50–54	510,195 (55.3)	93,644 (51.8)	63,392 (52.1)	20,240 (40)	3,085 (51.3)	789 (40.4)	691,345 (53.9)
55-	88,917 (8.8)	14,813 (7.6)	8,624 (6.6)	2,592 (4.9)	517 (7.9)	129 (6.2)	115,592 (8.3)
Smoking							
Never	923,518 (96.4)	176,371 (93.7)	117,896 (93.5)	48,550 (95)	6,019 (95.7)	1,907 (96.1)	1,274,261 (95.7)
Past	9,755 (1)	3,223 (1.7)	2,320 (1.8)	713 (1.4)	82 (1.3)	32 (1.6)	16,125 (1.2)

(Continued)

TABLE 1 (Continued)

	Non-MHT	TIB	CEPM	Oral Estrogen	CEPP	Transdermal estrogen	Total
Current	25,047 (2.6)	8,560 (4.5)	5,902 (4.7)	1,852 (3.6)	189 (3)	45 (2.3)	41,595 (3.1)
Alcohol (per week)							
None	820,873 (85.3)	146,881 (77.5)	96,196 (75.8)	41,050 (79.7)	5,242 (82.7)	1,658 (82.4)	1,111,900 (83.1)
~2/week	120,759 (12.6)	36,109 (19.1)	26,181 (20.6)	9,095 (17.7)	968 (15.3)	312 (15.5)	193,424 (14.5)
3~6/week	14,997 (1.6)	4,951 (2.6)	3,572 (2.8)	993 (1.9)	90 (1.4)	31 (1.5)	24,634 (1.9)
Daily	5,380 (0.6)	1,519 (0.8)	924 (0.7)	385 (0.7)	40 (0.6)	10 (0.5)	8,258 (0.6)
Physical exercise (per week)							
None	631,239 (65.5)	112,640 (59.5)	76,031 (59.9)	30,716 (59.7)	3,624 (57.2)	1,057 (52.8)	855,307 (63.8)
1~2	158,439 (16.4)	36,118 (19.1)	24,736 (19.5)	9,891 (19.2)	1,236 (19.5)	430 (21.5)	230,850 (17.2)
3~4	87,866 (9.1)	21,741 (11.5)	14,821 (11.7)	5,713 (11.1)	813 (12.8)	297 (14.8)	131,251 (9.8)
5~6	29,422 (3.1)	7,126 (3.8)	4,846 (3.8)	1,808 (3.5)	245 (3.9)	86 (4.3)	43,533 (3.2)
Daily	56,610 (5.9)	11,713 (6.2)	6,466 (5.1)	3,358 (6.5)	417 (6.6)	132 (6.6)	78,696 (5.9)
Period from menopause to inclusion (years)							
<5	396,090 (39.1)	113,704 (58.2)	88,755 (68.1)	27,783 (52.3)	3,347 (51.2)	1,055 (50.7)	630,734 (45.1)
5~9	212,604 (21)	44,936 (23)	25,830 (19.8)	13,287 (25)	1,568 (24)	546 (26.2)	298,771 (21.4)
10~	403,085 (39.8)	36,836 (18.8)	15,661 (12)	12,073 (22.7)	1,616 (24.7)	480 (23.1)	469,751 (33.6)

TIB, Tibolone; CEPM, Combined estrogen plus progestin by manufacturer; CEPP, Combined estrogen plus progestin by physician; BMI, Body mass index; CCI, Charlson comorbidity index; MHT, menopausal hormone therapy; SES, socioeconomic status. Data are expressed as the number (%) or median [25 percentile, 75 percentile].

TABLE 2 Characteristics of women with menopausal hormone therapy, Korea National Health Insurance Data, 2002–2019.

MHT characteristics	TIB	CEPM	Oral Estrogen	CEPP	Transdermal estrogen	Total MHT
Median duration (months)	25 [11–58]	25 [11–58]	15 [9–40]	16 [9–35]	13 [8–24]	23 [10–55]
Duration (years)						
<5	147,530 (75.5)	98,633 (75.7)	43,962 (82.7)	5,694 (87.2)	1,982 (95.2)	297,801 (76.9)
5–9.9	35,208 (18)	24,201 (18.6)	6,380 (12)	648 (9.9)	94 (4.5)	66,531 (17.2)
≥10	12,738 (6.5)	7,412 (5.7)	2,801 (5.3)	189 (2.9)	5 (0.2)	23,145 (6)
Duration of previous other MHT (years)						
<5	190,078 (97.2)	128,128 (98.4)	52,353 (98.5)	5,439 (83.3)	2,055 (98.8)	378,053 (97.6)
5–9.9	4,823 (2.5)	1,938 (1.5)	709 (1.3)	811 (12.4)	25 (1.2)	8,306 (2.1)
≥10	575 (0.3)	180 (0.1)	81 (0.2)	281 (4.3)	1 (0)	1,118 (0.3)
Last dosage of tibolone (per day)						
1.25 mg	1,859 (1)					
2.5 mg	193,416 (99)					
over 5 mg	181 (0.1)					
Prescribed specialty						
Gynecology	65,811 (33.7)	59,661 (45.8)	21,946 (41.3)	1,566 (24)	514 (24.7)	149,498 (38.6)
Non-gynecology	129,665 (66.3)	70,585 (54.2)	31,197 (58.7)	4,965 (76)	1,567 (75.3)	237,979 (61.4)

MHT, menopausal hormone therapy; TIB, Tibolone; CEPM, Combined estrogen plus progestin by manufacturer; CEPP, Combined estrogen plus progestin by physician. Data are expressed as the number (%) or median [25 percentile, 75 percentile].

influential study, the Women's Health Initiative Memory Study, reported a significantly increased TD risk for using conjugated equine estrogen (CEE) + medroxyprogesterone acetate and CEE alone (Shumaker et al., 2003). Similar results were noted in the Kronos Early Estrogen Prevention Cognitive and Affective Ancillary Study (Gleason et al., 2015). Early versus Late Intervention Trial with Estradiol-Cognitive Endpoints indicated no beneficial or harmful effects of MHT on dementia risk (Henderson, 2011; Henderson et al., 2016). Until recently, prescribing MHT purely for dementia prevention was not recommended in the absence of other clinical indications for MHT, such as vasomotor

TABLE 3 Incidence of dementia according to tibolone exposure status at recruitment, Korea National Health Insurance Data, 2002–2019.

	Non-MHT	TIB	CEPM	Oral Estrogen	CEPP	Transdermal estrogen	Total
Median period from menopause to inclusion (years)	7 [2–14.5]	3 [1–8]	2 [0–6]	4 [1–9]	4.5 [1–9.5]	4.5 [1–9]	5.5 [2–12.5]
AD dementia							
Not present	948,489 (93.7)	189,732 (97.1)	127,885 (98.2)	51,205 (96.4)	6,263 (95.9)	2,018 (97)	1,325,592 (94.7)
Present	63,290 (6.3)	5,744 (2.9)	2,361 (1.8)	1,938 (3.6)	268 (4.1)	63 (3)	73,664 (5.3)
Non-AD dementia							
Not present	962,844 (95.2)	186,420 (95.4)	125,803 (96.6)	50,866 (95.7)	6,243 (95.6)	2,011 (96.6)	1,334,187 (95.3)
Present	48,935 (4.8)	9,056 (4.6)	4,443 (3.4)	2,277 (4.3)	288 (4.4)	70 (3.4)	65,069 (4.7)
Total dementia							
Not present	914,926 (90.4)	182,104 (93.2)	124,063 (95.3)	49,447 (93)	6,037 (92.4)	1,966 (94.5)	1,278,543 (91.4)
Present	96,853 (9.6)	13,372 (6.8)	6,183 (4.7)	3,696 (7)	494 (7.6)	115 (5.5)	120,713 (8.6)

MHT, menopausal hormone therapy; TIB, Tibolone; CEPM, Combined estrogen plus progestin by manufacturer; CEPP, Combined estrogen plus progestin by physician; AD, Alzheimer's disease. Data are expressed as the number (%) or median [25 percentile, 75 percentile].

symptoms (hot flashes and night sweats) and sleep disturbances (Baber et al., 2016; Marjoribanks et al., 2017; North American Menopause Society, 2017). Our study, which adjusted for other dementia risk factors, also showed that hormone therapy increased the overall risk of dementia in elderly menopausal women. Moreover, our results (Supplementary Table S2) are consistent with the content of the current hormone therapy guidelines, explicitly emphasizing the potential benefits of hormone therapy for younger postmenopausal patients and those who commence treatment within 10 years of menopause onset.

The most important finding of this study was that MHT had different effects on the incidence of AD and non-AD dementia. In this study, TIB, CEPM, and estrogen-only use were associated with an increased risk of non-AD dementia, consequently increasing overall TD incidence. Excluding CEPP, most oral hormone therapies (TIB, CEPM, and estrogen) showed an increased tendency towards non-AD dementia occurrence. This is believed to be due to the accumulation of prothrombotic metabolites that may occur during the metabolic process of oral hormone therapy, leading to atherosclerotic effects, ischemia, and vascular cognitive decline (Henderson and Popat, 2011; Scarabin-Carre et al., 2012). Although TIB is known to show a cardiovascular protection effect through its metabolite (Del Rio et al., 2020), long-term use of TIB may also be associated with increased cardiovascular risks, such as CRP elevation and hyperthermia, which could lead to brain vessel wall injury (Formoso et al., 2016; Zhu et al., 2021). A similar mechanism can explain this as the analysis result that topical estrogen is not associated with non-AD dementia occurrence. In other words, topical estrogen, which has a hepatic first-pass effect, is not metabolized in the liver and does not produce various metabolites that can show adverse vascular effects (Simon, 2012; Beck et al., 2017; Oliver-Williams et al., 2019).

Previous studies have reported the neuroprotective effects of estrogen on the brain, cognition, and dementia (Yaffe et al., 2000; Carlson et al., 2001; Lui et al., 2003). Estrogen improves synapse formation in the hippocampus and increases choline acetyltransferase activity in the basal forebrain of rats (Aubele and Kritzer, 2011). Moreover, estrogen improves prefrontal lobe-dependent executive functioning in women (Krug et al., 2006). In addition to its cognitive effects, estrogen has neuroprotective effects, such as inhibiting amyloid-beta depositions in the brain, primarily forming neuropathological lesions in patients with AD (Sohrabji, 2005). However, an increased level of free estradiol may indicate atherothrombogenic stroke risk in older postmenopausal women (Henderson and Popat, 2011). Insulin resistance, dyslipidemia, and inflammation are the potential mediators of this association (Scarabin-Carre et al., 2012). Although the risk of ischemic stroke differs by age, recent studies of MHT on stroke incidence suggest that the risk of ischemic stroke increases with oral MHT use in women aged >60 years (Bushnell et al., 2014). Considering the incidence of all-cause dementia, most non-AD cases appear to be vascular dementia cases (O'Brien and Thomas, 2015). However, the precise mechanism by which MHT affects brain vessels and influences the incidence of ischemic stroke has not been established (Miller et al., 2003; Khalil, 2013). Considering vascular dementia and the increased risk of ischemic stroke due to MHT, our findings indicate that MHT specifically increases the risk of vascular dementia rather than AD dementia. Consequently, the incidence of overall dementia increased among postmenopausal women. Although there are several studies regarding the risk of AD and vascular dementia after using MHT (Savolainen-Peltonen et al., 2019; Vinogradova et al., 2021), the present study's findings are notable because they considered vascular dementia, which may be anticipated to occur at elevated rates in the presence of increased cerebral thrombosis risk in MHT users. This result is consistent with the finding of an earlier study that showed an overall increase of 20–40% in the risk of ischemic stroke.

The strength of our study was that it was a large-scale Asian population retrospective cohort study with a sufficient number of participants ($N = 2{,}506{,}272$) compared with the current observational study. It included many TIB users, and TIB is widely used in Europe, Asia, and Korea (Huang et al., 2010). Although we conducted a large-scale study of MHT effects on dementia, several issues limit the generalizability of our findings: the patients in our data were only Korean. Therefore, the generalization of the results to other ethnic or racial groups should be made with caution. There may have been detection bias in this study. Moreover, we did not have additional biomarkers indicating AD pathology, such as cerebrospinal fluid

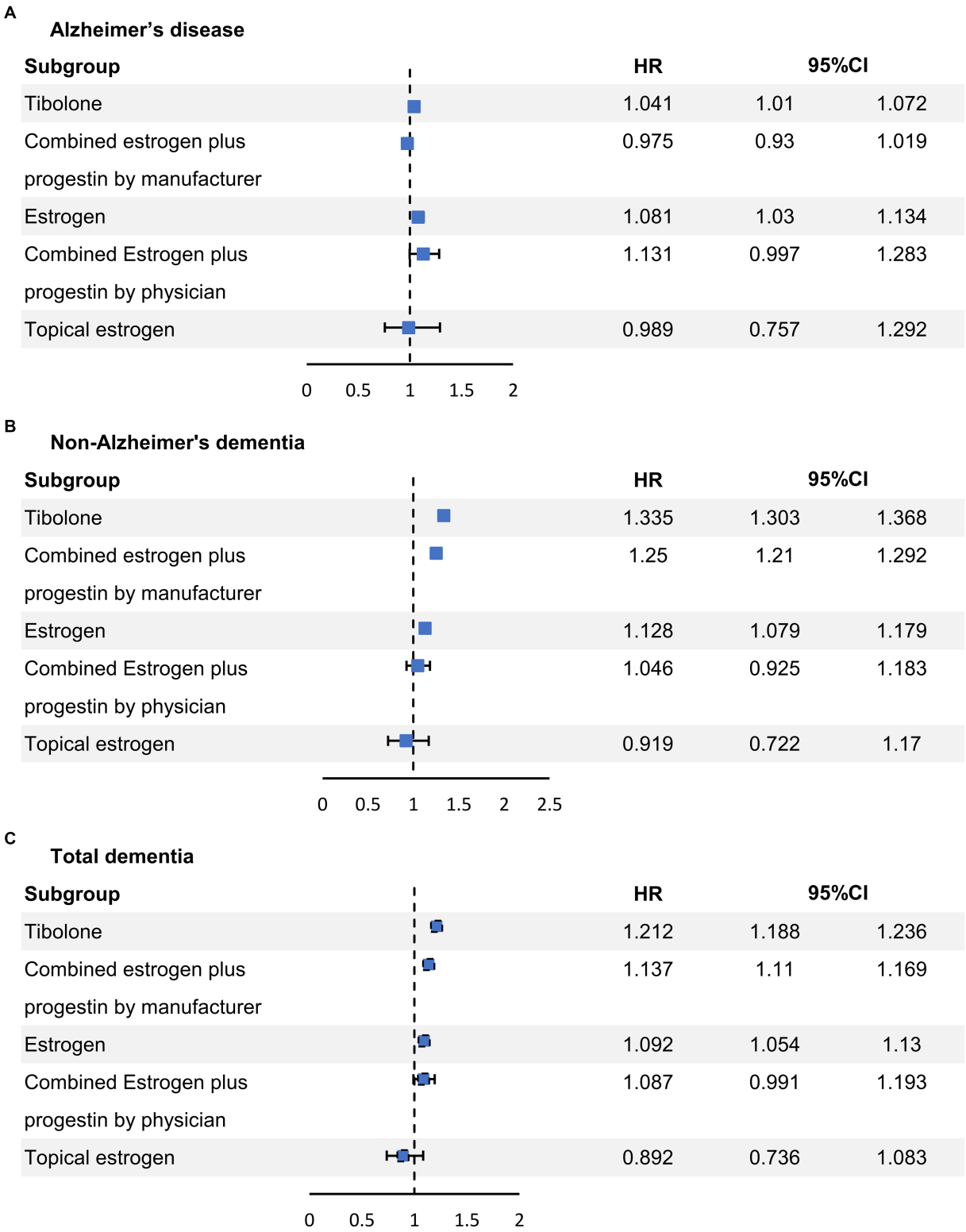

FIGURE 2
Conditional logistic regression analysis of the risk of each dementia phenotype based on the formulation of the MHT. MHT, menopausal hormone therapy. CI, confidence interval; HR, hazard ratio.

biomarkers, genetic testing, molecular imaging or neuropathological data from the participants. Also, although age is regarded to be an important parameter in estimating the risk of AD, our study has a limitation in that it did not reconcile younger skew of age and older age for development of AD. We assumed that non-AD dementia represents overall vascular dementia; however, other etiologies, such as Parkinson's disease, may occur alongside non-AD dementia. Therefore, it is impossible to confirm the diagnosis code for any

dementia, and our analysis may have led to a more inaccurate diagnosis. We assumed that most patients adhered to their physicians' instructions as this study's information on drug prescriptions was derived from claims data. However, menopausal women may not have adhered to their MHT prescriptions, which could have potentially introduced biases. It is also important to note that many Koreans prefer phytoestrogens over MHT (Kim Y. et al., 2022). Finally, although we analyzed as much data as possible, more information was needed regarding laboratory measurements, including serum hormone levels and blood pressure, which could be potential confounders.

This investigation showed that MHT had different effects on the incidence of AD and non-AD dementia, suggesting that it specifically increases the risk of non-AD dementia but that transdermal estrogen is not associated with dementia risk. The different effects of MHT should be considered in appropriately selected patients according to individual situations, such as underlying cardiovascular risk status.

Data availability statement

The data analyzed in this study was obtained from The National Health Information Database of the National Health Insurance Service in South Korea. The datasets are not readily available because, due to NHIS's privacy policy, only researchers have access to the data for a limited period of time. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Institutional Review Board of the Sanggye Paik Hospital (approval number: SGPAIK-2020-08-002). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

J-SY and JP: conception and design of the study, acquisition of data, and final approval of the manuscript. J-SY, JL, and JP: analysis and interpretation of the data, drafting and revising the manuscript for content. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1213481/full#supplementary-material

References

Aubele, T., and Kritzer, M. F. (2011). Gonadectomy and hormone replacement affects in vivo basal extracellular dopamine levels in the prefrontal cortex but not motor cortex of adult male rats. *Cereb. Cortex* 21, 222–232. doi: 10.1093/cercor/bhq083

Baber, R. J., Panay, N., and Fenton, A.IMS Writing Group (2016). 2016 IMS recommendations on women's midlife health and menopause hormone therapy. *Climacteric* 19, 109–150. doi: 10.3109/13697137.2015.1129166

Beck, K. L., Anderson, M. C., and Kirk, J. K. (2017). Transdermal estrogens in the changing landscape of hormone replacement therapy. *Postgrad. Med.* 129, 632–636. doi: 10.1080/00325481.2017.1334507

Bolormaa, E., Choe, S. A., Son, M., Ki, M., and Paek, D. (2022). Income-based disparities in the risk of distant-stage cervical cancer and 5-year mortality after the introduction of a National Cancer Screening Program in Korea. *Epidemiol. Health* 44:e2022066. doi: 10.4178/epih.e2022066

Bushnell, C., McCullough, L. D., Awad, I. A., Chireau, M. V., Fedder, W. N., Furie, K. L., et al. (2014). Guidelines for the prevention of stroke in women: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 45, 1545–1588. doi: 10.1161/01.str.0000442009.06663.48

Carlson, M. C., Zandi, P. P., Plassman, B. L., Tschanz, J. T., Welsh-Bohmer, K. A., Steffens, D. C., et al. (2001). Hormone replacement therapy and reduced cognitive decline in older women: the Cache County study. *Neurology* 57, 2210–2216. doi: 10.1212/wnl.57.12.2210

Del Rio, J. P., Molina, S., Hidalgo-Lanussa, O., Garcia-Segura, L. M., and Barreto, G. E. (2020). Tibolone as hormonal therapy and neuroprotective agent. *Trends Endocrinol. Metab.* 31, 742–759. doi: 10.1016/j.tem.2020.04.007

Derreberry, T. M., and Holroyd, S. (2019). Dementia in Women. *Med. Clin. North Am.* 103, 713–721. doi: 10.1016/j.mcna.2019.02.004

Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet* 366, 2112–2117. doi: 10.1016/S0140-6736(05)67889-0

Formoso, G., Perrone, E., Maltoni, S., Balduzzi, S., Wilkinson, J., Basevi, V., et al. (2016). Short-term and long-term effects of tibolone in postmenopausal women. *Cochrane Database Syst. Rev.* 10:CD008536. doi: 10.1002/14651858.CD008536.pub3

Gleason, C. E., Dowling, N. M., Wharton, W., Manson, J. E., Miller, V. M., Atwood, C. S., et al. (2015). Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-cognitive and affective study. *PLoS Med.* 12:e1001833; discussion e1001833. doi: 10.1371/journal.pmed.1001833

Han, M., Chang, J., Choi, S., Cho, Y., Lee, G., and Park, S. M. (2021). Association of tibolone and dementia risk: a cohort study using Korean claims data. *Gynecol. Endocrinol.* 37, 567–571. doi: 10.1080/09513590.2020.1843624

Henderson, V. W. (2011). Gonadal hormones and cognitive aging: a midlife perspective. *Womens Health (Lond)* 7, 81–93. doi: 10.2217/whe.10.87

Henderson, V. W., and Popat, R. A. (2011). Effects of endogenous and exogenous estrogen exposures in midlife and late-life women on episodic memory and executive functions. *Neuroscience* 191, 129–138. doi: 10.1016/j.neuroscience.2011.05.059

Henderson, V. W., St John, J. A., Hodis, H. N., McCleary, C. A., Stanczyk, F. Z., Shoupe, D., et al. (2016). Cognitive effects of estradiol after menopause: a randomized trial of the timing hypothesis. *Neurology* 87, 699–708. doi: 10.1212/WNL.0000000000002980

Huang, K. E., and Baber, R.Asia Pacific Tibolone Consensus, G (2010). Updated clinical recommendations for the use of tibolone in Asian women. *Climacteric* 13, 317–327. doi: 10.3109/13697131003681458

Imtiaz, B., Tuppurainen, M., Rikkonen, T., Kivipelto, M., Soininen, H., Kroger, H., et al. (2017). Postmenopausal hormone therapy and Alzheimer disease: a prospective cohort study. *Neurology* 88, 1062–1068. doi: 10.1212/WNL.0000000000003696

Jang, J. W., Park, J. H., Kim, S., Lee, S. H., Lee, S. H., and Kim, Y. J. (2021). Prevalence and incidence of dementia in South Korea: a Nationwide analysis of the National Health Insurance Service senior cohort. *J. Clin. Neurol.* 17, 249–256. doi: 10.3988/jcn.2021.17.2.249

Khalil, R. A. (2013). Estrogen, vascular estrogen receptor and hormone therapy in postmenopausal vascular disease. *Biochem. Pharmacol.* 86, 1627–1642. doi: 10.1016/j.bcp.2013.09.024

Kim, L., Kim, J. A., and Kim, S. (2014). A guide for the utilization of Health Insurance Review and Assessment Service national patient samples. *Epidemiol. Health* 36:e2014008. doi: 10.4178/epih/e2014008

Kim, Y., Kim, D. W., Kim, K., Choe, J. S., and Lee, H. J. (2022). Usual intake of dietary isoflavone and its major food sources in Koreans: Korea National Health and nutrition examination survey 2016-2018 data. *Nutr. Res. Pract.* 16, S134–S146. doi: 10.4162/nrp.2022.16.S1.S134

Kim, Y. J., Soto, M., Branigan, G. L., Rodgers, K., and Brinton, R. D. (2021). Association between menopausal hormone therapy and risk of neurodegenerative diseases: implications for precision hormone therapy. *Alzheimers Dement (N Y)* 7:e12174. doi: 10.1002/trc2.12174

Kim, J. H., Yim, D. H., Choi, I. A., Lee, J., Park, H., and Eom, S. Y. (2022). Impact of clinical association between gout and dementia: a Nationwide population-based cohort study in Korea. *Arthritis Care Res.* 75, 1088–1094. doi: 10.1002/acr.24959

Kong, J. W., and Park, J. Y. (2022). Understanding suicide risk in people with dementia and family caregivers in South Korea: a systematic review. *Behav. Sci. (Basel)* 12:97. doi: 10.3390/bs12040097

Krug, R., Born, J., and Rasch, B. (2006). A 3-day estrogen treatment improves prefrontal cortex-dependent cognitive function in postmenopausal women. *Psychoneuroendocrinology* 31, 965–975. doi: 10.1016/j.psyneuen.2006.05.007

Lee, S. R., Cho, M. K., Cho, Y. J., Chun, S., Hong, S. H., Hwang, K. R., et al. (2020). The 2020 menopausal hormone therapy guidelines. *J Menopausal Med* 26, 69–98. doi: 10.6118/jmm.20000

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., et al. (2020). Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet* 396, 413–446. doi: 10.1016/S0140-6736(20)30367-6

Lui, L. Y., Stone, K., Cauley, J. A., Hillier, T., and Yaffe, K. (2003). Bone loss predicts subsequent cognitive decline in older women: the study of osteoporotic fractures. *J. Am. Geriatr. Soc.* 51, 38–43. doi: 10.1034/j.1601-5215.2002.51007.x

Marjoribanks, J., Farquhar, C., Roberts, H., Lethaby, A., and Lee, J. (2017). Long-term hormone therapy for perimenopausal and postmenopausal women. *Cochrane Database Syst. Rev.* 2017:CD004143. doi: 10.1002/14651858.CD004143.pub5

McCarrey, A. C., and Resnick, S. M. (2015). Postmenopausal hormone therapy and cognition. *Horm. Behav.* 74, 167–172. doi: 10.1016/j.yhbeh.2015.04.018

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R. Jr., Kawas, C. H., et al. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 263–269. doi: 10.1016/j.jalz.2011.03.005

Miller, A. P., Chen, Y. F., Xing, D., Feng, W., and Oparil, S. (2003). Hormone replacement therapy and inflammation: interactions in cardiovascular disease. *Hypertension* 42, 657–663. doi: 10.1161/01.HYP.0000085560.02979.0C

Modelska, K., and Cummings, S. (2002). Tibolone for postmenopausal women: systematic review of randomized trials. *J. Clin. Endocrinol. Metab.* 87, 16–23. doi: 10.1210/jcem.87.1.8141

Noh, Y., Jeong, H. E., Kim, H. J., Ko, H., Nah, E. H., and Shin, J. Y. (2019). The multi-institutional health screening records database of South Korea: description and evaluation of its characteristics. *Yonsei Med. J.* 60, 1216–1222. doi: 10.3349/ymj.2019.60.12.1216

North American Menopause Society (2017). The 2017 hormone therapy position statement of The North American Menopause Society. *Menopause* 24, 728–753. doi: 10.1097/GME.0000000000000921

O'Brien, J. T., and Thomas, A. (2015). Vascular dementia. *Lancet* 386, 1698–1706. doi: 10.1016/S0140-6736(15)00463-8

Oliver-Williams, C., Glisic, M., Shahzad, S., Brown, E., Pellegrino Baena, C., Chadni, M., et al. (2019). The route of administration, timing, duration and dose of postmenopausal hormone therapy and cardiovascular outcomes in women: a systematic review. *Hum. Reprod. Update* 25, 257–271. doi: 10.1093/humupd/dmy039

Park, J. H., Eum, J. H., Bold, B., and Cheong, H. K. (2013). Burden of disease due to dementia in the elderly population of Korea: present and future. *BMC Public Health* 13:293. doi: 10.1186/1471-2458-13-293

Pinkerton, J. V. (2020). Hormone therapy for postmenopausal women. *N. Engl. J. Med.* 382, 446–455. doi: 10.1056/NEJMcp1714787

Quan, H., Li, B., Couris, C. M., Fushimi, K., Graham, P., Hider, P., et al. (2011). Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. *Am. J. Epidemiol.* 173, 676–682. doi: 10.1093/aje/kwq433

Savolainen-Peltonen, H., Rahkola-Soisalo, P., Hoti, F., Vattulainen, P., Gissler, M., Ylikorkala, O., et al. (2019). Use of postmenopausal hormone therapy and risk of Alzheimer's disease in Finland: nationwide case-control study. *BMJ* 364:l665. doi: 10.1136/bmj.l665

Scarabin-Carre, V., Canonico, M., Brailly-Tabard, S., Trabado, S., Ducimetiere, P., Giroud, M., et al. (2012). High level of plasma estradiol as a new predictor of ischemic arterial disease in older postmenopausal women: the three-city cohort study. *J. Am. Heart Assoc.* 1:e001388. doi: 10.1161/JAHA.112.001388

Seong, S. C., Kim, Y. Y., Park, S. K., Khang, Y. H., Kim, H. C., Park, J. H., et al. (2017). Cohort profile: the National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS) in Korea. *BMJ Open* 7:e016640. doi: 10.1136/bmjopen-2017-016640

Shumaker, S. A., Legault, C., Kuller, L., Rapp, S. R., Thal, L., Lane, D. S., et al. (2004). Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative memory study. *JAMA* 291, 2947–2958. doi: 10.1001/jama.291.24.2947

Shumaker, S. A., Legault, C., Rapp, S. R., Thal, L., Wallace, R. B., Ockene, J. K., et al. (2003). Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative memory study: a randomized controlled trial. *JAMA* 289, 2651–2662. doi: 10.1001/jama.289.20.2651

Simon, J. A. (2012). What's new in hormone replacement therapy: focus on transdermal estradiol and micronized progesterone. *Climacteric* 15, 3–10. doi: 10.3109/13697137.2012.669332

Sohrabji, F. (2005). Estrogen: a neuroprotective or proinflammatory hormone? Emerging evidence from reproductive aging models. *Ann. N. Y. Acad. Sci.* 1052, 75–90. doi: 10.1196/annals.1347.006

Tam, M. T., Dosso, J. A., and Robillard, J. M. (2021). The impact of a global pandemic on people living with dementia and their care partners: analysis of 417 lived experience reports. *J. Alzheimers Dis.* 80, 865–875. doi: 10.3233/JAD-201114

Vinogradova, Y., Dening, T., Hippisley-Cox, J., Taylor, L., Moore, M., and Coupland, C. (2021). Use of menopausal hormone therapy and risk of dementia: nested case-control studies using QResearch and CPRD databases. *BMJ* 374:n2182. doi: 10.1136/bmj.n2182

WHO expert consultation (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363, 157–163. doi: 10.1016/S0140-6736(03)15268-3

Yaffe, K., Haan, M., Byers, A., Tangen, C., and Kuller, L. (2000). Estrogen use, APOE, and cognitive decline: evidence of gene-environment interaction. *Neurology* 54, 1949–1954. doi: 10.1212/wnl.54.10.1949

Yoo, K. Y. (2008). Cancer control activities in the Republic of Korea. *Jpn. J. Clin. Oncol.* 38, 327–333. doi: 10.1093/jjco/hyn026

Yun, E. H., Hong, S., Her, E. Y., Park, B., Suh, M., Choi, K. S., et al. (2020). Trends in participation rates of the National Cancer Screening Program among cancer survivors in Korea. *Cancers (Basel)* 13:81. doi: 10.3390/cancers13010081

Zhu, Y., Zhu, Y., Chen, Y., Yan, Q., Baradwan, S., Gaman, M. A., et al. (2021). The effect of tibolone treatment on fasting blood sugar, insulin, insulin resistance and endothelial function in postmenopausal women: a meta-analysis of randomized controlled trials. *Exp. Gerontol.* 155:111586. doi: 10.1016/j.exger.2021.111586

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Sex differences in the structural rich-club connectivity in patients with Alzheimer's disease

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Background and objectives: Alzheimer's disease (AD) is more prevalent in women than in men; however, there is a discrepancy in research on sex differences in AD. The human brain is a large-scale network with hub regions forming a central core, the rich-club, which is vital to cognitive functions. However, it is unknown whether alterations in the rich-clubs in AD differ between men and women. We aimed to investigate sex differences in the rich-club organization in the brains of patients with AD.

Methods: In total, 260 cognitively unimpaired individuals with negative amyloid positron emission tomography (PET) scans, 281 with prodromal AD (mild cognitive impairment due to AD) and 285 with AD dementia who confirmed with positive amyloid PET scans participated in the study. We obtained high-resolution T1-weighted and diffusion tensor images and performed network analysis.

Results: We observed sex differences in the rich-club and feeder connections in patients with AD, suggesting lower structural connectivity strength in women than in men. We observed a significant group-by-sex interaction in the feeder connections, particularly in the thalamus. In addition, the connectivity strength of the thalamus in the feeder connections was significantly correlated with general cognitive function in only men with prodromal AD and women with AD dementia.

Conclusion: Our findings provide important evidence for sex-specific alterations in the structural brain network related to AD.

KEYWORDS

sex differences, structural brain network, network analysis, rich-club organization, thalamus

1. Introduction

Alzheimer's disease (AD), the most common type of dementia in older adults, is characterized by progressive cognitive decline (Ewers et al., 2011; Scheltens et al., 2016).

Epidemiological evidence suggests that the prevalence of AD dementia is higher in women than in men (Mazure and Swendsen, 2016). In addition to the higher prevalence of AD dementia in women, a longitudinal study demonstrated that cognitive progression in women with amnestic mild cognitive impairment (MCI) was twice as fast as that in men even after correcting for *APOE* genotypes (Lin et al., 2015). In addition, the annual conversion rate of MCI to AD dementia is higher in women than in men (Tifratene et al., 2015).

Cognitive abilities also demonstrate variations based on sex. Research indicates that men and women patients with MCI, even when experiencing similar levels of hippocampal atrophy and temporal lobe glucose metabolism, exhibit different verbal memory performance (Sundermann et al., 2016). Furthermore, there are notable sex differences in neuropsychiatric symptoms associated with AD dementia. Male AD dementia patients tend to display apathy, agitation, and socially inappropriate or abusive behavior, while female patients are more susceptible to depression, mood instability, and affective symptoms (Mazure and Swendsen, 2016; Ferretti et al., 2018).

However, the unpinning neuro-mechanisms responsible for the sex-related differences in AD remain unidentified. Only a few studies have reported on sex differences in the brain structures or cognitive decline in AD dementia (Ardekani et al., 2016; Koran et al., 2017). Women with positive AD biomarkers (Amyloid-β and tau) demonstrate faster rates of hippocampal atrophy and cognitive decline than do men (Koran et al., 2017). Conversely, some studies have demonstrated no distinct sex differences in post-mortem amyloid β or neurofibrillary tangle burden of patients with AD dementia (Barnes et al., 2005) or by the measurement of cerebrospinal fluid amyloid-β (Aβ42) and tau levels (Mattsson et al., 2017). The results exhibit some discrepancies. Therefore, more comprehensive research is required to investigate sex differences in the brain of patients with AD.

The human brain is organized into a large-scale network (Hagmann et al., 2007; Gong et al., 2009) characterized by an optimal balance between the integration and segregation of information (Latora and Marchiori, 2001). Hub regions, such as the superior frontal cortex, superior parietal cortex, and precuneus, play a pivotal role in the hierarchical organization of the brain (van den Heuvel and Sporns, 2011, 2013). These regions are highly interconnected with each other and form a central core, the rich-club (van den Heuvel and Sporns, 2011, 2013; van den Heuvel et al., 2013). Since rich-club connections link spatially distant regions, extensive long-distance white matter fibers to interconnect them are required. This incurs a high biological cost, it can be balanced out by having a substantial concentration of rich-club connections, thereby improving efficient communication (Van Den Heuvel et al., 2012).

In rich-club organization, the nodes are divided into rich-club and non-rich-club nodes, and then their connections are further classified into three categories including rich-club, feeder, and local connections (van den Heuvel and Sporns, 2011, 2013). The rich-club organization may play a pivotal role in cognitive function in the human brain (Griffa and Van Den Heuvel, 2018), and these rich-club brain regions are known to be involved in the integration of neural information (Kim and Min, 2020). Therefore, the investigation of rich-club organizations may provide structural network-based information relevant to the disease.

Disruptions in the structural brain network contribute to the pathophysiology of AD (Yan et al., 2018). Prior investigations into brain network connectivity in individuals with AD dementia have revealed disruptions in the overall level of structural connectivity, as well as significant lesions observed in hub regions located in the frontal, temporal, and parietal cortices (Dai et al., 2015; Fischer et al., 2015). In addition, a previous research related to the rich club organization in AD dementia have proposed that these structural network disruptions are particularly prevalent in regions that are more distantly connected within the brain (Daianu et al., 2015). Another study also found that disturbances in the rich-club organization have a dynamic and potent impact on the connectivity among peripheral regions in individuals with mild MCI (Yan et al., 2018). Moreover, these disrupted connectivity extend to the rich-club regions in the brains of patients with AD dementia (Yan et al., 2018). Notably, in comparison to rich-club connections, feeder and local connections are shown to exhibit earlier and more severe impairments in patients with AD dementia (Daianu et al., 2015).

However, whether alterations in the rich-club organization in the AD spectrum differ between sexes remains relatively unknown. Although previous studies have investigated the sex differences of rich-club organizations in healthy young participants (Wang et al., 2019), no studies have yet explored this aspect within AD patients. Furthermore, even though several prior investigations have examined the rich-club organization in AD dementia (Dai et al., 2015; Yan et al., 2018), these studies have not taken into account the topological attributes of the rich-club organization. Because the topological features of the rich-club organization provides valuable insights into the fundamental principles governing brain connectivity related to cognitive function, investigating topological characteristics of rich-club organization is also important to understand the sex-difference in the rich-club organization in patients with AD.

Therefore, we aimed to investigate sex differences in the rich-club organization of the brain in AD and to explore the association between sex differences in the relationship between rich-club organization and cognition.

2. Materials and methods

2.1. Participants

The study cohort included 826 participants; 260 of them were cognitively unimpaired (CU), 281 with prodromal AD, and 285 with AD dementia.

All participants underwent a comprehensive assessment including, neuropsychological evaluation, amyloid positron emission tomography (PET) using either flutemetamol (FMM) or florbetaben (FBB), and brain T1-weighted magnetic resonance imaging (MRI). Furthermore, each participant underwent clinical interviews encompassing clinical dementia ratings (CDR) (Morris, 1997; Choi et al., 2001), neurological and neuropsychological examinations, as well as laboratory tests. Neuropsychological evaluation utilized the Seoul Neuropsychological Screening Battery 2nd edition (SNSB-II) which encompasses five cognitive domains: attention, memory, visuospatial, language and frontal executive function (Ryu and Yang,

2023). Additionally, laboratory assessments included a complete blood count, blood chemistry, thyroid function tests, syphilis serology, and vitamin B12/folate levels. To confirm the absence of structural lesions, including cerebral infarctions, brain tumors, vascular malformations, and hippocampal sclerosis, brain MRI was conducted.

Individuals with CU status met the following criteria: (1) absence of medical history likely to impact cognitive function, as per Christensen's health screening criteria (Christensen et al., 1991); and (2) absence of objective cognitive impairment across various domains, determined by a comprehensive neuropsychological test battery (scoring above −1.0 standard deviations (SD) of age-matched and education-matched norms in memory, and above −1.5 SD in other cognitive domains), as assessed using SNSB-II (Ryu and Yang, 2023). For the purposes of this study, only CU individuals with negative amyloid PET scans were included. Participants diagnosed with amnestic MCI needed to satisfy the subsequent criteria (Petersen et al., 2014): (1) self-reported cognitive complaints by participants or caregivers; (2) objective cognitive impairment in at least one memory test (scoring below −1.0 SD of age-matched and education-matched norms in memory); (3) absence of significant impairment in activities of daily living; and (4) no diagnosis of dementia. In this study, only individuals with MCI and positive amyloid PET scans were chosen (Albert et al., 2011), referred to as prodromal AD henceforth. Participants with AD dementia were categorized as having probable AD dementia with positive amyloid PET scan, based on the National Institute on Aging-Alzheimer's Association Research Criteria for AD (McKhann et al., 2011).

Written informed consent was obtained from all participants. The Institutional Review Board of Samsung Medical Center approved the study protocol, and all methods were performed according to the approved guidelines.

2.2. Imaging data acquisition: MRI/PET

2.2.1. MRI data acquisition

Standardized 3-D T1 turbo field echo images were acquired from all participants at the Samsung Medical Center using an identical 3.0T MRI scanner (Philips Achieva; Philips Healthcare, Andover, MA, USA). The 3-D T1 parameters were as follows: sagittal slice thickness, 1.0 mm over contiguous slices with 50% overlap; no gap; repetition time (TR), 9.9 ms; echo time (TE), 4.6 ms; flip angle, 8°; and matrix size, 240 × 240 pixels reconstructed to 480 × 480 over a field of view (FOV) of 240 mm. In the whole-brain diffusion-weighted MRI examinations, we collected sets of axial diffusion-weighted single-shot echo-planar images using the following parameters: 128 × 128 acquisition matrix, $1.72 \times 1.72 \times 2\,mm^3$ voxels; reconstructed to $1.72 \times 1.72 \times 2\,mm^3$; 70 axial slices; $220 \times 220\,mm^2$ FOV; TE 60 ms, TR 7,383 ms; flip angle 90°; slice gap 0 mm; and b-factor of $600\,s/mm^2$. Using the baseline image without diffusion weighting (reference volume), diffusion-weighted images were acquired from 45 directions. All axial sections were acquired parallel to the anterior commissure-posterior commissure line.

2.2.2. Amyloid β PET data acquisition

The participants underwent FMM and FBB PET at the Samsung Medical Center using a Discovery STe PET/CT scanner (GE Medical Systems, Milwaukee, WI, USA) in 3-D scanning mode that examined 3.3-mm-thick 47 slices spanning the entire brain. According to the protocols for the ligands proposed by the manufacturers, a 20-min emission PET scan in dynamic mode (consisting of 4 × 5 min frames) was performed 90 min after injecting a mean dose of 185 MBq FMM or 311.5 MBq FBB. We reconstructed 3-D PET images in a 128 × 128 × 47 matrix with a voxel size of 2 × 2 mm × 3.27 mm using the ordered-subsets expectation maximization algorithm (FMM iterations = 4 and subset = 20; FBB iterations = 4 and subset = 20). The cut-off values of amyloid β positivity were 1.03 for FMM and 1.1 for FBB, which were defined by an iterative outlier method in both FMM and FBB PET (Kim et al., 2021).

2.3. Network construction

To construct the white matter networks, we followed the DTI process described by Lee et al. The processing details have been described previously (Lee et al., 2018). Briefly, the T1-weighted image was linearly registered to the reference volume of the diffusion image using FMRIB's Linear Image Registration Tool and nonlinearly registered to ICBM152 in the Montreal Neurological Institute space, where the automated anatomical labeling (AAL) template was defined using fast nonlinear image registration in the FSL program. The AAL atlas was mapped to an individual's diffusion space using the T1 transformation parameters. We corrected all diffusion-weighted images for eddy current distortion and head motion. A diffusion toolkit was used to perform the deterministic tractography. After fiber tracking, the networks were constructed using AAL templates for a 90 × 90 connectivity matrix.

2.4. Assessment of global network metrics

Network analysis was performed using the Brain Connectivity Toolbox.[1] We computed the connectivity strength, global efficiency (E_{glob}), local efficiency (E_{loc}), and clustering coefficient in each matrix to assess the graph metrics of the global topological organization of the whole-brain structural connectivity network. The connectivity strength is defined as the sum of all streamlines connecting nodes (Rubinov and Sporns, 2010). Global efficiency is a measure of the network integration and defined as the average inverse shortest path length between all pairs of nodes (Rubinov and Sporns, 2010). The mean clustering coefficient of a network is calculated as the average of the clustering coefficients across all nodes (Rubinov and Sporns, 2010).

2.5. Assessment of rich-club organization

Rich-club nodes were selected according to previously published guidelines (van den Heuvel and Sporns, 2011; Yoon et al., 2016). The selected rich-club nodes included the superior frontal cortex (SFC), superior parietal cortex (SPC), precuneus, hippocampus, putamen, and thalamus. Briefly, we computed the normalized rich-club coefficient to demonstrate the existence of

1 http://www.brain-connectivity-toolbox.net

rich-club organization in a network. This is because random networks demonstrate increasing network connections because nodes with a higher degree have a higher probability of being interconnected by chance. The rich-club coefficient, Φ (κ), is typically normalized relative to a set of comparable random networks of equal size and similar connectivity distribution (van den Heuvel and Sporns, 2011). Thus, for each network, $m = 1{,}000$ random networks were computed by shuffling the links, preserving the weights and degree sequence, and, thus, all node degrees, including the hubs in the network. Similar to that in other previous studies (van den Heuvel et al., 2013), a normalized rich-club coefficient Φ norm (κ) >1 over a range of κ indicated a rich-club organization in a network. A rich-club organization was identified by performing a two-tailed t-test with permutation testing (10,000 permutations) of the area under the curve (normalized weighted rich-club coefficient against degree), compared with a random network (Supplementary Figure S1).

Rich-club nodes are likely to be highly connected with each other, compared with connections expected by random chance (van den Heuvel and Sporns, 2011). All connections between the nodes in each structural matrix were categorized into one of the following groups: "rich-club connections," which are defined as the number of connections associating rich-club nodes, "feeder connections," which are defined as the number of connections associating rich-club to non-rich-club nodes, and "local connections," which are defined as the number of connections associating non-rich-club nodes (van den Heuvel and Sporns, 2011). To examine the region-specific alterations in the rich-club connections according to the cognitive status, we computed the connectivity strength of each rich-club node related to other nodes. Rich-club connections in the SFC, SPC, precuneus, putamen, and thalamus were calculated for each hemisphere and averaged into one value for each node.

For visualization, we converted the raw values of the connectivity strength of the rich-club organizations to a standardized Z-score using the mean scores and standard deviations (SD) of the CU group.

2.6. Statistical analyses

For baseline demographic data, we performed independent *t*-tests and chi-square tests for the continuous and dichotomous variables, respectively. We assessed the group differences in cognitive function as well as network characteristics using an analysis of covariance with the age and years of education as the covariates.

For sex effects on the global network metrics, including the connectivity strength, global efficiency, and the clustering coefficient of the whole-brain structural connectivity network, were compared between men and women using a general linear model (GLM) after adjusting for their age and years of education. We also used the GLM to examine the differences in the rich-club, feeder, and local connections between men and women, using the same covariates. Partial eta squared (η_p^2) was used to estimate the effect size. In addition, we examined the interaction effects between sex and cognitive groups on the rich-club organization. The Pearson's correlation analysis was used to examine the relationship between the rich-club organization and cognitive performance including the scores of Korean-Mini-Mental Status Examination (K-MMSE) and the sum of boxes of CDR. For the correlation analyses, we excluded outliers beyond the 5th to 95th percentile range of the K-MMSE scores or the sum of boxes of CDR.

We employed a permutation-based multiple testing method to investigate the effects of group and sex on the rich-club organization (Nichols and Holmes, 2002; Hurtz et al., 2014). In this approach, we randomly permutated the sex or group status of subjects while maintaining the age and levels of education values. Data were assessed against an empirical null distribution by running 10,000 synthesized permutations with a threshold of $p < 0.05$. A permutation-adjusted p value was computed based on the proportion of permutations with p values under the null distribution that was greater than the observed values from the actual data set (Westfall et al., 1993).

All analyses were performed using STATA 17.0 (StataCorpx, College Station, TX, USA).

3. Results

3.1. Basic information

3.1.1. Clinical characteristics

We observed no significant sex differences in the mean age, K-MMSE scores, CDR, or CDR sum of boxes among the whole participants (Table 1), whereas men exhibited higher educational levels than those of women in all groups.

According to each cognitive group (Table 2), there were no significant differences in the clinical severity and amyloid deposition in all groups. In addition, we observed no significant sex differences in the global network topologies, including the connectivity strength, clustering coefficient, and global efficiency, in the CU, prodromal AD, and AD dementia groups.

TABLE 1 Demographic characteristics.

	Women	Men	
	(*n* = 504)	(*n* = 322)	*p*-value
Age	69.92 ± 8.03	70.78 ± 8.54	0.147
Education	10.34 ± 4.73	13.69 ± 4.01	<0.001*
DM (n, %)	71 (14.09)	63 (19.57)	0.033*
HTN (n, %)	193 (38.29)	144 (44.72)	0.054
HL (n, %)	176 (34.92)	101 (31.36)	0.313
APOE4 carrier (n, %)	243 (44.64)	159 (46.27)	0.088
K-MMSE	23.72 ± 5.87	24.31 ± 5.11	0.143
CDR	0.68 ± 0.50	0.67 ± 0.43	0.713
Group (n, %)			0.503
CU	166 (32.94)	94 (29.19)	
Prodromal AD	166 (32.94)	115 (35.71)	
AD dementia	172 (34.13)	113 (35.09)	

APOE4, Apolipoprotein E4; CDR, Clinical dementia rating; K-MMSE, Korean-Mini-Mental Status Examination; CU, cognitively unimpaired; DM, diabetes mellitus; HL, hyperlipidemia; and HTN, hypertension. *p-value < 0.05.

TABLE 2 Clinical characteristics according to the cognitive group.

	CU			Prodromal AD			AD Dementia		
	Women (n = 166)	Men (n = 94)	p-value for sex	Women (n = 166)	Men (n = 115)	p-value for sex	Women (n = 172)	Men (n = 113)	p-value for sex
Age	69.10 ± 6.85	69.88 ± 7.88	0.411	71.28 ± 7.88	72.27 ± 7.54	0.292	69.40 ± 9.05	69.99 ± 9.81	0.599
Education	10.83 ± 4.75	13.11 ± 4.43	**0.0002***	10.48 ± 4.83	13.60 ± 3.77	**<0.001***	9.74 ± 4.59	14.28 ± 3.83	**<0.001***
DM (n, %)	29 (17.46)	20 (21.27)	0.389	20 (12.05)	26 (22.61)	**0.02***	22 (12.79)	17 (15.04)	0.560
Hypertension (n, %)	65 (39.16)	43 (45.74)	0.213	63 (37.95)	53 (46.08)	0.193	65 (38.01)	48 (42.47)	0.381
Hyperlipidemia (n, %)	66 (39.76)	31 (32.98)	0.336	60 (36.14)	32 (27.83)	0.129	50 (29.07)	38 (33.63)	0.376
APOE4 carrier (n, %)	19 (11.44)	20 (21.27)	**0.008***	101 (60.84)	72 (62.61)	0.206	105 (61.05)	57 (50.44)	0.200
Amyloid PET (SUVR)	0.92 ± 0.05	0.92 ± 0.05	0.613	1.40 ± 0.19	1.45 ± 0.22	0.029	1.49 ± 0.20	1.49 ± 0.16	0.984
Cognitive function and severity									
K-MMSE	28.27 ± 1.85	28.51 ± 1.35	0.271	24.6 ± 3.39	25.37 ± 3.15	0.068	17.92 ± 5.91	19.59 ± 5.09	**0.019***
CDR	0.40 ± 0.20	0.41 ± 0.20	0.905	0.53 ± 0.16	0.53 ± 0.17	0.851	1.11 ± 0.63	1.03 ± 0.51	0.265
CDR, sum of boxes	0.63 ± 0.47	0.63 ± 0.48	0.997	2.15 ± 1.68	1.80 ± 1.35	0.066	6.54 ± 3.44	6.23 ± 2.99	0.453
Global brain network properties									
Connectivity Strength	4.85 ± 0.68	4.96 ± 0.73	0.238	5.13 ± 0.55	5.15 ± 0.58	0.802	4.64 ± 0.61	4.62 ± 0.56	0.804
Clustering Coefficient	0.17 ± 0.02	0.18 ± 0.02	0.300	0.18 ± 0.02	0.19 ± 0.01	0.083	0.17 ± 0.02	0.17 ± 0.02	0.132
Global Efficiency	0.19 ± 0.02	0.20 ± 0.03	0.323	0.20 ± 0.02	0.20 ± 0.02	0.958	0.19 ± 0.02	0.19 ± 0.02	0.958

APOE4, Apolipoprotein E4; DM, diabetes mellitus; CDR, Clinical dementia rating; SUVR, Standardized Uptake Value Ratio; PET, positron emission tomography; and K-MMSE, Korean-Mini-Mental Status Examination. *$P < 0.05$.

3.2. Rich-club organization

3.2.1. Sex and group effects in the rich-club organization

Figure 1A shows sex differences of rich-club connections in each group (the mean values with SD). In a whole participants, women demonstrated lower connectivity strength of the rich-club connections (mean ± SD, −0.09 ± 0.76 vs. 0.04 ± 0.74, $\eta_p^2 = 0.01$, permutation-adjusted $p = 0.009$) and feeder connections (−0.15 ± 0.74 vs. −0.01 ± 0.73, $\eta_p^2 = 0.02$, permutation-adjusted $p = 0.001$) than did men. Particularly in the AD dementia group, women demonstrated a lower connectivity strength of the rich-club connections (−0.29 ± 0.89 vs. −0.0 ± 0.76, $\eta_p^2 = 0.03$, permutation-adjusted $p = 0.034$) and feeder connections (−0.36 ± 0.89 vs. −0.02 ± 0.94, $\eta_p^2 = 0.03$, permutation-adjusted $p = 0.01$) than did men.

Figure 1B shows the mean values with SE of connectivity strength in the rich-club organizations after adjusting age and years education. For the group effects, women in the AD dementia group demonstrated significantly lower rich-club ($\eta_p^2 = 0.04$, permutation-adjusted $p < 0.001$), feeder connections ($\eta_p^2 = 0.03$, permutation-adjusted $p < 0.001$), and local connections ($\eta_p^2 = 0.11$, permutation-adjusted $p < 0.001$) than those in the CU or prodromal AD group. However, men in the AD dementia group demonstrated only significant differences in the connectivity strength in the local connections, compared with those in the CU or prodromal AD group ($\eta_p^2 = 0.07$, permutation-adjusted $p < 0.001$). Conversely, there were no significant differences in the connectivity strength between the rich-club ($\eta_p^2 = 0.02$, permutation-adjusted $p = 0.160$) and feeder connections ($\eta_p^2 = 0.001$, permutation-adjusted $p = 0.360$), according to the cognitive status in men. We observed a significant group-by-sex interaction only in the feeder connections (*P for interaction* = 0.035).

3.2.2. Sex and group effects in the region-specific alterations of rich-club connections

Figure 2 indicates sex and group effects in the region-specific alterations of rich-club connections (mean ± SE). When we investigated the region-specific alterations in the rich-club connections between men and women, men showed significantly greater connectivity strength of the SPC with other rich-club nodes (0.43 ± 0.09 vs. 0.15 ± 0.07, $\eta_p^2 = 0.01$, permutation-adjusted $p = 0.021$) as well as that of the putamen with other rich-club nodes (0.19 ± 0.12 vs. −0.09 ± 0.08, $\eta_p^2 = 0.01$, permutation-adjusted $p = 0.039$) in the prodromal AD (Figures 2B,E). In AD dementia group, the connectivity strength between the thalamus and other rich-club nodes was significantly higher in the men compared to that of women (0.56 ± 0.11 vs. 0.02 ± 0.08, $\eta_p^2 = 0.02$, permutation-adjusted $p = 0.001$) (Figure 2F).

For the group effects, both men and women showed higher connectivity strength of the precuneus in the prodromal AD group compared to that in CU ($\eta_p^2 = 0.08$, permutation-adjusted $p < 0.001$ for women; $\eta_p^2 = 0.16$, permutation-adjusted $p = <0.001$ for men) but lower connectivity strength of the precuneus in AD dementia ($\eta_p^2 = 0.08$, permutation-adjusted $p < 0.001$ for women; $\eta_p^2 = 0.16$, permutation-adjusted $p < 0.001$ for men) compared to CU or prodromal AD groups (Figure 2C). Furthermore, both women and men demonstrate decreased connectivity strength of the SPC ($\eta_p^2 = 0.08$, permutation-adjusted $p < 0.001$ for women; $\eta_p^2 = 0.12$, permutation-adjusted $p < 0.001$ for men) in AD dementia group compared to CU or prodromal AD (Figure 2B). Especially among women, the connectivity strength of the putamen in AD dementia was decreased compared to CU ($\eta_p^2 = 0.02$, permutation-adjusted $p = 0.02$) (Figure 2E), whereas among men, the connectivity strength of the thalamus in AD dementia was increased among men compared to CU or prodromal AD groups ($\eta_p^2 = 0.01$, permutation-adjusted

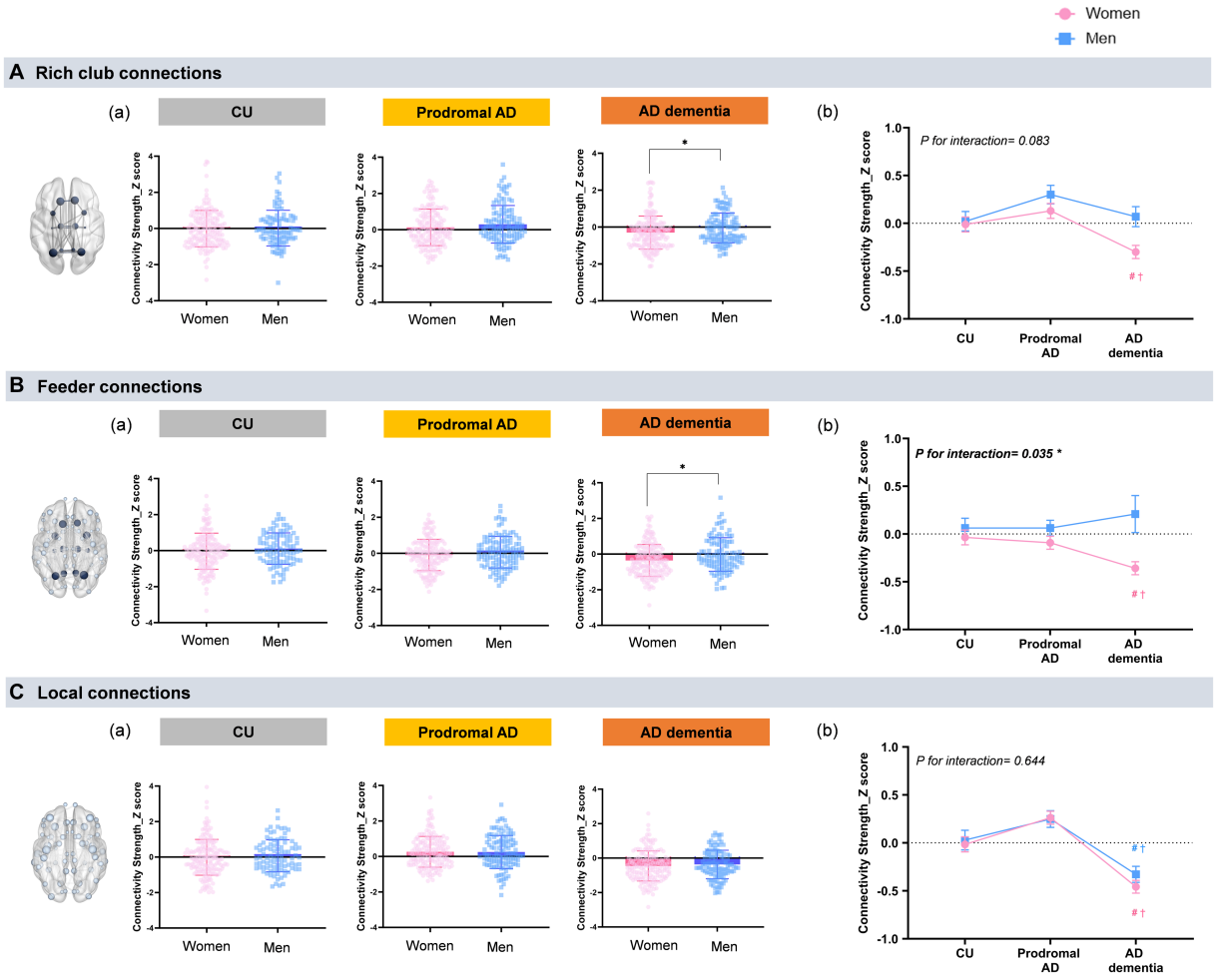

FIGURE 1
Sex and group differences in the rich-club organization. **(a)** Shows sex differences of rich-club connections in each group (the mean values with SD). A general linear model was used to examine the differences in the rich-club, feeder, and local connections between men and women with age and education of years as covariates. The rich-club nodes included the superior frontal cortex (SFC), superior parietal cortex (SPC), precuneus, hippocampus, putamen, and thalamus. *$P < 0.05$ for sex-differences. **(A)** Rich club connections: Only the AD dementia group demonstrates sex differences in the rich-club connections, which indicate women show a lower connectivity strength of the rich-club connections than men (-0.29 ± 0.89 vs. -0.0 ± 0.76, $\eta_p^2 = 0.03$, permutation-adjusted $p = 0.034$). **(B)** Feeder connections: Only women with AD dementia show lower connectivity strength of the feeder connections (-0.36 ± 0.89 vs. -0.02 ± 0.94, $\eta_p^2 = 0.03$, permutation-adjusted $p = 0.01$) than men. There were no sex-differences in the other groups. **(C)** Local connections: No sex-related differences are observed in the local connections in the all cognitive groups. **(b)** Shows the mean values with SE of connectivity strength in the rich-club organizations that were obtained by GLM after adjusting age and years education. #$p < 0.05$: CU vs. AD; †$p < 0.05$ for prodromal AD vs. AD dementia. **(A)** Rich club connections: Women with AD dementia demonstrate significantly decreased connectivity strength in the rich-club connections ($\eta_p^2 = 0.04$, permutation-adjusted $p < 0.001$) compared with CU or prodromal AD, whereas there were no group differences of rich-club connections in men ($\eta_p^2 = 0.02$, permutation-adjusted $p = 0.160$). There were no significant group-by-sex interaction in the rich-club connections (*P for interaction = 0.083*). **(B)** Feeder connections: Women with AD dementia also show significantly decreased connectivity strength in the feeder connections ($\eta_p^2 = 0.03$, permutation-adjusted $p < 0.001$) compared to those in CU or in prodromal AD, while there were no group differences of feeder connections in men ($\eta_p^2 = 0.001$, permutation-adjusted $p = 0.360$). A significant group-by-sex interaction is observed in the feeder connections (*P for interaction = 0.035*). **(C)** Local connections: Both women and men with AD dementia show lower connectivity strength in the local connections ($\eta_p^2 = 0.106$, permutation-adjusted $p < 0.001$) compared with CU or prodromal AD. There were no significant group-by-sex interaction in the rich-club connections (*P for interaction = 0.644*).

$p = 0.02$) (Figure 2F). There was a significant group by sex interaction on the connectivity strength of putamen with other rich-club node ($\eta_p^2 = 0.013$, $p = 0.026$).

3.2.3. Sex and group effects in the region-specific alterations of feeder connections

In the region-specific alterations of feeder connections (Figure 3), there was significant sex-differences in the connectivity strength of the thalamus among the AD dementia group (0.03 ± 0.07 vs. 0.44 ± 0.9, $\eta_p^2 = 0.02$, permutation-adjusted $p = 0.026$).

For the group effects, likewise the rich-club connections, both men and women with AD dementia showed decreased connectivity strength of SPC ($\eta_p^2 = 0.07$, permutation-adjusted $p < 0.001$ for women; $\eta_p^2 = 0.13$, permutation-adjusted $p < 0.001$ for men) and that of precuneus ($\eta_p^2 = 0.07$, permutation-adjusted $p < 0.001$ for women; $\eta_p^2 = 0.03$, permutation-adjusted $p = 0.01$ for men) in the feeder connections compared to CU and prodromal AD (Figures 2B,C). Among women, the connectivity strength of hippocampus in the feeder connection was decreased in AD dementia ($\eta_p^2 = 0.03$, permutation-adjusted $p < 0.001$) compared to CU or prodromal AD. Among men, the connectivity

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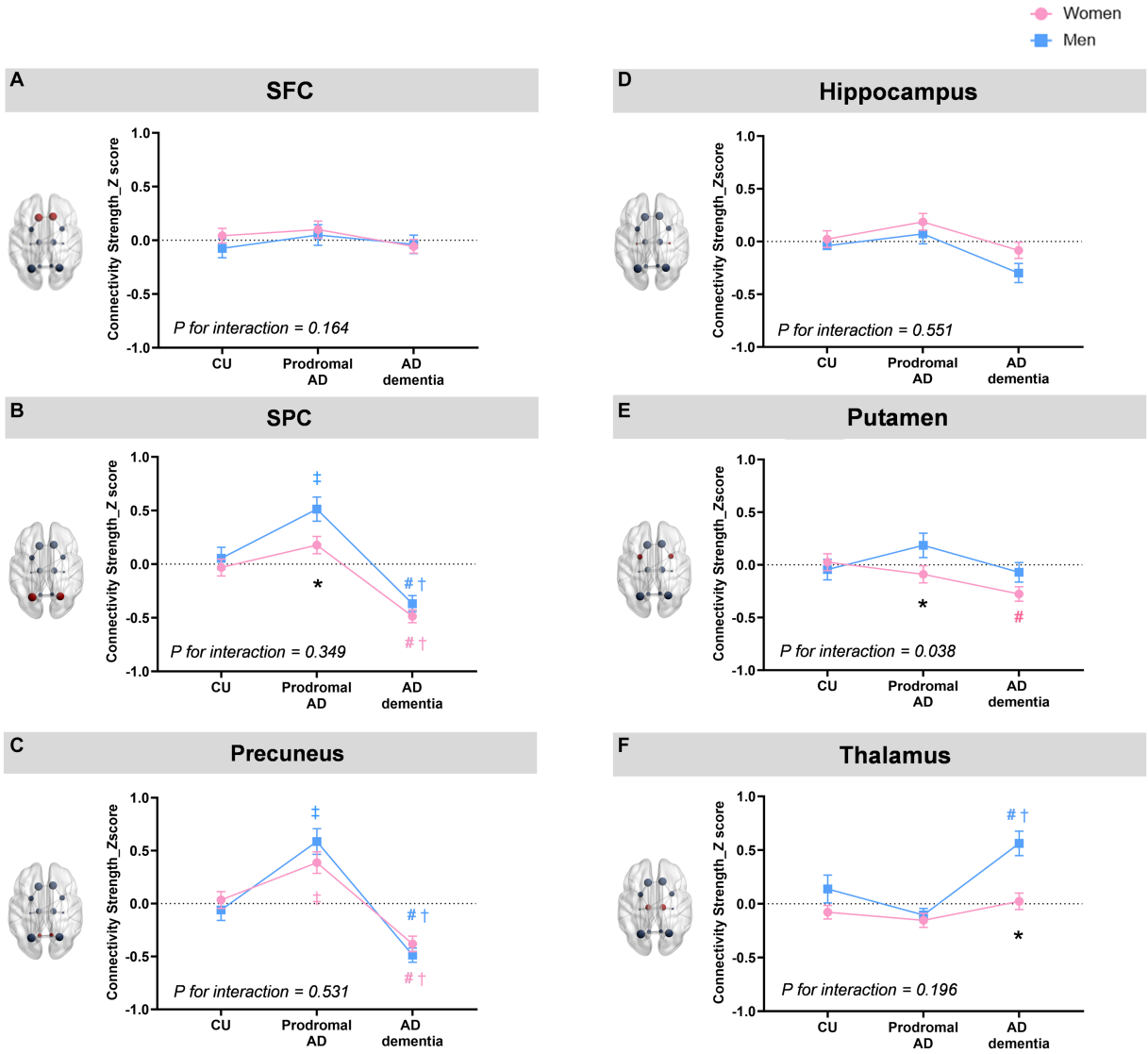

FIGURE 2
Sex and group effects in the region-specific alterations of rich-club connections. When we investigated the region-specific alterations in the rich-club connections between men and women, men show significantly greater connectivity strength of the SPC with other rich-club nodes (mean ± SE,0.43 ± 0.09 vs. 0.15 ± 0.07, η_p^2 = 0.01, permutation-adjusted p = 0.021) as well as that of the putamen with other rich-club nodes (0.19 ± 0.12 vs. −0.09 ± 0.08, η_p^2 = 0.01, permutation-adjusted p = 0.039) in the prodromal AD **(B,E)**. In AD dementia group, the connectivity strength between the thalamus and other rich-club nodes was significantly higher in the men compared to that women (0.56 ± 0.11 vs. 0.02 ± 0.08, η_p^2 = 0.02, permutation-adjusted p = 0.001) **(F)**. For the group effects, both men and women showed higher connectivity strength of the precuneus in the prodromal AD group compared to that in CU (η_p^2 = 0.08, permutation-adjusted p <0.001 for women; η_p^2 = 0.16, permutation-adjusted p = <0.001 for men) but lower connectivity strength in AD dementia (η_p^2 = 0.08, permutation-adjusted p <0.001 for women; η_p^2 = 0.16, permutation-adjusted p <0.001 for men) compared to CU or prodromal AD groups **(C)**. Furthermore, both women and men demonstrate decreased connectivity strength of the SPC (η_p^2 = 0.08, permutation-adjusted P <0.001 for women; η_p^2 = 0.12, permutation-adjusted P <0.001 for men) in AD dementia group compared to CU or prodromal AD **(B)**. Especially among women, the connectivity strength of the putamen in AD dementia was decreased compared to CU (η_p^2 = 0.02, permutation-adjusted p = 0.02) **(E)**, whereas among men, the connectivity strength of the thalamus in AD dementia was increased among men compared to CU or prodromal AD groups (η_p^2 = 0.01, permutation-adjusted P = 0.02) **(F)**. There was a significant group by sex interaction on the connectivity strength of putamen with other rich-club node (η_p^2 = 0.013, P = 0.026). * *Adjusted p* < 0.05 for sex-difference; # *Adjusted p* < 0.05: CU vs. AD dementia; ‡ *Adjusted p* < 0.05 for CU vs. Prodromal AD; † *Adjusted p* < 0.05 for prodromal AD vs. AD dementia.

strength of SPC in the feeder connections was increased in the prodromal AD compared to CU (η_p^2=0.13, permutation-adjusted p<0.001).

There was a significant group by sex interaction only on the connectivity strength of thalamus in the feeder connections (η_p^2=0.010, p=0.025).

3.2.4. Sex differences in the correlation between the cognitive performance and rich-club organization

The region-specific rich-club connections of the putamen were negatively correlated with the CDR-sum of boxes only in women with AD dementia (r=−0.21, permutation-adjusted p=0.006)

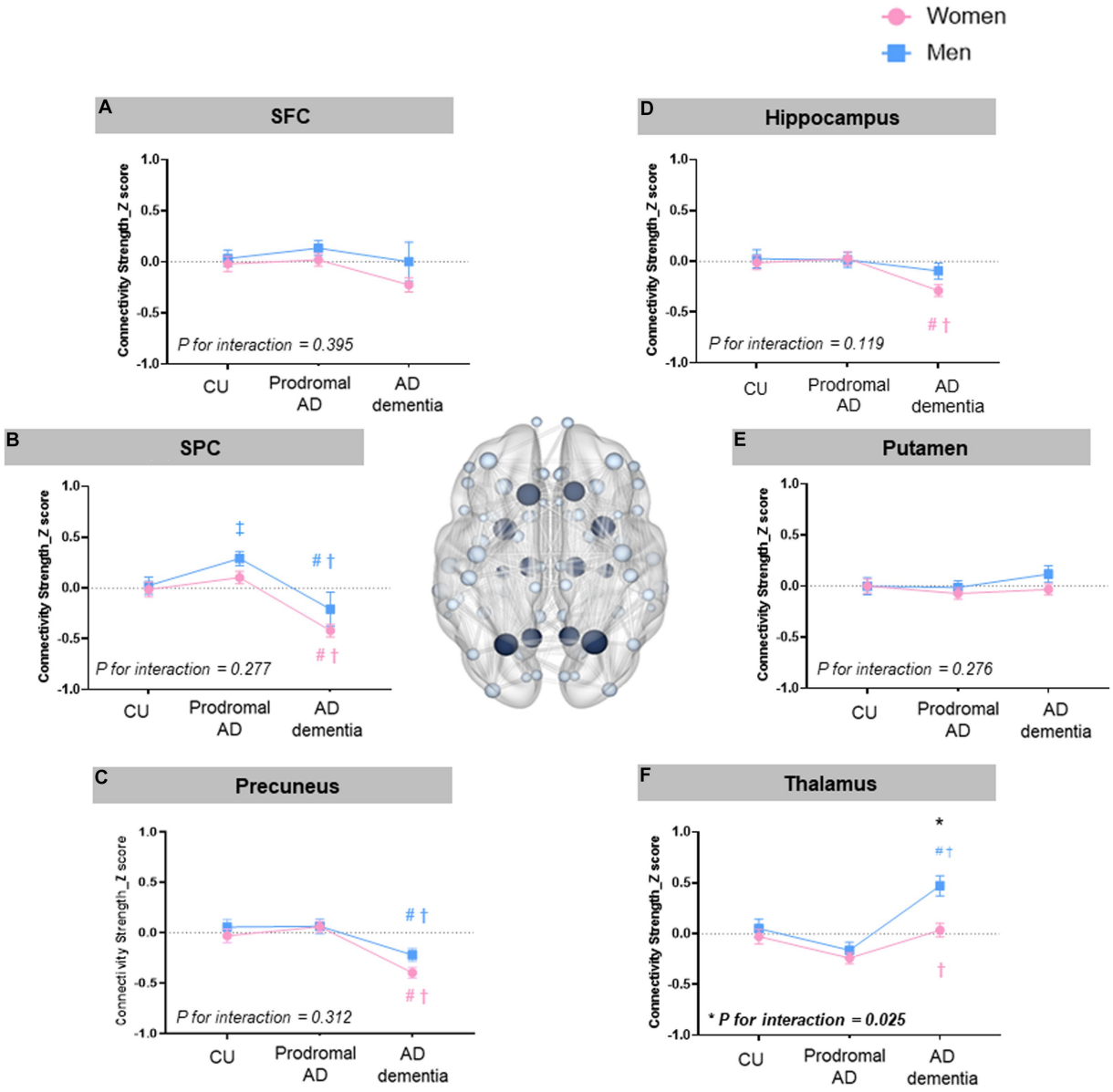

FIGURE 3
Sex and group effects in the region-specific alterations of feeder connections. There were significant sex-differences in the connectivity strength of the thalamus among the AD dementia group (mean ± SE, 0.03 ±0.07 for women vs. 0.44 ± 0.9 for men, η_p^2 = 0.02, permutation-adjusted p = 0.026). For the group effects, both men and women with AD dementia showed decreased connectivity strength of SPC (η_p^2 = 0.07, permutation-adjusted p <0.001 for women; η_p^2 = 0.13, permutation-adjusted p <0.001 for men) and that of precuneus (η_p^2 = 0.07, permutation-adjusted P <0.001 for women; η_p^2 = 0.03, permutation-adjusted p =0.01 for men) compared to CU and prodromal AD (Figures 2B,C). Especially among women, the connectivity strength of hippocampus in the feeder connection was decreased in AD dementia (η_p^2 = 0.03, permutation-adjusted p <0.001) compared to CU or prodromal AD. Among men, the connectivity strength of SPC in the feeder connections was increased in the prodromal AD compared to CU (η_p^2 = 0.13, permutation-adjusted p <0.001). There was a significant group by sex interaction only on the connectivity strength of thalamus in the feeder connections (η_p^2=0.010, p=0.025). * *Adjusted p* < 0.05 for sex-difference; # *Adjusted p* < 0.05: CU vs. AD dementia ; ‡ *Adjusted p* < 0.05 for CU vs. Prodromal AD; † *Adjusted p* <0.05 for prodromal AD vs. AD dementia.

(Supplementary Figure S2). There were no significant correlations between cognitive function and rich-club connections of the putamen in other cognitive group.

However, the region-specific feeder connections of the thalamus are positively correlated with the K-MMSE scores (r=0.23, permutation adjusted p=0.006) and negatively correlated with the CDR sum of boxes (r=−0.19, permutation adjusted p=0.016) in men with prodromal AD, whereas there is no significant correlation in women with prodromal AD. In the AD dementia, the connectivity strength of the thalamus in the feeder connections are negatively correlated with the K-MMSE scores (r=−0.39, adjusted p<0.001) only in women (Figure 4).

4. Discussion

This novel study demonstrated sex differences in the alterations of connectivity strength in the rich-club organization in AD spectrum disease, which was confirmed by amyloid PET.

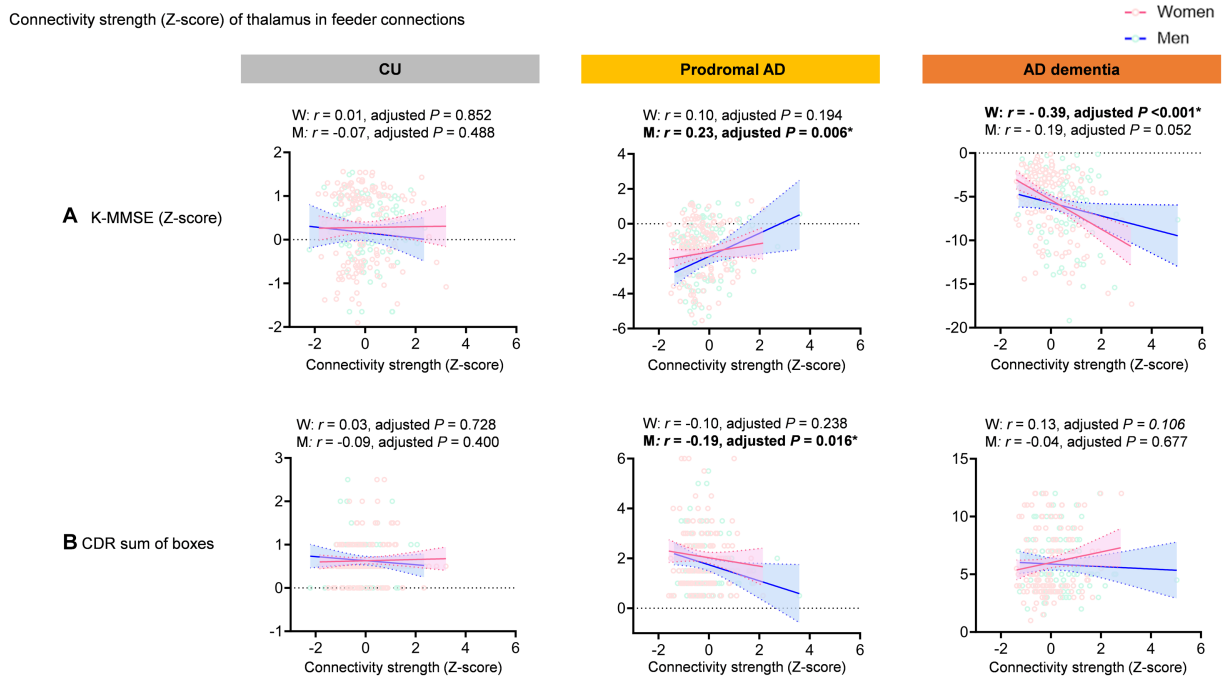

FIGURE 4
Correlation between feeder connections of the thalamus and cognitive function. The graph shows scatter plot with line of best fit (95% confidence interval). The region-specific feeder connections of the thalamus are positively correlated with the K-MMSE scores ($r = 0.23$, permutation adjusted $p = 0.006$) and negatively correlated with the CDR sum of boxes ($r = -0.19$, permutation adjusted $p = 0.016$) in men with prodromal AD, whereas there is no significant correlation in women with prodromal AD. In the AD dementia, the connectivity strength of the thalamus in the feeder connections are negatively correlated with the K-MMSE scores ($r = -0.39$, adjusted $p < 0.001$) only in women.

In the current study, we observed a significant sex difference in the rich-club and feeder connections only in patients with AD dementia. In the AD dementia group, women demonstrated lower connectivity strength of the rich-club and feeder connections than did men. Consistent with our findings, a previous study investigating gender differences in the rich-club organization reported that men demonstrated greater connectivity strength of rich-club nodes demonstrating an advantage in network efficiency in men compared to women (Wang et al., 2019), although this previous study was conducted in young healthy participants.

In terms of group effects of rich-club organizations, we found that both men and women with AD dementia demonstrated decreased structural connectivity of the local connections, compared with patients with CU or prodromal AD. Previous studies have shown that disruptions in the structural connectivity in rich-club organizations may originate from peripheral regions, such as local connections (Daianu et al., 2015; Yan et al., 2018), which are in line with our findings.

Interestingly, compared with CU or prodromal AD, only women with AD dementia demonstrated decreased connectivity strengths of the rich-club and feeder connections, while no differences of rich-club and feeder connections were observed in men with AD dementia. Given that women with prodromal AD and AD dementia demonstrated a similar amount of amyloid deposition with men, our findings may suggest that men may have more preserved rich-club and feeder connections of structural network. This finding may explain greater vulnerability of structural brain network to amyloid deposition in women, which is consistent with previous findings. Earlier research indicates that while the quantity of AD pathology is similar in men and women, the correlation between AD pathology and clinical symptoms in AD dementia is notably stronger in women compared to men (Barnes et al., 2005). In fact, each unit of AD pathology increases the odds of clinical symptoms over 20 times in women, while it only results in a three-fold increase in men. The authors therefore suggested that AD pathology is more likely to be clinically expressed as dementia in women than in men. In addition, previous studies have shown that women with AD have 1 ~ 1.5% higher rate of brain atrophy compared to men (Hua et al., 2010), and women demonstrated increased rate of tau accumulation than men (Smith et al., 2020). The exact causes for the heightened vulnerability of the rich-club organization in women with AD dementia remain uncertain. However, potential factors like sex differences in tau accumulation, comorbidities, cognitive reserve, neurotrophic factors, inflammation, and synapse biology have been proposed as potential contributors to these differences (Ferretti et al., 2018; Laws et al., 2018; Toro et al., 2019).

Significantly, we noticed a group-specific effect related to sex, specifically in the feeder connections, where women exhibited fewer feeder connections compared to men. This finding aligns with a previous study on gender differences in rich-club organization, which reported larger connectivity values, including connectivity strength, in men compared to women for feeder connections. This suggests that disparities in peripheral regions play a pivotal role in understanding gender differences in rich-club organization (Wang et al., 2019).

To uncover potential differences of rich-club organizations between men and women, our study also examined the topological

properties of rich-club organization. When we investigated the region-specific alterations in the rich-club connections between men and women, it is noteworthy that men showed significantly higher connectivity strength of the SPC as well as that of the putamen in the rich-club connections than women in the prodromal AD, while the higher connectivity strength of the thalamus in men than women in patients with AD dementia. Consisted with our findings, a previous study showed that the SPC was one of the regions which showed the most significant differences in network topology between women and men based on the 1,053 postmortem brain samples in AD (Sun et al., 2019). In addition, higher rich-club connections of the putamen in men with prodromal AD could be explained by the previous study showing the faster striatal AB accumulation in women than men and more pronounced tau accumulation in women than men (Kim et al., 2022), which may further induce more structural network abnormality in women than men.

It is also noteworthy that both men and women showed higher connectivity strength of the precuneus in the rich-club connections among the prodromal AD group compared to that in CU, but showed lower connectivity strength of the precuneus in AD dementia compared with that in CU or prodromal AD groups. Furthermore, both women and men demonstrate decreased connectivity strength of the SPC in AD dementia group compared to CU or prodromal AD. Alterations of rich-club connections in the precuneus as well as the SPC among prodromal AD or AD dementia could be explained by the facts that the precuneus and the SPC are one of the main regions which are mainly involved in AD (Jacobs et al., 2012; Ota et al., 2022). Consistent with our findings, alterations of structural connectivity in precuneus or SPC among patients with AD were reported in the previous study (Wang et al., 2016; Cao et al., 2020; Prawiroharjo et al., 2020).

Surprisingly, we observed enhanced connectivity strength in the precuneus of patients with prodromal AD. The implications of these structural changes are still a subject of debate. Despite the different methodologies, some researchers propose that increased structural connectivity could indicate a compensatory process in neurodegenerative diseases (Mole et al., 2016; Sheng et al., 2021), consistent with our findings.

Further studies are needed to elucidate to what extent these findings reflect real biological changes of these increased structural connectivity in patients with AD.

It should be noted that we found the significant sex-differences in the connectivity strength of thalamus in the rich-club and feeder connections, indicating that men with AD dementia had greater connectivity strength of thalamus in the rich-club and feeder connections than those in women. No studies have been conducted on the structural connectivity related to the thalamus in AD; however, women with AD dementia have smaller anterior thalamic volumes than do men (Callen et al., 2004), which could be consistent with our findings.

Furthermore, men with AD dementia demonstrated increased region-specific alterations in the feeder connections in the thalamus, compared with men with CU or prodromal AD. Although increased structural connectivity strength could be regarded as a compensatory process as mentioned above, there is limited information about the pathological basis of structural network compensation in AD (Jackson et al., 2019; Sheng et al., 2021); this warrants further research related to the structural connectivity of the thalamus in AD to elucidate sex differences in the thalamus of patients with AD.

Notably, the feeder connections of the thalamus were differently correlated with the cognitive scores in men and women. In men with prodromal AD, the feeder connections of the thalamus were positively correlated with the K-MMSE scores and negatively correlated with the CDR sum of boxes. Considering the thalamus receives and combines neural signals from various parts of the neocortex and is involved in coordinating this information (Aggleton et al., 2016; Alderson et al., 2017), the severity of disruption in the connectivity strength of the thalamus from feeder connections is supposedly correlated with cognitive dysfunction in men with prodromal AD. This finding is supported by previous findings suggestion that cognitive function is associated with connectivity of the thalamus in AD (Aggleton et al., 2016).

However, contrary to our expectations, region-specific feeder connections of the thalamus were negatively correlated with the K-MMSE scores only in women with AD dementia, suggesting that increased thalamic connectivity strength in such patients is associated with poor cognitive function.

The thalamus consists of multiple nuclei that are highly interconnected with the cortical and subcortical areas of the brain (Perry et al., 2021). One possible explanation for this negative correlation between the connectivity strength and cognitive function in women with AD dementia is that increased connectivity strength between certain thalamic nuclei and the cerebral cortex is associated with the inhibitory function of cognition. For example, the suppression of activity in the medial dorsal nucleus of the thalamus may play a vital role in facilitating information processing from the hippocampal formation to the prefrontal cortex, specifically in the memory domain (Yang et al., 2019; Perry et al., 2021). Therefore, the increased connectivity strength of the medial dorsal nucleus in the thalamus is supposedly associated with increased memory impairment in patients with AD dementia.

The current study had some limitations. First, we could not determine the causal relationship between alterations in the brain networks and could not examine cognitive decline because of the cross-sectional nature of the study design. Second, the participants did not have major psychiatric diseases, such as depression or anxiety; therefore, these potential confounding factors were not included in the analysis, which should be considered in future studies. Third, we analyzed the effects of *APOE ε4* on the connectivity strength of the rich-club organizations in patients with AD (Supplementary Figure S3); however, other genetic factors could also affect the structural connectivity of the rich-club organization in AD. Fourth, we performed amyloid PET to confirm amyloid pathology in the brain; nonetheless, we did not consider other pathologies, such as neurofibrillary tangles or Lewy bodies. Finally, although we utilized a permutation-based approach for multiple comparison correction, it is worth noting that this method might be less conservative compared to Bonferroni or False Discovery Rate correction. As a consequence, there is a possibility that it could lead to an increased risk of inflating the Type I error rate.

Nonetheless, the strength of this study is that it investigated the sex differences in the rich-club organizations in patients with AD confirmed by amyloid PET scans, which had not been examined before. Our findings provide important evidence for sex-specific

alterations in the structural brain network related to AD, suggesting the necessity of sex-specific strategies in clinical practice.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Institutional Review Board of Samsung Medical Center. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

GK, S-JK, J-KS, and SS contributed to the data conceptualization, analysis, and interpretation. GK and S-JK contributed to the drafting of the manuscript, analysis of the imaging data, and preparation of the figures. YB, YP, HJ, and JK contributed to the data interpretation. All authors have read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1209027/full#supplementary-material

References

Aggleton, J. P., Pralus, A., Nelson, A. J., and Hornberger, M. (2016). Thalamic pathology and memory loss in early Alzheimer's disease: moving the focus from the medial temporal lobe to Papez circuit. *Brain* 139, 1877–1890. doi: 10.1093/brain/aww083

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

Alderson, T., Kehoe, E., Maguire, L., Farrell, D., Lawlor, B., Kenny, R. A., et al. (2017). Disrupted thalamus white matter anatomy and posterior default mode network effective connectivity in amnestic mild cognitive impairment. *Front. Aging Neurosci.* 9:370. doi: 10.3389/fnagi.2017.00370

Ardekani, B. A., Convit, A., and Bachman, A. H. (2016). Analysis of the MIRIAD data shows sex differences in hippocampal atrophy progression. *J. Alzheimers Dis.* 50, 847–857. doi: 10.3233/JAD-150780

Barnes, L. L., Wilson, R. S., Bienias, J. L., Schneider, J. A., Evans, D. A., and Bennett, D. A. (2005). Sex differences in the clinical manifestations of Alzheimer disease pathology. *Arch. Gen. Psychiatry* 62, 685–691. doi: 10.1001/archpsyc.62.6.685

Callen, D. J., Black, S. E., Caldwell, C. B., and Grady, C. L. (2004). The influence of sex on limbic volume and perfusion in AD. *Neurobiol. Aging* 25, 761–770. doi: 10.1016/j.neurobiolaging.2003.08.011

Cao, R., Wang, X., Gao, Y., Li, T., Zhang, H., Hussain, W., et al. (2020). Abnormal anatomical Rich-Club organization and structural–functional coupling in mild cognitive impairment and Alzheimer's disease. *Front. Neurol.* 11:53. doi: 10.3389/fneur.2020.00053

Choi, S. H., Na, D. L., Lee, B. H., Hahm, D. S., Jeong, J. H., Yoon, S. J., et al. (2001). Estimating the validity of the Korean version of expanded clinical dementia rating (CDR) scale. *J. Korean Neurol. Assoc.* 19, 585–591.

Christensen, K. J., Multhaup, K. S., Nordstrom, S., and Voss, K. (1991). A cognitive battery for dementia: development and measurement characteristics. *Psychol. Assess.* 3, 168–174. doi: 10.1037/1040-3590.3.2.168

Dai, Z., Yan, C., Li, K., Wang, Z., Wang, J., Cao, M., et al. (2015). Identifying and mapping connectivity patterns of brain network hubs in Alzheimer's disease. *Cereb. Cortex* 25, 3723–3742. doi: 10.1093/cercor/bhu246

Daianu, M., Jahanshad, N., Nir, T. M., Jack, C. R. Jr., Weiner, M. W., Bernstein, M. A., et al. (2015). Rich club analysis in the Alzheimer's disease connectome reveals a relatively undisturbed structural core network. *Hum. Brain Mapp.* 36, 3087–3103. doi: 10.1002/hbm.22830

Ewers, M., Sperling, R. A., Klunk, W. E., Weiner, M. W., and Hampel, H. (2011). Neuroimaging markers for the prediction and early diagnosis of Alzheimer's disease dementia. *Trends Neurosci.* 34, 430–442. doi: 10.1016/j.tins.2011.05.005

Ferretti, M. T., Iulita, M. F., Cavedo, E., Chiesa, P. A., Schumacher Dimech, A., Santuccione Chadha, A., et al. (2018). Sex differences in Alzheimer disease — the gateway to precision medicine. *Nat. Rev. Neurol.* 14, 457–469. doi: 10.1038/s41582-018-0032-9

Fischer, F. U., Wolf, D., Scheurich, A., and Fellgiebel, A. (2015). Altered whole-brain white matter networks in preclinical Alzheimer's disease. *Neuroimage Clin.* 8, 660–666. doi: 10.1016/j.nicl.2015.06.007

Gong, G., He, Y., Concha, L., Lebel, C., Gross, D. W., Evans, A. C., et al. (2009). Mapping anatomical connectivity patterns of human cerebral cortex using in vivo diffusion tensor imaging tractography. *Cereb. Cortex* 19, 524–536. doi: 10.1093/cercor/bhn102

Griffa, A., and Van Den Heuvel, M. P. (2018). Rich-club neurocircuitry: function, evolution, and vulnerability. *Dialogues Clin. Neurosci.* 20, 121–132. doi: 10.31887/dcns.2018.20.2/agriffa

Hagmann, P., Kurant, M., Gigandet, X., Thiran, P., Wedeen, V. J., Meuli, R., et al. (2007). Mapping human whole-brain structural networks with diffusion MRI. *PLoS One* 2:e597. doi: 10.1371/journal.pone.0000597

Hua, X., Hibar, D. P., Lee, S., Toga, A. W., Jack, C. R., Weiner, M. W., et al. (2010). Sex and age differences in atrophic rates: an ADNI study with n=1368 MRI scans. *Neurobiol. Aging* 31, 1463–1480. doi: 10.1016/j.neurobiolaging.2010.04.033

Hurtz, S., Woo, E., Kebets, V., Green, A. E., Zoumalan, C., Wang, B., et al. (2014). Age effects on cortical thickness in cognitively normal elderly individuals. *Dement. Geriatr. Cogn. Dis. Extra* 4, 221–227. doi: 10.1159/000362872

Jackson, J., Jambrina, E., Li, J., Marston, H., Menzies, F., Phillips, K., et al. (2019). Targeting the synapse in Alzheimer's disease. *Front. Neurosci.* 13:735. doi: 10.3389/fnins.2019.00735

Jacobs, H. I. L., Van Boxtel, M. P. J., Jolles, J., Verhey, F. R. J., and Uylings, H. B. M. (2012). Parietal cortex matters in Alzheimer's disease: an overview of structural, functional and metabolic findings. *Neurosci. Biobehav. Rev.* 36, 297–309. doi: 10.1016/j.neubiorev.2011.06.009

Kim, H.-R., Choe, Y. S., Moon, S. H., Kim, H. J., Jang, H., Na, D. L., et al. (2021). Finding the optimal cutoff value for amyloid β positivity using the iterative outlier method and concordance rate. *Precis Future Med* 5, 83–89. doi: 10.23838/pfm.2021.00023

Kim, J. P., Chun, M. Y., Kim, S. J., Jang, H., Kim, H. J., Jeong, J. H., et al. (2022). Distinctive temporal trajectories of Alzheimer's disease biomarkers according to sex and APOE genotype: importance of striatal amyloid. *Front. Aging Neurosci.* 14:829202. doi: 10.3389/fnagi.2022.829202

Kim, D. J., and Min, B. K. (2020). Rich-club in the brain's macrostructure: insights from graph theoretical analysis. *Comput. Struct. Biotechnol. J.* 18, 1761–1773. doi: 10.1016/j.csbj.2020.06.039

Koran, M. E. I., Wagener, M., and Hohman, T. J.Alzheimer's Neuroimaging Initiative (2017). Sex differences in the association between AD biomarkers and cognitive decline. *Brain Imaging Behav.* 11, 205–213. doi: 10.1007/s11682-016-9523-8

Latora, V., and Marchiori, M. (2001). Efficient behavior of small-world networks. *Phys. Rev. Lett.* 87:198701. doi: 10.1103/PhysRevLett.87.198701

Laws, K. R., Irvine, K., and Gale, T. M. (2018). Sex differences in Alzheimer's disease. *Curr. Opin. Psychiatry* 31, 133–139. doi: 10.1097/YCO.0000000000000401

Lee, W. J., Han, C. E., Aganj, I., Seo, S. W., and Seong, J. K. (2018). Distinct patterns of rich club organization in Alzheimer's disease and subcortical vascular dementia: a white matter network study. *J. Alzheimers Dis.* 63, 977–987. doi: 10.3233/JAD-180027

Lin, K. A., Choudhury, K. R., Rathakrishnan, B. G., Marks, D. M., Petrella, J. R., Doraiswamy, P. M., et al. (2015). Marked gender differences in progression of mild cognitive impairment over 8 years. *Alzheimers Dement* 1, 103–110. doi: 10.1016/j.trci.2015.07.001

Mattsson, N., Lönneborg, A., Boccardi, M., Blennow, K., and Hansson, O.Geneva Task Force for the Roadmap of Alzheimer's Biomarkers (2017). Clinical validity of cerebrospinal fluid Aβ42, tau, and phospho-tau as biomarkers for Alzheimer's disease in the context of a structured 5-phase development framework. *Neurobiol. Aging* 52, 196–213. doi: 10.1016/j.neurobiolaging.2016.02.034

Mazure, C. M., and Swendsen, J. (2016). Sex differences in Alzheimer's disease and other dementias. *Lancet Neurol.* 15, 451–452. doi: 10.1016/S1474-4422(16)00067-3

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Kawas, C. H., et al. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 263–269. doi: 10.1016/j.jalz.2011.03.005

Mole, J. P., Subramanian, L., Bracht, T., Morris, H., Metzler-Baddeley, C., and Linden, D. E. J. (2016). Increased fractional anisotropy in the motor tracts of Parkinson's disease suggests compensatory neuroplasticity or selective neurodegeneration. *Eur. Radiol.* 26, 3327–3335. doi: 10.1007/s00330-015-4178-1

Morris, J. C. (1997). Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. *Int. Psychogeriatr.* 9, 173–176. doi: 10.1017/S1041610297004870

Nichols, T. E., and Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25. doi: 10.1002/hbm.1058

Ota, M., Numata, Y., Kitabatake, A., Tsukada, E., Kaneta, T., Asada, T., et al. (2022). Structural brain network correlations with amyloid burden in elderly individuals at risk of Alzheimer's disease. *Psychiatry Res. Neuroimaging* 319:111415. doi: 10.1016/j.pscychresns.2021.111415

Perry, B. A. L., Lomi, E., and Mitchell, A. S. (2021). Thalamocortical interactions in cognition and disease: the mediodorsal and anterior thalamic nuclei. *Neurosci. Biobehav. Rev.* 130, 162–177. doi: 10.1016/j.neubiorev.2021.05.032

Petersen, R. C., Caracciolo, B., Brayne, C., Gauthier, S., Jelic, V., and Fratiglioni, L. (2014). Mild cognitive impairment: a concept in evolution. *J. Intern. Med.* 275, 214–228. doi: 10.1111/joim.12190

Prawiroharjo, P., Yamashita, K.-I., Yamashita, K., Togao, O., Hiwatashi, A., Yamasaki, R., et al. (2020). Disconnection of the right superior parietal lobule from the precuneus is associated with memory impairment in oldest-old Alzheimer's disease patients. *Heliyon* 6:e04516. doi: 10.1016/j.heliyon.2020.e04516

Rubinov, M., and Sporns, O. (2010). Complex network measures of brain connectivity: uses and interpretations. *NeuroImage* 52, 1059–1069. doi: 10.1016/j.neuroimage.2009.10.003

Ryu, H. J., and Yang, D. W. (2023). The Seoul neuropsychological screening battery (SNSB) for comprehensive neuropsychological assessment. *Dementia Neurocogn. Disord.* 22:1. doi: 10.12779/dnd.2023.22.1.1

Scheltens, P., Blennow, K., Breteler, M. M., de Strooper, B., Frisoni, G. B., Salloway, S., et al. (2016). Alzheimer's disease. *Lancet* 388, 505–517. doi: 10.1016/S0140-6736(15)01124-1

Sheng, X., Chen, H., Shao, P., Qin, R., Zhao, H., Xu, Y., et al. (2021). Brain structural network compensation is associated with cognitive impairment and Alzheimer's disease pathology. *Front. Neurosci.* 15:630278. doi: 10.3389/fnins.2021.630278

Smith, R., Strandberg, O., Mattsson-Carlgren, N., Leuzy, A., Palmqvist, S., Pontecorvo, M. J., et al. (2020). The accumulation rate of tau aggregates is higher in females and younger amyloid-positive subjects. *Brain* 143, 3805–3815. doi: 10.1093/brain/awaa327

Sun, L. L., Yang, S. L., Sun, H., Li, W. D., and Duan, S. R. (2019). Molecular differences in Alzheimer's disease between male and female patients determined by integrative network analysis. *J. Cell. Mol. Med.* 23, 47–58. doi: 10.1111/jcmm.13852

Sundermann, E. E., Biegon, A., Rubin, L. H., Lipton, R. B., Mowrey, W., Landau, S., et al. (2016). Better verbal memory in women than men in MCI despite similar levels of hippocampal atrophy. *Neurology* 86, 1368–1376. doi: 10.1212/wnl.0000000000002570

Tifratene, K., Robert, P., Metelkina, A., Pradier, C., and Dartigues, J. F. (2015). Progression of mild cognitive impairment to dementia due to AD in clinical settings. *Neurology* 85, 331–338. doi: 10.1212/WNL.0000000000001788

Toro, C. A., Zhang, L., Cao, J., and Cai, D. (2019). Sex differences in Alzheimer's disease: understanding the molecular impact. *Brain Res.* 1719, 194–207. doi: 10.1016/j.brainres.2019.05.031

Van Den Heuvel, M. P., Kahn, R. S., Goñi, J., and Sporns, O. (2012). High-cost, high-capacity backbone for global brain communication. *Proc. Natl. Acad. Sci.* 109, 11372–11377. doi: 10.1073/pnas.1203593109

van den Heuvel, M. P., and Sporns, O. (2011). Rich-club organization of the human connectome. *J. Neurosci.* 31, 15775–15786. doi: 10.1523/JNEUROSCI.3539-11.2011

van den Heuvel, M. P., and Sporns, O. (2013). Network hubs in the human brain. *Trends Cogn. Sci.* 17, 683–696. doi: 10.1016/j.tics.2013.09.012

van den Heuvel, M. P., Sporns, O., Collin, G., Scheewe, T., Mandl, R. C., Cahn, W., et al. (2013). Abnormal rich club organization and functional brain dynamics in schizophrenia. *JAMA Psychiat.* 70, 783–792. doi: 10.1001/jamapsychiatry.2013.1328

Wang, X. N., Zeng, Y., Chen, G. Q., Zhang, Y. H., Li, X. Y., Hao, X. Y., et al. (2016). Abnormal organization of white matter networks in patients with subjective cognitive decline and mild cognitive impairment. *Oncotarget* 7, 48953–48962. doi: 10.18632/oncotarget.10601

Wang, B., Zhan, Q., Yan, T., Imtiaz, S., Xiang, J., Niu, Y., et al. (2019). Hemisphere and gender differences in the rich-club organization of structural networks. *Cereb. Cortex* 29, 4889–4901. doi: 10.1093/cercor/bhz027

Westfall, P. H., Young, S. S., and Wright, S. P. (1993). On adjusting P-values for multiplicity. *Biometrics* 49, 941–945. doi: 10.2307/2532216

Yan, T., Wang, W., Yang, L., Chen, K., Chen, R., and Han, Y. (2018). Rich club disturbances of the human connectome from subjective cognitive decline to Alzheimer's disease. *Theranostics* 8, 3237–3255. doi: 10.7150/thno.23772

Yang, M., Logothetis, N. K., and Eschenko, O. (2019). Occurrence of hippocampal ripples is associated with activity suppression in the mediodorsal thalamic nucleus. *J. Neurosci.* 39, 434–444. doi: 10.1523/jneurosci.2107-18.2018

Yoon, S., Kim, J. E., Hwang, J., Kim, T. S., Kang, H. J., Namgung, E., et al. (2016). Effects of creatine monohydrate augmentation on brain metabolic and network outcome measures in women with major depressive disorder. *Biol. Psychiatry* 80, 439–447. doi: 10.1016/j.biopsych.2015.11.027

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Brain volumetric changes in menopausal women and its association with cognitive function: a structured review

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The menopausal transition has been proposed to put women at risk for undesirable neurological symptoms, including cognitive decline. Previous studies suggest that alterations in the hormonal milieu modulate brain structures associated with cognitive function. This structured review provides an overview of the relevant studies that have utilized MRI to report volumetric differences in the brain following menopause, and its correlations with the evaluated cognitive functions. We performed an electronic literature search using Medline (Ovid) and Scopus to identify studies that assessed the influence of menopause on brain structure with MRI. Fourteen studies met the inclusion criteria. Brain volumetric differences have been reported most frequently in the frontal and temporal cortices as well as the hippocampus. These regions are important for higher cognitive tasks and memory. Additionally, the deficit in verbal and visuospatial memory in postmenopausal women has been associated with smaller regional brain volumes. Nevertheless, the limited number of eligible studies and cross-sectional study designs warrant further research to draw more robust conclusions.

KEYWORDS

menopause, MRI, cognition, women, structural imaging, brain

1. Introduction

Menopause, a condition unique to women, typically occurs during the fifth decade of life and is diagnosed retrospectively 12 months after cessation of menstruation (Shuster et al., 2010). Clinically, it can be confirmed by evaluating the circulating levels of follicle-stimulating hormone (FSH) and/or anti-Müllerian hormone (AMH; Santoro and Johnson, 2019). The decline in the ovarian follicular reserve to a very low threshold is the main driver of the menopausal transition during midlife (Broekmans et al., 2004). During the perimenopausal stage, the menstrual cycle length becomes irregular, accompanied by changes in circulating estrogens and progesterone (Taffe and Dennerstein, 2002). It is common for the estrogen levels to fluctuate and be unpredictable but eventually reach a steady low state after completion of the menopausal transition (Hall, 2015). A scientific consensus in 2011 has led to the development of a gold

standard criterion to distinguish the progress in the menopausal transition defined by the Stages of Reproductive Aging Workshop + 10 (STRAW + 10) based on the menstrual cycle pattern changes and FSH levels (Harlow et al., 2012).

While menopause is a normal biological process, the physiological changes during this critical period can be challenging to the individual. Diminished gonadal steroid hormone certainly has implications in the central nervous system, as many hallmark manifestations of menopause are neurological in nature, including forgetfulness, insomnia, depression, subjective memory complaints, and cognitive decline (Giannini et al., 2021). The brain is an important target for gonadal hormone effects. Localization of the classical estrogen receptors (ERα/β) and progesterone receptors (PRA/B) have been reported in regions important for cognition, including the hippocampus (Guerra-Araiza et al., 2003; Bean et al., 2014; Scudiero and Verderame, 2017), the medial prefrontal cortex (Brinton et al., 2008; Almey et al., 2014), the basal forebrain (Hammond and Gibbs, 2011), and the striatum (Enterría-Morales et al., 2016). Estrogen and progesterone have been regarded as neuroprotective hormones. Estrogen mediates morphological and neurochemical changes of the neural processes by stimulating brain-derived neurotrophic factor (BDNF; Luine and Frankfurt, 2013) and by transcription factors (Arevalo et al., 2015), cell signaling (Yang et al., 2020), neuronal growth (Scharfman and MacLusky, 2006; Bustamante-Barrientos et al., 2021), dendritic spine densities (Handley et al., 2022), synaptic organization (Wang et al., 2018), and regulation of cholinergic systems (Kwakowsky et al., 2016). Similarly, progesterone induce neuroprotection by activating the mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) signaling pathways (Singh and Su, 2013; Liu et al., 2022), inhibiting excitotoxicity (Luoma et al., 2011), promoting myelin repair (Engman et al., 2018), and exerting anti-inflammatory effects (Aryanpour et al., 2017; De Nicola et al., 2018). Several studies have demonstrated the detrimental effects of menopause on cognitive function (Sullivan and Fugate, 2001; Epperson et al., 2013; Weber et al., 2014; Lee et al., 2020). Among the cognitive domains affected are attention, working memory (Maki et al., 2021), verbal memory (Kilpi et al., 2020), and executive function (Wegesin and Stern, 2007). Nevertheless, analysis from the Study of Women's Health Across the Nation (SWAN) cohort reported cognitive decline only during the perimenopausal stage, and these changes were reversed during postmenopause (Greendale et al., 2009). Specifically, there is impairment in verbal memory scores during early and late perimenopause, while there is a lack of improvement in speed processing in the late perimenopausal phase compared with the pre- and postmenopausal phases (Greendale et al., 2009). However, there has been insufficient research to determine whether the detrimental effects of the menopausal transition on cognitive functions are time limited. To ascertain whether the cognitive decline is transient or permanent, it would be useful to replicate these findings in studies with a larger number of repeated assessments over a wider age span.

Neuroimaging studies using MRI in women have revealed that fluctuations in gonadal hormones at different reproductive stages could influence region-specific structural changes in the brain (Rehbein et al., 2021). At puberty, circulating estrogen levels are positively correlated with gray matter (GM) volumes in the middle frontal, inferior temporal, middle occipital, and parahippocampal gyri (Neufang et al., 2009; Peper et al., 2009), and negatively correlated with volume changes in the prefrontal, orbitofrontal, parietal, temporal, and anterior cingulate cortices (Peper et al., 2009; Koolschijn et al., 2014). There are also apparent structural alterations across different menstrual cycle phases, pregnancy, and the postpartum period. As such, variations in hormonal levels during the menstrual phase affect the hippocampal (Protopopescu et al., 2008; Lisofsky et al., 2015) and amygdalar (Ossewaarde et al., 2013) volumes, and there are pregnancy-related changes in regions subserving social cognition (Hoekzema et al., 2017). Considering that endogenous gonadal hormones have been associated with changes in brain structure, researchers have studied the efficacy of hormone replacement therapy (HRT) on brain volumes at menopause and its correlation with cognitive status (Kantarci et al., 2016, 2018). Several modifying factors, including the time of initiation, duration, and type of HRT and the critical window hypothesis, are important to contemplate when determining the outcome of HRT on brain volumes.

Given that gonadal hormone levels affect an array of brain cellular, morphological, and organizational changes, it is foreseeable that there are volume changes in the brains of postmenopausal women. However, most of the research related to structural and morphological brain changes in postmenopausal women has focused on age or hormone treatment. Few studies have examined structural brain changes related to the individual neurobiological mechanisms of menopause. Thus far, there have been no comprehensive structured review on this area of research; hence, we aimed to address this gap in the present review. Our primary objective was to summarize the current literature on structural brain changes as measured by MRI from cross-sectional and longitudinal studies associated with the menopause status. Our secondary objective was to determine whether the structural brain differences correlate with cognitive performance. We hypothesize that the postmenopausal period is associated with smaller volumes of brain regions associated with memory and executive function. These areas include structures in the frontal, temporal, and hippocampal regions, which are associated with poor cognitive performance.

2. Methods

2.1. Literature search

An electronic literature search was performed to identify studies done on differences in the structural brain of postmenopausal women. Two online databases—Medline via Ovid Medline and SCOPUS—were searched for papers published from 1945 to March 2022. The search strategy was limited to human studies and used a combination of the following sets of keywords: women or female*; elder or old or aging or age*; menopaus*; stuctur*; MRI*.

2.2. Selection of research articles

The outcomes generated from the two databases were retrieved to screen for eligibility. The studies were selected if they meet the following inclusion criteria:

i. Full research articles or original articles.
ii. Volumetric brain structure assessment was done using an MRI scanner.

iii. There was a single MRI session (cross-sectional) or multiple sessions (longitudinal).
iv. The participants did not have any medical conditions that affect the brain structure, including stroke, brain tumors, neurodegenerative diseases, or other medical illnesses that may significantly alter central nervous system functions.
v. The study population consisted of menopausal women (either peri- or postmenopausal), and the healthy controls comprised premenopausal women and men of the same age, to have a fair evaluation of the structural brain differences.
vi. Published in the English language.

The exclusion criteria were:

i. Narrative reviews, editorial, letters, dissertations, book chapters, books, conference proceedings, and lectures.
ii. Case–control studies, interventional studies, or animal studies.
iii. Studies using other imaging modalities such as single-photon emission computed tomography, diffusion tensor imaging (DTI), and magnetoencephalography.
iv. The participants had neurological disorders, such as dementia, Alzheimer's disease (AD), schizophrenia, and depression.
v. Studies without a premenopausal group and men as a comparison.
vi. Use of HRT as an intervention in clinical trials.

2.3. Data extraction and management

In the first phase, three independent reviewers screened all the titles of the articles and removed articles that were unrelated to the study. In the second phase, the reviewers removed duplicate articles from the two databases and obtained the abstracts of the remaining articles. For the third phase, the reviewers assessed the abstracts based on the inclusion and exclusion criteria. Finally, the reviewers obtained the full text of the remaining articles and determined whether they could be included. All reviewers agreed to the inclusion of the final articles in the review. Any disagreements were resolved through consensus. The reviewers extracted data from the articles and entered it into evidence tables. The extracted data were the study title, the publication date, the research design (cross-sectional or longitudinal MRI), participant characteristics (e.g., age, biological sex, and percentage of menopausal women), MRI measures [e.g., GM, white matter (WM), and regional brain volumes], findings (e.g., differences observed between groups and adjustment for covariates), and neuropsychological measures.

2.4. Assessing the quality of the studies

Fourteen included articles were qualitatively assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies designed by the National Institutes of Health (NIH; National Institutes of Health, 2021). Quality assessment tools are needed to assess the internal validity of research findings, which is recognized as a risk of bias by the Cochrane Collaboration (Boutron et al., 2021). The tool consists of 14 questions corresponding to various aspects of study validity, including the research question, the population definition, the participation rate, recruitment, data collection, the sample size, analyses, outcome measures, and confounders. The questions are shown in Table 1. Two reviewers (HAD and NZR) judged the quality of the studies by rating each article as good (low risk of bias), fair (moderate risk of bias), or poor (high risk of bias). Finally, the assessments given by the two reviewers were compared to reach a consensus.

3. Results

3.1. Search results

The online search yielded 3,202 potentially relevant articles, of which 102 articles were duplicates. We excluded 2,986 articles after screening the titles and abstracts based on the inclusion and exclusion criteria. Of the 114 full-text articles retrieved, we removed 50 articles after reading the full text and another 52 after data extraction. The reasons for exclusion were studies used functional MRI (fMRI; $n = 51$), hormonal treatment as an intervention in clinical trials ($n = 18$), lack of comparison with a premenopausal group or men ($n = 10$), one article was from the same project as another study ($n = 1$), and irrelevant to the main objective ($n = 22$). We included two additional articles after a manual search of the SCOPUS database. Of the 14 articles included in this review, one is longitudinal (Mosconi et al., 2018), and the remaining are cross-sectional. Figure 1 shows the flow chart of study selection.

3.2. Quality assessments of the studies

The quality assessments of the included studies are shown in Table 1. We rated two studies as good (14%) and the rest as fair (86%). The cross-sectional study design in most of the studies does not allow identifying a causal association between the menopause status and brain volume. In addition, we noted a deficiency in the information regarding the statistical justification of sample size or estimates of the effect size. Furthermore, seven studies did not provide sufficient details on the participation rate; this increases the risk of selection bias, meaning that the study population may not be representative of the target population. Other limitations included underreporting whether the MRI rater was blinded to the participants' menopause status. Most authors considered the effects of multiple potential confounders on the outcomes, including age, the type of menopause, the duration of menopause, HRT use, apolipoprotein E4 (ApoE4) carrier status, education level, and vascular risk factors. However, only three studies (Mosconi et al., 2017, 2018; Schelbaum et al., 2021) comprehensively controlled all these confounders, whereas the remaining studies marginally controlled several confounders. Indeed, the only eligible longitudinal study showed a high follow-up rate and adequate reassessment of the menopause status 3 years after baseline (Mosconi et al., 2018). In the current context, the level of exposure was regarded as the phase of menopause. When both peri- and postmenopausal women were present included in a study, there were apparent brain structure differences. The common strengths of most

TABLE 1 Quality assessments of the included studies.

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Quality rating (good, fair, or poor)
den Heijer et al. (2003)	Yes	Yes	Yes	Yes	NR	NA	NA	NA	NR	NA	Yes	Yes	NA	Yes	Fair
Sullivan et al. (2005)	Yes	Yes	NR	Yes	NR	NA	NA	NA	NR	NA	Yes	NR	NA	Yes	Fair
Cowell et al. (2007)	Yes	Yes	Yes	Yes	NR	NA	NA	NA	NR	NA	Yes	CD	NA	Yes	Fair
Goto et al. (2011)	Yes	Yes	Yes	Yes	NR	NA	NA	NA	Yes	NA	Yes	NR	NA	Yes	Good
Mosconi et al. (2017)	Yes	Yes	NR	Yes	NR	NA	NA	Yes	Yes	NA	Yes	NR	NA	Yes	Fair
Lu et al. (2018)	Yes	Yes	NR	Yes	NR	NA	NA	NA	Yes	NA	Yes	Yes	NA	Yes	Fair
Kim et al. (2018)	Yes	Yes	NR	Yes	NR	NA	NA	NA	Yes	NA	Yes	NR	NA	Yes	Fair
Mosconi et al. (2018)	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	Good
Baek et al. (2019)	Yes	Yes	NR	Yes	NR	NA	NA	NA	Yes	NA	Yes	NR	NA	NR	Fair
Seitz et al. (2019)	Yes	Yes	Yes	Yes	NR	NA	NA	Yes	Yes	NA	Yes	NR	NA	Yes	Fair
Rahman et al. (2020)	Yes	Yes	Yes	Yes	NR	NA	NA	Yes	Yes	NA	Yes	NR	NA	Yes	Fair
Than et al. (2021)	Yes	Yes	NR	Yes	NR	NA	NA	NA	Yes	NA	Yes	yes	NA	Yes	Fair
Schelbaum et al. (2021)	Yes	Yes	Yes	Yes	NR	NA	NA	Yes	Yes	NA	Yes	NR	NA	Yes	Fair
Zhang et al. (2021)	Yes	Yes	NR	Yes	NR	NA	NA	NA	Yes	NA	Yes	NR	NA	Yes	Fair

Q1: Was the research question or objective in this paper clearly stated?; Q2: Was the study population clearly specified and defined?; Q3: Was the participation rate of eligible persons at least 50%?; Q4: Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were the inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?; Q5: Was a sample size justification, power description, or variance and effect estimates provided?; Q6: For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?; Q7: Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?; Q8: For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?; Q9: Were the exposure measures (independent variables) clearly defined, valid, reliable and implemented consistently across all study participants?; Q10: Was the exposure(s) assessed more than once over time?; Q11: Were the outcome measures (dependent variables) clearly defined, valid, reliable and implemented consistently across all study participants?; Q12: Were the outcome assessors blinded to the exposure status of participants?; Q13: Was loss to follow-up after baseline 20% or less?; Q14: Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)? CD: cannot determined; NA: not applicable; NR: not reported; Q: questions. Quality was rated as poor if the percentage of "yes" answers was ≤ 40%, fair (41–69%), and good (≥ 70%). Questions with "NA" as an answer were not counted in the total quality rating scores.

included studies were an accurate description of the study objective and population, uniformity in sample recruitment, and reliable staging of the menopause status.

3.3. Study descriptions

3.3.1. Study population

The study population consisted of women at different reproductive stages, and five studies also included men (den Heijer et al., 2003; Mosconi et al., 2017, 2018; Rahman et al., 2020; Schelbaum et al., 2021; Table 2). The authors determined the postmenopausal population according to the STRAW+10 criteria (Mosconi et al., 2017, 2018; Kim et al., 2018; Lu et al., 2018; Baek et al., 2019; Seitz et al., 2019; Rahman et al., 2020; Schelbaum et al., 2021; Zhang et al., 2021), interviews (Goto et al., 2011), a self-reported questionnaire (Than et al., 2021), or unspecified criteria (den Heijer et al., 2003; Sullivan et al., 2005; Cowell et al., 2007). Some of the researchers measured additional sex steroid hormones, specifically FSH, to support the menopause status (Kim et al., 2018; Lu et al., 2018; Baek et al., 2019; Zhang et al., 2021). According to the STRAW +10 criteria, there are

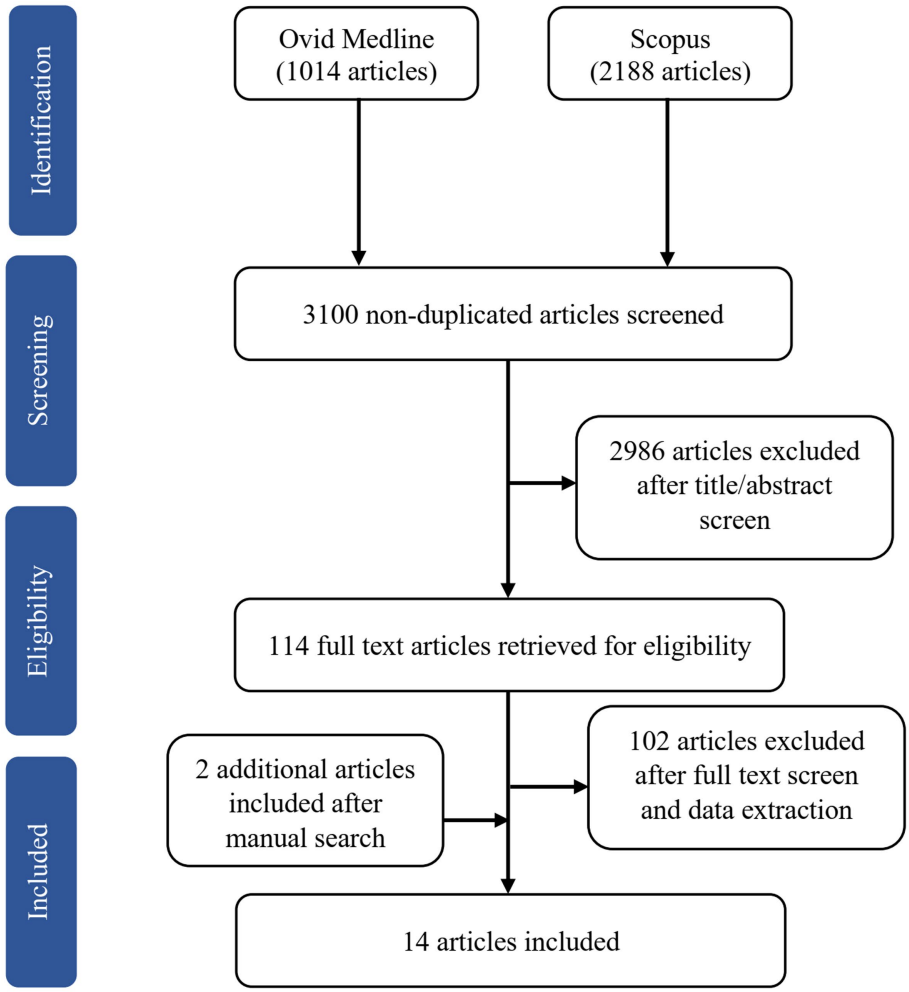

FIGURE 1
Flowchart illustrating the literature search and exclusion process.

three reproductive phases in women, namely reproductive, the menopausal transition, and postmenopause, with a total of 10 stages centered around the final menstruation phase (FMP) at Stage 0 (Table 3). Each study included postmenopausal participants except for Lu et al. (2018) and Zhang et al. (2021), in which the target population was perimenopausal women. Due to a lack of in-depth menopause assessment, Cowell et al. (2007) consolidated peri- and postmenopausal women in a single group, whereas Than et al. (2021) combined pre- and perimenopausal women.

3.3.2. Menopause characteristics

We noted that additional characterization of postmenopausal participants, including age at menopause, duration after menopause, and type of menopause, was scarce. However, two studies reported the mean age at menopause (Goto et al., 2011; Schelbaum et al., 2021), while one study recruited women who had been in postmenopause for 5.5 ± 2.5 years (Kim et al., 2018). In addition, Rahman et al. (2020), Schelbaum et al. (2021), and Than et al. (2021) reported the percentage of participants who underwent oophorectomy and hysterectomy, but they did not elaborate on whether the surgery was performed before or after the natural age of menopause.

3.3.3. Age effect

All postmenopausal women in this review ranged from 50 to 70 years of age. To minimize the age effects, the studies included age as a confounder during analysis (Mosconi et al., 2017, 2018; Kim et al., 2018; Rahman et al., 2020; Schelbaum et al., 2021), used it as a grouping factor at the sample level (Cowell et al., 2007; Goto et al., 2011), used age-matched counterparts (den Heijer et al., 2003; Seitz et al., 2019; Zhang et al., 2021), or measured the total brain volume (TBV) in age-restricted (< 58 years old) subgroup populations (Than et al., 2021). On the other hand, Lu et al. (2018) confirmed the effect of menopause by performing a correlation analysis between age and the brain regions of interest (ROIs) of samples from all women. The authors considered brain regions that were not correlated with age but that showed volume differences between groups to be an exclusive effect of menopause. Rather than controlling for the age variable, Than et al. (2021) examined the age-dependent effects of menopause on regional brain volumes. On the contrary, three studies did not consider age as a covariate (Sullivan et al., 2005; Cowell et al., 2007; Baek et al., 2019).

TABLE 2 Study descriptions.

	den Heijer et al. (2003)	Sullivan et al. (2005)	Cowell et al. (2007)	Goto et al. (2011)	Mosconi et al. (2017)	Lu et al. (2018)	Kim et al. (2018)	Mosconi et al. (2018)	Baek et al. (2019)	Seitz et al. (2019)	Rahman et al. (2020)	Than et al. (2021)	Schelbaum et al. (2021)	Zhang et al. (2021)
Study design	CS	CS	CS	CS	CS	CS	CS	LG	CS	CS	CS	CS	CS	CS
Study population	Post-M and Men	Post-M and Pre-M	Peri-M/ Post-M merged and Pre-M	Post-M and Pre-M	Post-M, Peri-M, Pre-M, and Men	Peri -M and Pre-M	Post-M and Pre-M	Post-M, Peri-M, Pre-M, and Men	Post-M and Pre-M	Post-M, Peri-M, and Pre-M	Post-M, Peri-M, Pre-M, and Men	Post-M and Pre-M/Peri-M merged	Post-M, Peri-M, Pre-M, and Men	Peri-M and Pre-M
Adjusted for age?	Matched for age	No	No	No, age as a grouping factor at the sample level	Yes	Sig. brain differences between Peri-M and Pre-M were correlated with age to exclude age effects	Yes	Yes	No	Matched for age	Yes.	TBV: age and M interaction in subgroup analyses (<58 years old) RBV: age and M interaction	Yes	Matched for age
Menopause assessment	-	-	-	Interview	STRAW +10 criteria	STRAW + 10 criteria	STRAW + 10 criteria	STRAW + 10 criteria	STRAW + 10 criteria	STRAW + 10 criteria	STRAW + 10 criteria	Self-reported questionnaires	STRAW + 10 criteria	STRAW + 10 criteria
Age at menopause	-	-	-	45.7 ± 2.9, 50.6 ± 3.1, 50.7 ± 4.3, and 49.6 ± 3.6 years for each Post-M in their 40, 50, 60, and 70 s, resp.	-	MT phase	-	-	-	-	-	-	51 ± 3 years	MT phase
Years after menopause	-	-	-	-	-	MT phase	5.5 ± 2.5 years	-	-	-	-	-	-	MT phase
Types of menopause	-	-	-	-	-	SM excluded	SM excluded	-	-	-	17% of Post-M and 4% of Peri-M had HYS	W > 60 years old with BO were regarded as Post-M	6% HYS, 6% pHYS, and 1% O	-
HRT use	HRT users excluded	41% of Post-M (active or past user)	46% of Post-M (active or past user)	-	14% of Post-M (active or past user)	HRT users excluded	HRT users excluded	HRT users excluded	HRT users excluded	HRT users excluded	26% of Post-M and 11% of Peri-M (active or past user)	7% of Post-M and 1.7% of Pre-M/ Peri-M (active or past user)	4% past users and 27% current user	HRT users excluded

(Continued)

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TABLE 2 (Continued)

	den Heijer et al. (2003)	Sullivan et al. (2005)	Cowell et al. (2007)	Goto et al. (2011)	Mosconi et al. (2017)	Lu et al. (2018)	Kim et al. (2018)	Mosconi et al. (2018)	Baek et al. (2019)	Seitz et al. (2019)	Rahman et al. (2020)	Than et al. (2021)	Schelbaum et al. (2021)	Zhang et al. (2021)
Education levels	1° ed. Of Post-M and Men was 37 and 23%, resp.	14.7 ± 3.6 years	≥ 13 years	-	≥ 12 years, adjusted	≥ 15 years	-	≥ 12 years	Post-M: 1° ed. (26%) 2° ed. (68%) 3° ed. (5%)	Post-M: HS (25%) C (28%) UNI (6%)	≥ 12 years	-	W: 17 ± 2 years	≥ 12 years
									Pre-M: 2° ed. (68%) 3° ed. (5%)	Peri-M: HS (24%) C (34%) UNI (7%)			Men: 18 ± 2 years	
										Pre-M: HS (18%) C (30%) UNI (12%)				
CMR	BMI, smoking status, and alcohol consumption	-	-	No difference in DI, SI, and BG level, and HTN	No difference in BMI, HTN, WTH, BP, FG, and CHOL in all groups	-	-	No difference in HTN and BMI in all groups	-	Each group was matched for BMI	No difference in WTH, TG, and IR, except ↓ CHOL/HDL in Post-M compared with Pre-M and Peri-M	Post-M had ↑ no. of CMR compared with Pre-M/Peri-M	Smoking status, HTN, and WTH were adjusted	-
					Men had ↑ IR and ↑ TG with ↓ HDL:LDL ratio compared with women			Men had ↑ IR and ↑ CHOL/HDL compared with women			Men had ↑ IR and CHOL/HDL compared with women			
Brain regions investigated based on *a priori* hypothesis?	Yes, HIP	Yes, HIP and TL	Yes, PFC	Yes, HIP	Yes, FL (med., inf., and PFC), PCC, TL (lat. And med.), and PL (inf. And sup.)	No	No	Yes, HIP	No	Yes, HIP, DLPFC, ACC, IPL, and PHC	No	No	Yes, FL, TL, HIP, PHC, amygdala, fusiform gyrus, PCC, BA, and EC	Yes, SCV

(Continued)

10.3389/fnagi.2023.1158001

TABLE 2 (Continued)

	den Heijer et al. (2003)	Sullivan et al. (2005)	Cowell et al. (2007)	Goto et al. (2011)	Mosconi et al. (2017)	Lu et al. (2018)	Kim et al. (2018)	Mosconi et al. (2018)	Baek et al. (2019)	Seitz et al. (2019)	Rahman et al. (2020)	Than et al. (2021)	Schelbaum et al. (2021)	Zhang et al. (2021)
ApoE4 carrier	27% in Post-M and 32% in Men	-	-	-	36, 46, 57, and 52% in Post-M, Peri-M, Pre-M, and Men, resp.	-	-	42, 50, 47, and 50% in Post-M, Peri-M, Pre-M, and Men, resp.	-	-	50, 30, and 44% in Post-M, Peri-M, and Pre-M, resp.	29% in Post-M and 25% in Pre-M/Peri-M	47% in both sexes	-

ACC, Anterior cingulate cortex; ApoE4, Apolipoprotein E 4; BA, Basal ganglia; BO, Bilateral oophorectomy; BG, Blood glucose; BMI, Body mass index; BP, Blood pressure; C, College; CHOL, Cholesterol; CMR, Cardiometabolic risk factors; CS, Cross-sectional; DI, Drinking index; DLPFC, Dorsolateral prefrontal cortex; EC, Entorhinal cortex; FG, Fasting glucose; FL, Frontal lobe; GM, Gray matter; HDL, High-density lipoprotein; HIP, Hippocampus; HRT, Hormone replacement therapy; HS, High school; HTN, Hypertension; HYS, Hysterectomy; inf., Inferior; IPL, Inferior parietal lobule; IR, Insulin resistance; lat., lateral; LDL, Low-density lipoprotein; LG, Longitudinal; M, Menopause; med., medial; MT, Menopause transition; O, Oophorectomy; PCC, Posterior cingulate cortex/precuneus; Peri-M, Perimenopausal women; pHYS, Partial hysterectomy; Post-M, Postmenopausal women; Pre-M, Premenopausal women; PFC, Prefrontal cortex; PHC, Parahippocampus; PL, Parietal lobe; resp., respectively; RBV, Regional brain volume; SCV, Subcortical volume; SI, Smoking index; Sig., Significant; SM, Surgical menopause; STRAW + 10, Stages of Reproductive Aging Workshop + 10; sup., superior; TBV, Total brain volume; TG, Triglyceride; TL, Temporal lobe; UNI, University; W, Women; WTH, Waist to hip ratio; 1° ed., Primary education; 2° ed., Secondary education; and 3° ed., Tertiary education.

3.3.4. Use of HRT

A history or current use of HRT among menopausal women varied among of the studies, and none of the studies specifically reported the types of HRT used. Some studies excluded women if they were current HRT users during the experiment (den Heijer et al., 2003; Mosconi et al., 2018; Seitz et al., 2019) or had a history of HRT use 1 month prior to the scans (Kim et al., 2018; Lu et al., 2018; Baek et al., 2019). Conversely, several studies included HRT users; the percentage of postmenopausal women actively taking HRT or past users was 41% (Sullivan et al., 2005), 46% (Cowell et al., 2007), 14% (Mosconi et al., 2017), 26% (Rahman et al., 2020), and 7% (Than et al., 2021); 11% for perimenopausal women (Rahman et al., 2020); and 1.7% for pre-/perimenopausal women (Than et al., 2021). One particular study that also included HRT users distinguished between current or past users, 4 and 27%, respectively (Schelbaum et al., 2021).

3.3.5. Education levels

The participants included in the studies had ≥12 years of education (Sullivan et al., 2005; Cowell et al., 2007; Mosconi et al., 2017, 2018; Lu et al., 2018; Rahman et al., 2020; Schelbaum et al., 2021; Zhang et al., 2021). Baek et al. (2019) reported that 26 and 68% of postmenopausal women had completed primary and secondary education, respectively, and only 5% had completed a university-level education. They reported that 79% of premenopausal women had completed a university-level education. In one study, the percentage of participants with primary education was similar in both men and women (den Heijer et al., 2003). Seitz et al. (2019) matched the academic status from secondary until tertiary education for the pre-, peri-, and postmenopausal women as well as the men. Three studies did not provide the educational background of the participants (Goto et al., 2011; Kim et al., 2018; Than et al., 2021).

3.3.6. Cardiometabolic risk factors

The cardiometabolic risk factors (CMR) include hypertension, hyperlipidemia, obesity, insulin resistance, smoking, and alcohol consumption. Several studies examined the CMR among the participants (den Heijer et al., 2003; Goto et al., 2011; Mosconi et al., 2017, 2018; Rahman et al., 2020; Schelbaum et al., 2021; Than et al., 2021). Six studies did not provide details on the CMR (Sullivan et al., 2005; Cowell et al., 2007; Kim et al., 2018; Lu et al., 2018; Baek et al., 2019; Zhang et al., 2021). In one study, the postmenopausal group had significantly more CMR than the pre-/perimenopausal group (Than et al., 2021); however, the much larger (7-fold) size of the former group could have contributed to this difference. In contrast, in one study, the cholesterol/high-density lipoprotein (HDL) ratio was lower in postmenopausal women compared with the pre- and perimenopausal women (Rahman et al., 2020). However, other studies reported similar CMR among the participants (Goto et al., 2011; Mosconi et al., 2017, 2018; Rahman et al., 2020; Table 2). Interestingly, in two studies, male subjects were hyperlipidemic and had higher insulin resistance compared with women from different reproductive phases (Mosconi et al., 2017, 2018).

3.3.7. ApoE4 carrier status

The ApoE4 carrier status was determined in multiple studies. Interestingly, these studies reported no significant difference in the

TABLE 3 The stages of reproductive aging workshop +10 staging system for reproductive aging in women.

Menarche					FMP (0)					
↓					↓					
Stage	-5	-4	-3b	-3a	-2	-1	+1a	+1b	+1c	+2
Terminology	Reproductive				Menopausal transition		Postmenopause			
	Early	Peak	Late		Early	Late	Early			Late
					Perimenopause					
Duration	Variable				Variable	1–3 years	2 years (1 + 1)		3–6 years	Remaining lifespan
Principal criteria										
Menstrual cycle	Variable to regular	Regular	Regular	Subtle changes in flow/ length	Variable length Persistent ≥7-day difference in length of consecutive cycles	Interval of amenorrhea of ≥60 days				
Supportive criteria										
Endocrine FSH AMH Inhibin B			Low Low	Variable* Low Low	↑ Variable* Low Low	↑ ≥ 25 IU/L** Low Low	↑ Variable* Low Low		Stabilizes Very low Very low	
Antral follicle count			Low	Low	Low	Low	Very low		Very low	
Descriptive characteristics										
Symptoms						Vasomotor symptoms Likely	Vasomotor symptoms Most likely			Increasing symptoms of urogenital atrophy

Source: Harlow et al. (2012). *Blood draw on cycle days 2–5. **Approximate expected level based on assays using current international pituitary standard. AMH. Anti-Müllerian hormone; FSH, Follicle-stimulating hormone; FMP, Final menstruation phase; ↑, Elevated.

percentage of carriers between the groups (den Heijer et al., 2003; Mosconi et al., 2017, 2018; Rahman et al., 2020; Schelbaum et al., 2021; Than et al., 2021). The authors considered the ApoE4 status to be a covariate and adjusted for it as a confounder during analysis.

3.3.8. Brain ROIs

Several studies investigated specific brain regions based on *a priori* hypotheses, including the frontal, parietal, temporal, prefrontal, anterior, and posterior cingulate cortices; the precuneus; the amygdala; the putamen; the hippocampus; and the parahippocampus (den Heijer et al., 2003; Sullivan et al., 2005; Cowell et al., 2007; Goto et al., 2011; Mosconi et al., 2017, 2018; Seitz et al., 2019; Schelbaum et al., 2021). These studies aimed to examine specific brain regions implicated in cognitive functions during menopause. The remaining studies explored GM alterations between groups with whole-brain analyses without predefining specific brain regions.

3.4. Structural neuroimaging

3.4.1. TBV and GM and WM volumes

The TBV refers to the overall volume or size of the entire brain, which includes both the GM and WM. Two studies reported significant reductions in the TBV as well as global GM and WM volumes in postmenopausal women compared with premenopausal women after controlling for age (Kim et al., 2018; Than et al., 2021; Table 4). These findings were supported by subgroup analysis restricted to populations <58 years old, which showed an interaction between age and menopause, indicating that for every 1-year increase in age, postmenopausal women had three times lower average predicted volume than pre-/perimenopausal group. This association remained significant after adjusting for other factors such as the ApoE4 status, HRT use, and CMR for both GM volume and TBV but not for WM volume (Than et al., 2021).

3.4.2. Cortical structures

3.4.2.1. Frontal regions

The frontal region undergoes structural changes during the menopausal transition, with reductions in GM and WM volumes (Table 4). Mosconi et al. (2017) found that both GM and WM volumes of the frontal region decreased significantly with each progression of the menopausal phase and relative to age-matched men. In the frontal region, there were volume differences in the orbital part of the right superior frontal gyrus, the lateral prefrontal cortex, the anterior cingulate cortex, the superior and inferior frontal gyri, and the supplementary motor area (SMA; Cowell et al., 2007; Kim et al., 2018; Lu et al., 2018; Baek et al., 2019; Schelbaum et al., 2021). The authors

TABLE 4 Findings from the volumetric neuroimaging studies.

Study	Population characteristics		Brain regions measured	Menopause effects on brain volumes	Covariates
	Group (*n*)	Mean ± SD age or (age range), years			
den Heijer et al. (2003)	Post-M (210)	70 ± 8	Hippocampal volume	Post-M < Men	-
	Men (202)	69 ± 8			
Sullivan et al. (2005)	Post-M (27)	(20–85)	Hippocampal volume	Post-M = Pre-M	ICV
	Pre-M (17)		GM and WM volumes in the temporal lobe		
Cowell et al. (2007)	Peri-M (5) and Post-M (15)	(50–72)	Lateral prefrontal volume	Post-M and Peri-M < Pre-M	ICV
	Pre-M (16)	(20–49)	Medial prefrontal volume	Post-M and Peri-M = Pre-M	
Goto et al. (2011)	Post-M in their 50s (59)	55.4 ± 2.7	Bilateral hippocampal volume	Post-M in their 50s < Pre-M	ICV
	Post-M in their 60s (49)	64.2 ± 2.6			
	Post-M in their 70s (17)	74.1 ± 3.0			
	Pre-M (46)	45.1 ± 2.9			
Mosconi et al. (2017)	Post-M (14)	57 (52–60)	GM and WM volumes in the posterior cingulate/precuneus, frontal, temporal, and parietal regions	Post-M and Peri-M < Men	ICV, age, education, and ApoE4
	Peri-M (13)	50 (40–56)			
	Pre-M (15)	48 (40–55)	GM and WM volume in the frontal regions	Post-M < Peri-M < Pre-M	
	Men (18)	52 (42–60)			
Lu et al. (2018)	Peri-M (25)	51.6 ± 1.63	GM volume in the left putamen, right pallidum, right inferior parietal gyrus, right superior frontal gyrus (orbital part), and right postcentral gyrus	Peri-M < Pre-M	Age and ICV
	Pre-M (32)	47.75 ± 1.55			
Kim et al. (2018)	Post-M (20)	55.7 ± 2.4	Total GM and WM volumes	Post-M < Pre-M	Age
	Pre-M (20)	39.9 ± 8.1	GM volume in the inferior frontal gyrus, supplementary motor area, superior temporal gyrus, and olfactory cortex		
Mosconi et al. (2018)	Post-M (12)	58 ± 2 (55–60)	Rate of hippocampal volume change	Post-M: ↑ volume loss	ICV, age, education, ApoE4 status, and CMR
	Peri-M (14)	53 ± 4 (45–60)			
	Pre-M (15)	47 ± 5 (40–55)		Peri-M, Pre-M, and Men: minimal to no change	
	Men (18)	52 ± 6 (42–60)			
Baek et al. (2019)	Post-M (19)	55.5 ± 2.6	GM volume in the insula, putamen, parahippocampal gyrus, amygdala, and anterior cingulate gyrus	Post-M < Pre-M	-
	Pre-M (19)	40.2 ± 6.7			

(Continued)

TABLE 4 (Continued)

Study	Population characteristics		Brain regions measured	Menopause effects on brain volumes	Covariates
	Group (*n*)	Mean ± SD age or (age range), years			
Seitz et al. (2019)	Post-M (32)	50.59 ± 2.23	Volume of the hippocampus, anterior cingulate cortex, inferior parietal cortex and dorsolateral prefrontal cortex	Post-M = Peri-M = Pre-M	Age
	Peri-M (29)	49.83 ± 1.91			
	Pre-M (33)	49.24 ± 1.71			
Rahman et al. (2020)	Post-M (41)	58 ± 3	GM volume of the hippocampus, parahippocampal gyrus, amygdala, insula, and caudate	Post-M < Peri-M < Pre-M < Men	Age and ICV
	Peri-M (28)	51 ± 4			
	Pre-M (16)	44 ± 4	Regional WM volumes		
	Men (36)	52 ± 8			
Than et al. (2021)	Post-M (1,827)	63.9 (54.2–73.6)	TBV and GM and WM volumes	Post-M < Pre-M/Peri-M	Covariates for age and menopause interaction: ApoE4, CMR, and HRT
			TBV and GM volume	Post-M < Pre-M/Peri-M	
			Volumes of the frontal, temporal, parietal, occipital, and opercular cortices, and the paracingulate gyrus		
	Post-M (1,827)	63.9 (54.2–73.6)	WM volume	Post-M = Pre-M/Peri-M	
			Putamen	Post-M > Pre-M/Peri-M	
Schelbaum et al. (2021)	Post-M (49)	W: 52 ± 6 (40–65)	GM volume in the fusiform gyrus, amygdala, hippocampus, parahippocampus, and frontal and temporal regions	Post-M and Peri-M < Men	Age, ICV, hysterectomy status, and HRT use
	Peri-M (35)				
	Pre-M (15)	M: 52 ± 7 (40–65)	GM volume in frontotemporal regions	Post-M and Peri-M < Pre-M	
	Men (29)				
Zhang et al. (2021)	Peri-M (45)	47.38 ± 1.65	Amygdalar volume	Peri-M < Pre-M	Age and education level
	Pre-M (54)	46.89 ± 1.69	Hippocampal and basal ganglia volumes	Peri-M = Pre-M	

ApoE4, Apolipoprotein E4; CMR, Cardiometabolic risk factors; GM, Gray matter; HRT, Hormone replacement therapy; ICV, Intracranial volume; M, Men; Peri-M, Perimenopausal women; Post-M, Postmenopausal women; Pre-M, Premenopausal women; SD, Standard deviation; TBV, Total brain volume; W, Women; WM, White matter; >, more than; <, less than; =, equal to.

found these differences between peri- and postmenopausal women compared with premenopausal women and men. In another study, the authors found that the interaction between the menopause status and age further influences the association between age and the paracingulate gyrus volume in postmenopausal women (Than et al., 2021). However, some studies did not find significant differences in the anterior cingulate and dorsolateral prefrontal cortex volume (Seitz et al., 2019) as well as in the medial prefrontal volume among women, stratified by menopausal status (Cowell et al., 2007), indicating the complexity and variability of the effects of menopause on the frontal region. These findings collectively suggest that menopause is associated with marked volume differences in the frontal region.

3.4.2.2. Temporal regions

Several studies investigated the impact of menopause on the temporal cortex. In one study, women had a smaller temporal cortex volume than men, and this effect was more evident in peri- and postmenopausal women (Mosconi et al., 2017). Specifically, the included studies reported that postmenopausal women have smaller volumes in the superior temporal gyrus, olfactory cortex, the entorhinal cortex, the fusiform gyrus, the superior and inferior temporal gyri, and the parahippocampus compared with premenopausal women (Kim et al., 2018; Baek et al., 2019;Rahman et al., 2020; Schelbaum et al., 2021). Furthermore, Than et al. (2021) reported an interaction between

TABLE 5 Assessments of neuropsychological measures and brain volumetric changes.

Study	Neuropsychological tests	Menopause effects on cognition	Relationship between cognition and brain volume in Peri-M/ Post-M
den Heijer et al. (2003)	Delayed recall	Post-M > Men	↑ Delayed recall scores but ↓ hippocampal volume
Mosconi et al. (2017)	DSSS	Peri-M and Post-M > Men	Cognitive function unaffected but ↓ GM volume in the frontal, posterior cingulate, precuneus, temporal, and parietal regions
	Paired associates delayed recall	Post-M = Peri-M = Pre-M = Men	
	Paragraph delayed recall		
	Designs score		
	Object naming		
Mosconi et al. (2018)	Paragraph recall	Post-M and Peri-M < Men	↓ Paragraph recall scores and block design tasks with ↓ hippocampus volume
	Block design	Post-M < Pre-M, Peri-M, and Men	
Rahman et al. (2020)	Paired associates delayed recall	Post-M < Pre-M and Peri-M	↓ Paired associates delayed recall scores with ↓ GM and WM volumes
	DSSS	Post-M = Peri-M = Pre-M = Men	
	Paragraph immediate and delayed recall		
	Designs score		
	Object naming		
	Vocabulary score		
Schelbaum et al. (2021)	RAVLT	Post-M = Peri-M = Pre-M = Men	Cognitive function unaffected but ↓ GM volume in the medial temporal lobe
	WMS-LM delayed recall test		
	Trail Making Test Part B		
	Object naming		
Zhang et al. (2021)	Two-back task	Peri-M < Pre-M	↓ Reaction time and accuracy rate in both tests with ↓ amygdalar volume
	Stroop test		

ACC, Anterior cingulate cortex; DSSS, Digit symbol substitution score; GM, Gray matter; Peri-M, Perimenopausal women; Post-M, Postmenopausal women; Pre-M, Premenopausal women; RAVLT, Rey auditory verbal learning test; WAIS, Wechsler adult intelligence scale; WM, White matter; WMS-LM, Wechsler Memory Scale logical memory; >, more than; <, less than; =, equal to; ↑, increased; ↓, decreased.

the menopause status and age: Postmenopausal women had a smaller temporal volume than pre-/perimenopausal women. However, Sullivan et al. (2005) did not find differences in the temporal lobe volume between pre- and postmenopausal women. These findings suggest that menopause can have significant effects on the temporal cortex.

3.4.2.3. Parietal regions

In a large cohort study, an interaction between the menopause status and age influenced volume reductions in parietal regions, with postmenopausal women exhibiting lower volumes compared with the pre−/perimenopausal group (Than et al., 2021). Both peri- and postmenopausal women experience changes in different subregions of the parietal cortex. The posterior cingulate cortex and precuneus were smaller in peri- and postmenopausal women compared with age-matched men (Mosconi et al., 2017; Schelbaum et al., 2021). Additionally, the right postcentral and right inferior parietal gyri were reduced in perimenopausal women compared with premenopausal woman (Lu et al., 2018). On the contrary, Seitz et al. (2019) reported no volumetric difference in the inferior parietal lobule among women of different menopausal statuses. These findings suggest that menopause may contribute to structural changes in the parietal cortex, potentially impacting cognitive functions associated with this region.

3.4.2.4. Occipital regions

There has been limited attention regarding menopause-related structural changes to the occipital cortex, which is responsible for visual processing and perception. Nevertheless, Than et al. (2021) observed a steeper negative association between age and occipital volume in postmenopausal women compared with pre-/perimenopausal women.

3.4.2.5. Insula and opercular regions

The insula and opercular regions are adjacent brain structures with diverse functions including interoception, emotion processing, and sensorimotor integration (Gogolla, 2017). Although they have not been as extensively studied in the context of menopause compared with other brain regions, there is evidence suggesting structural changes in these areas. Both peri- and postmenopausal women exhibited lower volumes in the insula compared with premenopausal women and men (Baek et al., 2019; Rahman et al., 2020). Moreover, Than et al. (2021) described that the menopause status interacted with age, resulting in a lower opercular cortex volume in postmenopausal women compared with pre−/perimenopausal women.

TABLE 6 Brain structure and neuropsychological measures outcome in menopause.

	den Heijer et al. (2003)	Sullivan et al. (2005)	Cowell et al. (2007)	Goto et al. (2011)	Mosconi et al. (2017)	Mosconi et al. (2018)	Kim et al. (2018)	Lu et al. (2018)	Baek et al. (2019)	Seitz et al. (2019)	Rahman et al. (2020)	Than et al. (2021)	Schelbaum et al. (2021)	Zhang et al. (2021)	Overall score
A. Structural parameters															
1. TBV and GM volumes	.	.	.	.	.	.	−1	.	.	.	.	−1	.	.	**−2/2***
2. WM volumes	.	.	.	.	.	.	−1	.	.	.	.	0	.	.	**−1/2***
3. Cortical volumes															
(i) *Frontal regions*	.	.	−1	.	−1	.	−1	−1	−1	0	.	−1	−1	.	**−7/8***
(ii) *Temporal regions*	.	0	.	.	−1	.	−1	.	−1	.	−1	−1	−1	.	**−6/7***
(iii) *Parietal regions*	.	.	.	.	−1	.	.	−1	.	0	.	−1	−1	.	**−4/5***
(iv) *Occipital regions*	.	.	.	.	.	.	.	.	.	.	.	−1	.	.	**−1/1***
(v) *Insula and opercular regions*	.	.	.	.	.	.	.	.	−1	.	−1	−1	.	.	**−3/3***
4. Subcortical volume															
(i) *Hippocampus*	−1	0	.	−1	.	−1	.	.	.	0	−1	.	−1	0	**−5/8***
(ii) *Basal ganglia (pallidum/putamen/caudate)*	.	.	.	.	.	.	.	−1	−1	.	−1	+1	−1	0	**−3/6***
(iii) *Amygdala*	.	.	.	.	.	.	.	.	−1	.	−1	.	−1	−1	**−4/4***
B. Neuropsychological measures															
1. Memory (immediate and delayed recall of a paragraph, paired associates, and two-back task)	+1	.	.	.	0	−1	.	.	.	.	−1	.	0	−1	−2/6
2. Visuospatial (block design test)	.	.	.	.	.	−1	.	.	.	.	.	.	.	.	**−1/1***
3. Executive function (Stroop test)	.	.	.	.	.	.	.	.	.	.	.	.	.	−1	**−1/1***
4. Other tests	.	.	.	.	0	.	.	.	.	.	0	.	0	.	0/3

*Menopause is concluded to have a negative impact on the structural and neurophysiological parameters if the proportion of the summed score is at least more than half of the denominator for each parameter.

−1: negative effects of menopause on structural or neuropsychological measures; 0: no effects of menopause on structural or neuropsychological measures; +1: positive effects of menopause on structural or neuropsychological measures. GM, Gray matter; TBV, Total brain volume; and WM, white matter.

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3.4.3. Subcortical structures

3.4.3.1. Hippocampus

The hippocampus, a vital structure in the subcortical region of the brain, has been widely studied in the context of menopause. Several studies have investigated the impact of menopause on the hippocampal volume, and the authors have reported variable findings. In support of our hypothesis, both cross-sectional and longitudinal studies have reported significant differences in hippocampal volume between groups. Initial work by den Heijer et al. (2003) reported that older women had a significantly lower mean hippocampal volume than men. Further supporting these findings, Goto et al. (2011) demonstrated a smaller bilateral hippocampal volume in postmenopausal women compared with premenopausal women. Interestingly, Goto et al. (2011) also found that after menopause, the hippocampal volume remained relatively stable, showing minimal changes from the 50 to 70 s. Moreover, postmenopausal women had the smallest hippocampal volume, followed by peri- and premenopausal women, while men had the largest volume (Rahman et al., 2020; Schelbaum et al., 2021). In the longitudinal study, postmenopausal women had the highest rate of hippocampal volume atrophy. Over the 3-year of follow-up, there was an average of 3.3% hippocampal atrophy in postmenopausal women, while there was <1% atrophy in the other groups (Mosconi et al., 2018). However, several studies did not observe any significant difference in the hippocampal volume depending on the menopause status (Sullivan et al., 2005; Seitz et al., 2019; Zhang et al., 2021).

3.4.3.2. Basal ganglia

The basal ganglia is a group of subcortical structures that comprises the caudate, putamen, and pallidum. Supporting our hypothesis, three studies reported smaller putamen volumes in peri- and postmenopausal women compared with premenopausal women and men (Lu et al., 2018; Baek et al., 2019; Schelbaum et al., 2021). Additionally, when comparing women of different reproductive phases, the caudate volume was lowest in postmenopausal women and lower relative to men (Rahman et al., 2020). Nevertheless, Zhang et al. (2021) did not observe any significant difference in the caudate, putamen, and pallidum volumes between peri- and premenopausal women. Interestingly, Than et al. (2021) reported a higher putamen volume in postmenopausal women than in pre-/perimenopausal women. These findings suggest complex and varied alterations in the volumes of specific basal ganglia structures during menopause.

3.4.3.3. Amygdala

Several studies have examined the impact of menopause on the amygdalar volume. Supporting our hypothesis, multiple independent studies have demonstrated a significantly smaller amygdalar volumes in peri- and postmenopausal women compared with premenopausal women and men (Baek et al., 2019; Rahman et al., 2020; Schelbaum et al., 2021; Zhang et al., 2021).

3.5. Neuropsychological measures

Several of the included studies explored cognitive performance through various neuropsychological tools, yielding intriguing findings. The researchers employed verbal memory tests including paired associates delayed recall, paragraph recall, and word recall. In addition, they conducted visuospatial tasks through block design tests. However, verbal memory tests are particularly relevant and consistently revealed worse performance in postmenopausal compared with pre- and perimenopausal women (Table 5). After adjusting for age and education, Rahman et al. (2020) found that postmenopausal women scored lower in paired associates delayed recall tests than pre- and perimenopausal women. Further supporting this finding, postmenopausal women had higher cognitive decline rates than the pre- and perimenopausal women and men in the paragraph recall and block design tests in the longitudinal study (Mosconi et al., 2018). Conversely, den Heijer et al. (2003) observed a higher number of words recalled in women compared with men.

Apart from verbal memory tests, Zhang et al. (2021) explored executive function and working memory performance among perimenopausal women using the Stroop test and the two-back task, respectively. The study revealed that perimenopausal women showed a lower accuracy and a longer reaction time than premenopausal women in both of these tests.

On the other hand, Mosconi et al. (2017) reported that women at different reproductive stages and age-matched men had comparable performance in various neuropsychological tools, including paired associates delayed recall, paragraph delayed recall, designs score, object naming, and the Wechsler Adult Intelligence Scale (WAIS) vocabulary. The only significant findings were the digit symbol substitution scores: Men scored lower than the other groups. Similarly, Rahman et al. (2020) observed no effects of menopause in the tests mentioned above except for paired associates delayed recall. Moreover, Schelbaum et al. (2021) measured memory and global cognitive scores assessing executive function and language, which were not affected by the menopause status. However, the authors found significant correlations between the medial temporal region volume and the memory and cognitive scores.

3.6. Overall scoring of the evidence

Table 6 summarizes the evidence from the 14 included publications regarding the effects of the menopause status on each structural and neuropsychological parameter. Studies that reported negative impacts of both peri- and postmenopause on each parameter are scored −1, while positive or no effects are scored +1 and 0, respectively. The sum of all the scores within each parameter is shown in the overall score column. The denominator in the overall score represents the total number of studies that measured the effects of menopause on the respective parameters. We inferred a negative effect of menopause when the proportion of the summed score is at least more than half of the denominator. As depicted in Table 6, the menopause status impacted the TBV; the global GM and WM volumes; the frontal, temporal, parietal, and insular cortical volumes; and the hippocampal, basal ganglia, and amygdalar volumes. In addition, menopause had an inconsistent influence on memory tests, while its effect on visuospatial abilities and executive functions was limited.

4. Discussion

This review of the 14 eligible neuroimaging studies revealed volumetric brain differences in menopausal women. Although the

effects of aging on the brain have been explored extensively, there have been few studies investigating the impact of ovarian aging on volumetric changes in the brain. It can be challenging to distinguish between the effects of chronological versus reproductive aging because the two processes are tightly interrelated. However, alteration in the hormonal environment during menopause may significantly influence the structure of specific brain regions. We found that volumetric brain alterations during the perimenopausal phase were not temporary: They progressed further in the postmenopausal phase (Mosconi et al., 2018). The most frequently observed volume differences are in the frontal cortex followed by the hippocampus and the temporal cortex. These regions have long been known to play a central role in various behavioral and cognitive functions (Rubin et al., 2014; Sigurdsson and Duvarci, 2016; Armstrong et al., 2020). Consistently, the authors used memory-related tasks to assess the cognitive function of menopausal women, although they reported mixed findings. While there is impairment in visuospatial ability and executive function, these tasks were only measured in one study and, therefore, provided a weak relationship.

4.1. Brain volume

We found consistent evidence for brain volume alterations, especially in the frontal, hippocampal, and temporal regions of postmenopausal women. Importantly, these regions—consisting of the prefrontal cortex, hippocampus, and the temporal area—form memory circuit regions (Warburton and Brown, 2010; Huijgen and Samson, 2015; Chen et al., 2016). Seven out of eight studies uniformly reported volume alterations in the frontal regions in postmenopausal women, indicating the most affected areas in menopausal women. There are inconsistencies between reports on the hippocampal volume whereby some authors reported that the menopause status did not cause volume differences. However, the majority of the articles (five out eight) supported the negative effects of menopause on hippocampal volume. In addition, multiple studies found differences in the global GM volumes and other brain regions, including the parietal cortex, the insula, the basal ganglia, and the amygdala.

The reduced volume in the frontal regions is consistent with changes reported in preclinical menopause models: decreased spine density in the dorsolateral prefrontal cortex (Hao et al., 2007) and on the pyramidal neurons of the medial prefrontal cortex (Wallace et al., 2006). Some researchers have asserted that the nature of cognitive decline in postmenopausal women is primarily a deficit in executive function (Keenan et al., 2001; Joffe et al., 2006). In functional neuroimaging studies, researchers have hypothesized that postmenopausal women require more effort to maintain normal cognitive performance (denoted by higher activity in the frontal regions) or exhibit changes in neural connectivity (Jacobs et al., 2016; Vega et al., 2016). However, it is not entirely clear whether the structural alterations precede functional impairment in menopause. Nevertheless, increased spontaneous neuronal activity in the frontal regions and reduced GM volume in the left gyrus rectus have been observed in perimenopausal women (Liu et al., 2021).

The hippocampus is vital for learning, memorizing, and encoding new information into long-term memory; hence, damage or atrophy in this area has clinical consequences (Urgolites et al., 2020). A decreased GM volume in the right medial temporal lobe among menopausal women is associated with subjective cognitive complaints (Conley et al., 2020). Furthermore, studies in ovariectomized animals have shown significant alterations in the structure and function of hippocampus, followed by poor memory performance (Su et al., 2012; Sbisa et al., 2017; Xiao et al., 2018). Throughout the lifespan, the hippocampus exhibits structural plasticity that is implicated in aging, disease, and physiological regulation (Bartsch and Wulff, 2015). A form of structural plasticity that occurs in the hippocampus is adult neurogenesis (Toda et al., 2019). Neurons in the subventricular zone of the dentate gyrus retain the capacity to divide (Gonçalves et al., 2016). Researchers have reported impaired hippocampal neurogenesis in ovariectomized rats; it could be reversed with estradiol treatment (Tanapat et al., 1999; Ormerod et al., 2003; Barha et al., 2009). However, while day 6 post-ovariectomy resulted in diminished neurogenesis (Tanapat et al., 1999), this effect was short lived as evaluation at day 28 post-ovariectomy revealed no effect on neurogenesis (Tanapat et al., 2005). These results indicate that there may be a compensatory mechanism that restores neurogenesis at longer periods post-ovariectomy. This is further corroborated by a transcriptomic findings where long-term ovariectomy upregulated the genes involved in neurogenesis (Sárvári et al., 2016). It is possible that the proposed explanation for hippocampal volume preservation in postmenopausal woman might be related to neurogenesis that occurs years later after the FMP.

The smaller volume in the temporal regions are consistent with functional imaging studies that have reported reduced cerebral blood flow to the temporal regions (Słopień et al., 2003), reduced resting neuronal activity of the superior temporal gyrus (Liu et al., 2021), regional homogeneity in the inferior temporal gyrus (Zhang et al., 2022), and reduced glucose metabolism in the middle and inferior temporal gyri of postmenopausal women (Mosconi et al., 2021). A decrease in the inferior temporal gyrus volume affects visual perception, language comprehension, and verbal fluency (Lin et al., 2020), while a reduced superior temporal gyrus volume affects speech sounds, language function, and social cognition (Ramos Nuñez et al., 2020). In addition, a decrease in smell acuity among postmenopausal women might be explained by a volume loss in the olfactory cortex (Doty et al., 2015).

The pallidum, putamen, and caudate nucleus are the subcortical nuclei that form the basal ganglia, which is primarily involved in motor controls, although its role in cognitive functions has been well established (Hélie et al., 2015; Moretti et al., 2017). Both the putamen and pallidum showed smaller volumes in menopausal women, although postmenopausal woman had a larger putamen volume than pre−/perimenopausal women (Table 6). Previous studies have reported structural changes in the basal ganglia in response to sex hormone fluctuations across the menstrual cycle. During the midluteal phase of the menstrual cycle, there is a positive correlation between the basal ganglia volume and progesterone levels (Pletzer et al., 2018). In addition, women who use oral contraceptives have a larger basal ganglia volume than women who do not (Pletzer et al., 2019). On the other hand, the basal ganglia volume is smaller when estradiol levels are relatively higher during the late follicular phase (Pletzer et al., 2018).

We noted negative impacts of menopause in all of the studies that measured the insular and amygdalar volumes (Table 6). The insula is important for cognition, decision-making, and somatosensory

function (Uddin et al., 2017). Alterations in its structure are associated with various deficits, including speech and language processing as well as understanding emotions and behavior (Menon and Uddin, 2010). Despite its relatively small size, the amygdala contains abundant ERs that mediate the action of estradiol on emotions and memory (Framorando et al., 2021). Consistently, Engman et al. (2018) observed increased functional amygdalar connectivity in women with higher estradiol levels.

4.2. Neuropsychological tests

Six studies investigated whether the observed structural brain differences are supported by evidence of altered cognitive function. Neuropsychological tests are often used to assess behavioral changes and have been used to diagnose cognitive impairment in people with neurological diseases. The tests include a wide range of approaches that evaluate different parts of the cognitive domains. One of the tests of verbal memory assesses the recall of either verbal lists (den Heijer et al., 2003) or short paragraphs (Mosconi et al., 2017, 2018; Rahman et al., 2020). In the included studies, reduced verbal memory performance in postmenopausal women was associated with smaller brain volumes, notably in the hippocampus (Mosconi et al., 2018; Rahman et al., 2020). This observation corresponds with a growing body of evidence associating verbal memory and the hippocampus (Beyer et al., 2013; Seitz et al., 2019; Witt et al., 2019). Interestingly, den Heijer et al. (2003) observed that postmenopausal women recalled more words despite having a lower hippocampal volume than men. However, when the authors divided the postmenopausal women into three groups according to their total, bioavailable, and free estradiol levels, the group with the highest bioavailable and free estradiol levels had the lowest scores in the delayed recall test and the smallest hippocampal volume. Although the findings of this study do not support the neuroprotective effects of estradiol, the reduced hippocampal volume was associated with poor memory performance (den Heijer et al., 2003).

On the other hand, two studies did not observe differences in the verbal memory scores, although there were structural brain differences between groups (Mosconi et al., 2017; Schelbaum et al., 2021). The preservation of verbal memory despite structural brain alterations of postmenopausal women in these studies can be illustrated in the context of the cognitive reserve (CR) hypothesis. Women have greater advantages on verbal memory than men due to sex-specific CR in the domain of verbal memory (Beinhoff et al., 2008; Sundermann et al., 2016a,b) combined with estrogen-mediated modulation of hippocampal function, including synapse and spine formation, signaling, and excitability (Spencer et al., 2008; Shanmugan and Epperson, 2014). However, the drawback of having better verbal memory includes missing an amnestic mild cognitive impairment diagnosis and delayed AD detection even when brain pathological changes are present (Sundermann et al., 2019). This further explains why women present a more rapid decline across a wide range of cognitive abilities after being diagnosed with AD. Based on the current review, the extent of the verbal memory advantage women have is still unclear given the inconclusive findings among the studies. Future studies should explore the interaction between verbal memory and menopause and its correlation with regional brain volumes on a larger scale.

Other cognitive domains affected by menopause are working memory and executive function, as evidenced by reduced performance in the two-back task and the Stroop test, respectively (Zhang et al., 2021). Furthermore, these tests presented significant correlations with the amygdalar volume: The two-back task accuracy had a positive correlation while the Stroop test reaction time showed a negative correlation (Zhang et al., 2021). The coordination of several interconnected brain regions is crucial for working memory and executive function, with the prefrontal cortex playing a central role (Barbey et al., 2013). Prior studies have highlighted the functional relationship between the amygdala and the bilateral prefrontal cortex (Kim et al., 2010, 2011). Furthermore, alterations in functional connectivity between the amygdala and the bilateral prefrontal cortex have been observed in postmenopausal women; these changes were associated with decreased executive functions (Zhang S. et al., 2018).

Visuospatial ability refers to the cognitive process that enables a person to visually identify targets in space and to interpret the relationship of objects in the environment in more than one dimension (de Bruin et al., 2016). fMRI scans shows activation in the occipital-temporal and frontal–parietal networks during visuospatial tasks (Gogos et al., 2010). In the current review, postmenopausal women had the greatest rate of decline in the block design test of all the reproductive states and men (Mosconi et al., 2018). Moreover, postmenopausal woman had the greatest rate of decline in glucose metabolism in the frontal cortex, a finding suggesting that the decline in the block design test may be potentially associated with alterations in glucose metabolism in the frontal cortex (Mosconi et al., 2018). These findings further support previous reports indicating that the block design test, used to assess visuospatial skills, is sensitive to changes in estrogen levels, particularly in the context of menopause (Duka et al., 2000; Mosconi et al., 2018; Karishma et al., 2020).

In contrast to verbal and working memory, executive function, and visuospatial ability, women of different reproductive states and men had similar scores in the WAIS-R object naming and vocabulary scores (Mosconi et al., 2017; Rahman et al., 2020). These subtests measure perceptual reasoning and the verbal comprehension index (Morin and Midlarsky, 2017; Scott et al., 2021). In addition, Schelbaum et al. (2021) reported no difference in global cognitive scores from which they assessed visual attention, task switching, language, and memory, although there was a positive correlation between the medial temporal lobe GM volume and the cognitive scores. The lack of score difference suggests that the preceding brain structural changes following menopause may not correlate with the cognitive performance in the aforementioned cognitive domains. This could be influenced by more education and a younger age despite menopause. Based on this information, brain structural changes seem to be more sensitive to the hormonal milieu, whereas cognitive performance deficits either manifest much later or may not be detectible with a single endpoint measure.

4.3. Effects of confounding variables

We had to consider several demographic variables across the studies, especially those that could significantly influence the outcome measures. These variables include age, age at menopause (early vs. late menopause), the type of menopause (natural vs. surgical), HRT use, education level, the CMR, and the ApoE4 carrier

status. A large body of evidence indicates that there is an age-associated reduction in global and regional brain volumes (Ritchie et al., 2015; Schippling et al., 2017), and these changes have a relationship with cognitive performance (Persson et al., 2016; Nave et al., 2019). In this regard, all but two of the included studies controlled for age to differentiate the effects of chronological age from the effects of menopause status on brain volume. This suggests that menopause significantly influences the decline in global and regional brain volume independent of the aging process. Furthermore, menopause could accelerate brain aging, as shown by a steeper decline in the age-associated reduction in brain volumes of postmenopausal women compared with pre−/perimenopausal women (Than et al., 2021). However, the volume changes in brain regions after menopause varies. Kim et al. (2018) reported a negative correlation between the SMA volume and years after menopause, while Goto et al. (2011) showed no significant difference in the hippocampal volume when they compared postmenopausal women in their 50, 60, and 70 s.

The median age of natural menopause is 51 years, preceded by 4–10 years of perimenopause (Gold et al., 2013). Nevertheless, women could have premature or early menopause if their FMP occurs before 40 and 45 years of age, respectively, due to various causes, including surgical and non-surgical (Shuster et al., 2010). Early menopause is associated with shorter lifetime exposure to endogenous sex hormones, which negatively impacts neurological health (Matyi et al., 2019; Hao et al., 2023) and the cardiovascular system (Zhu et al., 2019). In contrast, prolonged exposure to endogenous hormones exerts neuroprotective effects, as evidenced by a larger GM volume of the superior parietal lobule and left precuneus (Schelbaum et al., 2021). Unfortunately, the included studies did not provide the participants' age at the initiation of menopause. This lack of information may be attributed to several factors, including limited data availability and a focus on hormones or specific outcome measures rather than age at menopause when the authors designed their analyses. However, considering the relevance of age at menopause in relation to cardiovascular risk factors and the onset of dementia, it is crucial to include age at menopause as an important covariate in future studies.

Only three of the included studies included active HRT users (Sullivan et al., 2005;Cowell et al., 2007; Mosconi et al., 2017), while most of the included studies excluded them or controlled for HRT use. Studies that included postmenopausal women actively taking HRT showed smaller volumes in the frontal regions than premenopausal women and relative to men (Cowell et al., 2007; Mosconi et al., 2017), while the hippocampus and temporal lobe showed no volume differences between pre- and postmenopausal woman (Sullivan et al., 2005). The Women's Health Initiative Memory Study (WHIMS-MRI) reported that HRT was associated with GM reduction in the hippocampal and frontal regions (Resnick et al., 2009; Zhang et al., 2016). On the other hand, the dorsolateral prefrontal cortex volume was preserved in postmenopausal women who used a transdermal estradiol patch compared with the placebo group (Kantarci et al., 2016). The inconsistencies regarding the HRT effects on the brain structures gave rise to a speculative theory, termed the critical window hypothesis, which suggests that HRT is beneficial in the years immediately after menopause but may be deleterious when initiated ≥10 years after menopause (Whitmer et al., 2011; Maki, 2013; McCarrey and Resnick, 2015). In the current review, the absence of data on the HRT initiation time, formulation, and duration makes it difficult to conclude whether HRT is deleterious to the frontal lobe or effective in preserving the temporal lobe. In this context, it is unclear whether HRT provides neuroprotection; this issues requires further investigation.

Six of the included studies described the number of participants with the ApoE4 genotype, of which their distribution was similar between the groups and controlled as confounders (Table 2). Therefore, the brain volume alterations of postmenopausal women in these studies are independent of the ApoE4 status. The ε4 allele of the *APOE* gene is known to be the strongest genetic risk factor associated with sporadic AD (Serrano-Pozo et al., 2021). Previous studies have examined the ApoE4 by sex interaction, suggesting that female carriers are at higher risk of developing and accelerated AD progression, cognitive impairment, and lower brain volumes than male carriers (Sampedro et al., 2015; Neu et al., 2017; O'Bryant et al., 2022). Nevertheless, the effects of HRT on brain volume seemed to be more beneficial in ApoE4 carriers, who have been reported to have higher hippocampal, entorhinal and amygdala volumes (Yue et al., 2007; Saleh et al., 2023). Interestingly, the advantageous effect of starting HRT early or during the critical window on the hippocampal volume was limited to ApoE4 carriers (Saleh et al., 2023). This finding suggests that ApoE4 carriers are more sensitive to an HRT intervention, especially if it is initiated early.

The recruited subjects had at least completed primary school and had an average of 12 years of education. This level of education ensures a more homogenous socioeconomic status by minimizing low education variability that may influence the strength of the association between the menopause status and brain volume (Table 2). Education is neuroprotective as illustrated in the context of the CR theory. Essentially, individuals with higher CR can withstand advanced pathogenic mechanisms of neurodegenerative diseases or brain damage without showing any clinical manifestations (Meng and D'Arcy, 2012; Amieva et al., 2014). Education is widely used as an indirect indicator of CR, although other determinants such as occupational complexity, intelligence, and the participation rate in cognitively stimulating activities have also been considered. A cognitively stimulating environment promotes neurogenesis (Brown et al., 2003) and synaptic plasticity, and upregulates BDNF levels in animal models (Zhang M. et al., 2018). With evidence regarding the neuroprotective role of CR, future studies investigating its association with the structural and functional brain in menopausal women are needed to gain insight into predicting dementia risk.

Eight of the included studies described various CMR, including clinical (e.g., blood pressure, lipid profiles, obesity, glucose levels, and insulin resistance) and non-clinical (e.g., smoking and alcohol consumption) indicators (Table 2). Prior studies have reported clear evidence on the association between hypertension, hyperglycemia, and central obesity and brain structure alterations including smaller GM and total brain volumes, a thinner cortex, and larger ventricles than healthy controls (Song et al., 2020; Vergoossen et al., 2020). In the current review, while men had higher lipid levels and insulin resistance, they showed larger regional brain volumes than women after correcting for the intracranial volume (ICV; Mosconi et al., 2017, 2018; Rahman et al., 2020). Conversely, postmenopausal women who had lower lipid levels displayed smaller regional brain volumes than the pre- and perimenopausal women (Rahman et al., 2020). Concurrently, although there was no difference in the CMR among the pre-, peri-, and

postmenopausal groups, the latter exhibited the lowest volume in the frontal and hippocampal regions (Goto et al., 2011; Mosconi et al., 2017, 2018). It is important to note that the participants included in the studies were healthy, independent of clinical diagnoses of cardiometabolic diseases. Additional studies with larger samples that include various degrees of cardiometabolic diseases and their risk factors are needed to investigate its influence on the brain structure of menopausal women.

4.4. Methodological considerations

Based on the included studies, the frontal and temporal cortices and the hippocampus are the most consistently reported regions with changes based on ROI-based (hypotheses-driven) and whole-brain analyses (Table 6). Studies based on *a priori* hypotheses may be biased toward findings in brain regions that have previously been associated with aging- and AD-related ROI, while possible associations with other regions of the brain may be overlooked. Whole-brain studies overcome this problem, but subtle differences may go unnoticed due to strict adjustment for multiple comparisons. Indeed, three hypothesis-driven studies did not find volume differences in the hippocampus (Sullivan et al., 2005; Seitz et al., 2019; Zhang et al., 2021), as observed in exploratory whole-brain studies. These inconsistent findings may indicate that MRI is not sensitive enough to detect subcortical volume alterations or that other factors might influence to preserve the hippocampal volume independent of the menopause status.

The frontal, temporal, and parietal regions were the most frequently measured cortical volumes, reported in eight, seven, and five studies, respectively (Table 6). Interestingly, the impact of menopause on the frontal, temporal, and parietal subregions was not uniform across studies (Table 4). Each subregion of the cortex has a distinct cytoarchitecture that represents its functionality (Brodmann, 1909; Amunts et al., 1999). As such, higher cognitive tasks, including executive function, working memory, inhibitory control, and cognitive flexibility, depend on the integrity of the frontal cortex (Garcia-Alvarez et al., 2019). On the other hand, the temporal cortex has vast functions, with at least eight cognitive domains identified related to speech, hearing, visual, episodic memory, phonological processing, semantic, and social cognition (Bajada et al., 2017), while the parietal cortex is involved primarily in receiving and integrating sensory inputs (Freund, 2003). For MRI, the common approach to defining the parcellation in the brain is to use a variety of atlases spatially normalized to the stereotactic space (Thyreau and Taki, 2020; Lawrence et al., 2021). However, different parcellations across studies make assessing the reproducibility of menopause-related brain changes difficult. In the current review, three studies used manual parcellation (den Heijer et al., 2003; Sullivan et al., 2005; Cowell et al., 2007), while the remaining studies utilized various atlas-based methods or parcellation protocols (Goto et al., 2011; Mosconi et al., 2017, 2018; Kim et al., 2018; Lu et al., 2018; Baek et al., 2019; Seitz et al., 2019; Rahman et al., 2020; Schelbaum et al., 2021; Zhang et al., 2021). Challenges associated with different anatomical parcellations include inter-subject variability, nomenclature problems, and inconsistency in identifiable landmarks (Bohland et al., 2009; Moghimi et al., 2021). Therefore, standardized parcellation protocols across different populations would provide researchers with a valuable resource to evaluate future neuroimaging studies and serve as a guide for better interpretation (Bohland et al., 2009; Mandal et al., 2012).

Due to the small sample sizes of the included studies, most authors employed various correction methods for multiple comparisons, including the Bonferroni, false discovery rate, familywise error, bootstrapping, and cluster-level small volume corrections. However, three of the studies did not indicate the specific correction methods used (den Heijer et al., 2003; Sullivan et al., 2005; Cowell et al., 2007). Additionally, one study examined the results at a $p < 0.001$ threshold for a specific brain region based on *a priori* hypotheses without implementing any correction for multiple comparisons (Mosconi et al., 2017). Overall, the included studies from 2011 to 2021 recognized the importance of multiple comparison correction to reduce the risk of false positives. This recognition enhanced the reliability and validity of study findings, thereby improving study generalizability.

5. Limitations

Our review has several limitations. First, we could not completely rule out the effects of HRT use among the participants because most of the studies included HRT users (past or active); this inclusion may confound the association between menopause and brain volumes. Second, the age range of the postmenopausal women varied among the studies, which could explain the different outcomes regarding brain volume differences and the regions involved. Some studies recruited young postmenopausal women, while others used elderly postmenopausal participants. This is important because elderly postmenopausal women could have more structural alterations that may be compounded by chronological age. Third, while the majority of the studies used the STRAW+10 criteria to classify the menopause stage, there was a lack of sex hormone measurement to support the menopause status in most studies. Fourth, to allow for a uniform analysis, brain regions for the structural parameters in the evidence scoring table were allocated according to the classic anatomical lobe classification (frontal, temporal, parietal, and occipital), even though the specific subregions within the lobes were not similar across different studies. Fifth, we could not determine the reliability of structural and neuropsychological parameters that were only assessed in one study. Sixth, due to the small number of studies that measured cognitive performance and the few cognitive domains tested, we could not draw meaningful conclusions regarding the relationship between the effects of menopause and neuropsychological measures. Seventh, the predominant cross-sectional study design of the eligible studies does not allow determining causal or temporal relationships.

Future studies should include a thorough description of the types, duration, and initiation of HRT use; measurement of circulating ovarian hormone levels; control for age effects; standardization of experimental methods; a longitudinal study design in different ethnic populations; and correlation of the MRI data to sex-specific cognitive function. In addition, instead of focusing on specific subregions, whole-brain analysis could uncover more regions that are susceptible to menopause-related changes.

6. Perspectives and conclusion

Across the included studies, there is compelling evidence for menopause effects on the cortical and subcortical brain regions that are key to cognitive processes. It is challenging to state that there is a strong

association between the menopause status and brain volume differences because of the inconsistencies in the measured brain regions, the small number of eligible studies, and the cross-sectional nature of most of the studies. However, based on the evidence scoring table, ROI analyses have most frequently highlighted the involvement of the frontal and temporal regions. Additionally, the hippocampus has consistently emerged as a prominent affected area. In addition, memory-related tasks have been the most used task when assessing cognitive function in menopausal women, although there were inconsistent findings. The visuospatial ability and executive function tasks were only measured in one study and, therefore, provided a weak relationship. Although the current literature is limited by the heterogeneity in population characteristics, menopause-associated factors, and the potential confounding variables, it will be essential to conduct large, well-designed prospective neuroimaging studies to identify volumetric brain changes over time at the regional and whole-brain levels to draw stronger conclusions. Comprehensive and reliable neuroimaging findings, especially on specific subregions that are most prone to the effects of menopause, could provide a basis for additional related research especially on the neurobiological pathway mechanisms. Furthermore, providing coordinated data from a standardized stereotaxic space in neuroimaging studies could be used for a future meta-analysis.

Author contributions

NR, HD, MY, and HA contributed to the study conception. NR designed the methodology. NR, HD, and MY conducted formal analysis and wrote the original draft. HA, MY, NM, and MS provided comments and revisions to the draft. HD supervised the research activity and managed the funding sources. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Almey, A., Cannell, E., Bertram, K., Filardo, E., Milner, T. A., and Brake, W. G. (2014). Medial prefrontal cortical estradiol rapidly alters memory system bias in female rats: ultrastructural analysis reveals membrane-associated estrogen receptors as potential mediators. *Endocrinology* 155, 4422–4432. doi: 10.1210/en.2014-1463

Amieva, H., Mokri, H., le Goff, M., Meillon, C., Jacqmin-Gadda, H., Foubert-Samier, A., et al. (2014). Compensatory mechanisms in higher-educated subjects with Alzheimer's disease: a study of 20 years of cognitive decline. *Brain* 137, 1167–1175. doi: 10.1093/brain/awu035

Amunts, K., Schleicher, A., Brgel, U., Mohlberg, H., Uylings, H. B., and Zilles, K. (1999). Broca's region revisited: cytoarchitecture and intersubject variability. *J. Comp. Neurol.* 412, 319–341. doi: 10.1002/(SICI)1096-9861(19990920)412:2<319::AID-CNE10>3.0.CO;2-7

Arevalo, M.-A., Azcoitia, I., and Garcia-Segura, L. M. (2015). The neuroprotective actions of oestradiol and oestrogen receptors. *Nat. Rev. Neurosci.* 16, 17–29. doi: 10.1038/nrn3856

Armstrong, N. M., An, Y., Shin, J. J., Williams, O. A., Doshi, J., Erus, G., et al. (2020). Associations between cognitive and brain volume changes in cognitively normal older adults. *NeuroImage* 223:117289. doi: 10.1016/j.neuroimage.2020.117289

Aryanpour, R., Pasbakhsh, P., Zibara, K., Namjoo, Z., Beigi Boroujeni, F., Shahbeigi, S., et al. (2017). Progesterone therapy induces an M1 to M2 switch in microglia phenotype and suppresses NLRP3 inflammasome in a cuprizone-induced demyelination mouse model. *Int. Immunopharmacol.* 51, 131–139. doi: 10.1016/j.intimp.2017.08.007

Baek, H. S., Kim, G. W., Sundaram, T., Park, K., and Jeong, G. W. (2019). Brain morphological changes with functional deficit associated with sexual arousal in postmenopausal women. *Sex. Med.* 7, 480–488. doi: 10.1016/j.esxm.2019.06.013

Bajada, C. J., Haroon, H. A., Azadbakht, H., Parker, G. J. M., Lambon Ralph, M. A., and Cloutman, L. L. (2017). The tract terminations in the temporal lobe: their location and associated functions. *Cortex* 97, 277–290. doi: 10.1016/j.cortex.2016.03.013

Barbey, A. K., Koenigs, M., and Grafman, J. (2013). Dorsolateral prefrontal contributions to human working memory. *Cortex* 49, 1195–1205. doi: 10.1016/j.cortex.2012.05.022

Barha, C. K., Lieblich, S. E., and Galea, L. A. M. (2009). Different forms of Oestrogen rapidly upregulate cell proliferation in the dentate gyrus of adult female rats. *J. Neuroendocrinol.* 21, 155–166. doi: 10.1111/j.1365-2826.2008.01809.x

Bartsch, T., and Wulff, P. (2015). The hippocampus in aging and disease: from plasticity to vulnerability. *Neuroscience* 309, 1–16. doi: 10.1016/j.neuroscience.2015.07.084

Bean, L. A., Ianov, L., and Foster, T. C. (2014). Estrogen receptors, the hippocampus, and memory. *Neuroscientist* 20, 534–545. doi: 10.1177/1073858413519865

Beinhoff, U., Tumani, H., Brettschneider, J., Bittner, D., and Riepe, M. W. (2008). Gender-specificities in Alzheimer's disease and mild cognitive impairment. *J. Neurol.* 255, 117–122. doi: 10.1007/s00415-008-0726-9

Beyer, M. K., Bronnick, K. S., Hwang, K. S., Bergsland, N., Tysnes, O. B., Larsen, J. P., et al. (2013). Verbal memory is associated with structural hippocampal changes in newly diagnosed Parkinson's disease. *J. Neurol. Psychiatry* 84, 23–28. doi: 10.1136/jnnp-2012-303054

Bohland, J. W., Bokil, H., Allen, C. B., and Mitra, P. P. (2009). The brain atlas concordance problem: quantitative comparison of anatomical Parcellations. *PLoS One* 4:e7200. doi: 10.1371/journal.pone.0007200

Boutron, I., Page, M. J., Higgins, J. P. T., Altman, D. G., Lundh, A., and Hróbjartsson, A. (2021). "Considering bias and conflicts of interest among the included studies" in *Cochrane Handbook for Systematic Reviews of Interventions Version 6.2*. J.P.T. Higgins, J. Thomas, J. Chandler, M. Cumpston, T. Li, M.J. Page & V.A. Welch. 2nd ed. (Chichester: John Wiley & Sons).

Brinton, R. D., Thompson, R. F., Foy, M. R., Baudry, M., Wang, J., Finch, C. E., et al. (2008). Progesterone receptors: form and function in brain. *Front. Neuroendocrinol.* 29, 313–339. doi: 10.1016/j.yfrne.2008.02.001

Brodmann, K. (1909). *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien Dargestellt auf Grund des Zellenbaues*. Leipzig: Barth.

Broekmans, F. J., Faddy, M. J., Scheffer, G., and te Velde, E. R. (2004). Antral follicle counts are related to age at natural fertility loss and age at menopause. *Menopause* 11, 607–614. doi: 10.1097/01.Gme.0000123643.76105.27

Brown, J., Cooper-Kuhn, C. M., Kempermann, G., van Praag, H., Winkler, J., Gage, F. H., et al. (2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur. J. Neurosci.* 17, 2042–2046. doi: 10.1046/j.1460-9568.2003.02647.x

Bustamante-Barrientos, F. A., Méndez-Ruette, M., Ortloff, A., Luz-Crawford, P., Rivera, F. J., Figueroa, C. D., et al. (2021). The impact of estrogen and estrogen-like

molecules in neurogenesis and neurodegeneration: beneficial or harmful? *Front. Cell. Neurosci.* 15:636176. doi: 10.3389/fncel.2021.636176

Chen, J., Duan, X., Shu, H., Wang, Z., Long, Z., Liu, D., et al. (2016). Differential contributions of subregions of medial temporal lobe to memory system in amnestic mild cognitive impairment: insights from fMRI study. *Sci. Rep.* 6:26148. doi: 10.1038/srep26148

Conley, A. C., Albert, K. M., Boyd, B. D., Kim, S. G., Shokouhi, S., McDonald, B. C., et al. (2020). Cognitive complaints are associated with smaller right medial temporal gray-matter volume in younger postmenopausal women. *Menopause* 27, 1220–1227. doi: 10.1097/gme.0000000000001613

Cowell, P. E., Sluming, V. A., Wilkinson, I. D., Cezayirli, E., Romanowski, C. A., Webb, J. A., et al. (2007). Effects of sex and age on regional prefrontal brain volume in two human cohorts. *Eur. J. Neurosci.* 25, 307–318. doi: 10.1111/j.1460-9568.2006.05281.x

de Bruin, N., Bryant, D. C., MacLean, J. N., and Gonzalez, C. L. R. (2016). Assessing visuospatial abilities in healthy aging: a novel Visuomotor task. *Front. Aging Neurosci.* 8:7. doi: 10.3389/fnagi.2016.00007

de Nicola, A. F., Garay, L. I., Meyer, M., Guennoun, R., Sitruk-Ware, R., Schumacher, M., et al. (2018). Neurosteroidogenesis and progesterone anti-inflammatory/neuroprotective effects. *J. Neuroendocrinol.* 30:e12502. doi: 10.1111/jne.12502

den Heijer, T., Geerlings, M. I., Hofman, A., de Jong, F. H., Launer, L. J., Pols, H. A. P., et al. (2003). Higher estrogen levels are not associated with larger hippocampi and better memory performance. *Arch. Neurol.* 60, 213–220. doi: 10.1001/archneur.60.2.213

Doty, R. L., Tourbier, I., Ng, V., Neff, J., Armstrong, D., Battistini, M., et al. (2015). Influences of hormone replacement therapy on olfactory and cognitive function in postmenopausal women. *Neurobiol. Aging* 36, 2053–2059. doi: 10.1016/j.neurobiolaging.2015.02.028

Duka, T., Tasker, R., and McGowan, J. F. (2000). The effects of 3-week estrogen hormone replacement on cognition in elderly healthy females. *Psychopharmacology* 149, 129–139. doi: 10.1007/s002139900324

Engman, J., Sundström Poromaa, I., Moby, L., Wikström, J., Fredrikson, M., and Gingnell, M. (2018). Hormonal cycle and contraceptive effects on amygdala and salience resting-state networks in women with previous affective side effects on the pill. *Neuropsychopharmacology* 43, 555–563. doi: 10.1038/npp.2017.157

Enterría-Morales, D., López-López, I., López-Barneo, J., and d'Anglemont de Tassigny, X. (2016). Striatal GDNF production is independent to circulating estradiol level despite Pan-neuronal activation in the female mouse. *PLoS One* 11:e0164391. doi: 10.1371/journal.pone.0164391

Epperson, C. N., Sammel, M. D., and Freeman, E. W. (2013). Menopause effects on verbal memory: findings from a longitudinal community cohort. *J. Clin. Endocrinol. Metab.* 98, 3829–3838. doi: 10.1210/jc.2013-1808

For the Alzheimer's Disease Neuroimaging InitiativeSampedro, F., Vilaplana, E., de Leon, M. J., Alcolea, D., Pegueroles, J., et al. (2015). APOE-by-sex interactions on brain structure and metabolism in healthy elderly controls. *Oncotarget* 6, 26663–26674. doi: 10.18632/oncotarget.5185

Framorando, D., Moses, E., Legrand, L., Seeck, M., and Pegna, A. J. (2021). Rapid processing of fearful faces relies on the right amygdala: evidence from individuals undergoing unilateral temporal lobectomy. *Sci. Rep.* 11:426. doi: 10.1038/s41598-020-80054-1

Freund, H.-J. (2003). Somatosensory and motor disturbances in patients with parietal lobe lesions. *Adv. Neurol.* 93, 179–193.

Garcia-Alvarez, L., Gomar, J. J., Sousa, A., Garcia-Portilla, M. P., and Goldberg, T. E. (2019). Breadth and depth of working memory and executive function compromises in mild cognitive impairment and their relationships to frontal lobe morphometry and functional competence. *Alzheimer's Dement. Diagn. Assess. Dis. Monit.* 11, 170–179. doi: 10.1016/j.dadm.2018.12.010

Giannini, A., Caretto, M., Genazzani, A. R., and Simoncini, T. (2021). Neuroendocrine changes during menopausal transition. *Endocrine* 2, 405–416. doi: 10.3390/endocrines2040036

Gogolla, N. (2017). The insular cortex. *Curr. Biol.* 27, R580–R586. doi: 10.1016/j.cub.2017.05.010

Gogos, A., Gavrilescu, M., Davison, S., Searle, K., Adams, J., Rossell, S. L., et al. (2010). Greater superior than inferior parietal lobule activation with increasing rotation angle during mental rotation: an fMRI study. *Neuropsychologia* 48, 529–535. doi: 10.1016/j.neuropsychologia.2009.10.013

Gold, E. B., Crawford, S. L., Avis, N. E., Crandall, C. J., Matthews, K. A., Waetjen, L. E., et al. (2013). Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am. J. Epidemiol.* 178, 70–83. doi: 10.1093/aje/kws421

Gonçalves, J. T., Schafer, S. T., and Gage, F. H. (2016). Adult neurogenesis in the Hippocampus: from stem cells to behavior. *Cells* 167, 897–914. doi: 10.1016/j.cell.2016.10.021

Goto, M., Abe, O., Miyati, T., Inano, S., Hayashi, N., Aoki, S., et al. (2011). 3 tesla MRI detects accelerated hippocampal volume reduction in postmenopausal women. *J. Magn. Reson. Imaging* 33, 48–53. doi: 10.1002/jmri.22328

Greendale, G. A., Huang, M.-H., Wight, R. G., Seeman, T., Luetters, C., Avis, N. E., et al. (2009). Effects of the menopause transition and hormone use on cognitive performance in midlife women. *Neurology* 72, 1850–1857. doi: 10.1212/WNL.0b013e3181a71193

Guerra-Araiza, C., Villamar-Cruz, O., González-Arenas, A., Chavira, R., and Camacho-Arroyo, I. (2003). Changes in progesterone receptor isoforms content in the rat brain during the Oestrous cycle and after Oestradiol and progesterone treatments. *J. Neuroendocrinol.* 15, 984–990. doi: 10.1046/j.1365-2826.2003.01088.x

Hall, J. E. (2015). Endocrinology of the menopause. *Endocrinol. Metab. Clin. N. Am.* 44, 485–496. doi: 10.1016/j.ecl.2015.05.010

Hammond, R., and Gibbs, R. B. (2011). GPR30 is positioned to mediate estrogen effects on basal forebrain cholinergic neurons and cognitive performance. *Brain Res.* 1379, 53–60. doi: 10.1016/j.brainres.2010.11.098

Handley, E. E., Reale, L. A., Chuckowree, J. A., Dyer, M. S., Barnett, G. L., Clark, C. M., et al. (2022). Estrogen enhances dendrite spine function and recovers deficits in neuroplasticity in the prpTDP-43A315T mouse model of amyotrophic lateral sclerosis. *Mol. Neurobiol.* 59, 2962–2976. doi: 10.1007/s12035-022-02742-5

Hao, W., Fu, C., Dong, C., Zhou, C., Sun, H., Xie, Z., et al. (2023). Age at menopause and all-cause and cause-specific dementia: a prospective analysis of the UK biobank cohort. *Hum. Reprod.* 38, 1746–1754. doi: 10.1093/humrep/dead130

Hao, J., Rapp, P. R., Janssen, W. G. M., Lou, W., Lasley, B. L., Hof, P. R., et al. (2007). Interactive effects of age and estrogen on cognition and pyramidal neurons in monkey prefrontal cortex. *Proc. Natl. Acad. Sci.* 104, 11465–11470. doi: 10.1073/pnas.0704757104

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., et al. (2012). Executive summary of the stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging. *Menopause* 19, 387–395. doi: 10.1097/gme.0b013e31824d8f40

Hélie, S., Ell, S. W., and Ashby, F. G. (2015). Learning robust cortico-cortical associations with the basal ganglia: an integrative review. *Cortex* 64, 123–135. doi: 10.1016/j.cortex.2014.10.011

Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., et al. (2017). Pregnancy leads to long-lasting changes in human brain structure. *Nat. Neurosci.* 20, 287–296. doi: 10.1038/nn.4458

Huijgen, J., and Samson, S. (2015). The hippocampus: a central node in a large-scale brain network for memory. *Rev. Neurol.* 171, 204–216. doi: 10.1016/j.neurol.2015.01.557

Jacobs, E. G., Weiss, B., Makris, N., Whitfield-Gabrieli, S., Buka, S. L., Klibanski, A., et al. (2016). Reorganization of functional networks in verbal working memory circuitry in early midlife: the impact of sex and menopausal status. *Cereb. Cortex* 27, bhw127–bhw2870. doi: 10.1093/cercor/bhw127

Joffe, H., Hall, J. E., Gruber, S., Sarmiento, I. A., Cohen, L. S., Yurgelun-Todd, D., et al. (2006). Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause* 13, 411–422. doi: 10.1097/01.gme.0000189618.48774.7b

Kantarci, K., Tosakulwong, N., Lesnick, T. G., Zuk, S. M., Gunter, J. L., Gleason, C. E., et al. (2016). Effects of hormone therapy on brain structure: a randomized controlled trial. *Neurology* 87, 887–896. doi: 10.1212/wnl.0000000000002970

Kantarci, K., Tosakulwong, N., Lesnick, T. G., Zuk, S. M., Lowe, V. J., Fields, J. A., et al. (2018). Brain structure and cognition 3 years after the end of an early menopausal hormone therapy trial. *Neurology* 90, e1404–e1412. doi: 10.1212/wnl.0000000000005325

Karishma, K. J., Sahni, R. K., Singh, A., and Grewal, S. (2020). Effect of menopause on cognition in post-menopausal women. *Brain Disord. Ther.* 9:257. doi: 10.35248/2168-975X.20.9.257

Keenan, P. A., Ezzat, W. H., Ginsburg, K., and Moore, G. J. (2001). Prefrontal cortex as the site of estrogen's effect on cognition. *Psychoneuroendocrinology* 26, 577–590. doi: 10.1016/s0306-4530(01)00013-0

Kilpi, F., Soares, A. L. G., Fraser, A., Nelson, S. M., Sattar, N., Fallon, S. J., et al. (2020). Changes in six domains of cognitive function with reproductive and chronological ageing and sex hormones: a longitudinal study in 2411 UK mid-life women. *BMC Womens Health* 20:177. doi: 10.1186/s12905-020-01040-3

Kim, M. J., Gee, D. G., Loucks, R. A., Davis, F. C., and Whalen, P. J. (2010). Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. *Cereb. Cortex.* 21, 1667–1673. doi: 10.1093/cercor/bhq237

Kim, M. J., Loucks, R. A., Palmer, A. E., Brown, A., Solomon, K. A., Marchante, A. N., et al. (2011). The structural and functional connectivity of the amygdala: from Normal emotion to pathological anxiety. *Behav. Brain Res.* 223, 403–410. doi: 10.1016/j.bbr.2011.04.025

Kim, G.-W., Park, K., and Jeong, G.-W. (2018). Effects of sex hormones and age on brain volume in post-menopausal women. *J. Sex. Med.* 15, 662–670. doi: 10.1016/j.jsxm.2018.03.006

Koolschijn, P. C. M. P., Peper, J. S., and Crone, E. A. (2014). The influence of sex steroids on structural brain maturation in adolescence. *PLoS One* 9:e83929. doi: 10.1371/journal.pone.0083929

Kwakowsky, A., Milne, M. R., Waldvogel, H. J., and Faull, R. L. (2016). Effect of estradiol on Neurotrophin receptors in basal forebrain cholinergic neurons: relevance for Alzheimer's disease. *Int. J. Mol. Sci.* 17:2122. doi: 10.3390/ijms17122122

Lawrence, R. M., Bridgeford, E. W., Myers, P. E., Arvapalli, G. C., Ramachandran, S. C., Pisner, D. A., et al. (2021). Standardizing human brain parcellations. *Sci. Data* 8:78. doi: 10.1038/s41597-021-00849-3

Lee, K. S., Jung, M. S., Kim, M., Cha, K., and Chung, E. (2020). Impact of cognitive aging on health-related quality of life in menopausal women. *Osong. Public Health Res. Perspect.* 11, 185–193. doi: 10.24171/j.phrp.2020.11.4.07

Lin, Y. H., Young, I. M., Conner, A. K., Glenn, C. A., Chakraborty, A. R., Nix, C. E., et al. (2020). Anatomy and white matter connections of the inferior temporal gyrus. *World Neurosurg.* 143, e656–e666. doi: 10.1016/j.wneu.2020.08.058

Lisofsky, N., Mårtensson, J., Eckert, A., Lindenberger, U., Gallinat, J., and Kühn, S. (2015). Hippocampal volume and functional connectivity changes during the female menstrual cycle. *NeuroImage* 118, 154–162. doi: 10.1016/j.neuroimage.2015.06.012

Liu, C., Gao, W., Zhao, L., and Cao, Y. (2022). Progesterone attenuates neurological deficits and exerts a protective effect on damaged axons via the PI3K/AKT/mTOR-dependent pathway in a mouse model of intracerebral hemorrhage. *Aging* 14, 2574–2589. doi: 10.18632/aging.203954

Liu, N., Zhang, Y., Liu, S., Zhang, X., and Liu, H. (2021). Brain functional changes in perimenopausal women: an amplitude of low-frequency fluctuation study. *Menopause* 28, 384–390. doi: 10.1097/gme.0000000000001720

Lu, W., Guo, W., Hou, K., Zhao, H., Shi, L., Dong, K., et al. (2018). Grey matter differences associated with age and sex hormone levels between premenopausal and perimenopausal women: a voxel-based morphometry study. *J. Neuroendocrinol.* 30:e12655. doi: 10.1111/jne.12655

Luine, V., and Frankfurt, M. (2013). Interactions between estradiol, BDNF and dendritic spines in promoting memory. *Neuroscience* 239, 34–45. doi: 10.1016/j.neuroscience.2012.10.019

Luoma, J. I., Kelley, B. G., and Mermelstein, P. G. (2011). Progesterone inhibition of voltage-gated calcium channels is a potential neuroprotective mechanism against excitotoxicity. *Steroids* 76, 845–855. doi: 10.1016/j.steroids.2011.02.013

Maki, P. M. (2013). Critical window hypothesis of hormone therapy and cognition: a scientific update on clinical studies. *Menopause* 20, 695–709. doi: 10.1097/GME.0b013e3182960cf8

Maki, P. M., Springer, G., Anastos, K., Gustafson, D. R., Weber, K., Vance, D., et al. (2021). Cognitive changes during the menopausal transition: a longitudinal study in women with and without HIV. *Menopause* 28, 360–368. doi: 10.1097/gme.0000000000001725

Mandal, P. K., Mahajan, R., and Dinov, I. D. (2012). Structural brain atlases: design, rationale, and applications in normal and pathological cohorts. *J. Alzheimers Dis.* 31, S169–S188. doi: 10.3233/JAD-2012-120412

Matyi, J. M., Rattinger, G. B., Schwartz, S., Buhusi, M., and Tschanz, J. T. (2019). Lifetime estrogen exposure and cognition in late life: the Cache County study. *Menopause* 26, 1366–1374. doi: 10.1097/gme.0000000000001405

McCarrey, A. C., and Resnick, S. M. (2015). Postmenopausal hormone therapy and cognition. *Horm. Behav.* 74, 167–172. doi: 10.1016/j.yhbeh.2015.04.018

Meng, X., and D'Arcy, C. (2012). Education and dementia in the context of the cognitive reserve hypothesis: a systematic review with Meta-analyses and qualitative analyses. *PLoS One* 7:e38268. doi: 10.1371/journal.pone.0038268

Menon, V., and Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct. Funct.* 214, 655–667. doi: 10.1007/s00429-010-0262-0

Moghimi, P., Dang, A.T., Netoff, T.I., Lim, K.O., and Atluri, G. (2021). A review on MR based human brain parcellation methods. arXiv [Preprint]. doi: 10.48550/arXiv.2107.03475

Moretti, R., Caruso, P., Crisman, E., and Gazzin, S. (2017). Basal ganglia: their role in complex cognitive procedures in experimental models and in clinical practice. *Neurol. India* 65, 814–825. doi: 10.4103/neuroindia.NI_850_16

Morin, R. T., and Midlarsky, E. (2017). Predictors of WAIS–R vocabulary in late life: differences by race. *J. Clin. Exp. Neuropsychol.* 39, 833–841. doi: 10.1080/13803395.2016.1269155

Mosconi, L., Berti, V., Dyke, J., Schelbaum, E., Jett, S., Loughlin, L., et al. (2021). Menopause impacts human brain structure, connectivity, energy metabolism, and amyloid-beta deposition. *Sci. Rep.* 11:10867. doi: 10.1038/s41598-021-90084-y

Mosconi, L., Berti, V., Quinn, C., McHugh, P., Petrongolo, G., Varsavsky, I., et al. (2017). Sex differences in Alzheimer risk: brain imaging of endocrine vs chronologic aging. *Neurology* 89, 1382–1390. doi: 10.1212/wnl.0000000000004425

Mosconi, L., Rahman, A., Diaz, I., Wu, X., Scheyer, O., Hristov, H. W., et al. (2018). Increased Alzheimer's risk during the menopause transition: a 3-year longitudinal brain imaging study. *PLoS One* 13:e0207885. doi: 10.1371/journal.pone.0207885

National Institutes of Health (2021). Study quality assessment tools 2014. Available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools (Accessed March 1, 2022).

Nave, G., Jung, W. H., Karlsson Linnér, R., Kable, J. W., and Koellinger, P. D. (2019). Are bigger brains smarter? Evidence from a large-scale preregistered study. *Psychol. Sci.* 30, 43–54. doi: 10.1177/0956797618808470

Neu, S. C., Pa, J., Kukull, W., Beekly, D., Kuzma, A., Gangadharan, P., et al. (2017). Apolipoprotein E genotype and sex risk factors for Alzheimer disease: a meta-analysis. *JAMA Neurol.* 74, 1178–1189. doi: 10.1001/jamaneurol.2017.2188

Neufang, S., Specht, K., Hausmann, M., Gunturkun, O., Herpertz-Dahlmann, B., Fink, G. R., et al. (2009). Sex differences and the impact of steroid hormones on the developing human brain. *Cereb. Cortex* 19, 464–473. doi: 10.1093/cercor/bhn100

O'Bryant, S. E., Barber, R. C., Philips, N., Johnson, L. A., Hall, J. R., Subasinghe, K., et al. (2022). The link between APOE4 presence and neuropsychological test performance among Mexican Americans and non-Hispanic whites of the multiethnic health & aging brain study—health disparities cohort. *Dement. Geriatr. Cogn. Disord.* 51, 26–31. doi: 10.1159/000521898

Ormerod, B. K., Lee, T. T., and Galea, L. A. (2003). Estradiol initially enhances but subsequently suppresses (via adrenal steroids) granule cell proliferation in the dentate gyrus of adult female rats. *J. Neurobiol.* 55, 247–260. doi: 10.1002/neu.10181

Ossewaarde, L., van Wingen, G. A., Rijpkema, M., Bäckström, T., Hermans, E. J., and Fernández, G. (2013). Menstrual cycle-related changes in amygdala morphology are associated with changes in stress sensitivity. *Hum. Brain Mapp.* 34, 1187–1193. doi: 10.1002/hbm.21502

Peper, J. S., Brouwer, R. M., Schnack, H. G., van Baal, G. C., van Leeuwen, M., van den Berg, S. M., et al. (2009). Sex steroids and brain structure in pubertal boys and girls. *Psychoneuroendocrinology* 34, 332–342. doi: 10.1016/j.psyneuen.2008.09.012

Persson, N., Ghisletta, P., Dahle, C. L., Bender, A. R., Yang, Y., Yuan, P., et al. (2016). Regional brain shrinkage and change in cognitive performance over two years: the bidirectional influences of the brain and cognitive reserve factors. *NeuroImage* 126, 15–26. doi: 10.1016/j.neuroimage.2015.11.028

Pletzer, B., Harris, T., and Hidalgo-Lopez, E. (2018). Subcortical structural changes along the menstrual cycle: beyond the hippocampus. *Sci. Rep.* 8:16042. doi: 10.1038/s41598-018-34247-4

Pletzer, B., Harris, T., and Hidalgo-Lopez, E. (2019). Previous contraceptive treatment relates to grey matter volumes in the hippocampus and basal ganglia. *Sci. Rep.* 9:11003. doi: 10.1038/s41598-019-47446-4

Protopopescu, X., Butler, T., Pan, H., Root, J., Altemus, M., Polanecsky, M., et al. (2008). Hippocampal structural changes across the menstrual cycle. *Hippocampus* 18, 985–988. doi: 10.1002/hipo.20468

Rahman, A., Schelbaum, E., Hoffman, K., Diaz, I., Hristov, H., Andrews, R., et al. (2020). Sex-driven modifiers of Alzheimer risk. A multimodality brain imaging study. *Neurology* 95, e166–e178. doi: 10.1212/wnl.0000000000009781

Ramos Nuñez, A. I., Yue, Q., Pasalar, S., and Martin, R. C. (2020). The role of left vs. right superior temporal gyrus in speech perception: An fMRI-guided TMS study. *Brain Lang.* 209:104838. doi: 10.1016/j.bandl.2020.104838

Rehbein, E., Hornung, J., Sundström Poromaa, I., and Derntl, B. (2021). Shaping of the female human brain by sex hormones: a review. *Neuroendocrinology* 111, 183–206. doi: 10.1159/000507083

Resnick, S. M., Espeland, M. A., Jaramillo, S. A., Hirsch, C., Stefanick, M. L., Murray, A. M., et al. (2009). Postmenopausal hormone therapy and regional brain volumes: the WHIMS-MRI study. *Neurology* 72, 135–142. doi: 10.1212/01.wnl.0000339037.76336.cf

Ritchie, S. J., Dickie, D. A., Cox, S. R., Valdes Hernandez, M. d. C., Corley, J., Royle, N. A., et al. (2015). Brain volumetric changes and cognitive ageing during the eighth decade of life. *Hum. Brain Mapp.* 36, 4910–4925. doi: 10.1002/hbm.22959

Rubin, R. D., Watson, P. D., Duff, M. C., and Cohen, N. J. (2014). The role of the hippocampus in flexible cognition and social behavior. *Front. Hum. Neurosci.* 8:742. doi: 10.3389/fnhum.2014.00742

Saleh, R. N. M., Hornberger, M., Ritchie, C. W., and Minihane, A. M. (2023). Hormone replacement therapy is associated with improved cognition and larger brain volumes in at-risk APOE4 women: results from the European prevention of Alzheimer's disease (EPAD) cohort. *Alzheimers Res. Ther.* 15:10. doi: 10.1186/s13195-022-01121-5

Santoro, N., and Johnson, J. (2019). Diagnosing the onset of menopause. *JAMA* 322, 775–776. doi: 10.1001/jama.2019.6250

Sárvári, M., Kalló, I., Hrabovszky, E., Solymosi, N., and Liposits, Z. (2016). Ovariectomy alters gene expression of the hippocampal formation in middle-aged rats. *Endocrinology* 158, 69–83. doi: 10.1210/en.2016-1516

Sbisa, A. M., Gogos, A., and van den Buuse, M. (2017). Spatial working memory in the touchscreen operant platform is disrupted in female rats by ovariectomy but not estrous cycle. *Neurobiol. Learn. Mem.* 144, 147–154. doi: 10.1016/j.nlm.2017.07.010

Scharfman, H. E., and MacLusky, N. J. (2006). Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Front. Neuroendocrinol.* 27, 415–435. doi: 10.1016/j.yfrne.2006.09.004

Schelbaum, E., Loughlin, L., Jett, S., Zhang, C., Jang, G., Malviya, N., et al. (2021). Association of Reproductive History with Brain MRI biomarkers of dementia risk in midlife. *Neurology* 97, e2328–e2339. doi: 10.1212/wnl.0000000000012941

Schippling, S., Ostwaldt, A.-C., Suppa, P., Spies, L., Manogaran, P., Gocke, C., et al. (2017). Global and regional annual brain volume loss rates in physiological aging. *J. Neurol.* 264, 520–528. doi: 10.1007/s00415-016-8374-y

Scott, T. M., Morlett Paredes, A., Taylor, M. J., Umlauf, A., Artiola i Fortuny, L., Heaton, R. K., et al. (2021). Demographically-adjusted norms for the WAIS-R block

design and arithmetic subtests: results from the neuropsychological norms for the US-Mexico border region in Spanish (NP-NUMBRS) project. *Clin. Neuropsychol.* 35, 419–432. doi: 10.1080/13854046.2019.1707285

Scudiero, R., and Verderame, M. (2017). Gene expression profile of estrogen receptors alpha and beta in rat brain during aging and following high fat diet. *C. R. Biol.* 340, 372–378. doi: 10.1016/j.crvi.2017.08.001

Seitz, J., Kubicki, M., Jacobs, E. G., Cherkerzian, S., Weiss, B. K., Papadimitriou, G., et al. (2019). Impact of sex and reproductive status on memory circuitry structure and function in early midlife using structural covariance analysis. *Hum. Brain Mapp.* 40, 1221–1233. doi: 10.1002/hbm.24441

Serrano-Pozo, A., Das, S., and Hyman, B. T. (2021). APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *Lancet Neurol.* 20, 68–80. doi: 10.1016/S1474-4422(20)30412-9

Shanmugan, S., and Epperson, C. N. (2014). Estrogen and the prefrontal cortex: towards a new understanding of estrogen's effects on executive functions in the menopause transition. *Hum. Brain Mapp.* 35, 847–865. doi: 10.1002/hbm.22218

Shuster, L. T., Rhodes, D. J., Gostout, B. S., Grossardt, B. R., and Rocca, W. A. (2010). Premature menopause or early menopause: Long-term health consequences. *Maturitas* 65, 161–166. doi: 10.1016/j.maturitas.2009.08.003

Sigurdsson, T., and Duvarci, S. (2016). Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. *Front. Syst. Neurosci.* 9:190. doi: 10.3389/fnsys.2015.00190

Singh, M., and Su, C. (2013). Progesterone and neuroprotection. *Horm. Behav.* 63, 284–290. doi: 10.1016/j.yhbeh.2012.06.003

Słopień, R., Junik, R., Meczekalski, B., Halerz-Nowakowska, B., Maciejewska, M., Warenik-Szymankiewicz, A., et al. (2003). Influence of hormonal replacement therapy on the regional cerebral blood flow in postmenopausal women. *Maturitas* 46, 255–262. doi: 10.1016/S0378-5122(03)00144-0

Song, R., Xu, H., Dintica, C. S., Pan, K.-Y., Qi, X., Buchman, A. S., et al. (2020). Associations between cardiovascular risk, structural brain changes, and cognitive decline. *J. Am. Coll. Cardiol.* 75, 2525–2534. doi: 10.1016/j.jacc.2020.03.053

Spencer, J. L., Waters, E. M., Romeo, R. D., Wood, G. E., Milner, T. A., and McEwen, B. S. (2008). Uncovering the mechanisms of estrogen effects on hippocampal function. *Front. Neuroendocrinol.* 29, 219–237. doi: 10.1016/j.yfrne.2007.08.006

Su, J., Sripanidkulchai, K., Hu, Y., Wyss, J. M., and Sripanidkulchai, B. (2012). The effect of ovariectomy on learning and memory and relationship to changes in brain volume and neuronal density. *Int. J. Neurosci.* 122, 549–559. doi: 10.3109/00207454.2012.690795

Sullivan, M. E., and Fugate, W. N. (2001). Midlife women's attributions about perceived memory changes: observations from the Seattle midlife Women's health study. *J. Womens Health Gend. Med.* 10, 351–362. doi: 10.1089/152460901750269670

Sullivan, E. V., Marsh, L., and Pfefferbaum, A. (2005). Preservation of hippocampal volume throughout adulthood in healthy men and women. *Neurobiol. Aging* 26, 1093–1098. doi: 10.1016/j.neurobiolaging.2004.09.015

Sundermann, E. E., Biegon, A., Rubin, L. H., Lipton, R. B., Mowrey, W., Landau, S., et al. (2016a). Better verbal memory in women than men in MCI despite similar levels of hippocampal atrophy. *Neurology* 86, 1368–1376. doi: 10.1212/WNL.0000000000002570

Sundermann, E. E., Maki, P., Biegon, A., Lipton, R. B., Mielke, M. M., Machulda, M., et al. (2019). Sex-specific norms for verbal memory tests may improve diagnostic accuracy of amnestic MCI. *Neurology* 93, e1881–e1889. doi: 10.1212/wnl.0000000000008467

Sundermann, E. E., Maki, P. M., Rubin, L. H., Lipton, R. B., Landau, S., Biegon, A., et al. (2016b). Female advantage in verbal memory: evidence of sex-specific cognitive reserve. *Neurology* 87, 1916–1924. doi: 10.1212/WNL.0000000000003288

Taffe, J. R., and Dennerstein, L. (2002). Menstrual patterns leading to the final menstrual period. *Menopause* 9, 32–40. doi: 10.1097/00042192-200201000-00006

Tanapat, P., Hastings, N. B., and Gould, E. (2005). Ovarian steroids influence cell proliferation in the dentate gyrus of the adult female rat in a dose- and time-dependent manner. *J. Comp. Neurol.* 481, 252–265. doi: 10.1002/cne.20385

Tanapat, P., Hastings, N. B., Reeves, A. J., and Gould, E. (1999). Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J. Neurosci.* 19, 5792–5801. doi: 10.1523/jneurosci.19-14-05792.1999

Than, S., Moran, C., Beare, R., Vincent, A. J., Collyer, T. A., Wang, W., et al. (2021). Interactions between age, sex, menopause, and brain structure at midlife: a UK biobank study. *J. Clin. Endocrinol. Metab.* 106, 410–420. doi: 10.1210/clinem/dgaa847

Thyreau, B., and Taki, Y. (2020). Learning a cortical parcellation of the brain robust to the MRI segmentation with convolutional neural networks. *Med. Image Anal.* 61:101639. doi: 10.1016/j.media.2020.101639

Toda, T., Parylak, S. L., Linker, S. B., and Gage, F. H. (2019). The role of adult hippocampal neurogenesis in brain health and disease. *Mol. Psychiatry* 24, 67–87. doi: 10.1038/s41380-018-0036-2

Uddin, L. Q., Nomi, J. S., Hébert-Seropian, B., Ghaziri, J., and Boucher, O. (2017). Structure and function of the human insula. *J. Clin. Neurophysiol.* 34, 300–306. doi: 10.1097/WNP.0000000000000377

Urgolites, Z. J., Wixted, J. T., Goldinger, S. D., Papesh, M. H., Treiman, D. M., Squire, L. R., et al. (2020). Spiking activity in the human hippocampus prior to encoding predicts subsequent memory. *Proc. Natl. Acad. Sci.* 117, 13767–13770. doi: 10.1073/pnas.2001338117

Vega, J. N., Zurkovsky, L., Albert, K., Melo, A., Boyd, B., Dumas, J., et al. (2016). Altered brain connectivity in early postmenopausal women with subjective cognitive impairment. *Front. Neurosci.* 10:433. doi: 10.3389/fnins.2016.00433

Vergoossen, L. W. M., Jansen, J. F. A., Backes, W. H., and Schram, M. T. (2020). Cardiometabolic determinants of early and advanced brain alterations: insights from conventional and novel MRI techniques. *Neurosci. Biobehav. Rev.* 115, 308–320. doi: 10.1016/j.neubiorev.2020.04.001

Wallace, M., Luine, V., Arellanos, A., and Frankfurt, M. (2006). Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. *Brain Res.* 1126, 176–182. doi: 10.1016/j.brainres.2006.07.064

Wang, S., Zhu, J., and Xu, T. (2018). 17β-estradiol (E2) promotes growth and stability of new dendritic spines via estrogen receptor β pathway in intact mouse cortex. *Brain Res. Bull.* 137, 241–248. doi: 10.1016/j.brainresbull.2017.12.011

Warburton, E. C., and Brown, M. W. (2010). Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. *Neuropsychologia* 48, 2262–2272. doi: 10.1016/j.neuropsychologia.2009.12.022

Weber, M. T., Maki, P. M., and McDermott, M. P. (2014). Cognition and mood in perimenopause: a systematic review and meta-analysis. *J. Steroid Biochem. Mol. Biol.* 142, 90–98. doi: 10.1016/j.jsbmb.2013.06.001

Wegesin, D. J., and Stern, Y. (2007). Effects of hormone replacement therapy and aging on cognition: evidence for executive dysfunction. *Aging Neuropsychol. Cogn.* 14, 301–328. doi: 10.1080/13825580600802893

Whitmer, R. A., Quesenberry, C. P., Zhou, J., and Yaffe, K. (2011). Timing of hormone therapy and dementia: the critical window theory revisited. *Ann. Neurol.* 69, 163–169. doi: 10.1002/ana.22239

Witt, J.-A., Coras, R., Becker, A. J., Elger, C. E., Blümcke, I., and Helmstaedter, C. (2019). When does conscious memory become dependent on the hippocampus? The role of memory load and the differential relevance of left hippocampal integrity for short- and long-term aspects of verbal memory performance. *Brain Struct. Funct.* 224, 1599–1607. doi: 10.1007/s00429-019-01857-1

Xiao, Q., Luo, Y., Lv, F., He, Q., Wu, H., Chao, F., et al. (2018). Protective effects of 17β-estradiol on hippocampal myelinated fibers in Ovariectomized middle-aged rats. *Neuroscience* 385, 143–153. doi: 10.1016/j.neuroscience.2018.06.006

Yang, Y., Zhao, L., Li, N., Dai, C., Yin, N., Chu, Z., et al. (2020). Estrogen exerts neuroprotective effects in vascular dementia rats by suppressing autophagy and activating the Wnt/β-catenin signaling pathway. *Neurochem. Res.* 45, 2100–2112. doi: 10.1007/s11064-020-03072-5

Yue, Y., Hu, L., Tian, Q. J., Jiang, J. M., Dong, Y. L., Jin, Z. Y., et al. (2007). Effects of long-term, low-dose sex hormone replacement therapy on hippocampus and cognition of postmenopausal women of different apoE genotypes. *Acta Pharmacol. Sin.* 28, 1129–1135. doi: 10.1111/j.1745-7254.2007.00618.x

Zhang, T., Casanova, R., Resnick, S. M., Manson, J. E., Baker, L. D., Padual, C. B., et al. (2016). Effects of hormone therapy on brain volumes changes of postmenopausal women revealed by optimally-discriminative voxel-based morphometry. *PLoS One* 11:e0150834. doi: 10.1371/journal.pone.0150834

Zhang, S., Fan, W., Hu, H., Wen, L., Gong, M., Liu, B., et al. (2021). Subcortical volume changes in early menopausal women and correlation with neuropsychological tests. *Front. Aging Neurosci.* 13:738679. doi: 10.3389/fnagi.2021.738679

Zhang, Y., Fu, W. Q., Liu, N. N., and Liu, H. J. (2022). Alterations of regional homogeneity in perimenopause: a resting-state functional MRI study. *Climacteric* 21, 460–466. doi: 10.1080/13697137.2021.2014808

Zhang, S., Hu, J., Fan, W., Liu, B., Wen, L., Wang, G., et al. (2018). Aberrant cerebral activity in early postmenopausal women: a resting-state functional magnetic resonance imaging study. *Front. Cell. Neurosci.* 12:454. doi: 10.3389/fncel.2018.00454

Zhang, M., Zhai, Y., Sun, Y., Zhang, W., Li, Q., Brann, D., et al. (2018). Swimming improves cognitive reserve in ovariectomized rats and enhances neuroprotection after global cerebral ischemia. *Brain Res.* 1692, 110–117. doi: 10.1016/j.brainres.2018.05.020

Zhu, D., Chung, H.-F., Dobson, A. J., Pandeya, N., Giles, G. G., Bruinsma, F., et al. (2019). Age at natural menopause and risk of incident cardiovascular disease: a pooled analysis of individual patient data. *Lancet Public Health* 4, e553–e564. doi: 10.1016/S2468-2667(19)30155-0

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Sex-specific relationship between non-alcoholic fatty liver disease and amyloid-β in cognitively unimpaired individuals

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Background: Non-alcoholic fatty liver disease (NAFLD) is known to be associated with a high risk of clinically diagnosed Alzheimer's disease (AD). Additionally, the prevalence of NAFLD and AD is higher in elderly females than in males. However, a sex-specific association between NAFLD and amyloid-beta (Aβ) deposition remains unclear. Therefore, we investigated the sex-specific relationship between NAFLD and Aβ deposition in a large-sized cohort of cognitively unimpaired (CU) individuals.

Methods: We enrolled 673 (410 [60.9%] females and 263 [39.1%] males) CU individuals aged ≥45 years who underwent Aβ positron emission tomography (PET). The presence of NAFLD, assessed using the hepatic steatosis index, and the severity of NAFLD, assessed using the Fibrosis-4 index, were considered predictors. Aβ deposition on PET was considered as an outcome.

Results: Females had a higher frequency of NAFLD than males (48 and 23.2%, $p < 0.001$). Among females, the presence of NAFLD ($\beta = 0.216$, $p < 0.001$) was predictive of increased Aβ deposition, whereas among males, the presence of NAFLD ($\beta = 0.191$, $p = 0.064$) was not associated with Aβ deposition. Among females, the presence of NAFLD with low ($\beta = 0.254$, $p = 0.039$), intermediate ($\beta = 0.201$, $p = 0.006$), and high fibrosis ($\beta = 0.257$, $p = 0.027$) was predictive of increased Aβ deposition. Aβ deposition also increased as the severity of NAFLD increased in females (p for trend = 0.001).

Conclusion: We highlight the marked influence of NAFLD and its severity on the risk of Aβ deposition in relation to sex. Furthermore, our findings suggest that sex-specific strategies regarding the management of NAFLD are necessary for the prevention of Aβ deposition.

KEYWORDS

NAFLD, amyloid-beta (Aβ), sex, Alzheimer's disease, preclinical stage of Alzheimer's disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent chronic liver disease (Younossi et al., 2016) that has detrimental effects on multiple extrahepatic health conditions and liver-related outcomes. NAFLD is closely associated with metabolic syndromes, including diabetes and insulin resistance and complications of metabolic syndromes such as cardiovascular diseases (Gaggini et al., 2013). Thus, NAFLD is considered a hepatic manifestation of metabolic syndrome. Meanwhile, Alzheimer's disease (AD) is characterized by the amyloid-beta (Aβ) deposition in the brain. Aβ deposition starts 15–20 years before the onset of cognitive impairment. Since Aβ positron emission tomography (PET) allowed us to detect Aβ deposition in living individuals, 15–30% of cognitively unimpaired (CU) individuals are found to have Aβ deposition (Sperling et al., 2020). Aβ deposition is also known to be associated with poor metabolic health (Gottesman et al., 2017). Specifically, metabolic syndromes, including diabetes, insulin resistance, hyperlipidemia, and increased weight variability, are closely related to Aβ deposition in CU individuals (Reed et al., 2014; Willette et al., 2015; Kang et al., 2022).

Many studies show that NAFLD is associated with a higher risk of clinically diagnosed AD (Shang et al., 2021; Kim et al., 2022). However, no previous studies have investigated the association between NAFLD and Aβ deposition in CU individuals. Aβ deposition in CU individuals represents the preclinical stage of AD (Shim and Morris, 2011). This preclinical stage has received considerable attention because early intervention may increase the possibility of therapeutic success. In addition, the prevention of Aβ deposition in CU individuals is regarded as primary prevention. Thus, investigation of the relationship between NAFLD and Aβ deposition in CU individuals has important implications for primary prevention, especially considering the paucity of known prevention methods.

Previous studies have suggested that there were differences in the effects of metabolic syndrome on brain health between females and males (Lee et al., 2018; Kim et al., 2019). In addition, the prevalence of NAFLD and AD was higher in elderly females than in elderly males (Alzheimer's Association, 2016; Lonardo et al., 2019; Tobari and Hashimoto, 2020). Therefore, the effects of NAFLD on Aβ deposition may differ depending on sex. However, the sex-specific relationship between NAFLD and Aβ deposition remains unclear.

In the present study, we aimed to investigate the sex-specific relationship between the presence of NAFLD, as defined using the hepatic steatosis index (HSI), and Aβ deposition in a large-sized cohort of CU individuals. Furthermore, we aimed to identify the sex-specific relationship between NAFLD severity, assessed using the Fibrosis-4 (FIB-4) index, and Aβ deposition. Given that brain health is more vulnerable to metabolic syndrome in females than in males, the effect of NAFLD on Aβ deposition may be more prominent in females than in males.

Methods

Study participants

We enrolled 673 cognitively unimpaired (CU) participants ≥45 years of age who underwent Aβ PET at the memory clinic in the Department of Neurology at Samsung Medical Center in Seoul, Korea, between August 2015 and November 2021. These participants comprised volunteers who applied for comprehensive dementia evaluation advertised in the local community, memory clinic, and paper; spouses of patients who visited the memory clinic; and participants with subjective cognitive decline. All participants underwent standardized neuropsychological test battery using the Seoul Neuropsychological Screening Battery 2nd edition (SNSB-II; Kang et al., 2021), brain magnetic resonance imaging (MRI), and laboratory tests, including liver function tests. All participants met the following criteria: (1) no medical history that was likely to affect cognitive function based on Christensen's health screening criteria (Christensen et al., 1991); (2) no objective cognitive impairment in any cognitive domain on a comprehensive neuropsychological test battery (above at least −1.0 standard deviation (SD) of age-adjusted norms on any cognitive test); and (3) independence in activities of daily living. We excluded the participants with history of viral hepatitis or alcohol-liver disease, severe WMH (cap or band >10 mm and longest diameter of deep white matter lesion >25 mm), and structural lesions including cerebral infarction, intracranial hemorrhage, brain tumors, and hydrocephalus on MRI.

This study was approved by the Institutional Review Board of Samsung Medical Center approved. Written informed consent was obtained from all the participants.

Fatty liver assessment

Fatty liver was defined using the HSI, which includes serum alanine aminotransferase (ALT, IU/L), serum aspartate aminotransferase (AST, IU/L), body mass index (BMI, kg/m^2), sex, and presence of diabetes. The HSI was calculated using the following variables: HSI = 8 × (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus).

Hepatic steatosis index has been created and validated for the detection of NAFLD in large populations, not only in Asians (Lee et al., 2010; Murayama et al., 2021) but also in Europeans (Meffert et al., 2014; Khani et al., 2022). In these validation studies in Asians, the area under the receiver operator characteristic curve was greater than 0.8. According to a validation study in a Korean population (Lee et al., 2010), fatty liver was defined as an HSI > 36 for the present study.

Liver fibrosis assessment

Liver fibrosis was assessed using the Fibrosis-4 (FIB-4) index in participants with fatty liver. The FIB-4 index was calculated using the following formula: age (years) × AST (IU/L)/[platelet count (10^9/L) × ALT (IU/L)$^{1/2}$]. The FIB-4 index correlates highly with biopsy-proven advanced liver fibrosis (Lee et al., 2021). Furthermore, previous studies have demonstrated that the FIB-4 index was closely associated with liver disease-specific mortality (Unalp-Arida and Ruhl, 2017) and cardiovascular disease (Schonmann et al., 2021). Liver fibrosis was categorized according to the following cut-off values: ≤ 1.3, low fibrosis; > 1.3 and < 2.67, intermediate fibrosis; ≥ 2.67, high fibrosis.

Aβ PET acquisition

All the participants underwent Aβ PET [^{18}F-florbetaben (FBB) and ^{18}F-flutemetamol (FMM)] PET using a Discovery STe PET/CT scanner (GE Medical Systems, Milwaukee, WI, United States). For FBB or FMM PET, a 20-min emission PET scan in dynamic mode (consisting of 4 × 5 min frames) was performed 90 min after the injection of a mean dose of 311.5 MBq FBB or 197.7 MBq FMM, respectively. Three-dimensional PET images were reconstructed in a 128 × 128 × 48 matrix with 2 mm × 2 mm × 3.27 mm voxel size using the ordered-subsets expectation maximization algorithm (FBB, iteration = 4 and subset = 20; FMM, iteration = 4 and subset = 20).

Aβ PET quantification using Centiloid values

Amyloid-beta uptake was defined according to Aβ PET quantification using Centiloid values. We performed a direct comparison of FBB-FMM Centiloid (dcCL) method previously developed by our group (Cho et al., 2020) to standardize the quantification of Aβ PET images obtained using different ligands. The dcCL method for FBB and FMM PET enables the transformation of the standardized uptake value ratio (SUVR) of FBB and FMM PETs to dcCL values directly without conversion to the ^{11}C-labeled Pittsburgh compound SUVR.

There are three steps for obtaining dcCL values (Cho et al., 2020): (1) preprocessing of PET images, (2) determination of the global cortical target volume of interest (CTX VOI), and (3) conversion of dcSUVR to dcCL values. First, to preprocess the Aβ PET images, PET images were co-registered to each participant's MR image and then normalized to a T1-weighted MNI-152 template using the SPM8 unified segmentation method. We used T1-weighted MR image correction with the N3 algorithm only for intensity non-uniformities, without applying corrections to the PET images for brain atrophy or partial volume effects. Second, we used the FBB-FMM CTX VOI, defined as the area of AD-specific brain Aβ deposition in our previous study (Cho et al., 2020). Briefly, to exclude areas of aging-related brain Aβ deposition, the FBB-FMM CTX VOI was generated by comparing SUVR parametric images (with the whole cerebellum as a reference area) between 20 typical patients with Alzheimer's disease-related cognitive impairment (AD-CTX) and 16 healthy elderly participants (EH-CTX) who underwent both FBB and FMM PET scans. To generate the FBB-FMM CTX VOI, the average EH-CTX image was subtracted from the average AD-CTX image. We then defined the FBB-FMM CTX VOI as the area of AD-related brain Aβ accumulation common to both FBB and FMM PET. Finally, the dcSUVR values of the FBB-FMM CTX VOI were converted into dcCL units using the dcCL conversion equation. The dcCL equation was derived from the FBB-FMM CTX VOI separately for FBB and FMM PET and applied to FBB and FMM dcSUVR.

To determine the participants' dcCL cut-off-based Aβ positivity, we applied the optimal cut-off value derived using *k*-means cluster analysis in 527 independent samples of participants with normal cognition. The cut-off value was set at 27.08, representing the 95th percentile of the lower cluster, and the whole cerebellum was used as a reference region.

Figure 1 presents two representative cases with respect to Aβ uptake and Aβ positivity on PET scan.

Standardized neuropsychological test battery

All participants underwent the SNSB-II (Kang et al., 2021), which includes standardized and validated tests of various cognitive functions. The SNSB-II evaluates many cognitive factors, including verbal and visual memory, visuo-constructive function, language, praxis, components of Gerstmann syndrome (acalculia, agraphia, right/left disorientation, and finger agnosia), and frontal/executive functions. We chose to use six cognitive measures, which are representative and important neuropsychological tests, to evaluate cognitive function in five cognitive domains as follows: (1) Memory: Seoul Verbal Learning Test and Rey–Osterrieth Complex Figure Test (RCFT) delayed recall; (2) Language: Korean version of the Boston Naming Test; (3) Visuospatial function: RCFT copy; (4) Frontal executive function: Stroop Test color reading; and (5) Attention: Digit Span Test backward.

Statistical analyses

All statistical analyses were performed separately in males and females. Independent *t*-tests and chi-square tests were used to

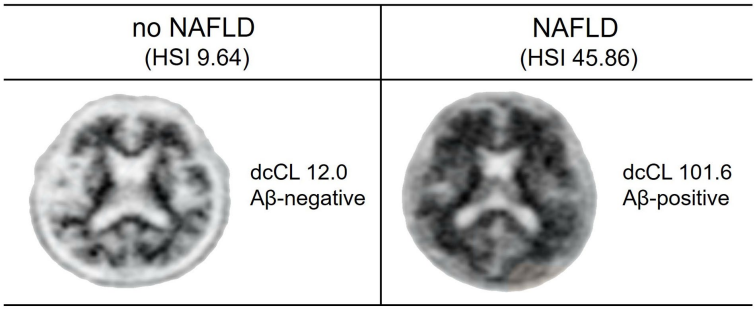

FIGURE 1
Aβ uptake and positivity in study participants. Two representative cases of Aβ PET are shown. One participant without NAFLD has low Aβ uptake (dcCL, 12.0) and Aβ-negative on PET scan. Another participant with NAFLD has high Aβ uptake (dcCL, 101.6) and Aβ-positive on PET scan.

compare the demographic and clinical characteristics of the participants.

To investigate the association between the presence of NAFLD and Aβ deposition, we performed linear regression analyses in males and females, with the presence of NAFLD as a predictor, and quantified dcCL values as an outcome after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and Apolipoprotein E4 (*APOE4*) genotype. To investigate the association between the severity of NAFLD and Aβ deposition, we performed linear regression analyses in males and females, with the severity of NAFLD (no NAFLD, NAFLD with low fibrosis, NAFLD with intermediate fibrosis, and NAFLD with high fibrosis) as a predictor and quantified dcCL values as an outcome, after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. We also performed a linear trend test using linear regression analysis in males and females, with the severity of NAFLD as a continuous variable, after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. To evaluate the interaction of sex and NAFLD on Aβ deposition, we performed a linear regression analysis, with NAFLD and sex together as the main effect and NAFLD × sex as an interaction effect, after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype in all participants.

A sensitivity analysis using cut-off-based categorization rather than quantified dcCL values was performed to further validate the relationship between NAFLD and Aβ deposition. We used logistic regression analysis with NAFLD as a predictor and Aβ positivity as an outcome after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype.

All reported *p* values were two-sided, and the significance level was set at 0.05. All the analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States).

TABLE 1 Demographics and clinical information of participants.

	Females (n = 410)	Males (n = 263)	p value
Demographics			
Age, years	70.7 ± 7.7	71.8 ± 7.2	0.049
Education, years	10.9 ± 4.7	13.8 ± 3.9	< 0.001
APOE4 genotype	103 (25.1%)	70 (26.6%)	0.732
Hypertension	178 (43.4%)	138 (52.5%)	0.027
Diabetes	63 (15.4%)	66 (25.1%)	0.002
Hyperlipidemia	202 (49.3%)	121 (46.0%)	0.455
BMI	23.8 ± 3.1	24.7 ± 2.6	< 0.001
Liver profiles			
Presence of NAFLD	197 (48.0%)	61 (23.2%)	< 0.001
NAFLD severity			< 0.001
Low fibrosis	27 (6.6%)	2 (0.8%)	
Intermediate fibrosis	140 (34.1%)	44 (16.7%)	
High fibrosis	30 (7.3%)	15 (5.7%)	
Aβ deposition			
dcCL values	19.3 ± 30.7	23.7 ± 38.7	0.117
*Aβ positivity	102 (24.9%)	70 (26.6%)	0.679

Values are presented as mean ± standard deviation. *Aβ positivity was determined using optimal cut-off values of dcCL. Aβ, Amyloid-beta; *n*, Number of patients whose data were available for analysis; APOE4, Apolipoprotein E4; BMI, Body mass index; dcCL, Direct comparison of FBB-FMM Centiloid; and NAFLD, non-alcoholic fatty liver disease.

Results

Clinical characteristics of participants

Among the 673 participants, 410 were females and 263 were males (Table 1). Females had a lower mean age (70.7 ± 7.7 and 71.8 ± 7.2, $p = 0.049$), education years (10.9 ± 4.7 and 13.8 ± 3.9, $p < 0.001$), and BMI (23.8 ± 3.1 and 24.7 ± 2.6, $p < 0.001$) than males. There were no differences between males and females in the mean dcCL values (19.29 ± 30.68 and 23.73 ± 38.74, $p = 0.117$), the frequency of Aβ + (24.9 and 26.6%, $p = 0.679$), and the frequency of *APOE4* genotype (25.1 and 26.6%, $p = 0.732$). Females had a lower frequency of hypertension (43.4 and 52.5%, $p = 0.027$) and diabetes (15.4 and 25.1%, $p = 0.002$) than males. However, the frequency of NAFLD was higher in females than in males (48 vs. 23.2%, $p < 0.001$).

Effects of NAFLD on Aβ deposition

As illustrated in Figure 2A, among females, the presence of NAFLD (β = 0.216, $p < 0.001$) was predictive of increased dcCL (Table 2). However, among males, the presence of NAFLD (β = 0.191, $p = 0.064$) was not associated with dcCL values (Table 2). There was no interaction effect between sex and the presence of NAFLD (sex × NAFLD, $p = 0.109$) on dcCL values.

Regarding the severity of NAFLD, among females, the presence of NAFLD with low (β = 0.254, $p = 0.039$), intermediate (β = 0.201, $p = 0.006$), and high fibrosis (β = 0.257, $p = 0.027$) was predictive of increased dcCL values (Table 2; Figure 3A). However, among males, there were no differences in the dcCL values between those without and with NAFLD with low (β = 0.139, $p = 0.917$), intermediate (β = 0.262, $p = 0.077$), or high fibrosis (β = 0.018, $p = 0.917$; Table 2).

In the linear trend test, as illustrated in Figure 3A, the dcCL values increased as the severity of NAFLD (low, intermediate, and high fibrosis) increased in females (p for trend = 0.001), which was not the case with the males (p for trend = 0.136). There was no interaction effect between sex and NAFLD severity (sex × severity of NAFLD, $p = 0.073$) on dcCL values.

Sensitivity analysis

Regarding the categorical values of Aβ, among females, presence of NAFLD [odds ratio (OR) = 2.32, $p = 0.005$] was independently associated with higher Aβ positivity (Figure 2B), whereas, among males, the presence of NAFLD (OR = 1.83, $p = 0.165$) was not associated with Aβ positivity (Figure 2B). There was no interaction effect between sex and the presence of NAFLD (sex × NAFLD, $p = 0.143$) on Aβ positivity. The relationship between the severity of NAFLD and Aβ positivity showed a similar trend. Aβ positivity became higher as the severity of NAFLD (low, intermediate, and high fibrosis) increased in females (p for trend = 0.014), whereas Aβ

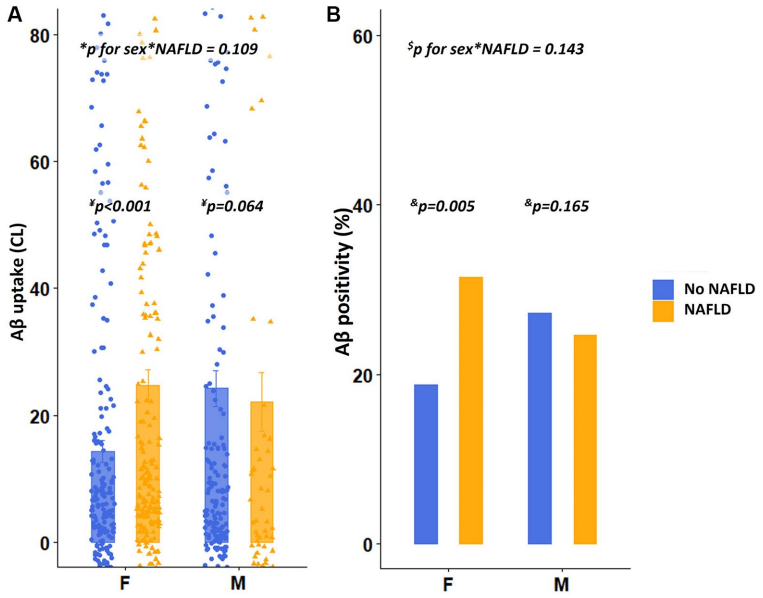

FIGURE 2
Differences in Aβ uptake and positivity according to the presence of NAFLD. **(A)** Values depicted in the bar plot represent the presence of NAFLD on the X-axis and Aβ uptake (CL) on the Y-axis. **(B)** Values depicted in the bar plot represent the presence of NAFLD on the X-axis and Aβ positivity (%) on the Y-axis. *p for interaction was estimated using the linear regression analyses, including the presence of NAFLD as the main effect and sex × presence of NAFLD as an interaction effect after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. ¥p value was estimated using linear regression analyses, with the presence of NAFLD as a predictor, after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. $$p$ for interaction was estimated using logistic regression analyses, including the presence of NAFLD as a main effect and sex × severity of NAFLD as an interaction effect after controlling for age, education years, and *APOE4* genotype. &p value was estimated using logistic regression analyses, with the severity of NAFLD as a predictor after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. Aβ, Amyloid; *APOE4*, Apolipoprotein E4; BMI, Body mass index; dcCL, Direct comparison of FBB-FMM Centiloid; and NAFLD, Non-alcoholic fatty liver disease.

TABLE 2 **Relationship between NAFLD and Aβ deposition.**

		Females		Males	
		*Beta (SE)	p	*Beta (SE)	p
Presence	NAFLD	0.216 (0.060)	< 0.001	0.191 (0.103)	0.064
¥Severity	Low fibrosis	0.254 (0.123)	0.039	0.139 (0.440)	0.917
	Intermediate fibrosis	0.201 (0.064)	0.006	0.262 (0.117)	0.077
	High fibrosis	0.257 (0.108)	0.027	0.018 (0.170)	0.917

*Beta was obtained using linear regression analyses, with the presence or severity of NAFLD separately as a predictor, after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and APOE4 genotype. ¥p values were obtained after FDR correction. Aβ, amyloid; APOE4, Apolipoprotein E4; BMI, body mass index; FDR, false discovery rate; NAFLD, Non-alcoholic fatty liver disease; and SE, standard error.

positivity was not associated with the severity of NAFLD in males (p for trend = 0.365, Figure 3B). There was no interaction effect between sex and NAFLD severity (sex × NAFLD severity, $p = 0.090$) on Aβ positivity.

Discussion

In the present study, we systematically investigated the sex-specific relationships between NAFLD and Aβ deposition in a large number of CU individuals. We found that the presence of NAFLD, as defined using HSI, was associated with higher Aβ deposition in females but not in males. The severity of NAFLD, as defined using FIB-4, was also associated with higher Aβ deposition in females but not in males. Thus, our findings suggest that there may be a sex-specific relationship between NAFLD and Aβ deposition. Therefore, our results may contribute to the design of sex-specific strategies for NAFLD management to prevent Aβ deposition in CU individuals.

In the present study, females had a higher incidence of NAFLD than males. Considering our female participants to be in postmenopausal status, our findings are consistent with previous studies showing that the frequency of NAFLD is higher in postmenopausal females than in males (Lonardo et al., 2019; Tobari and Hashimoto, 2020). However, the frequency of NAFLD is higher in males, when compared to females of pre-menopausal age group (Lonardo et al., 2019; Tobari and Hashimoto, 2020). This difference in the results before and after menopause could be explained by the influence of estrogen. Estrogen promotes well-metabolic conditions by regulating energy homeostasis, enhancing insulin release, modulating the role of growth hormones, and preventing inflammation (Rettberg et al., 2014). Hormone therapy has been reported to have a protective effect against NAFLD in postmenopausal females (McKenzie et al., 2006).

Our first major finding was that NAFLD was associated with higher Aβ deposition in females. Previous studies have demonstrated that NAFLD is closely related to a greater risk of cognitive impairment and the clinical diagnosis of AD in the elderly population (Seo et al., 2016; Jeong et al., 2022). However, to the best

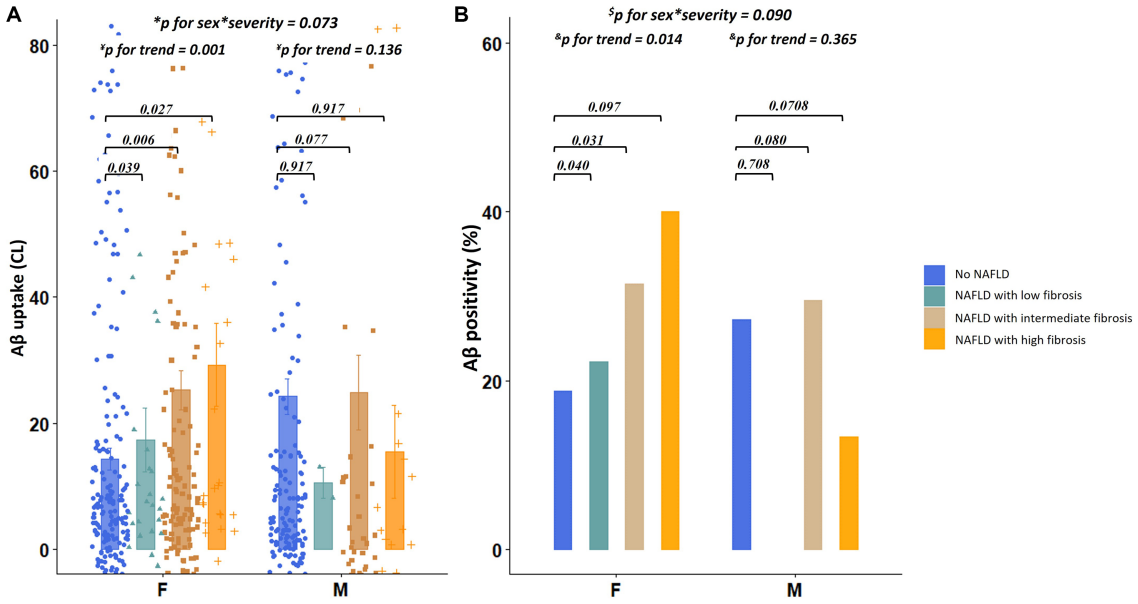

FIGURE 3
Differences in Aβ uptake and positivity according to the severity of NAFLD. **(A)** Values depicted in the bar plot represent the severity of NAFLD (no NAFDL, NAFLD with low fibrosis, NAFLD with intermediate fibrosis, and NAFLD with high fibrosis) on the X-axis and Aβ uptake (CL) on the Y-axis. **(B)** Values depicted in the bar plot represent the severity of NAFLD (no NAFDL, NAFLD with low fibrosis, NAFLD with intermediate fibrosis, and NAFLD with high fibrosis) on the X-axis and Aβ positivity (%) on the Y-axis. *p for interaction was estimated using linear regression analyses, including the severity of NAFLD as the main effect and sex × severity of NAFLD as an interaction effect after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. ¥p for trend was estimated using linear regression analyses, with the severity of NAFLD as a continuous variable after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. $p for interaction was estimated using logistic regression analyses, including the presence of NAFLD as the main effect and sex × severity of NAFLD as an interaction effect after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. &p for trend was estimated using logistic regression analyses, with the severity of NAFLD as a continuous variable after controlling for age, education years, hypertension, diabetes, hyperlipidemia, BMI, and *APOE4* genotype. Aβ, Amyloid; *APOE4*, Apolipoprotein E4; BMI, Body mass index; dcCL, Direct comparison of ^{18}F-florbetaben-^{18}F-flutemetamol Centiloid; and NAFLD, Non-alcoholic fatty liver disease.

of our knowledge, the association between NAFLD and Aβ deposition on PET has not been found in CU individuals. Several mechanisms may explain the association between NAFLD and Aβ deposition. First, NAFLD-related chronic inflammation may contribute to the activation of microglial cells in the brain, leading to increased levels of inflammatory cytokines, eventually resulting in increased Aβ deposition. An animal study found that NAFLD-induced high-fat diet aggravates neuroinflammation, accompanied by increased Aβ deposition (Kim et al., 2016). Additionally, another study suggested that increased lipocalin-2 levels related to NAFLD may induce the breakdown of the blood–brain barrier and increase the levels of inflammatory cytokines in the brain (Mondal et al., 2020). Alternatively, the low expression of low-density lipoprotein receptor-related protein (LRP-1) observed in patients with NAFLD may lead to decreased Aβ clearance (Tamaki et al., 2007). The stimulation of LRP-1 expression in the hepatocyte decreases Aβ deposition in the brain of AD mice model, which improves cognitive function (Sehgal et al., 2012).

An intriguing finding of the present study is that the relationship between NAFLD and Aβ deposition is valid in females but not in males. Although the exact mechanism remains uncertain, multifactorial factors, including sex hormones and socio-behaviors, may underlie the sex-specific relationship between NAFLD and Aβ deposition. Estrogen deficiency in postmenopausal females with NAFLD may be a risk factor for Aβ deposition. Estrogen may also protect from Aβ deposition through anti-inflammatory properties and neurotrophic effects (Morinaga et al., 2007; Anderson et al., 2017). Furthermore, estrogen deficiencies may be attributed to blood–brain barrier breakdown, suggesting that postmenopausal females are more vulnerable to a highly systemic-inflammatory state (Maggioli et al., 2016). However, further comprehensive studies are necessary to identify the exact mechanism underlying the interactions between sex, NAFLD, Aβ deposition, and inflammation.

Our second major finding was that the severity of NAFLD might be associated with Aβ deposition. In line with our findings, recent studies have shown that advanced liver fibrosis, which determines the severity of NAFLD, is predictive of cognitive decline and development (Weinstein et al., 2019; Solfrizzi et al., 2020). A link between NAFLD severity and Aβ deposition may occur through systemic inflammation. The interleukin signaling pathway is an important shared pathomechanism between NAFLD and AD (Karbalaei et al., 2018). Furthermore, animal studies have reported that systemic inflammation precipitates Aβ deposition, which eventually causes AD (Krstic et al., 2012; Carret-Rebillat et al., 2015).

The strength of the present study is that we systematically investigated the effects of NAFLD on Aβ deposition in a large-sized cohort of CU individuals. Especially, enrollment of CU individuals

ameliorated the potential reverse effects of dementia on NAFLD. However, this study has several limitations that should be addressed. First, we could not assess NAFLD using imaging or histological confirmation. However, this argument is mitigated to some degree, considering that HSI is a widely used surrogate marker of NAFLD and is well-validated (Lee et al., 2010; Meffert et al., 2014; Murayama et al., 2021; Khani et al., 2022). Second, since this was a cross-sectional study, it was difficult to guarantee the causality of Aβ deposition due to NAFLD. Third, although Aβ deposition is a necessary but not sufficient condition for AD, we did not assess the other AD biomarkers including phosphorylated tau. Finally, our participants were mainly recruited from individuals who underwent comprehensive dementia evaluation in the memory clinic. This study may have resulted in the enrollment of healthier or more "health-seeking" individuals, which may also limit the generalizability of this study to other community-based individuals. Nevertheless, our study is noteworthy, suggesting that the prevention and management of NAFLD might be important for the primary prevention of Aβ deposition in CU individuals. Furthermore, CU females were more vulnerable to developing NAFLD and had more prominent effects of NAFLD on Aβ deposition than males. Thus, screening for early detection of NAFLD is necessary to identify individuals at high risk of Aβ deposition, especially females.

In conclusion, we highlighted that the presence of NAFLD was predictive of a higher Aβ burden in females but not in males. The severity of NAFLD is also associated with a higher Aβ burden in females. Therefore, our results suggest that sex-specific strategies are required for NAFLD management to prevent Aβ deposition in CU individuals.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Institutional Review Board of Samsung Medical Center. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. HY: Formal analysis, Writing – review & editing. BC: Data curation, Writing – review & editing. JK: Data curation, Writing – review & editing. HJ: Data curation, Writing – review & editing. HK: Data curation, Writing – review & editing. MK: Data curation, Writing – review & editing. KO: Data curation, Writing – review & editing. S-BK: Data curation, Writing – review & editing. DN: Data curation, Writing – review & editing. YC: Data curation, Writing – review & editing. SS: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alzheimer's Association (2016). 2016 Alzheimer's disease facts and figures. *Alzheimers Dement.* 12, 459–509. doi: 10.1016/j.jalz.2016.03.001

Anderson, L. J., Liu, H., and Garcia, J. M. (2017). Sex differences in muscle wasting. *Adv. Exp. Med. Biol.* 1043, 153–197. doi: 10.1007/978-3-319-70178-3_9

Carret-Rebillat, A. S., Pace, C., Gourmaud, S., Ravasi, L., Montagne-Stora, S., Longueville, S., et al. (2015). Neuroinflammation and Aβ accumulation linked to systemic inflammation are decreased by genetic PKR down-regulation. *Sci. Rep.* 5:8489. doi: 10.1038/srep08489

Cho, S. H., Choe, Y. S., Kim, H. J., Jang, H., Kim, Y., Kim, S. E., et al. (2020). A new Centiloid method for (18)F-florbetaben and (18)F-flutemetamol PET without conversion to PiB. *Eur. J. Nucl. Med. Mol. Imaging* 47, 1938–1948. doi: 10.1007/s00259-019-04596-x

Christensen, K. J., Multhaup, K. S., Nordstrom, S., and Voss, K. (1991). A cognitive battery for dementia: development and measurement characteristics. *Psychol. Assess. J. Consult. Clin. Psychol.* 3, 168–174. doi: 10.1037/1040-3590.3.2.168

Gaggini, M., Morelli, M., Buzzigoli, E., DeFronzo, R. A., Bugianesi, E., and Gastaldelli, A. (2013). Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients* 5, 1544–1560. doi: 10.3390/nu5051544

Gottesman, R. F., Schneider, A. L., Zhou, Y., Coresh, J., Green, E., Gupta, N., et al. (2017). Association between midlife vascular risk factors and estimated brain amyloid deposition. *JAMA* 317, 1443–1450. doi: 10.1001/jama.2017.3090

Jeong, S., Oh, Y. H., Choi, S., Chang, J., Kim, S. M., Son, J. S., et al. (2022). Association of non-alcoholic fatty liver disease with incident dementia later in life among elder adults. *Clin. Mol. Hepatol.* 28, 510–521. doi: 10.3350/cmh.2021.0332

Kang, S. H., Kim, J. H., Chang, Y., Cheon, B. K., Choe, Y. S., Jang, H., et al. (2022). Independent effect of body mass index variation on amyloid-β positivity. *Front. Aging Neurosci.* 14:924550. doi: 10.3389/fnagi.2022.924550

Kang, S. H., Park, Y. H., Kim, J. P., Kim, J. S., Kim, C. H., Jang, H., et al. (2021). Cortical neuroanatomical changes related to specific neuropsychological deficits in subcortical vascular cognitive impairment. *NeuroImage Clin.* 30:102685. doi: 10.1016/j.nicl.2021.102685

Karbalaei, R., Allahyari, M., Rezaei-Tavirani, M., Asadzadeh-Aghdaei, H., and Zali, M. R. (2018). Protein-protein interaction analysis of Alzheimer's disease and NAFLD based on systems biology methods unhide common ancestor pathways. *Gastroenterol. Hepatol, Bed Bench* 11, 27–33.

Khani, V., Momeni Moghaddam, A., and Hatami, B. (2022). Comparison of hepatic steatosis index as noninvasive diagnostic tool and liver ultrasound for non-alcoholic steatosis in the adult population. *Gastroenterol. Hepatol, Bed Bench* 15, 360–365. doi: 10.22037/ghfbb.v15i4.2480

Kim, D. G., Krenz, A., Toussaint, L. E., Maurer, K. J., Robinson, S. A., Yan, A., et al. (2016). Non-alcoholic fatty liver disease induces signs of Alzheimer's disease (AD) in wild-type mice and accelerates pathological signs of AD in an AD model. *J. Neuroinflammation* 13:1. doi: 10.1186/s12974-015-0467-5

Kim, S. E., Lee, J. S., Woo, S., Kim, S., Kim, H. J., Park, S., et al. (2019). Sex-specific relationship of cardiometabolic syndrome with lower cortical thickness. *Neurology* 93, e1045–e1057. doi: 10.1212/wnl.0000000000008084

Kim, G. A., Oh, C. H., Kim, J. W., Jeong, S. J., Oh, I. H., Lee, J. S., et al. (2022). Association between non-alcoholic fatty liver disease and the risk of dementia: a nationwide cohort study. *Liver Int.* 42, 1027–1036. doi: 10.1111/liv.15244

Krstic, D., Madhusudan, A., Doehner, J., Vogel, P., Notter, T., Imhof, C., et al. (2012). Systemic immune challenges trigger and drive Alzheimer-like neuropathology in mice. *J. Neuroinflammation* 9:151. doi: 10.1186/1742-2094-9-151

Lee, J. H., Kim, D., Kim, H. J., Lee, C. H., Yang, J. I., Kim, W., et al. (2010). Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Digest. Liver Dis.* 42, 503–508. doi: 10.1016/j.dld.2009.08.002

Lee, J. S., Kim, S., Yoo, H., Park, S., Jang, Y. K., Kim, H. J., et al. (2018). Trajectories of physiological brain aging and related factors in people aged from 20 to over-80. *J. Alzheimer's Dis.* 65, 1237–1246. doi: 10.3233/jad-170537

Lee, J., Vali, Y., Boursier, J., Spijker, R., Anstee, Q. M., Bossuyt, P. M., et al. (2021). Prognostic accuracy of FIB-4, NAFLD fibrosis score and APRI for NAFLD-related events: a systematic review. *Liver Int.* 41, 261–270. doi: 10.1111/liv.14669

Lonardo, A., Nascimbeni, F., Ballestri, S., Fairweather, D., Win, S., Than, T. A., et al. (2019). Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology* 70, 1457–1469. doi: 10.1002/hep.30626

Maggioli, E., McArthur, S., Mauro, C., Kieswich, J., Kusters, D. H. M., Reutelingsperger, C. P. M., et al. (2016). Estrogen protects the blood-brain barrier from inflammation-induced disruption and increased lymphocyte trafficking. *Brain Behav. Immun.* 51, 212–222. doi: 10.1016/j.bbi.2015.08.020

McKenzie, J., Fisher, B. M., Jaap, A. J., Stanley, A., Paterson, K., and Sattar, N. (2006). Effects of HRT on liver enzyme levels in women with type 2 diabetes: a randomized placebo-controlled trial. *Clin. Endocrinol.* 65, 40–44. doi: 10.1111/j.1365-2265.2006.02543.x

Meffert, P. J., Baumeister, S. E., Lerch, M. M., Mayerle, J., Kratzer, W., and Völzke, H. (2014). Development, external validation, and comparative assessment of a new diagnostic score for hepatic steatosis. *Am. J. Gastroenterol.* 109, 1404–1414. doi: 10.1038/ajg.2014.155

Mondal, A., Bose, D., Saha, P., Sarkar, S., Seth, R., Kimono, D., et al. (2020). Lipocalin 2 induces neuroinflammation and blood-brain barrier dysfunction through liver-brain axis in murine model of nonalcoholic steatohepatitis. *J. Neuroinflammation* 17:201. doi: 10.1186/s12974-020-01876-4

Morinaga, A., Hirohata, M., Ono, K., and Yamada, M. (2007). Estrogen has anti-amyloidogenic effects on Alzheimer's beta-amyloid fibrils in vitro. *Biochem. Biophys. Res. Commun.* 359, 697–702. doi: 10.1016/j.bbrc.2007.05.158

Murayama, K., Okada, M., Tanaka, K., Inadomi, C., Yoshioka, W., Kubotsu, Y., et al. (2021). Prediction of nonalcoholic fatty liver disease using noninvasive and non-imaging procedures in Japanese health checkup examinees. *Diagnostics* 11:132. doi: 10.3390/diagnostics11010132

Reed, B., Villeneuve, S., Mack, W., DeCarli, C., Chui, H. C., and Jagust, W. (2014). Associations between serum cholesterol levels and cerebral amyloidosis. *JAMA Neurol.* 71, 195–200. doi: 10.1001/jamaneurol.2013.5390

Rettberg, J. R., Yao, J., and Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Front. Neuroendocrinol.* 35, 8–30. doi: 10.1016/j.yfrne.2013.08.001

Schonmann, Y., Yeshua, H., Bentov, I., and Zelber-Sagi, S. (2021). Liver fibrosis marker is an independent predictor of cardiovascular morbidity and mortality in the general population. *Digest. Liver Dis.* 53, 79–85. doi: 10.1016/j.dld.2020.10.014

Sehgal, N., Gupta, A., Valli, R. K., Joshi, S. D., Mills, J. T., Hamel, E., et al. (2012). *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *Proc. Natl. Acad. Sci. U. S. A.* 109, 3510–3515. doi: 10.1073/pnas.1112209109

Seo, S. W., Gottesman, R. F., Clark, J. M., Hernaez, R., Chang, Y., Kim, C., et al. (2016). Nonalcoholic fatty liver disease is associated with cognitive function in adults. *Neurology* 86, 1136–1142. doi: 10.1212/wnl.0000000000002498

Shang, Y., Nasr, P., Ekstedt, M., Widman, L., Stål, P., Hultcrantz, R., et al. (2021). Non-alcoholic fatty liver disease does not increase dementia risk although histology data might improve risk prediction. *JHEP Rep.* 3:100218. doi: 10.1016/j.jhepr.2020.100218

Shim, Y. S., and Morris, J. C. (2011). Biomarkers predicting Alzheimer's disease in cognitively Normal aging. *J. Clin. Neurol.* 7, 60–68. doi: 10.3988/jcn.2011.7.2.60

Solfrizzi, V., Scafato, E., Custodero, C., Loparco, F., Ciavarella, A., Panza, F., et al. (2020). Liver fibrosis score, physical frailty, and the risk of dementia in older adults: the Italian longitudinal study on aging. *Alzheimers Dement.* 6:e12065. doi: 10.1002/trc2.12065

Sperling, R. A., Donohue, M. C., Raman, R., Sun, C. K., Yaari, R., Holdridge, K., et al. (2020). Association of Factors with Elevated Amyloid Burden in clinically Normal older individuals. *JAMA Neurol.* 77, 735–745. doi: 10.1001/jamaneurol.2020.0387

Tamaki, C., Ohtsuki, S., and Terasaki, T. (2007). Insulin facilitates the hepatic clearance of plasma amyloid beta-peptide (1 40) by intracellular translocation of low-density lipoprotein receptor-related protein 1 (LRP-1) to the plasma membrane in hepatocytes. *Mol. Pharmacol.* 72, 850–855. doi: 10.1124/mol.107.036913

Tobari, M., and Hashimoto, E. (2020). Characteristic features of nonalcoholic fatty liver disease in Japan with a focus on the roles of age, sex and body mass index. *Gut Liver* 14, 537–545. doi: 10.5009/gnl19236

Unalp-Arida, A., and Ruhl, C. E. (2017). Liver fibrosis scores predict liver disease mortality in the United States population. *Hepatology* 66, 84–95. doi: 10.1002/hep.29113

Weinstein, G., Davis-Plourde, K., Himali, J. J., Zelber-Sagi, S., Beiser, A. S., and Seshadri, S. (2019). Non-alcoholic fatty liver disease, liver fibrosis score and cognitive function in middle-aged adults: the Framingham study. *Liver Int.* 39, 1713–1721. doi: 10.1111/liv.14161

Willette, A. A., Johnson, S. C., Birdsill, A. C., Sager, M. A., Christian, B., Baker, L. D., et al. (2015). Insulin resistance predicts brain amyloid deposition in late middle-aged adults. *Alzheimers Dement.* 11, 504–510.e501. doi: 10.1016/j.jalz.2014.03.011

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., and Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64, 73–84. doi: 10.1002/hep.28431

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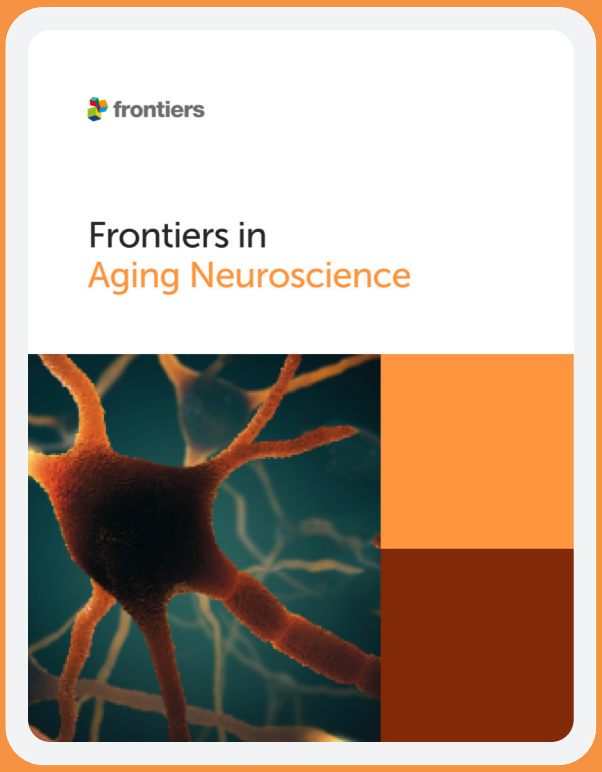

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