RISK FACTORS FOR ALZHEIMER'S DISEASE

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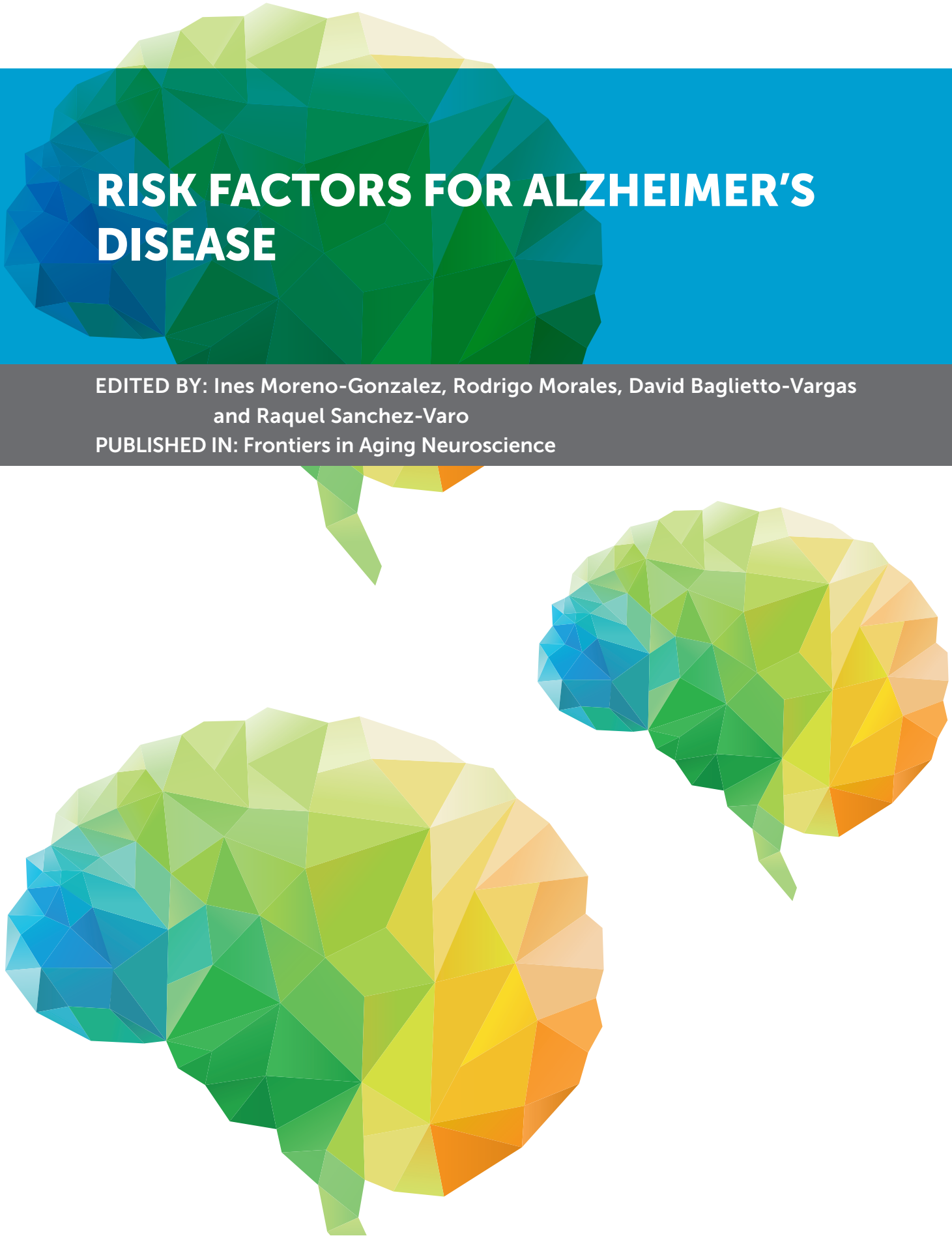

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RISK FACTORS FOR ALZHEIMER'S DISEASE

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EDITORIAL
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Editorial: Risk Factors for Alzheimer's Disease

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Keywords: Alzheimer's disease, risk factors, modifiable, genetic variance, peripheral

Editorial on the Research Topic

Risk Factors for Alzheimer's Disease

Late-onset sporadic Alzheimer's disease (AD) is a multifactorial disease in which several risk factors contribute to the onset and disease progression. Risk factors for AD include aging, sex, lifestyle, comorbidities, and genetic factors. Considering the progressive aging of the population, the prevention or delay of cognitive dysfunction by targeting modifiable risk factors is becoming a therapeutic approach of growing interest. In this Research Topic, we have compiled a series of manuscripts that describe emerging risk factors and the use of those for early diagnosis. In this line, Edwards et al. clearly summarize the relationship between treatable comorbidities, lifestyle habits and AD development. In this review, they expose data dealing with the preventive effects of moderate physical activity, a balanced diet and treatment of sleep disturbances. The effect of smoking and alcohol consumption to promote dementia is also debated as well as comorbid medical conditions linked to this neurodegenerative disease such as cerebrovascular diseases or depression. In this regard, Amtul et al. analyzed the effect of cerebrovasculature alterations, such as ischemia, and its causative link with the development of AD. It is remarkable the epidemiological data on how stress, as a common cause of depression, has an important role on inducing cognitive impairment, positing stress in the eye of the storm. In the same line, Ouanes and Popp remark the relationship between high cortisol levels and increased risk of cognitive decline and dementia. In fact, elevated cortisol not only affects AD pathology but can also worsen sleep and cardiovascular diseases. Modulation of glucocorticoids and hypothalamic-pituitary-adrenal (HPA) axis functioning may be considered as a pharmacological target. Conversely, there is an increasing number of reports indicating that long-term administration of benzodiazepines and related hypnotic drugs to treat depression or insomnia may induce cognitive dysfunction as a side effect, especially in elderly population. Ettcheto et al. debate on the relevance of sleep disorders in cognition and compile epidemiological clinical data, proposing that benzodiazepines may act as contributing factors to prompt cognitive decline in aged AD patients rather than patients that may be at higher risk are usually recommended to take them.

Although genetic variants are considered the primary cause for familial forms of AD, recent evidences indicate that genetic variants are also a prominent risk factor for sporadic AD cases, in addition to the apolipoprotein E (ApoE ε4 allele). Fernandez et al., Pietzuch et al., and Zhang et al. highlight several causative mechanisms by which APOE4 mediates both amyloid beta (Aβ) and tau pathology, altering brain connectivity and modulating the inflammatory process and glucose. Furthermore, these toxic effects triggered by ApoE are not only related to an isoform-specific manner (mainly the ε4 allele), but new evidences also suggest that multiple ApoE-related functions are specific to a particular cell type in the brain. In this regard, lipid dysregulation, deficient glucose metabolism and pro-inflammatory response in glia cells mediated

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by APOE are new exploratory means by which APOE may contribute to the development of AD and more deep understanding of these pathological processes could turn into new promising therapeutic targets for AD. Zhang et al. also emphasize other associated genes, such as the bridging integrator 1(BIN1) and the sortilin-related receptor 1 (SORL1), which modulate the amyloid and tau aggregates via alteration endosomal trafficking.

An interesting emerging model explaining the early events triggering AD involves microbial infection (Moir et al., 2018). Here, AD-associated Aβ would emerge after pathogen neuroinvasion. These misfolded protein aggregates known to have anti-microbial activity would act as a primary barrier of defense, helping to clear pathogens from the brain (Soscia et al., 2010). Complementary to this hypothesis, bacterial infection also leads to peripheral inflammation and blood-brain barrier (BBB) dysfunction. Interestingly, both events are known AD risk factors and could act in conjunction or separately to initiate the deleterious molecular pathways leading to AD. These facts are nicely discussed by Giridharan et al.. Moreover, the authors postulate that some outcomes derived from infection (e.g., sepsis) may posit risks for dementia. Several lines of investigation supporting this hypothesis are critically discussed in this article. Following the role of peripheral tissue alterations and AD, research on liver dysfunction as a potential risk factor for dementia has been importantly neglected. Most of the Aβ generated in the body is cleared by the liver (Hone et al., 2003) and eliminated by the urine (Ghiso et al., 1997). In that sense, it is surprising that little research has been done in this area. Estrada et al. summarize direct and indirect evidence supporting the role of liver damage specifically focused in non-alcoholic fatty liver disease and related BBB dysfunction in the progression of AD. Linked to the review by Giridharan et al. the authors also describe the role of certain pathogens in liver health and their possible involvement in AD by altering peripheral Aβ clearance.

At present, no pre-symptomatic diagnostic tests are available for AD. In that sense, several non-invasive approaches linking peripheral changes and dementia have been explored. Positive associations have the potential benefit to help in clinical trials enrolment and implementation of preventive treatments in the future. Nishizawa et al. describe that direct measurement of abdominal visceral fat is inversely related to cognitive decline. This result supports previous epidemiological data and has the advantage of being directly performed by weighting the actual adipose tissue at short post-mortem time windows. Similarly, Liu et al. communicate that grip strength is linked to cognitive function in a large Chinese cohort. Although attractive, it is clear that confounding factors other than motor tests or physical functions may be responsible for the observed outcomes.

In addition to the different risk factors that have been identified and that provide prospects to determine the potential to develop AD, novel clinical approaches and biomarkers are emerging in an effort to monitor the onset and progression of AD pathology and therefore, be used as early diagnostic assessments. In this line, Qi et al. propose the evaluation of the functional connectivity in the medial temporal lobe as a measurement of initial memory impairment related with AD, providing a novel imaging biomarker to predict and diagnose AD. On a similar line, Amtul et al. provide with new evidences about the usefulness of imaging microvascular alterations to determine the severity of AD pathology. In a very compiling study, Sapkota et al. present a variety of biomarkers for AD, including salivary biomarkers in conjunction with other risk markers, and combine them in a multi-modal array analysis to determine their collective ability to discern and even predict the clinical status of AD patients and mild cognitive impairment from cognitively normal individuals.

Overall, all these studies point out the necessity to identify potential risk factors of AD, emphasizing the interrelation of multiple of these factors and the utility of combining a variety of markers to better predict and early diagnose AD. Although modifiable risk may be somehow prevented or controlled, genetic variations combined with other potential factors may hinder AD prevention. Further understanding on the interrelationship of these biomarkers will facilitate personalized therapeutic interventions in individuals at risk.

AUTHOR CONTRIBUTIONS

IM-G, RM, DB-V and RS-V have contributed to manuscript writing and editing. IM-G coordinated, reviewed, and approved the final version. All authors have made a substantial intellectual contribution to this manuscript and approved it for publication.

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Alzheimer's Biomarkers From Multiple Modalities Selectively Discriminate Clinical Status: Relative Importance of Salivary Metabolomics Panels, Genetic, Lifestyle, Cognitive, Functional Health and Demographic Risk Markers

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Background: Among the neurodegenerative diseases of aging, sporadic Alzheimer's disease (AD) is the most prevalent and perhaps the most feared. With virtually no success at finding pharmaceutical therapeutics for altering progressive AD after diagnosis, research attention is increasingly directed at discovering biological and other markers that detect AD risk in the long asymptomatic phase. Both early detection and precision preclinical intervention require systematic investigation of multiple modalities and combinations of AD-related biomarkers and risk factors. We extend recent unbiased metabolomics research that produced a set of metabolite biomarker panels tailored to the discrimination of cognitively normal (CN), cognitively impaired and AD patients. Specifically, we compare the prediction importance of these panels with five other sets of modifiable and non-modifiable AD risk factors (genetic, lifestyle, cognitive, functional health and bio-demographic) in three clinical groups.

Method: The three groups were: CN (n = 35), mild cognitive impairment (MCI; n = 25), and AD (n = 22). In a series of three pairwise comparisons, we used machine learning technology random forest analysis (RFA) to test relative predictive importance of up to 19 risk biomarkers from the six AD risk domains.

Results: The three RFA multimodal prediction analyses produced significant discriminating risk factors. First, discriminating AD from CN was the AD metabolite panel and two cognitive markers. Second, discriminating AD from MCI was the AD/MCI metabolite panel and two cognitive markers. Third, discriminating MCI from CN was the MCI metabolite panel and seven markers from four other risk modalities: genetic, lifestyle, cognition and functional health.

Conclusions: Salivary metabolomics biomarker panels, supplemented by other risk markers, were robust predictors of: (1) clinical differences in impairment and dementia

and even; (2) subtle differences between CN and MCI. For the latter, the metabolite panel was supplemented by biomarkers that were both modifiable (e.g., functional) and non-modifiable (e.g., genetic). Comparing, integrating and identifying important multi-modal predictors may lead to novel combinations of complex risk profiles potentially indicative of neuropathological changes in asymptomatic or preclinical AD.

Keywords: Alzheimer's disease, mild cognitive impairment, cognitively normal, salivary metabolomics, biomarkers, genetics, cognition, victoria longitudinal study

INTRODUCTION

Epidemiological projections point in the direction of increased worldwide prevalence and growing burden of neurodegenerative disease, especially Alzheimer's disease (AD; Prince et al., 2015; Alzheimer's Association, 2016; Wimo et al., 2017). Given the lack of success in developing therapeutics to reverse the course of neurodegeneration in aging after diagnosis (Cummings et al., 2014), research and clinical attention has shifted to multimodal risk detection in asymptomatic phases (Sperling et al., 2011) so as to promote early risk management or prevention (Anstey et al., 2015). Early detection of sporadic AD may require systematic attention to multiple modalities of biomarkers and risk factors, perhaps beyond (but including) the established neurobiological and clinical hallmarks of the disease (e.g., beta amyloid; Barnes and Yaffe, 2011). Accordingly, recent research has focused on testing panels, dosages, and interactions of multiple biomarkers, examining their synergistic, modifying, or complementary influences on phenotypes, pre-clinical trajectories or clinical status (Edwards et al., 2015; McFall et al., 2015a; Iturria-Medina et al., 2016; Sapkota et al., 2017; Sapkota and Dixon, 2018). Arguably, identifying perturbations in profiles of biomarkers in asymptomatic periods of impairment or AD may provide a promising opportunity for developing precision or programmatic interventions that could delay or prevent clinical diagnosis (Imtiaz et al., 2014; Hampel et al., 2017). However, translational progress may be optimized when one or more of three conditions are available: (1) a roster of established multi-modal modifiable risk biomarkers are included; (2) these risk biomarkers can be estimated with valid but relatively non-invasive technology; and (3) comparative prediction and discrimination data are available (Anstey et al., 2015; Olanrewaju et al., 2015; Casanova et al., 2016).

We adopt a multi-modal comparative approach to determining the relative importance of multiple established risk biomarkers of cognitive impairment and AD. The six AD risk biomarker clusters include: (1) novel metabolomics biomarker panels; (2) selected AD genetic risk polymorphisms (e.g., *Apolipoprotein E* (*APOE*)); (3) functional health (e.g., vascular); (4) lifestyle engagement (e.g., physical activity); (5) cognitive performance (e.g., memory); and (6) bio-demographic factors (e.g., sex). A total of 19 risk biomarkers are available for testing simultaneously in three pairwise competitive analyses conducted with machine learning technology random forest analyses (RFA). This approach identifies the predictors that contribute most significantly to the discrimination of the clinical groups. In the present study, these groups include the benchmark cognitively normal (CN) as well as mild cognitive impairment (MCI) and AD groups. The predictors vary in the extent to which they are likely to be modifiable, an important consideration for potential downstream intervention (Barnes and Yaffe, 2011; Anstey et al., 2013b, 2015; Norton et al., 2014; Livingston et al., 2017). We limited our predictors to those that are likely to require relatively non-invasive assessment techniques. Accordingly, in the present study, both metabolomics and genetic markers were developed from salivary samples. A central aim of this study was to examine the extent to which newly discovered metabolomics biomarker panels would emerge as important predictors of MCI and AD in the competitive context of a broad range of other established and relatively non-invasive AD risk factors.

Two of the present biomarker clusters are derived from salivary samples collected in the context of longitudinal study of aging. Saliva is of interest in research on biomarkers of neurodegenerative diseases and aging for several reasons. It is a premier non-invasive biofluid, easily collected and stored (Wong, 2006) and increasingly acknowledged for its potential as a source fluid for genomic, metabolomics and candidate biomarker studies (Wishart et al., 2013; Liang et al., 2015). Its viability for DNA extraction and genotyping is well established and effectively applied in genetics of aging and dementia (McFall et al., 2016; Sapkota et al., 2017). Recently, metabolomics technology has advanced such that salivary samples have provided source fluids for biomarker discovery in AD (Liang et al., 2015; Figueira et al., 2016). In our previous work, we have used salivary samples for genotyping, biomarker network and interaction analyses (McFall et al., 2016; Sapkota et al., 2017), and metabolomics-based discovery of biomarkers of impairment and AD (Zheng et al., 2012; Huan et al., 2018). Although not yet comprehensively compared across biofluid modalities, salivary biomarkers may enable better accessibility to a wider range of worldwide and diversity samples than as yet available via more traditional biofluids (blood, cerebral spinal fluid; Hu et al., 2010; Thambisetty and Lovestone, 2010; Mousavi et al., 2014; Trushina and Mielke, 2014; Liang et al., 2016; Simpson et al., 2016; Toledo et al., 2017).

The first set of biomarkers was developed in a previous salivary metabolomics analysis of CN, MCI and AD samples. Metabolomics is a global approach to detecting perturbations in metabolic pathways that can reflect early and subtle disease-related changes in the central nervous system (Kaddurah-Daouk and Krishnan, 2009). It evaluates the metabolic state of the organism. The metabolome represents the end

and transitional products of interactions between genes, proteins, and the environment (Xia et al., 2013; Jové et al., 2014; González-Domínguez et al., 2015). The result of a metabolomics analysis is an empirically and quantitatively derived set of metabolites that discriminate between two clinical groups and provide targets for further analyses of mechanisms, associations and clinical applications (Mishur and Rea, 2012; Ibáñez et al., 2013; Enche Ady et al., 2017). In the present study, we assemble a set of discovered and verified discriminant metabolite panels (comprised of more than one biomarker) from a recent salivary AD metabolomics study (Huan et al., 2018). Specifically, we developed putatively identified metabolite panels discriminating CN, MCI and AD groups (Huan et al., 2018). Unique metabolite biomarker panels were developed for each pairwise comparison and all panels displayed very high sensitivity for the comparisons. The AD biomarker panel (discriminating AD from CN) was comprised of three metabolites (Methylguanosine, Histidinyl-Phenylalanine, Choline-cytidine) that were associated with the phenylalanine and histamine biosynthesis pathways (Huan et al., 2018). The AD/MCI panel was comprised of three metabolites (Amino-dihydroxybenzene, Glucosylgalactosyl hydroxylysine $-$ H_2O, Aminobutyric acid + H_2) that were provisionally associated with lipid metabolism pathways[1]. The MCI/CN panel was comprised of two metabolites (Glucosylgalactosyl hydroxylysine $-$ H_2O, Glutamine-carnitine) and provisionally associated with carnitine synthesis, oxidation of branched chain fatty acid, lipid and fatty acid metabolism pathways[2]. Details of the metabolomics procedures used in the earlier study are available elsewhere (Zheng et al., 2012; Huan et al., 2018) and are summarized in the present "Materials and Methods" section.

The genetic biomarker modality was also derived from salivary samples. We selected AD genetic risk markers from an available pool with relatively known properties and application in multi-modal biomarker research (Williams et al., 2010; Karch et al., 2014; Karch and Goate, 2015; Huynh and Mohan, 2017). All four were detected in genome-wide association studies and frequently linked to AD and cognitive decline (Harold et al., 2009; Lambert et al., 2009; Chibnik et al., 2011). The four genetic markers are: *APOE* rs7412, (rs429358; Brainerd et al., 2011; Dixon et al., 2014; Runge et al., 2014; Mahoney-Sanchez et al., 2016), *Complement receptor 1* (*CR1*; rs6656401; Crehan et al., 2012; Fonseca et al., 2016), *Clusterin* (*CLU*; rs11136000; Thambisetty et al., 2013; McFall et al., 2016) and *Phosphatidylinositol-binding clathrin assembly protein* (*PICALM*; rs3851179; Barral et al., 2012; Xiao et al., 2012; Ferencz et al., 2014; Morgen et al., 2014). *APOE* is the most established genetic risk factor for AD and is involved in lipid transport and metabolism (Liu et al., 2013). *CR1* may be involved in rate of $A\beta_{42}$ clearance in AD (Lambert et al., 2009). *CLU* has been associated with regulation of lipid transport, Aβ clearance, and brain atrophy (Karch and Goate, 2015). *PICALM* is involved in Aβ peptide production and connected to Aβ metabolism and plaque formation (Xiao et al., 2012).

[1]http://www.hmdb.ca/metabolites/HMDB0000585
[2]http://www.hmdb.ca/metabolites/HMDB0000062

The remaining sets of AD risk factors have been examined in observational research, reported in reviews, and linked to early AD detection and potential prevention (Livingston et al., 2017). The functional health predictor domain included three dementia-related biomarkers: pulse pressure (PP), body mass index (BMI) and gait timed walk; Qiu et al., 2003; Dahl et al., 2013; Mielke et al., 2013; Emmerzaal et al., 2015; McDade et al., 2016; McFall et al., 2016; MacDonald et al., 2017). PP, a reliable proxy of arterial stiffness has been considered a better predictor of poor vascular health compared to systolic blood pressure alone (Raz et al., 2011; Nation et al., 2013) and linked to (1) AD biomarkers in CN and AD risk (Nation et al., 2013; McFall et al., 2016); (2) MCI (Yaneva-Sirakova et al., 2012); (3) cerebral small vessel disease (Singer et al., 2014); and (4) cognitive decline (McFall et al., 2015b). Lower late-life BMI and higher mid-life BMI has consistently been linked to increased dementia risk (Emmerzaal et al., 2015). Potential mechanisms (Emmerzaal et al., 2015) include: (1) greater inflammation (Yaffe et al., 2004); (2) structural brain changes (Pannacciulli et al., 2006); and (3) higher cholesterol levels in mid-life and lower levels in late-life (Mielke et al., 2005). The lifestyle activity predictor domain included four markers of everyday engagement, with higher levels often associated with AD risk reduction and lower levels with risk elevation. A standard self-report instrument represented levels of everyday integrative cognitive, novel cognitive, physical and social activities (Deary et al., 2006; Bherer et al., 2013; Wang et al., 2013; Vemuri et al., 2014; Thibeau et al., 2017). Cognitively stimulating lifestyle activities (Vemuri et al., 2012) and physical activities (Chen et al., 2016; Falck et al., 2017) have been shown to delay AD onset. Specifically, physical activities may lead to improvements in neurogenesis as a result of increased cerebral blood flow in the dentate gyrus (Chen et al., 2016). The cognitive performance predictor domain included four measures: episodic memory (as early cognitive manifestation associated with hippocampal dysfunction), EF (Stroop, which tests the ability to inhibit cognitive interferences; Scarpina and Tagini, 2017), speed (simple reaction time, the level of which reflects slower or faster processing speed potential indicator of early normal or preclinical cognitive decline (McFall et al., 2015a), and global cognition (assessed with the Mini-Mental State Exam (MMSE)). The bio-demographic domain included age, sex and education (Li and Singh, 2014; Schneeweis et al., 2014; Jack et al., 2015; Cadar et al., 2016; Riedel et al., 2016; Sachdev et al., 2016). Age is the most important non-modifiable risk factor for developing AD with large number of sporadic AD cases occurring after 65 years (Guerreiro and Bras, 2015). Sex differences have been observed in AD with significantly higher prevalence in women than men (Mazure and Swendsen, 2016). Lifestyle experiences and choices (i.e., diet, exercise) vary by sex and may have an indirect influence on the brain over the lifespan (Mazure and Swendsen, 2016). Education has widely been used as a proxy for cognitive reserve (Tucker and Stern, 2011; Stern, 2012, 2017). Adults with higher cognitive reserve (higher education levels) may have greater tolerance to AD pathology than those with lower cognitive reserve (lower education levels; Stern, 2012).

For each of three comparative RFA prediction models we included up to 19 predictors. RFA is a machine-learning-based data exploration technique that combines large numbers of regression tree predictions from a random sample of participants and variables (Strobl et al., 2009; McDermott et al., 2017). It accommodates multiple predictors and smaller sample sizes, producing a solution that features a rank ordering of the top important predictors of the target clinical condition. The general objective was to examine and compare the extent to which new salivary metabolite biomarker panels fared in the competitive context of other AD biomarkers in predicting clinical status in pairwise comparisons across three groups: CN, MCI and AD.

MATERIALS AND METHODS

Participants

Participants were community-dwelling older adult volunteers from the Victoria Longitudinal Study (VLS), an ongoing multi-cohort investigation of biomedical, genetic, metabolic, functional, neurocognitive and other aspects of aging, impairment and dementia. This study was carried out in accordance with the recommendations of the Human Research Ethics Guidelines, University of Alberta with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Human Research Ethics Board. Detailed information on overall VLS recruitment, research design, and participant characteristics are available elsewhere (Dixon and de Frias, 2004; McFall et al., 2015a). For the present study, the CN and MCI participants were drawn from a subset of the main cohorts that participated in the VLS biofluid and genetics initiative (2009–2012). The AD patients were recruited from the Geriatric and Cognitive Clinic at the Glenrose Rehabilitation Hospital (Edmonton). All participants (N = 82) received a small honorarium for their contributions. The present research includes adults classified as CN (n = 35; age 64–75 years; 62.9% female), MCI (n = 25; age 64–75 years; 60% female), and diagnosed AD (n = 22; age 52–91; 72.7% female). Participant demographic characteristics are presented in **Table 1**.

Classification and Diagnosis

To select CN and MCI participants, we initially applied exclusionary criteria (no diagnosed dementia, cardiovascular disease, stroke history, or psychiatric illness, MMSE $\geq$ 24) and inclusionary criteria (two waves (4.5 years) of longitudinal data, complete data on a separate cognitive reference battery). We implemented an established and objective four-step cognitive classification procedure that requires strict adherence to specific assessment and selection rules (Dixon et al., 2007, 2014; de Frias et al., 2009; Dolcos et al., 2012; Huan et al., 2018). We conducted the full classification procedure at each of two waves (about 4.5 years apart). At both waves, eligible participants completed a five-domain cognitive battery, including measures of key domains: perceptual speed, inductive reasoning, episodic memory, verbal fluency and semantic memory. The procedure was as follows. Source participants were: (1) stratified into two age (64–73 and 74–95) and education (0–12 years and 13 + years) groups; (2) placed in appropriate age x education subgroups; (3) analyzed for mean cognitive scores on all tests; and (4) evaluated by score within respective age x education subgroups. We applied a moderate criterion to establish higher or lower ("impaired") group based on one standard deviation below the subgroup mean for any cognitive test. For participants to be classified as CN or MCI they were required to be objectively stable in their classification at both waves (at least 4.5 years). The procedure resulted in n = 25 MCI participants, who we then matched (age, sex) with CN adults and supplemented with randomly selected additional participants (n = 35). Overall, this approach emphasizes objective and stable classification, reducing the risk of false assignments and enhancing homogeneity of the groups (Dolcos et al., 2012; Bondi et al., 2014; Dixon et al., 2014). AD patients were recruited from the Geriatric and Cognitive Neurology clinics at the Glenrose Hospital in Edmonton, Alberta. The clinical diagnosis of AD was based on the Diagnostic and Statistical Manual of Mental Disorders (4th Edition) criteria for Dementia of the Alzheimer Type. Clinical assessments were performed as part of routine clinical evaluation, which included caregiver report of cognitive decline and impaired functional status, mental status evaluation of the patient (including the MMSE and Montreal Cognitive Assessment) and a physical and neurological examination. All patients had routine laboratory assessment for causes of dementia, including blood work and brain imaging according to Canadian Consensus Guidelines (Gauthier et al., 2012). Imaging excluded significant vascular pathology; however, cerebrospinal or other amyloid biomarkers were not available. AD patients did not have vascular dementia based on a modified ischemic score >4. Medical comorbidity was recorded using the modified Cumulative Illness Rating Scale.

TABLE 1 | Clinical characteristics of CN, MCI and AD groups[a].

Characteristics	CN	MCI	AD
N (total = 82)	35	25	22
Age (years)[b]	69.94 (3.80)	70.40 (3.38)	77.09 (11.20)
Gender (M/F)	13/22	10/15	6/16
Education, years[b]	15.69 (2.69)	14.68 (2.94)	11.59 (3.23)
Mini-Mental State Exam[b]	28.46 (1.42)	27.39 (3.14)	21.32 (4.76)

CN, Cognitively Normal; MCI, Mild Cognitive Impairment; AD, Alzheimer's disease. [a]Exclusionary, diagnostic, and classification criteria applied. [b]Values are mean (standard deviation).

Salivary Samples

Salivary samples were collected and prepared according to the manufacturer's protocol. Participants were instructed not to eat one hour before testing and light washing was permitted prior to saliva collection. One saliva sample was collected per participant. The time of day for saliva collection varied throughout across participants. At a regular point in the data collection for each participant, the saliva collection task was announced, instructions were delivered, and the device was displayed and described. As the overall procedure was not time-limited, there was sufficient time for full samples from

all individuals. We used the Oragene® • DNA Self-Collection Kit OG-500 (DNA Genotek Inc., Ottawa, ON, Canada). Whole saliva was collected, placed inside the kit, and shaken. The kit contained an Oragene DNA-preserving solution. The ingredients of Oragene solution include ethyl alcohol (>24%) and Tris-HCl buffer (pH 8). As provided by established procedures, samples stored at room temperature were analyzed for DNA extraction, genotyping and the metabolomics analyses. Our previous pilot study included an analysis of five different saliva samples from CN adults collected at varying times of the day and stored at different temperatures to examine performance of the metabolomics profiling method. We observed that metabolites detected for each individual sample significantly discriminated the individuals despite small metabolite variations that may have been present for samples collected at different times of the day. In addition, there were relatively minor metabolite variations across a range of storage temperatures (room temperature, −20°C, −80°C; Zheng et al., 2012). All saliva samples were then preserved in −80°C for long-term storage and follow-up studies.

Alzheimer's Predictors From Six Risk Domains

In this section, we describe the procedures for obtaining the risk and biomarker data. These included two AD biomarker clusters using salivary samples: (1) salivary metabolites; and (2) genetic polymorphisms. The remaining domains were: (3) functional health; (4) lifestyle activity; (5) cognition; and (6) bio-demographic. We recruited diagnosed AD patients with mild form of dementia and limited available time for the testing session than the other two groups. Thus, we reduced the cognitive and physical load of our testing sessions for them. The total number of predictors differed between the clinical status discrimination analyses because the AD group was not tested on PP, BMI and lifestyle activities.

Metabolomics Procedure and Metabolite Panel Development

The metabolomics analyses leading to the present biomarker panels were performed in a previous study (Huan et al., 2018). In the study establishing the present biomarker panels, we applied a salivary metabolomics workflow with a differential chemical isotope labeling based liquid chromatography-mass spectrometry (LC-MS) platform using dansylation derivatization for an in-depth profiling of the amine/phenol submetabolome (Huan et al., 2018). This was adapted and extended from earlier pilot work on saliva metabolome profiling (Zheng et al., 2012). Five microliters saliva sample was aliquoted out from each individual sample and labeled with ^{12}C-DnsCl. A pooled sample was prepared by mixing small aliquots of individual samples and then labeled with ^{13}C-DnsCl. The ^{12}C-labeled individual sample was then mixed with ^{13}C-labeled pooled sample in a 1:1 amount ratio after the total concentration of the labeled metabolites was determined by LC-ultraviolet. The ^{12}C-/^{13}C- ion pairs belonging to the labeled amine/phenol submetabolome were extracted from raw LC-MS data by a peak pair picking program, IsoMS (Zhou et al., 2014). Missing values in the ion pair list was retrieved using Zero-fill (Huan and Li, 2015a) by searching and filling in the missing values from the raw MS data. Accurate intensity ratios of the ion pairs were reconstructed by their chromatographic peak ratios using IsoMS-Quant (Huan and Li, 2015b). After the LC-MS data processing, multivariate statistical analysis of the LC-MS data was conducted using SIMCA-P + 12.0 (Umetrics, Umeå, Sweden).

The metabolite biomarker panels were determined as follows (Huan et al., 2018). Pairwise statistical comparisons used orthogonal partial least squares-discriminant analysis (OPLS-DA) and volcano plot analyses. The diagnostic power of the common metabolites that were highly ranked with both statistical tools was then evaluated by receiver operating characteristic (ROC) analysis and linear SVM model using MetaboAnalyst (Xia et al., 2012). For positive or definitive metabolite identification, the peak pairs were matched against a Dns-standards library (Huan et al., 2015) by retention time and accurate mass. In addition, putative metabolite identification was performed based on accurate mass match of the peak pairs found to the metabolites in the Human Metabolome Database (Wishart et al., 2013) and the Evidence-based Metabolome Library using MyCompoundID (Li et al., 2013), with a mass tolerance of 5 ppm.

As a final step in the discovery phase, a machine learning linear SVM tool in MetaboAnalyst (Xia et al., 2012) was used to develop a diagnostic model for each of the three pairwise comparisons with: (1) 63 metabolites discriminating AD vs. CN; (2) 47 metabolites discriminating AD vs. MCI; and (3) two metabolites discriminating MCI vs. CN. In a follow-up validation phase, the diagnostic performance was further evaluated in a small (n = 27) but independent data set drawn from the same population. Specifically, validation was tested with similarly classified or diagnosed CN (age 68–75 years, 50% female), MCI; age 67–75 years, 50% female), and AD; age 53–91 years, 71.4% female) groups (Huan et al., 2018). The final diagnostic model best discriminated: (a) AD from CN with the AD metabolite panel (Methylguanosine, Histidinyl-Phenylalanine, Choline-cytidine); (b) AD from MCI with the AD/MCI metabolite (Amino-dihydroxybenzene, Glucosylgalactosyl hydroxylysine − H_2O, Aminobutyric acid + H_2); and (c) MCI from CN with the MCI metabolite panel (Glucosylgalactosyl hydroxylysine − H_2O, Glutamine-carinitine; Huan et al., 2018). The additive score is comprised of the sum of all the values for each metabolite in the three diagnostic models and was used as the final metabolite panel in the present clinical status prediction analyses. Higher score indicated higher metabolite concentration in the diseased group.

Genetic Markers

DNA was manually extracted from 0.8 ml of saliva sample mix using the manufacturer's protocol with adjusted reagent volumes. Genotyping was carried out by using a PCR-RFLP strategy to analyze the allele status for *APOE* (rs7412, rs429358), *CR1* (rs6656401), *CLU* (rs11136000) and *PICALM* (rs3851179). Genotyping was successful for the targeted SNPs for all present participants (McFall et al., 2015a). We included all three allelic combinations coded from 1 (lowest risk) to 3 (highest risk) for *CR1* (G/G = 1, G/A = 2, A/A = 3), CLU (T/T = 1, T/C = 2,

C/C = 3), and *PICALM* (C/C = 1, C/T = 2, T/T = 3). For *APOE*, the study sample did not include any ε2/ε4 carriers and, therefore, the remaining five allelic combinations were coded from 1 (lowest risk) to 5 (highest risk; ε2/ε2 = 1, ε2/ε3 = 2, ε3/ε3 = 3, ε3/ε4 = 4, ε4/ε4 = 5).

Functional Health

In this category we included PP, BMI and gait (timed walk) as predictors. PP was calculated with systolic minus diastolic blood pressure (McFall et al., 2016). BMI was obtained from measurements of weight in kilograms and height in centimeters. BMI was calculated by taking the weight in kilograms divided by the square of one's height in meters (kg/m^2; MacDonald et al., 2011; Besser et al., 2014). A timed walking test was used to measure gait speed for all participants. The CN and MCI groups began the walking task from a standing position, a standard procedure for individuals with no mobility or dementia concerns. Specifically, the CN and MCI groups were measured by asking participants to walk a distance of 3 m, turn around, and walk back (MacDonald et al., 2017). AD patients began the task from a sitting position in an arm chair (i.e., the Timed Up and Go Test, a standard task in dementia research). Participants were seated in an armchair, and asked to get up and walk 3 m, turn around, walk and sit back in the chair. The time taken to complete this task was measured with a stopwatch in seconds. PP, BMI and timed walk were included as continuous variables.

Lifestyle Activity

The commonly used VLS Activity Lifestyle Questionnaire has 67 items measuring seven types of lifestyle engagement (Hultsch et al., 1999; Dolcos et al., 2012; Small et al., 2012) From the full inventory, we extracted items (n = 50) associated with the key dementia-related lifestyle aspects (cognitive, physical, and social). We evaluated two types of cognitive activities: (1) integrative information processing measured (n = 12) such as playing a musical instrument or household repairs, and (2) novel information processing (n = 27) such as completing jigsaw puzzles or reading the newspaper (Runge et al., 2014; Sapkota et al., 2017). Physical activity (n = 4) included jogging or gardening (Thibeau et al., 2017). Social activity (n = 7) included volunteering or visiting friends (Brown et al., 2016). The frequency of participation is rated on a 9-point scale with never (0), less than once a year (1), about once a year (2), 2 or 3 times a year (3), about once a month (4), 2 or 3 times a month (5), about once a week (6), 2 or 3 times a week (7), and daily (8). All the items were summed for each domain with higher scores representing greater frequency of activity (e.g., Small et al., 2012; Runge et al., 2014; Sapkota et al., 2017; Thibeau et al., 2017).

Cognition

The cognitive performance domain was represented by four standardized tests covering key aspects of performance known to be associated with differential normal and impaired aging, as well as dementia. First, to represent memory we used the standard VLS Word Recall task (Dixon and de Frias, 2004). From a pool of six equivalent lists, two different but comparable lists of 30 English words (i.e., six taxonomic categories with five words each) were used. Participants were given 2 min to study the list and 5 min to write down their answers. The total numbers of words correctly recalled from each list was averaged and used as the final score (Josefsson et al., 2012). Second, to measure EF (inhibition) we used the Stroop test (Scarpina and Tagini, 2017). This test consists of the standard three parts (Parts A, B and C), with the measures based on latencies. The score is the standardized Stroop interference index ([Part C− Part A]/ Part A), with a lower index reflecting better performance (MacLeod, 1991; de Frias et al., 2009; Diamond, 2013). Third, to measure speed we used the SRT task (Dixon et al., 2007). In this computer-based nonverbal response time task participants press a key on the response console with the index finger of their dominant hand at every occurrence of the target stimulus as quickly as possible. Response latencies were recorded to a precision of ±1 ms as the final score (McFall et al., 2015a). Fourth, for global cognition, we used examined the MMSE (Folstein et al., 1975), which measures performance on a scale of 0–30.

Bio-Demographic

The VLS personal data inventory was used to determine type and level of demographic risk (Anstey et al., 2013a; Sachdev et al., 2016). We examined education (total number of school years; Amieva et al., 2014; Cadar et al., 2016), age (in years; Small et al., 2011; Papenberg et al., 2015), and sex (male vs. female; Altmann et al., 2014; Li and Singh, 2014; McDermott et al., 2017).

Statistical Analyses

RFA is a machine learning technology that applies a nonparametric approach to assess a large number of predictors in both complex and small data sets (Strobl et al., 2009; Kuhn and Johnson, 2013). These applications include biomarker predictions related to AD (Kaup et al., 2015; McDermott et al., 2017). We used RFA from the Party package (Hothorn et al., 2005) in RStudio version 1.0.136 (2017). The analysis combines regression trees based on a random selection of participants and variables. The regression trees are all combined and then used to rank variables according to their importance in predicting an outcome. The RFA party package accounts for any potential correlated predictor variables (Strobl et al., 2009). Any missing values were imputed using the missForest package (Stekhoven and Bühlmann, 2012). All the forests in our analyses examined 5,000 trees and a random sample of 10 predictors was tested at each potential split. The analyses ranked relative predictive importance based on standard statistical operations and procedural recommendations (Strobl et al., 2009). The metric for these rankings is termed "variable importance," which specifies how important each factor is in discriminating two groups relative to all other factors in the model. Goodness of model fit for each RFA analyses was examined with area under the ROC curve (C-statistics). The C-statistic ranges from 0.50 to 1.00 can be interpreted as equivalent to the Area under the Curve in a ROC analysis where higher values are associated with better predictive models. Any variables with negative, zero, or small positive values are determined as not important predictors; these are represented to the left of the vertical dashed line. Variables

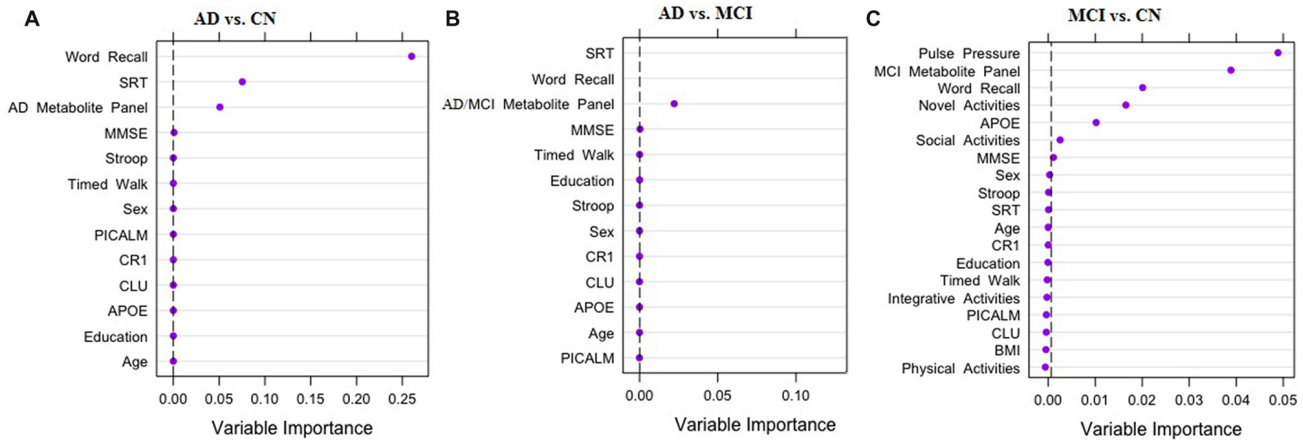

FIGURE 1 | Results of random forest analyses (RFA) for three pairwise comparisons. The three panels of the figure display strongest predictors for discriminating clinical status: **(A)** Alzheimer's disease (AD) vs. Cognitively Normal (CN); **(B)** AD vs. Mild Cognitive Impairment (MCI); **(C)** MCI vs. CN. Dashed black line is the cut off for variable importance in discriminating clinical status relative to other factors in the model. *APOE, Apolipoprotein E* (rs7412, rs429358); *CR1, Complement receptor 1* (rs6656401); *CLU, Clusterin* (rs11136000); *PICALM, Phosphatidylinositol-binding clathrin assembly protein* (rs3851179); BMI, Body Mass Index; SRT, Simple Reaction Time; MMSE, Mini-Mental State Exam.

right of the vertical dashed line with high positive values are considered to be important predictors (Strobl et al., 2009).

RFA was used to determine the most important predictors for discrimination in three pairwise groups (AD vs. MCI, AD vs. CN, MCI vs. CN). First, we tested which of the 13 risk factors were the most important predictors for discriminating clinical status for: (1) AD vs. CN and (2) AD vs. MCI. Second, we tested which of the 19 risk factors were the most important predictors for discriminating clinical status for (3) MCI vs. CN.

RESULTS

Across the three multi-modal prediction analyses, we observed significant discrimination for the pairwise comparisons of the three clinical groups with predictors from the six AD biomarker risk domains (see **Figure 1**). First, for the AD vs. CN analysis, three important discriminative predictors were identified (C-statistic: 1.00). As shown in **Figure 1A**, the top predictors included two cognitive measures (speed and memory) and the AD metabolite panel. Specifically: (1) poorer memory performance; (2) slower speed performance; and (3) higher levels (greater risk) of the AD metabolite panel discriminated AD from CN group at a high level of importance. Second, for the AD vs. MCI analysis the same two cognitive predictors and the AD/MCI metabolite panel were identified as important predictors (C-statistic: 0.99). As can be seen in **Figure 1B**, A different order of importance was observed: (1) slower speed performance; (2) poorer memory performance; and (3) higher levels (greater risk) of the AD/MCI metabolite panel were the most important factors discriminating the AD and MCI groups. Third, as shown in **Figure 1C**, in the MCI vs. CN analysis, seven of the 19 predictors were identified as important in discriminating the groups (C-statistic = 0.94). Notably, the seven predictors represented five (of the six) risk domains (see **Figure 1C**). Specifically, the most important predictors for discriminating MCI from CN were: (1) higher PP; (2) higher levels of the MCI metabolite panel; (3) poorer memory performance; (4) lower frequency of novel cognitive activity; (5) elevated *APOE* risk; (6) decreased social activity; and (7) lower MMSE score.

DISCUSSION

We examined and compared neurodegenerative disease status predictions by selected modifiable and non-modifiable AD risk factors representing six prominent modalities. The relative prediction patterns were examined for the pairwise discrimination of the three groups (CN, MCI and AD). An important aim was to test the extent to which recently discovered salivary metabolomics biomarker panels (Huan et al., 2018) would perform in the competitive context of other biomarkers and risk factors of AD. Given the dynamic, insidious and multi-factorial nature of AD, it is likely that multiple modalities of risk biomarkers may contribute to the diagnosis of the disease. A corresponding emerging interest is in determining viable combinations of predictors for use in timely (early) detection and targeted (precise) intervention. Our results supported both the multi-modal predictor expectation and the potential valuable role that salivary-based biomarkers discovered through metabolomics analyses may play in identifying important components of AD biomarker batteries.

In our earlier metabolomics analyses, we detected salivary metabolite panels that were most accurate in discriminating the three groups (Huan et al., 2018). In this study, we examined how these panels performed in discriminating these groups in the competitive context of other known AD biomarkers or risk factors. In each of the three pairwise comparisons, the RFA results showed that the relevant metabolite panel was among the top important predictors. In fact, the AD and AD/MCI metabolite panels and the same two cognitive

performance measures—i.e., speed and memory—in a different order discriminated AD from CN and AD from MCI. For the AD-CN comparison, the important predictors were memory, followed by speed and the AD metabolite panel. The AD metabolite panel represents pathways involved in AD protein regulation (Huan et al., 2018). For the AD-MCI comparison, speed and memory were among the most important predictors, and the associated AD/MCI metabolite panel also contributed at an important level. Dipeptides identified in both the AD metabolite panel and AD/MCI metabolite panel may reinforce the role of protein dysregulation in AD as a result of degraded proteins from amyloid or tau (Huan et al., 2018). Memory decline and impairment is a cardinal marker of preclinical dementia and is described as an oft-reported clinical symptom of aging and impairment (McKhann et al., 2011). Poorer memory performance in AD is consistent with key memory-related structural changes observed in the aging and impaired brain (Bartsch and Wulff, 2015). Specifically, hippocampal atrophy rates are comparatively greater in MCI than CN older adults and whole brain atrophy rates are greater in AD patients than MCI (Henneman et al., 2009). Slower speed performance has shown to be an early marker of lower and steeper cognitive decline (McFall et al., 2015a) and may be associated with poorer executive functioning and memory performance as well as increased dementia risk (Bäckman et al., 2005). Slower speed performance is also positively correlated with white matter tracts especially in the parietal and temporal cortices, and the left middle frontal gyrus (Turken et al., 2008).

Much attention in recent years has been on the detection of early signs—and their biomarker predictors—of transitions from CN to mildly impaired aging (Albert et al., 2011; Brainerd et al., 2013). Recently, this transition has also been investigated with unbiased metabolomics procedures (Zheng et al., 2012; Figueira et al., 2016; Liang et al., 2016; Huan et al., 2018). Two related challenges are that: (1) neither group is diagnosable with AD and (2) the exact probabilities of individual future conversion to AD are unknown. Moreover, both groups are likely to be in fluctuant, even overlapping, states of brain and cognitive aging—as indicated by the phenomenon of reversion (Manly et al., 2008; Koepsell and Monsell, 2012). Reviews of this challenge have led to the recommendation that multiple biomarkers and longitudinal data are advisable for differential classification. In the present study, these two groups were exactingly and objectively classified based on longitudinal data. Specifically, both groups were comprised of participants who were independently classified in status on two separate waves (about 4 years apart), underscoring the validity of the CN classification and the chronicity of the cognitively impaired classification. Our results reflect the challenge and relevance of considering multiple modalities of risk, and the apparent validity of carefully characterized groups. The RFA results showed that seven factors (representing five modalities) were found to be important predictors of impairment. The important predictors in order of significance were PP, MCI metabolite panel, memory, novel activities, *APOE*, social activities and MMSE. Elevated PP has been linked to cognitive impairment in MCI potentially in association with large artery stiffness (Yaneva-Sirakova et al., 2012; McFall et al., 2016). Our previously discovered MCI metabolite panel was the second important predictor of MCI status. This panel could be used in future targeted studies focusing on the differences and early markers of early memory impairment, as distinguished from normal memory decline. Notably, we employed a broad performance-based classification scheme that complements the standard clinical approach to MCI classification (Petersen et al., 2014). Two aspects of lifestyle activities—specifically, lower frequency of novel cognitive activities and decreased social engagement—predicted MCI group membership, in the context of the CN benchmark. The discriminative associations for these markers were in the expected direction, indicating that poorer lifestyle activities predicted probability of cognitive impairment (Verghese et al., 2006; Hughes et al., 2013). As previously reported in the literature (Brainerd et al., 2011; Dixon et al., 2014), AD genetic risk, as represented by *APOE* ε4+ genotypes predicted membership in the cognitive impairment group, in the context of the CN benchmark. Finally, poorer global cognition in the MCI group, an indication of future risk of dementia (O'Bryant et al., 2008), suggests that the MCI group maybe on an accelerated path to dementia onset compared to the CN group.

Overall, the results are consistent with the general perspective that risk markers from multiple modalities contribute to the prediction, classification or diagnosis of cognitive statuses such as MCI and AD. The results are also consistent with our expectation that new metabolite panels, derived from salivary metabolomics analyses, can be confirmed as among the better predictors of clinical status—but not the only predictor, especially for the crucial discrimination of CN and the impairment group. Along with notable strengths, we acknowledge several limitations. First, as a function of leveraging our earlier metabolomics study, our present sample sizes are relatively small. Although not perfect, this fact is statistically accommodated in the machine learning prediction analyses we used. Specifically, RFA are well suited to deal with small sample sizes because a large number of trees can be used in RFA models (Strobl et al., 2009). Larger number of trees allows for a large variety of predictor variable combinations to account for small sample sizes. Moreover, RFA outperforms other non-machine learning techniques (i.e., regression, and factor analysis) in that it accommodates: (1) small samples size in highly complex datasets (Maroco et al., 2011); (2) highly correlated datasets; and (3) large number of regression trees with specified set of predictors. The latter compensates for power issues as frequently observed in other statistical models with small sample sizes. We take the average of all 5,000 trees to employ a bagged variable importance measure—a procedure that leads to more stability and reduces the risk of over-fitting of the data. Nevertheless, some over-fitting of prediction models may occur, so further validation research is recommended. We specifically recommend follow-up validation studies, appropriate statistical evaluation, and larger sample sizes. Second, we deliberately incorporated a large number of predictors from multiple modalities—and all are established in a variety of independent research projects—but not all possible and potentially relevant predictors were included. For example, future research should include other standard AD biomarkers, such as cerebrospinal fluid β-amyloid (1–42),

total tau, and phospho-tau-181 (Humpel, 2011), as well as other AD-specific neuroimaging biomarkers (e.g., hippocampal volume). Third, in order to more broadly generalize our results, we recommend studies recruit samples that are more demographically diverse, include alternative biospecimens or validate with autopsy confirmed AD cases. As noted, the metabolite panels used here were established in previous metabolomics research using the same groups. Future validation work would also benefit from targeting and testing these panels in different populations.

We tested a multi-modal array of risk biomarkers for their relative predictive power in discriminating three clinical status groups. This provides empirical evidence confirming the view that such multi-modal approaches can be valuable in research on neurodegenerative disease. In addition, the results also confirm that metabolomics procedures can produce biomarker panels that have relevance in the competitive context of other known risk factors for AD. Future work should examine such novel metabolite panels in the context of additional AD biomarkers. The results also show that modifiable risk factors can be important predictors of clinical status, even in the context of biomarkers from metabolomics and genomic approaches. At present, they appear to be especially relevant for the crucial discrimination of normal and impaired groups. The overall results lead to indications of potential use for validation and translation of non-invasive metabolite panels in pertinent combinations with established multi-modal biomarkers for early dementia risk detection and intervention programs. Early and precise risk detection can lead to personalized risk management and other intervention strategies for older adults at elevated risk for AD (Barnes and Yaffe, 2011; Anstey et al., 2014; Olanrewaju et al., 2015).

DATA AVAILABILITY

Datasets are available upon request from the corresponding authors.

AUTHOR CONTRIBUTIONS

RD is the director of the VLS and was responsible for all data and sample collection. RD and SS designed and planned the present research and statistical analyses. SS and TH assembled and validated the data set. LL is the director of the metabolomics lab and led TH, TT and JZ in performing the metabolomics analyses that contributed the metabolite biomarker panels. SS performed the data assembly and the present statistical analyses, with contributions from RD and TH. SS wrote the drafts of the article, with assistance from RD, LL, RC and TH. RC performed the clinical diagnoses of AD and consulted on the MCI classifications.

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Primary Disruption of the Memory-Related Subsystems of the Default Mode Network in Alzheimer's Disease: Resting-State Functional Connectivity MRI Study

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Background: Recent studies have indicated that the default mode network (DMN) comprises at least three subsystems: The medial temporal lobe (MTL) and dorsal medial prefrontal cortex (DMPFC) subsystems and a core comprising the anterior MPFC (aMPFC) and posterior cingulate cortex (PCC). Additionally, the disruption of the DMN is related to Alzheimer's disease (AD). However, little is known regarding the changes in these subsystems in AD, a progressive disease characterized by memory impairment. Here, we performed a resting-state functional connectivity (FC) analysis to test our hypothesis that the memory-related MTL subsystem was predominantly disrupted in AD.

Method: To reveal specific subsystem changes, we calculated the strength and number of FCS in the DMN intra- and inter-subsystems across individuals and compared the FC of the two groups. To further examine which pairs of brain regional functional connections contributed to the subsystem alterations, correlation coefficients between any two brain regions in the DMN were compared across groups. Additionally, to identify which regions made the strongest contributions to the subsystem changes, we calculated the regional FC strength (FCS), which was compared across groups.

Results: For the intra-subsystem, decreased FC number and strength occurred in the MTL subsystem of AD patients but not in the DMPFC subsystem or core. For the inter-subsystems, the AD group showed decreased FCS and number between the MTL subsystem and PCC and a decreased number between the PCC and DMPFC subsystem. Decreased inter-regional FCS were found within the MTL subsystem in AD patients relative to controls: The posterior inferior parietal lobule (pIPL) showed decreased FC with the hippocampal formation (HF), parahippocampal cortex (PHC) and ventral MPFC (vMPFC). Decreased inter-regional FCS of the inter-subsystems were also found in AD patients: The HF and/or PHC showed decreased FC with dMPFC and TPJ, located in the DMPFC subsystem, and with PCC. AD patients also showed decreased

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FC between the PCC and TLC of the dMPFC subsystem. Furthermore, the HF and PHC in the MTL subsystem showed decreased regional FCS.

Conclusion: Decreased intrinsic FC was mainly associated with the MTL subsystem of the AD group, suggesting that the MTL subsystem is predominantly disrupted.

Keywords: resting-state fMRI, default network, subsystems, functional connectivity, Alzheimer's disease

INTRODUCTION

Since its name was proposed more than a decade ago (Raichle et al., 2001), the default mode network (DMN) has been characterized by its high level of metabolic activity during passive states and low activity in externally directed conditions (Shulman et al., 1997; Gusnard and Raichle, 2001; Raichle et al., 2001). Additionally, the robust activity correlations within the DMN at rest and during task performance have been confirmed in many papers (Greicius et al., 2003; Fox et al., 2005; Buckner et al., 2008; Grigg and Grady, 2010; Spreng and Grady, 2010; Allen et al., 2011). Although the precise function of the DMN is still debated, most literature has revealed that the DMN shows increased activity not only during rest but also during tasks, as long as experimental conditions involve aspects of self-generated thought (Andrews-Hanna et al., 2014), such as autobiographical memory (Svoboda et al., 2006; Spreng and Grady, 2010), future projection (Addis et al., 2007), mind wandering (Christoff et al., 2009; Smallwood et al., 2013), and social cognition (Mar, 2010). These features of self-generated thought suggest that they comprise multiple-component processes (Andrews-Hanna et al., 2014), which serve to prepare for upcoming events (Baird et al., 2011), form a sense of self-identity and continuity across time (Smallwood et al., 2011; Prebble et al., 2013), and navigate the social world (Immordino-Yang et al., 2012; Mar et al., 2012; Ruby et al., 2013). Recently, converging evidence has revealed that the DMN has a parallel level of complexity in functional-anatomical organization to support multiple-component processes of self-generated thought (Buckner et al., 2008; Sestieri et al., 2011; Andrews-Hanna, 2012; Kim, 2012; Roy et al., 2014). Andrews-Hanna et al. (2010b) explored the intrinsic functional organization of the DMN by using functional connectivity magnetic resonance imaging (fcMRI), graph analysis and hierarchical clustering techniques. The authors suggested that the DMN comprised two distinct subsystems: a dorsal medial prefrontal cortex (DMPFC) subsystem consisting of the dMPFC, the temporal parietal junction (TPJ), the lateral temporal cortex (LTC), and the temporal pole (TempP), and an MTL subsystem comprising the ventral medial prefrontal cortex (vMPFC), the posterior inferior parietal lobule (pIPL), the retrosplenial cortex (Rsp), the hippocampal formation (HF), and the parahippocampal cortex (PHC). Both subsystems converge on a core that includes the anterior MPFC (aMPFC) and the posterior cingulate cortex (PCC). The authors then proved that the two subsystems had relatively distinct functions by designing an experiment. The DMPFC subsystem was preferentially activated during decisions about one's present situation or mental state, and the MTL subsystem was selectively activated during self-relevant predictions about one's future.

Previous work has consistently shown that compared with healthy subjects, patients with Alzheimer's disease (AD) have reduced FC within the DMN when at rest (Greicius et al., 2004; Rombouts et al., 2005; Celone et al., 2006; Wang K. et al., 2006) and less pronounced deactivation during cognitive tasks (Lustig et al., 2003; Celone et al., 2006). In addition, several studies of cortical hubs that performed functional and structural connectivity analysis combined with graph theoretical analysis revealed that most of the hubs are located within the DMN, such as the PCC, the medial prefrontal cortex (MPFC), and the HF (Hagmann et al., 2008; Buckner et al., 2009; Yu et al., 2017). It has been suggested that these cortical hubs in the DMN are preferentially affected in AD (Yu et al., 2017). The abovementioned studies have suggested that DMN impairment is related to AD. Moreover, although several studies have found that reductions in FC only exist in some regions, such as the PCC and the medial temporal lobe (MTL), in patients with AD (Zhou et al., 2008; Bai et al., 2009), other studies have reported widespread reductions in FC as the disease progresses (Damoiseaux et al., 2012). AD is a progressive dementia, and the predominant symptom is memory impairment, although disturbances in other cognitive functions also occur (Krajcovicova et al., 2014). Based on the above findings, distinct subsystems in the DMN engage in differential cognitive processes involving memory construction and self-oriented cognition, and these cognitive processes are influenced by AD (Greicius et al., 2004; Buckner et al., 2008, 2009; Zhou et al., 2010; Menon, 2011). Therefore, we hypothesized that the memory-related MTL subsystem was predominantly disrupted in AD and that other subsystems in the DMN may be disrupted to a certain degree. To test these hypotheses, rs-fMRI was performed on 24 patients with mild to moderate AD and on 27 healthy elderly subjects. Eleven regions of interest (ROIs) in the DMN subsystem for FC analysis were selected based on published data by Andrews-Hanna et al. (2010b). The DMN FC matrices were constructed by estimating the Pearson correlation between the time series of pairs of ROIs in the subsystems. To reveal the specific disrupted subsystem, we analyzed the strength and number of FCS in the intra- and inter-subsystems of the DMN. Then, to examine which pairs of regional FCS contributed to changes in the subsystems, each pair of correlation coefficients in the DMN was compared across groups.

In the present study, a regional FCS analysis was also used to identify regions that made the strongest contributions to the changes in the subsystems. Regional FCS is defined as the mean of the correlation strength of one ROI with all other target regions in a brain network and is referred to as the "degree centrality" of a

weighted network in graph theory (Buckner et al., 2009; Zuo et al., 2012; Wang L. et al., 2013). Brain ROIs with higher FCS values usually indicate their central roles in the functional integrity of the brain network (Wang L. et al., 2013).

MATERIALS AND METHODS

Participants

A total of 55 subjects were enrolled from Shanghai Huashan Hospital. All participants were categorized into a control group (n = 27) and an AD patient group (n = 28). AD patients were diagnosed by a qualified neurologist using criteria for amnestic AD (Fennema-Notestine et al., 2009), with mini-mental state examination (MMSE) scores between 12 and 27 (inclusive) and clinical dementia rating (CDR) scores of 1 or 2. The control groups had MMSE scores between 26 and 30 (inclusive) and CDR scores of 0. The data of 4 subjects (4 patients with AD) were excluded due to excessive motion, severe brain atrophy, hydrocephalus or large areas of cerebral infarction. Details regarding the clinical and demographic data of the remaining 51 subjects are shown in **Table 1**. There were no significant differences in terms of sex or age between the two groups.

Image Acquisition and Preprocessing

All subjects underwent whole-brain MRI scanning on a 3.0T SIEMENS Verio. Resting-state BOLD functional MRI data were collected using an echo-planar imaging (EPI) sequence. The scanning parameters were TR = 2000 ms, TE = 35 ms, FOV = 25.6 cm × 25.6 cm, flip angle = 90°, matrix = 256 × 256, slices = 33, thickness = 4 mm, and gap = 4 mm.

Unless specifically stated otherwise, all of the preprocessing was performed using statistical parametric mapping (SPM8[1]). The first 5 images were discarded in consideration of magnetization equilibrium. The remaining 155 images were corrected for the acquisition time delay among different slices, and then, the images were realigned to the first volume for head-motion correction. The fMRI images were further spatially normalized to the Montreal Neurological Institute (MNI) EPI template and resampled to a 2-mm cubic voxel. Several sources of spurious variance, including the estimated motion parameters, the linear drift, and the average time series in the cerebrospinal fluid and white matter regions, were removed from the data through linear regression. Finally, temporal bandpass filtering (0.03–0.06 HZ) was performed to reduce the effects of low-frequency drift and high-frequency noise (Liu et al., 2008; Zhang et al., 2012). The time course of head motion was obtained by estimating the translations in each direction and the rotations in angular motion about each axis for each of the 155 consecutive volumes. All of the subjects included in this study exhibited a maximum displacement of less than 3 mm (smaller than the size of a voxel in plane) at each axis and an angular motion of less than 3 for each axis. Data from two subjects were excluded due to excessive motion.

[1]http://www.fil.ion.ucl.ac.uk/spm

TABLE 1 | Demographics and clinical information.

Characteristics	HC (n = 27)	AD (n = 24)	P
Age	63.74 ± 7.80	67.54 ± 10.48	0.146[a]
Female/Male	11/16	13/11	0.406[b]
MMSE	28.84 ± 1.19	21.46 ± 1.67	<0.001[a]

Data are presented as the mean ± standard deviation (SD). [a]The P-value was obtained using a two-sample t-test. [b]The P-value was obtained using the Pearson chi-square test.

Functional Connectivity Analysis

fcMRI was performed using REST[2] (Song et al., 2011) to extract the time series for each ROI (spherical radius of 4 mm) (**Table 2**) by averaging the time courses of all voxels within an ROI. For each subject, we first calculated a Pearson's correlation and the significance level (i.e., p-value) between all given ROIs. Then, we obtained an 11 × 11 symmetric correlation matrix and the corresponding p-value matrix for each subject. To eliminate unreliable correlations, we retained only those correlations whose corresponding p-values met a statistical threshold $p < 0.001$ (0.05/55, Bonferroni correction); otherwise, we set the correlations to zero (Cruse et al., 2011; Wang et al., 2016). Then, correlation coefficients in a weighted 11 × 11 FC matrix were converted into z-values with the application of Fisher's r-to-z transformation. Fisher's Z-transformed correlation matrix was used for the subsequent analysis of each subject. The strength of the intra-subsystem FCS was defined as the total and mean of all positive-only inter-regional FCS within the selected subsystem, while the strength of the inter-subsystem FCS was defined as the total and mean of the positive-only FCS between any two ROIs of the two selected subsystems. In this paper, we calculated the strength of the FCS of the intra- and inter-subsystems of each subject and compared them

[2]http://www.restfmri.net

TABLE 2 | Regions of interest (ROIs) within the DMN subsystems for functional connectivity analysis from Andrews-Hanna et al. (2010b).

Region	Abbreviation	x	y	z
Core region				
Anterior medial prefrontal cortex	aMPFC	−6	52	−2
Posterior cingulate cortex	PCC	−8	−56	26
DMPFC subsystem				
Dorsal medial prefrontal cortex	dMPFC	0	52	26
Temporal parietal junction	TPJ	−54	−54	28
Lateral temporal cortex	LTC	−60	−24	−18
Temporal pole	TempP	−50	14	−40
MTL subsystem				
Ventral medial prefrontal cortex	vMPFC	0	28	−18
Posterior inferior parietal lobule	pIPL	−44	−74	32
Retrosplenial cortex	Rsp	−14	−52	8
Parahippocampal cortex	PHC	−28	−40	−12
Hippocampal formation	HF	−22	−20	−26

Coordinates are based on the Montreal Neurological Institute coordinate system.

across groups using two-sample *t*-tests. We also analyzed the differences in the numbers of positive-only FCS of the intra- and inter-subsystems between two groups using two-sample *t*-tests. Notably, to explore the specific changes in FC between the core regions and subsystems, we calculated the number and strength of FCS between the two core regions and subsystems separately. To examine which pairs of brain regional FCS contributed to the alterations in the subsystems of the DMN in AD, the correlation coefficients between any two ROIs in the DMN were also compared across groups using two-sample *t*-tests with false discovery rate (FDR) correction. We also listed the results with *p*-values less than 0.05, 0.01, and 0.001 to find possible differences in physiological significance. Furthermore, to identify which regions made the highest contributions to the changes in the subsystems, we compared the regional FCS across groups by using two-sample *t*-tests. The FCS of a given region was calculated using the following equation (Buckner et al., 2009; Zuo et al., 2012; Wang L. et al., 2013):

$$FCS(i) = \frac{1}{N-1} \sum_{j=1, j\neq i}^{N} z_{ij},\ r_{ij} > r_0$$

where N is the number of ROIs in the DMN (here $N = 11$), r_{ij} z_{ij} is the Fisher Z-transformed correlation coefficient, $r_{ij}$is the correlation coefficient between ROI i and ROI j, and r0 is a correlation threshold that was used to eliminate weak correlations possibly derived from noise (here, $r0 = 0$). The difference in regional FCS between the two groups revealed regions that were disrupted in patients with AD.

The Effects of Different Analysis Strategies

In our study, an FC matrix included positive and negative connections. In the positive-only strategy, we analyzed FC matrices by using the positive-only inter-regional FCS. For example, we assumed that no inter-regional FC existed if the inter-regional FC was non-positive. Given the controversies in the treatment of negative correlations in rs-fMRI network studies (Fox et al., 2009; Wang et al., 2011; Dai et al., 2015), we also calculated the strengths and numbers of positive and negative FCS of the intra- and inter-subsystems for the two groups and performed an FCS analysis including both positive and negative (absolute values) connections to assess the stability of our findings.

RESULTS

Group Differences in the Strengths and Numbers of FCS of the Subsystems and Core

To explore specific changes in the subsystems in AD patients, the total and mean FC strengths of the intra- and inter-subsystems of the DMN were calculated. Different analysis strategies consistently produced the same results. In this study, we show the results of the positive-only strategy.

The results of the former method are as follows: Patients with AD showed decreased total FC strength (FCS) in the two subsystems (see **Figure 1A**) between core regions and subsystems and between subsystems relative to the controls (see **Figure 1B**). Two-sample *t*-tests were performed to statically analyze the differences between the AD group and the control group. Significant differences between the two groups were found in the MTL subsystem ($t(49) = -3.00$, $p = 0.004$) but not in the core ($t(49) = 0.77$, $p = 0.45$) or the DMPFC subsystem ($t(49) = -1.31$, $p = 0.20$). Significantly decreased total FCS was also found between the PCC and the MTL subsystem ($t(49) = -2.41$, $p = 0.02$) but not found between the subsystems ($t(49) = -1.64$, $p = 0.11$), between the PCC and the dMPFC subsystem ($t(49) = -1.33$, $p = 0.19$), between the amPFC and the dMPFC subsystem ($t(49) = 0.77$, $p = 0.44$), or between the amPFC and the MTL subsystem ($t(49) = -1.01$, $p = 0.32$). For total FCS in the core, stronger correlations were observed in the AD group, although not significant (see **Figure 1A**). However, there was no significant difference in the mean strength of the FC of the inter- and intra-subsystems (see **Figures 2A,B**) between the two groups. For the FC matrix of each subject, we also calculated the total number of intra- and inter-subsystem FCS (see **Figure 3**). The statistical analysis (two-sample *t*-tests) revealed that the AD group showed a significantly smaller total number of FCS in the MTL subsystem ($t(49) = 3.74$, $p = 0.0005$) (see **Figure 3A**). Moreover, a significantly reduced number of FCS was also found between the PCC and the MTL subsystem ($t(49) = 3.12$, $p = 0.003$) and between the PCC and the DMPFC subsystem ($t(49) = 2.47$, $p = 0.02$) (see **Figure 3B**) in AD patients relative to controls.

Group Differences in the Inter-Regional FCS of the DMN

To examine which pairs of brain regional correlation coefficients contributed to the alteration in the subsystems of the DMN, correlation coefficients between pairs of ROIs in the DMN for each subject were compared across groups (see **Figure 4**). As shown in **Figure 4**, weaker correlations between ROIs in the DMN were observed in the AD group relative to the control group. Fewer inter-regional FCS were found within the MTL subsystem of AD patients than within that of controls: pIPL showed low FC with the hippocampus, the PHC and the vMPFC. In addition, fewer pairs of brain regional FCS of the inter-subsystems were found in AD patients. The hippocampus and the PHC showed decreased FC with the TPJ and the dMPFC, both of which were located in the DMPFC subsystem and the PCC. The PCC also showed decreased FC with the TLC, which exists in the dMPFC subsystem in AD. The functional connection between the hippocampus and the PCC is the only connection that was retained after FDR correction.

The Most Significantly Disrupted Regions Measured by FCS

To identify which regions made the strongest contributions to the changes in subsystems of the DMN, the differences in the regional FCS between the two groups were measured (see **Figure 5**). Different analysis strategies consistently produced

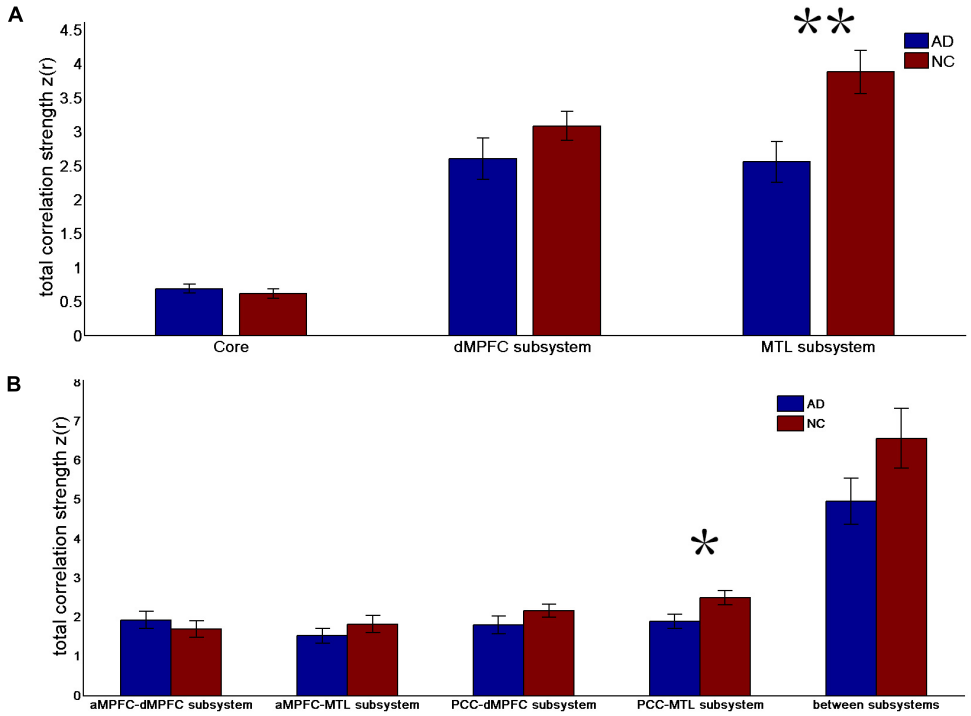

FIGURE 1 | Total correlation strength (positive-only) in the default network subsystems in the control and AD groups. **(A)** Total correlation strength within the core and subsystems of the two groups. **(B)** Total correlation strength between core regions (including the aMPFC and PCC) and subsystems separately and between the two subsystems in the two groups (*$p < 0.05$, **$p < 0.01$). Error bars represent the standard error of the mean.

the same results. Here, we present the results of the positive-only strategy. Between-group comparison analysis (two-sample *t*-tests) revealed that regions showing the most significant group differences in FCS were the HF ($t(49) = 2.76$, $p = 0.008$) and the PHC ($t(49) = 2.28$, $p = 0.03$).

DISCUSSION

Our results suggest that the memory-related MTL subsystem is predominantly disrupted in AD. First, we explored the changes in FC of the intra- and inter-subsystems in patients with AD. The strength and number of FCS were significantly decreased, specifically in the MTL subsystems but not in the core or the DMPFC subsystem. Moreover, the strength and number of FCS were significantly decreased between the MTL subsystems and the PCC. We also examined which pairs of brain regional functional connections contributed to the changes in the subsystems and found that the HF and the PHC showed extensively decreased FCS with other regions in the DMN. Furthermore, we identified the HF and the PHC as making the strongest contributions to the alterations in the subsystems. Additionally, our data show that in addition to the changes in the MTL subsystem, the core and DMPFC subsystems in the default network also have some degree of disruption. A significantly decreased number of FCS between the core and the DMPFC subsystem was found in AD patients. Inter-regional FC analysis revealed that there are a few pairs of decreased brain regional FCS between the subsystems and between the core and the dMPFC in patients with AD.

Predominantly Disrupted Memory-Related Subsystems in Patients With AD

As consistently reported in the literature, the MTL subsystem is reliably activated when engaged in autobiographical memory and episodic future thought (Andrews-Hanna et al., 2014). These spontaneous thoughts may allow individuals to construct and simulate scenarios, mentally organize their plans, and prepare for what may lie ahead (Immordino-Yang et al., 2012). Additionally, spontaneous thoughts may facilitate consolidation of the most self-relevant information into long-term memory. In the present study, we found that the MTL subsystem is predominantly disrupted. Within the MTL subsystem, the PIPL showed decreased FC with the HF, the PHC and the vMPFC. This finding was supported by the research of Wang L. et al. (2006) and Wang Z. et al. (2013). The HF and the PHC located in the MTL subsystem were mostly disrupted in AD patients. Consistent with our findings, previous researchers have proposed that pathological amyloid depositions and atrophy in AD patients are located in cortical regions such as the HF and the PHC (Buckner et al., 2005, 2009; Stricker et al., 2012). fMRI studies have revealed that AD patients have reduced activity in regions of the DMN. Greicius et al. (2004) found decreased resting-state activity in the hippocampus of AD patients. The PHC and

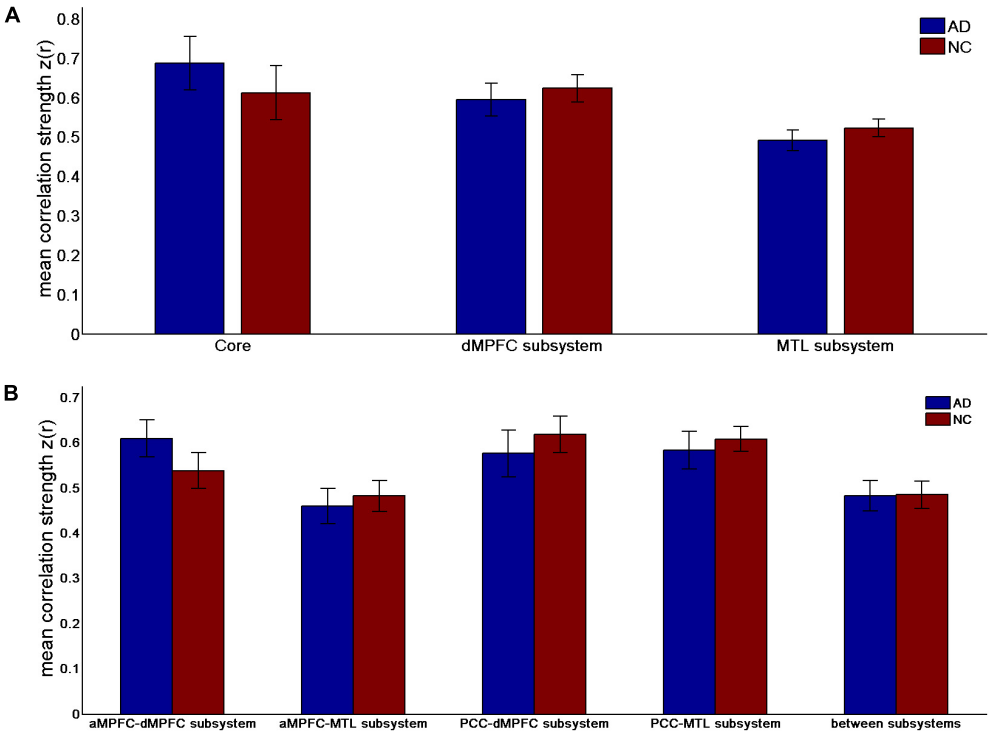

FIGURE 2 | Mean correlation strength (positive-only) in the default network subsystems in the control and AD groups. **(A)** Mean correlation strength within the core and subsystems in the two groups. **(B)** Mean correlation strength between core regions (including the aMPFC and PCC) and subsystems separately and between the two subsystems in the two groups. Error bars represent the standard error of the mean.

the HF possibly play significant roles in the MTL subsystem because the cognitive processes in which the MTL subsystem is involved might be supported by the MTL (including the PHC and the HF), which dynamically interacts with cortical regions within and outside the default network. Recent studies have supported the possibility of resting MTL activity or MTL-cortical FC related to individual differences in spontaneous episodic thoughts (Andrews-Hanna et al., 2010a), memory ability (Wig et al., 2008), and the consolidation of recent experiences (Tambini et al., 2010). Thus, the changes in the FC of the HF and the PHC in the DMN probably reflect cognitive impairment in recent episodic memory. Our findings support the hypothesis that the memory-related MTL subsystem of the DMN is primarily disrupted in AD.

In addition to the changes within the MTL subsystem itself, in the present study, we found that the MTL subsystem also showed decreased number and strength of FCS with the PCC in patients with AD. A number of studies have also suggested that the PCC plays an essential role in spatial orientation, self-appraisal and internal monitoring as well as memory processing (Gusnard and Raichle, 2001; Greicius et al., 2003; Whishaw and Wallace, 2003; Ries et al., 2006). As the core of the DMN, the PCC, which exhibits strong functional coherence with both subsystems (Andrews-Hanna et al., 2014) in coordination with the MTL subsystems, may facilitate memory processing. As consistently reported in previous literature, in the early stage of AD, FC between the PCC and the hippocampus, the anterior cingulate cortex, the MPFC and the precuneus are disrupted (Greicius et al., 2004; Wang L. et al., 2006; Sorg et al., 2007). It is possible that impairment between the PCC and the MTL subsystem may damage memory processing. These findings indicate that AD predominantly disrupts the memory-related MTL subsystem.

More Extensive Disruption of Subsystems in Patients With AD

In addition to the predominantly decreased FC associated with the memory-related MTL subsystem, we also found more extensively reduced FC in the inter-subsystems. Compared with the control group, the number of FCS between the PCC and the DMPFC subsystem was significantly reduced in AD, and inter-regional FC revealed that a few pairs of brain regional FCS between the subsystems and between the core region and the dMPFC subsystem exhibited significant decreases in the AD group. In line with our findings, previous studies have found that dissociated FC occurs between the PCC and the inferior temporal cortex in AD patients (Zhang et al., 2010). AD patients also have decreased hippocampus connectivity with the TPJ (Greicius et al., 2004) and the MPFC (Grady, 2001). These results provide powerful evidence for the possibility of the deterioration of all subsystems of the DMN as AD progresses, and previous studies support this possibility. A resting-state FC study suggested that early in the disease, regions of the

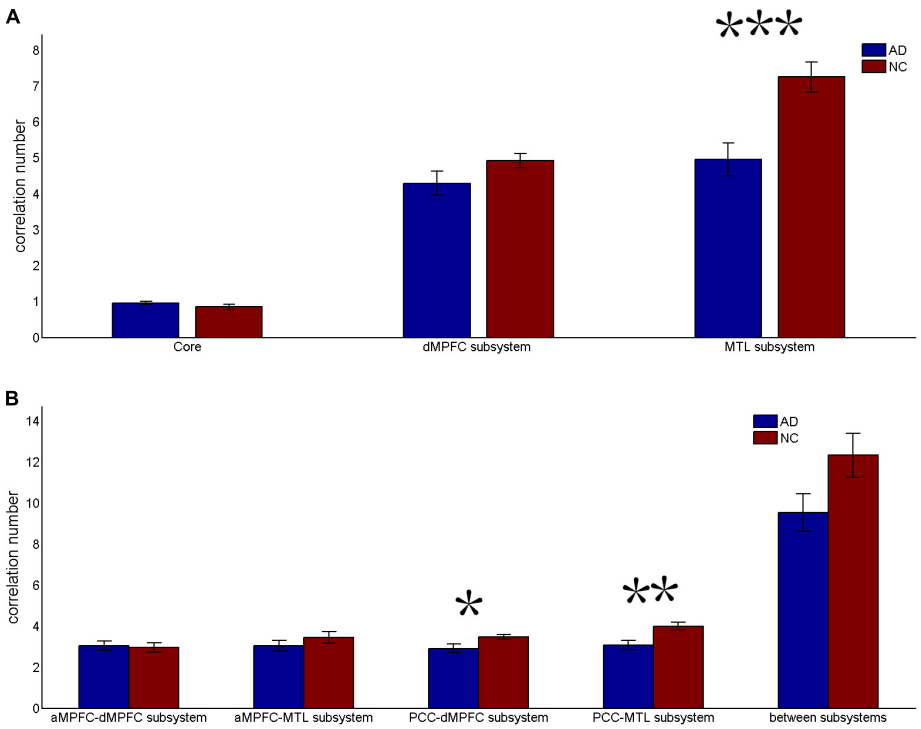

FIGURE 3 | Correlation number (positive-only) in the default network subsystems in the control and AD groups. **(A)** Correlation number within the core and subsystems in the two groups. **(B)** Correlation number between core regions (including the aMPFC and the PCC) and subsystems separately and between the two subsystems in the two groups (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). Error bars represent the standard error of the mean.

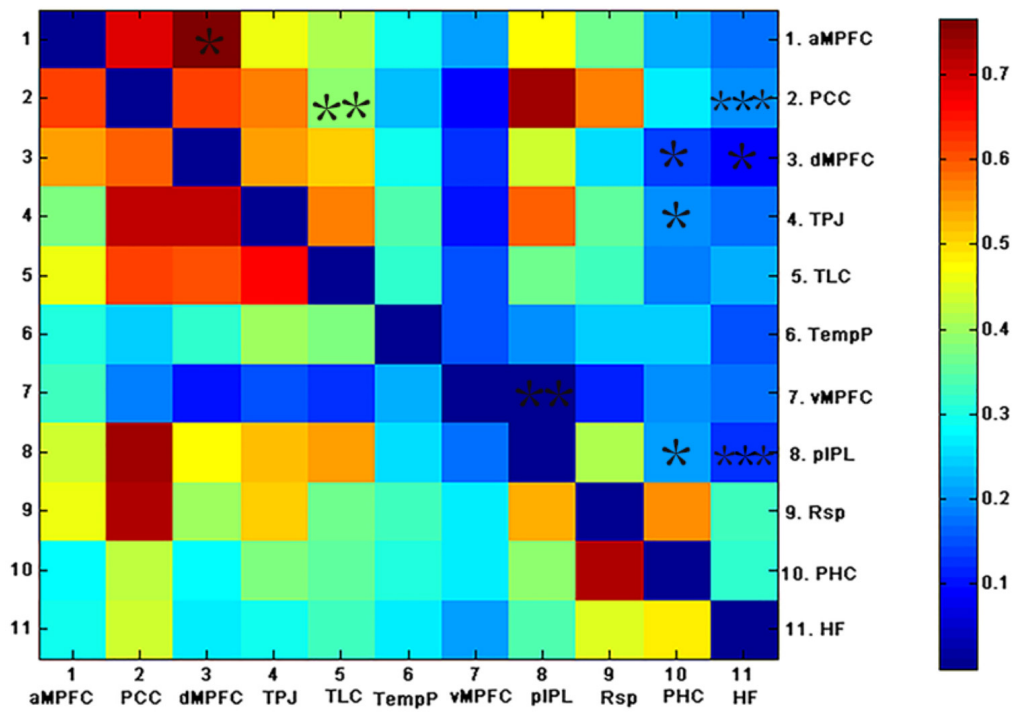

FIGURE 4 | The above matrices show the correlation coefficients between the ROIs in the DMN, as well as the group differences (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). The bottom and top triangles represent the group average correlation matrix of the control and AD groups, respectively. The color bar indicates the correlation coefficients between regions.

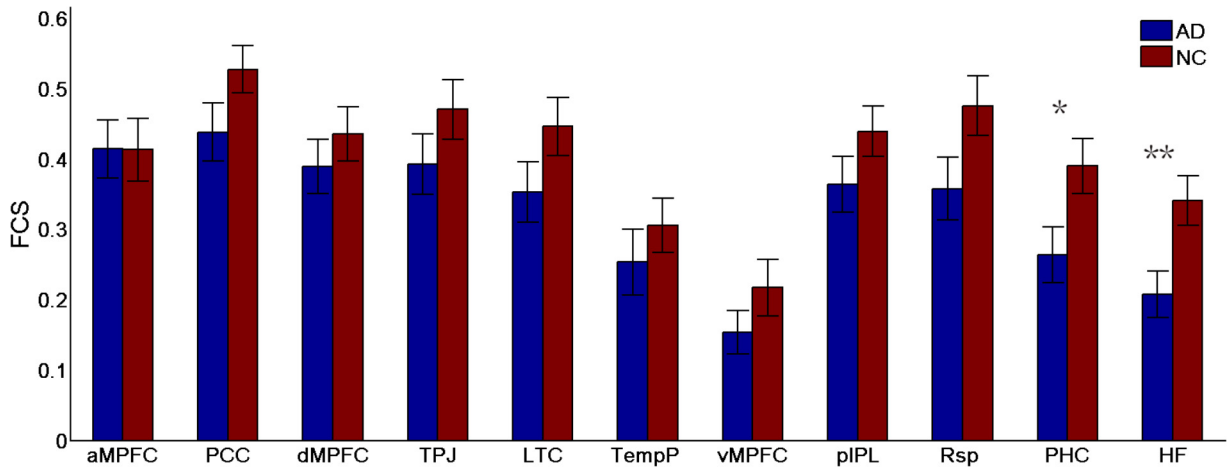

FIGURE 5 | Regional functional connectivity strength (FCS) in the control and AD groups (*$p < 0.05$, **$p < 0.01$). Error bars represent the standard error of the mean.

posterior DMN, including the PCC/Rsp, the IPL, and the LTC (Buckner et al., 2005), begin to disengage their connectivity; however, as the disease progresses, connectivity within all systems eventually deteriorates (Damoiseaux et al., 2012). Zhang et al. (2010) suggested that impairment in the PCC FC changes with AD progression. In contrast to the constructive functions of the MTL subsystem, the DMPFC subsystem may play an important role in examining the mental states of social agents (Andrews-Hanna, 2012), including the ability to reflect on, evaluate, or appraise social information. The PCC shares functional properties with both subsystems, possibly interacting with the DMPFC to facilitate examination of the mental state. The DMN subsystems supporting component processes of self-generated thought work together but independently to complete internal information processing and ultimately guide and motivate behavior. For example, the DMPFC subsystem may allow individuals to reflect on the mental states elicited by the stimulus, and the MTL subsystem may allow individuals to integrate this introspective information into a goal-directed plan (Andrews-Hanna, 2012). Therefore, more extensive disruption of the subsystems of the DMN in patients with AD in the DMN may account for additional cognitive impairments other than memory deficits to some degree. Our findings support previous studies indicating that the DMN is an important target network to be impaired (Buckner et al., 2009) and specify that different subsystems of the default network are not equivalently damaged in AD but that memory-related MTL subsystems are selectively damaged. As the disease progresses, other subsystems of the DMN may also have a certain degree of damage.

Limitations

Several limitations in the present work should be considered. First, our study had a small sample size. Second, our research focused only on subjects with mild to moderate AD, which did not allow us to determine the specific changes in the DMN subsystems during different stages of the disease. Third, we did not perform related behavioral tests. Specific self-referential cognitive ability changes in AD patients may be related to the corresponding FC in the DMN subsystems. Four, our processing of the functional matrix of each subject may remove some of the weaker connections that are actually present. Considering these limitations, future work should involve a longitudinal study (i.e., from mild cognitive impairment (MCI) to mild, moderate, and serious AD) and include more participants in each group to enhance stability of the results. In addition, we should explore the relationship between the changes in the DMN subsystems in the functional and structural network and specific self-referential cognitive ability. Furthermore, we will explore more reasonable ways to implement our research.

CONCLUSION

In summary, this study shows that the memory-related MTL subsystem of the DMN is predominantly disrupted in patients with AD and further that the core and DMPFC subsystems in the DMN are also disrupted to some degree. These findings indicate the different levels of disruption of distinct subsystems of the DMN, possibly providing novel insight into the potential pathogenesis of AD and a possible imaging biomarker for the early diagnosis of AD.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of "GCP and SFDA, Ethics Review Committee of Huashan Hospital affiliated to Fudan University" with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the "Ethics Review Committee of Huashan Hospital affiliated to Fudan University."

AUTHOR CONTRIBUTIONS

HQ and XZ designed the study. HQ analyzed and interpreted the data and drafted the manuscript. HQ and HL revised the manuscript and interpreted the data. XZ, HMH, and HJH acquired the data. All the authors read and approved the final manuscript.

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frontiers in Aging Neuroscience

REVIEW
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The Early Events That Initiate β-Amyloid Aggregation in Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by the development of amyloid plaques and neurofibrillary tangles (NFTs) consisting of aggregated β-amyloid (Aβ) and tau, respectively. The amyloid hypothesis has been the predominant framework for research in AD for over two decades. According to this hypothesis, the accumulation of Aβ in the brain is the primary factor initiating the pathogenesis of AD. However, it remains elusive what factors initiate Aβ aggregation. Studies demonstrate that AD has multiple causes, including genetic and environmental factors. Furthermore, genetic factors, many age-related events and pathological conditions such as diabetes, traumatic brain injury (TBI) and aberrant microbiota also affect the aggregation of Aβ. Here we provide an overview of the age-related early events and other pathological processes that precede Aβ aggregation.

Keywords: Alzheimer's disease, β-amyloid, aggregation, age, diabetes

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INTRODUCTION

Alzheimer's disease (AD) is the most frequent cause of dementia in the elderly. By 2030, the world's AD population will reach more than 70 million (McDade and Bateman, 2017). But no effective treatments can prevent, halt, or reverse AD so far. Pathologically, AD is characterized by the assemblies of extracellular β-amyloid (Aβ) plaques and cytoplasmic neurofibrillary tangles (NFTs) consisting of the microtubule-associated protein tau (Braak and Braak, 1991).

Aβ is generated by the sequential proteolytic cleavage of the much larger amyloid precursor protein (APP) by β-secretase and γ-secretase. In contrast, cleavage of APP by α-secretase precludes Aβ formation. The exact physiological function of Aβ remains unknown. In AD brain, Aβ adopts a highly ordered structure known as cross-β spine, or amyloid (Lührs et al., 2005). Many studies have shown a causal relationship between Aβ and the pathogenesis of AD. The NFTs mainly consist of aggregated tau that bears abnormal posttranslational modifications, including hyperphosphorylation, acetylation, ubiquitylation, truncation and so on. Compared to Aβ, tau deposits correlates better with the degree of cognitive impairment (Goedert and Spillantini, 2006). It is believed that tau functions primarily to stabilize microtubes, and its aggregation in AD causes deficits through a loss-of-function mechanism (Morris et al., 2011). However, recent studies have shown that tau may promote or enhance excitatory neurotransmission by modulating the distribution of synaptic activity-related signaling molecules (Morris et al., 2011).

Currently, the predominant framework of AD research is the amyloid hypothesis. According to the amyloid hypothesis (Hardy and Selkoe, 2002), Aβ is the pathological factor that initiates the onset and progression of AD. Thus, Aβ is proposed to be the target of primary prevention trials (McDade and Bateman, 2017). However, what initially

triggers the aggregation and accumulation of Aβ in AD is unclear. To stop the disease before it starts, we should find the earlier events that precede Aβ aggregation. This review highlights the relationship between risk factors of AD and Aβ aggregation to bring us closer to a comprehensive understanding of the pathogenesis of AD and prevention potential of early events in AD.

ASSEMBLY OF Aβ

Aβ is 40–42 amino acids in length and is formed by proteolytic cleavage of the much larger APP. APP is a transmembrane protein with a single membrane-spanning domain (Glenner and Wong, 1984a,b; Masters et al., 1985), which may have a trophic function (Thornton et al., 2006; Weyer et al., 2011). APP can be cleaved by β-secretase and γ-secretase generating the N terminus and the C terminus of Aβ respectively. During the amyloidogenic process, APP is first cleaved by β-secretase to release the C-terminal fragment (C99), and then C99 is further cleaved by γ-secretase to generate Aβ. In contrast, cleaved by α-secretase precluding Aβ formation. C99 is cleaved at different sites by γ-secretase, resulting in different Aβ profiles (Acx et al., 2014). The major species of Aβ profiles are 40 or 42 amino acids long, and Aβ42 is more aggregation-prone and believed to be the toxic building block of Aβ assemblies.

Aβ adopts a highly ordered structure known as cross-β spine or amyloid (Lührs et al., 2005). The formation of Aβ fibrils can be divided into three phase including nucleation phase, elongation phase and stationary phase (Iadanza et al., 2018). In nucleation phase, oligomeric Aβ forms a nucleus, which can recruit other monomers. As fibrils grow, they can shatter, producing new aggregation-prone species to elongate the fibril. Until nearly all free monomer is converted into a fibrillar form, a variety of insoluble fibrils, oligomers and soluble monomer achieve dynamic balance in the stationary phase. Oligomers are considered to be more pathogenic than mature fiber. However, which Aβ assemblies are most pathogenic is unresolved (Benilova et al., 2012). The fibrils also associate with each other, with other proteins, and with non-proteinaceous factors to form the plaques (Stewart et al., 2017).

Aβ plaques first develop in one or more parts of the basal temporal and orbitofrontal neocortex (Braak and Braak, 1991; Thal et al., 2002; Braak and Del Trecidi, 2015). They were then observed throughout the neocortex, in the hippocampal formation, amygdala, diencephalon and basal ganglia. In severe AD cases, Aβ brain plaque also appears in the mesencephalon, lower brainstem and cerebellar cortex.

Multiple lines of evidence indicate that APP and Aβ contribute causally to the pathogenesis of AD. However, the function of Aβ remains confused. There is evidence that Aβ regulates neuronal and synaptic activity, and that Aβ accumulation in the brain leads to a combination of abnormal network activity and synaptic depression, which can result in excitotoxicity (Palop and Mucke, 2010). Recent studies suggest that Aβ is an antimicrobial peptide, which may play a protective role in innate immunity, and infectious or sterile inflammatory stimuli may drive amyloidosis (Kumar et al., 2016).

THE AMYLOID HYPOTHESIS AND THE PRION HYPOTHESIS

The Amyloid Hypothesis

In 1992, Hardy and Higgins (1992) postulated that "Aβ . . . is the causative agent in AD pathology and that NFTs, cell loss, vascular damage and dementia follow as a direct result of this deposition." This hypothesis has dominated the AD field for more than two decades. A variety of clinical and laboratory evidence supports the hypothesis. The most reliable data supporting the initiator role of Aβ come from genetic studies. The mutations of APP, presenilin-1 (PS1), and PS2, which are involved in Aβ production, cause the autosomal dominant familial AD (fAD; Bettens et al., 2013). Besides, duplication of the APP locus on chromosome 21 in Down syndrome cause age-related dementia with brain parenchymal Aβ deposits (Prasher et al., 1998; Rovelet-Lecrux et al., 2006). Moreover, a rare APP mutation is protective against dementia because it inhibits the production of Aβ and the development of plaques in the brain (Jonsson et al., 2012).

There are also some observations that do not fit easily with the amyloid hypothesis. The main objections can be summed up as the anatomical and temporal discord between Aβ plaque deposition, neuronal death and clinical symptoms in AD (Musiek and Holtzman, 2015). Early neuronal loss regions (entorhinal cortex and hippocampus) are consistent more closely with tau pathology regions than Aβ deposition site (precuneus and frontal lobes), both spatially and temporally (Arriagada et al., 1992; Musiek and Holtzman, 2015). This anatomic disconnection is still not fully explained. However, some studies suggest that the appearance of high-grade cortical tau pathology requires the presence of Aβ aggregation (Price and Morris, 1999; Knopman et al., 2003; Petersen et al., 2006) and tau-mediated toxicity requires trigger from Aβ (West et al., 1994; Gómez-Isla et al., 1996). As for the temporal discrepancy, the neuropathology occurring before symptom onset can be explained as a preclinical AD (Bateman et al., 2012). It is well-accepted that Aβ plays a key role in AD, but it does not exert its effects in a vacuum. The Aβ toxicity involves a complicated network (Musiek and Holtzman, 2015).

The Prion Hypothesis

The amyloid hypothesis cannot adequately explain the progression of Aβ pathology over a long distance. The recent surge of studies shows the misfolded proteins, such as Aβ and tau, have prion-like properties. Therefore, the prion hypothesis was proposed to explain how amyloid aggregates propagate through anatomically connected brain areas. According to the prion hypothesis, Aβ and tau are similar to prion in the cross-β quaternary structure, the mechanism of self-propagation and cell-to-cell transmission, and the ability to form structurally diverse fibrils (strains; Guo and Lee, 2014). The amyloid formation can be divided into two processes, a slow nucleation

phase (the aggregation of the protein into seeds) and a growth phase (the growing fibril break to generate and spread new amyloid seeds; Jucker and Walker, 2013). Also, this seeding process could be homologous or heterologous (Morales et al., 2009), which means oligomers composed of one misfolded protein can promote the polymerization of another protein. This process is termed as "cross-seeding," which may play an essential and yet uncovered role in the origin of AD.

WHAT INITIATES Aβ AGGREGATION?

Genotype of Protein

fAD: APP, PS1, Down Syndrome

Although the cases of fAD account for only less than 1% of total AD, the research of fAD helps us to discover the causative gene defects, including APP and PS1. APP gene is located on chromosome 21. It is well-known that APP gene mutations, duplication of its gene or trisomy of chromosome 21 (Down's syndrome) cause fAD (Prasher et al., 1998). Thirty-nine missense mutations in the APP gene have been described in individuals from Early-onset fAD (Wang Q. et al., 2015), most of which are inside or surrounds the Aβ area. APP mutations either increase total Aβ production or lead to an increased proportion of Aβ42 (Citron et al., 1992; Suzuki et al., 1994). PS1 or PS2 is the catalytic subunit of the γ-secretase protein complex. Mutations in PS1 are the most frequent cause of fAD. The mutations increase the ratio of Aβ42 to Aβ40, which may result from reduced γ-secretase activity (Citron et al., 1997).

sAD: ApoE, BIN1 and TREM2

Most cases of AD are sporadic. Inherited forms of the ε4 allele of Apolipoprotein E (ApoE4) was identified as a major genetic risk factor for sAD. Furthermore, the bridging integrator 1 (BIN1 or amphiphysin2) is the second most important genetic susceptibility locus in late-onset AD after ApoE4 (Tan et al., 2013). Recently, rare mutations in triggering receptor expressed on myeloid cells (TREM2) has received much attention, because one of its variants, R47H, is reported to increase the risk for LOAD by 2–3 folds (Guerreiro et al., 2013).

ApoE

ApoE is a glycoprotein with a molecular weight of 34.2 kDa (Mahley, 1988), which has three isoforms, ApoE2, ApoE3 and ApoE4, in humans (Mahley and Huang, 2006). ApoE is mainly expressed in brain and liver. Astrocytes and neurons have long been recognized as the primary source of ApoE in the brain (Huang, 2006). The primary role of ApoE is to transport lipids and cholesterol in the body. Besides, ApoE also plays a role in mediating synaptogenesis, synaptic plasticity and neuroinflammation (Holtzman et al., 2012). Corder et al. (1993) reported that subjects with an ApoE ε4 allele had an earlier onset clinical dementia in families with AD. Poirier et al. (1993) further confirmed the association in a case-control study of sporadic AD. This conclusion was supported by a series of other reports (Amouyel et al., 1993; Noguchi et al., 1993; Myers et al., 1996), making the ApoE ε4 allele the most important genetic risk factor for AD. In contrast to APP, PS1 and PS2, the presence of ApoE ε4 is not sufficient to cause the disease. Indeed, despite decades of research, the pathophysiological pathway linking ApoE4 to AD remains unclear. To date, the studies suggest that ApoE4 may promote the pathogenesis of AD via Aβ-dependent and Aβ-independent mechanisms.

Aβ-Dependent Mechanisms. ApoE is associated with the formation of amyloid plaques. Lipid-free ApoE3 and ApoE4 can form stable complexes with Aβ peptides. ApoE4 forms complexes with Aβ more efficiently and rapidly than ApoE3 (Huang and Mahley, 2006). Further studies have shown that ApoE binds to residues 12–28 of Aβ and this binding modulates Aβ accumulation, hence affecting disease progression. Peptides that interrupt ApoE/Aβ binding reduced Aβ-related pathology and cognitive improvements in an APP/PS1 transgenic AD mouse model (Liu et al., 2017). On the other hand, lipidated ApoE3 binds Aβ with higher affinity than ApoE4 (Huang and Mahley, 2006), and further studies (Kim et al., 2009) demonstrate that altering ApoE lipidation changes its ability to mediate Aβ clearance or deposition in the brain. Furthermore, recent data describe a novel signal transduction pathway in neurons whereby ApoE activates a non-canonical MAP kinase cascade that enhances APP transcription and amyloid-β synthesis (Huang et al., 2017).

It has been reported that human ApoE regulates Aβ clearance. ApoE2 and ApoE3 clear Aβ more efficiently than ApoE4 (Bales et al., 1999). Also, a C-terminally truncated ApoE4 was found in AD brain, which inefficiently removes Aβ and acts in concert with Aβ to elicit neuronal and behavioral deficits in transgenic mice (Bien-Ly et al., 2011). Overall, ApoE4 may initiate Aβ accumulation through binding with Aβ and decreasing its clearance. Interesting, Wisniewski et al. (1995) isolated Aβ from senile plaques and found that a carboxyl-terminal fragment of ApoE was co-purified. *In vitro*, this fragment could form amyloid-like fibrils. The amyloid-like property of ApoE fragment is reminiscent of the cross-seeding hypothesis. Whether the ApoE fragment initiates Aβ aggregation though cross-seeding needs further investigation.

Aβ-Independent Mechanisms. ApoE4 also impairs synaptogenesis and decreases dendritic spine density. This effect is independent of Aβ accumulation (Dumanis et al., 2009; Brodbeck et al., 2011). Besides, the Aβ-independent roles of ApoE4 also include its detrimental effects on neuronal plasticity, aberrant proteolysis that generates neurotoxic fragments, stimulation of Tau phosphorylation and disruption of the cytoskeleton and impairment of mitochondrial function (Huang, 2010).

BIN1

Except for ApoE, some studies sought associations between biologically plausible candidate genes and risk of sAD. Among them, BIN1 gene has been identified as the most important genetic risk locus in LOAD after ApoE. Interestingly, although BIN1 mRNA level was found to be increased in AD brains, the

protein levels of the longest isoform of BIN1 was decreased, whereas the levels of the shorter BIN1 isoforms were increased (Chapuis et al., 2013; Holler et al., 2014). BIN1 affects AD risk through various pathways, mainly including tau pathology, APP endocytosis/intracellular trafficking, immune/inflammation of the brain, and calcium transients (Tan et al., 2013). Of those, tau pathology is the most studied aspect. BIN1 can interact with tau (Chapuis et al., 2013), and the decline of BIN1 isoform1 promotes the propagation of tau pathology (Calafate et al., 2016). BIN1 is important in the intercellular trafficking of APP, Aβ, ApoE and BACE1 (Tan et al., 2013; Miyagawa et al., 2016; Ubelmann et al., 2017). Miyagawa et al. (2016) found that depletion of BIN1 impaired endosomal trafficking and lysosomal degradation of BACE1, leading to elevated Aβ production. Ubelmann et al. (2017) also found that BACE1 was trapped in tubules of early endosomes and failed to recycle in axons after BIN1 depletion, eventually resulted in increased Aβ production. However, the precise role of BIN1 in the BACE1 recycling remains speculative.

TREM2

TREM2 is a transmembrane protein of the immunoglobulin superfamily that is expressed in mononuclear phagocytes, including microglial in brain (Colonna and Wang, 2016). The main function of TREM2 is regulating the microglial phagocytosis and response to inflammatory stimulation. And the individuals carrying the TREM2 variant R47H have an increased risk for AD by 2–3 folds (Guerreiro et al., 2013).

TREM2 binds to anionic ligands including phospholipids, bacterial LPS, sulfatides and DNA (Daws et al., 2003; Cannon et al., 2012; Wang Y. et al., 2015). Recently, Aβ (Zhao et al., 2018), clusterin (CLU; Yeh et al., 2016) and ApoE (Atagi et al., 2015) are also reported to bind the extracellular region of TREM2. The binding with Aβ oligomers mediates Aβ degradation and downstream signaling (Zhao et al., 2018). Additionally, R47H variant impairs Aβ binding (Zhao et al., 2018). The binding with CLU and ApoE also mediate uptake of lipoprotein-Aβ complexes by microglia. Uptake of lipoprotein-Aβ complexes was reduced in individuals carrying a TREM2 AD variant, R62H (Yeh et al., 2016).

TREM2 associated with the adaptor proteins DNAX-activation protein 10 (DAP10) and DAP12. TREM2-DAP10-DAP12 signaling modulates the energetic cellular metabolism by activating the mechanistic target of rapamycin (mTOR; Xing et al., 2015). TREM2-deficient microglia showed a metabolic defect (Ulland et al., 2017), which may result in the microglia ineffectively responding to stressful events, such as Aβ toxicity. Furthermore, some study crossed the TREM2-deficient mice with developed Aβ-plaque-driven mice, and they found microglia of TREM2-deficient mice failed to cluster around Aβ plaque (Wang Y. et al., 2015; Wang et al., 2016; Yuan et al., 2016; Mazaheri et al., 2017; Ulland et al., 2017). The clustering of microglia around Aβ plaque was of significance to limit the Aβ plaque spreading and protect surrounding neurons (Condello et al., 2015; Wang et al., 2016; Yuan et al., 2016). The study further found that the lack of TREM2 increased the Aβ plaque burden in the 5XFAD model of AD (Wang Y. et al., 2015). And the areas not covered by microglia had a high degree of neural dystrophy (Condello et al., 2015). Instead, elevated expression of TREM2 reduced neural dystrophy in the 5XFAD model of AD (Ulland et al., 2017; Lee et al., 2018). However, a TREM2 deficiency in APP/PS1 mice led to a dramatic reduction in Aβ plaque burden (Jay et al., 2015). The different outcomes may due to the use of different mouse models.

TREM2 also modulates the expression of activation markers in disease-associated microglia. TREM2-deficiency failed to upregulate some activation genes (Keren-Shaul et al., 2017). The partial defect of microglial activation may contribute to the development of AD. Recently, TREM2 was found to alter the degradative process in microglia. Zhao et al. (2018) show that TREM2 KO microglia cause defective clearance of Aβ by disrupting proteasome function. Conversely, Lee et al. (2018) reported that lysosomal degradation was involved in Aβ clearance.

Except for the gene mentioned above, there are several genes related to LOAD, such as PLD3, ABCA7, CASS4, CD33, CD2AP, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB5-DBR1, INPP5D, MS4A, MEF2C, NME8, PICALM, PTK2B, SLC24H4 RIN3, SORL1, ZCWPW1 (Karch and Goate, 2015). But the relationship with Aβ aggregation is not fully clarified.

Age-Related Process

Aging is the primary non-genetic risk for sporadic AD, but little is known about how aging affects Aβ generation. Recent summit addressed seven main hallmarks of the basic aging process (Kennedy et al., 2014), including decreased adaptation to stress, loss of proteostasis, stem cell exhaustion, metabolism, macromolecular damage, unfavorable epigenetic and impaired inflammaging. In this review article, we will focus on the mechanism of age-related pathologic process initiating Aβ aggregation.

Age-Related Neuronal Stress

Several stress-related signaling pathways are related to AD, such as oxidative stress (Arimon et al., 2015) and nitrosative stress (Guix et al., 2012).

Aging is usually accompanied by accumulation of reactive oxygen species (ROS; Finkel and Holbrook, 2000). Generally, ROS function as messenger and are kept at low level. Excessive amounts of ROS accumulation is defined as oxidative stress, which will damage various cell components. In the brain of AD, ROS production and the level of oxidative stress markers are elevated (Krstic and Knuesel, 2013). Furthermore, lipid peroxidation precedes Aβ deposition (Pratico et al., 2001), suggesting the oxidative stress may be an initiator of Aβ pathology. Further study found that lipid peroxidation product 4-hydroxynonenal (4-HNE) elevated γ-secretase activity and Aβ production, resulting in Aβ and neurodegenerative pathologies in AD (Gwon et al., 2012). Another study also found the β-secretase activity was affected by 4-HNE (Arimon et al., 2015). Oxidative stress was also reported to cause pathogenic PS1 conformational change

(Wahlster et al., 2013) and induce Aβ aggregation (Siegel et al., 2007). During the oxidative stress process, superoxide anions react with nitric oxide generating peroxynitrite, which causes nitrosative stress. Peroxynitrite-triggered nitrotyrosination is especially relevant in AD (Smith et al., 1997). Guix et al. (2012) found that the secretion of Aβ is enhanced in an *in vitro* model of neuronal aging. This is associated with an increase in γ-secretase complex formation. Moreover, the age-related nitrative stress promoted the nitrotyrosination of PS1, which is associated with an increased association of the two PS1 fragments, PS1-CTF and PS1-NTF. Further, it raised the Aβ42/Aβ40 ratio.

Repressor element 1-silencing transcription (REST) is also involved in the neuronal stress response. Lu T. et al. (2014) showed that elevated REST levels are associated with the preservation of the ordinary aged people from AD. REST is induced in the aging human brain and regulates a network of genes that mediate cell death, stress resistance and AD pathology. At early stages of AD, REST is lost from the nucleus, resulting in dysregulation of the gene network, including the γ-secretase complex members PS-2 and pen-2, which are implicated in Aβ generation. Interesting, the study also found that aging individuals who harbor substantial AD pathology do not appear to progress to dementia when neuronal REST levels are high.

Age-Related Inflammation

Aging is characterized by chronic, systemic, low-grade inflammation (Franceschi et al., 2017). Inflammation is considered to contribute to and exacerbate AD pathology (Gandy and Heppner, 2013; Sudduth et al., 2013; Sarlus and Heneka, 2017). The inflammation in AD includes microglia and astrocytes dysfunction (Xia et al., 2018). One study using viral mimic to stimulated the systemic immune system found the deposition of APP and its proteolytic fragments (Krstic et al., 2012). Some AD-related chronic disease, such as obesity and type 2 diabetes, will lead to the systemic inflammatory condition, which further increases the risk of AD (Takeda et al., 2010; Thaler et al., 2012).

Microglia play the most important role in inflammatory responses in AD. In physiological condition, microglia remove the apoptotic neurons and prune the synaptic connections to keep the normal development of CNS (Paolicelli et al., 2011; Hong et al., 2016). In response to neuropathological insults, including Aβ, microglia alter its morphology and proliferate, express inflammatory markers, phagocytose dead cells and myelin debris, secrete cytokines and neurotrophic factors (Lue et al., 2010). This process is termed as microglial activation. Furthermore, the activation plays a dual role in AD pathogenesis. On the one hand, microglia increase phagocytosis or clearance of Aβ. On the other hand, the persistent production of Aβ leads to the chronic activation of microglia and drive further amyloid deposition (Hickman et al., 2018). Microglia-Aβ interactions lead to NACHT-, LRR- and pyrin (PYD)-domain-containing protein 3 (NLRP3) inflammasome activation (Heneka et al., 2013). After activation, NLRP3 recruits the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), triggering ASC helical fibrillar assembly (Lu A. et al., 2014). ASC rapidly bind to Aβ and increase the formation of amyloid-β oligomers and aggregates by a cross-seeding mechanism (Venegas et al., 2017). Under what conditions the microglia play a positive function, and how can we keep the microglia inflammatory response in check and promote clearance of Aβ without accelerating Aβ aggregation remain to be investigated.

Astrocytes also participate in neuroinflammation in AD. Aβ deposition might be a potent trigger of astroglia activation in AD because the cells surround Aβ plaques (Medeiros and LaFerla, 2013). One study found that reducing astrocyte activation in APP/PS1 mice decreased the amyloid levels and ameliorated cognitive and synaptic function (Furman et al., 2012). The result suggested that astrocyte activation may play a deleterious role in AD. However, it has been confirmed that astrocytes can bind and degrade Aβ (Wyss-Coray et al., 2003). But in some mouse models of AD astrocytes show atrophy (Olabarria et al., 2010), which might result in reduced clearance of Aβ. Overall, inflammation plays a complex but important role in AD.

Age-Related Disturbances in Proteostasis

Aging is related to a functional decline in protein homeostasis (proteostasis) machinery, contributing to the development of protein misfolding in AD. The proteostasis network (PN) maintains protein homeostasis by controlling the levels of functional proteins and preventing their aggregation. The process is achieved by three branches of the PN, including protein synthesis, the chaperone pathways for the remodeling of misfolded proteins and protein disaggregates, and the protein degradation pathways (Hipp et al., 2014). mTOR pathway acts as a central pathway regulating protein synthesis (Saxton and Sabatini, 2017). Numerous studies have shown that inhibition of mTOR activity could extend lifespan among mammalian species, suggesting mTOR may regulate aging (Antikainen et al., 2017). Activation of mTOR has been recognized as a major event causing the onset of AD (Yates et al., 2013). The activation of mTOR downregulates the autophagy, resulting in reduced clearance of Aβ and elevated Aβ accumulation (Nixon, 2013), and elevates the expressions of β-secretase and γ-secretase by AMPK and IGF-1 pathway (Cai et al., 2015). Aβ aggregates are believed to be detoxified by DAF-16 regulated active aggregation activity that assembles small oligomers into large, less toxic structures and HSF-1 mediated disaggregation and degradation activity (Taylor and Dillin, 2011). Also, DAF-16 and HSF-1 and effector molecules such as kinase mTOR can regulate aging.

Diabetes

Numerous epidemiological studies suggest that diabetic patients have a significant risk of developing AD (Arvanitakis et al., 2004; Luchsinger et al., 2005; Fukazawa et al., 2013). T2DM increases the risk of dementia by 50%–150% (Strachan et al., 1997; Biessels et al., 2006). However, the underlying mechanisms with clinical relevance remain to be elucidated. Several mechanisms have been proposed, including insulin and insulin-like growth factor (IGF) resistance, glucose toxicity, oxidative stress and mitochondrial dysfunction. Here we focus on the potential mechanisms by which diabetes affects the initiation of Aβ aggregation.

Insulin and IGF Resistance

Insulin and IGF signaling is involved in synaptic plasticity, and the organization and function of the brain, playing neuromodulatory and neurotrophic roles (Baglietto-Vargas et al., 2016), and hence may play an essential role in learning and memory. Several studies suggest that insulin and IGF resistance participate in AD pathogenesis (Correia et al., 2011; Cholerton et al., 2013). AD patients have lower brain levels of insulin and insulin receptor (IR), and insulin signaling impairments have been documented in postmortem brain and animal models of AD (Steen et al., 2005; Lester-Coll et al., 2006; Moloney et al., 2010). These abnormalities were associated with increased APP mRNA expression (Steen et al., 2005). A novel study reveals that insulin deficiency alters APP processing by increasing the expression of BACE-1 and accompanied by increased translational upregulation of APP through the PERK-eIF2α phosphorylation pathway (Devi et al., 2012). Another study found that insulin resistance might alter APP processing through autophagy activation (Son et al., 2012). Besides, insulin signaling provides a physiological defense mechanism against Aβ oligomer-induced synapse loss through downregulating oligomer binding sites in neurons (De Felice et al., 2009). Insulin also has multiple anti-amyloidogenic effects on human neuronal cells, including preventing the translocation of the APP intracellular domain fragment into the nucleus, increasing the transcription of anti-amyloidogenic proteins, and increasing the α-secretase-dependent APP-processing pathway (Pandini et al., 2013). On other hand, Aβ oligomers can inhibit insulin signaling via the JNK/TNFα pathway (Bomfim et al., 2012), suggesting a positive feed-forward mechanism.

Hyperglycemia

Chronic hyperglycemia characterizes diabetes, and several lines of evidence suggest that hyperglycemia has toxic effects on the brain (Gispen and Biessels, 2000; Kerti et al., 2013). Epidemiological studies show that hyperglycemia individuals had a higher risk of AD and exhibited higher conversion rate from mild cognitive impairment (MCI) to AD, indicating that hyperglycemia might be responsible for AD onset and progression (Crane et al., 2013; Morris et al., 2014). Hyperglycemia may have toxic effects on neurons through several mechanisms, including the direct impact on Aβ, the formation of advanced glycation end products (AGEs; Umegaki, 2014), osmotic insult and oxidative stress.

Hyperglycemia directly raises interstitial fluid (ISF) Aβ levels via altering neuronal activity, which increases Aβ production. K_{ATP} channel impairments mediate hyperglycemia-induced neuronal excitability and increased ISF Aβ (Macauley et al., 2015). Hyperglycemia could also directly inhibit APP protein degradation and enhance Aβ production (Yang et al., 2013). Moreover, hyperglycemia accelerates Aβ aggregation through the formation of AGEs. AGEs are generated by a non-enzymatic reaction of glucose, free amino groups, lipids, and nucleic acids (Singh et al., 2001; Sims-Robinson et al., 2010). The receptors for AGEs (RAGE) are highly expressed in both microglia and neurons and are responsible for the pathological consequences (Lue et al., 2001). Aβ is a RAGE ligand, and Aβ-RAGE interaction exaggerates neuronal stress, accumulation of Aβ, impaired learning and memory and neuroinflammation (Chen et al., 2007). Additionally, AD patients with diabetes (ADD) have higher levels of AGEs than non-diabetic AD individuals (Valente et al., 2010). And RAGE is also demonstrated as a cofactor for Aβ-induced neuronal perturbation in an AD model (Arancio et al., 2004). Oxidative stress is also a key player in diabetes and AD (Moreira, 2012). Oxidative stress stimulates APP gene expression and modulates its processing via modulating γ- and β-secretases (Jolivalt et al., 2010; Oda et al., 2010), which contributes to Aβ aggregation.

Cross-Seeding

Islet amyloid polypeptide (IAPP) form β-sheet aggregates in the pancreas in type 2 diabetes (Pillay and Govender, 2013). Interestingly, IAPP deposits are also found in the brain tissue of patients with AD (Jackson et al., 2013). IAPP and Aβ share similar β-sheet secondary structures, and they are 25% identical in amino acid sequence and have a high binding affinity to each other (Andreetto et al., 2010). Emerging evidence indicates that cross-amyloid interactions may play a key role in Aβ aggregation, including the interaction of Aβ-tau (Guo et al., 2006), the Aβ-α-synuclein (Westermark et al., 1996), and the Aβ-IAPP interaction (Andreetto et al., 2010). The potential mechanisms of IAPP-induced AD development include the independently toxic effects, lose the physiological function of soluble IAPP in the brain, and interacting with Aβ (Zhang and Song, 2017). In this part, we will focus on the mechanism of cross-interaction of Aβ and IAPP.

In vitro Evidence

O'Nuallain et al. (2004) first studied the seeding efficiency between Aβ(1–40) and amyloid fibrils produced from IAPP. They found the IAPP fibrils are poor seeds for Aβ(1–40) elongation. But it is difficult to gauge whether low cross-seeding efficiency might be biologically significant. Later, Yang et al. (2013) found both nucleation and fibrillization of Aβ/hIAPP mixtures (1:1) were slower than pure Aβ or pure hIAPP. And they suggest that the cross-seeding of Aβ and hIAPP was less efficient than homologous seeding of pure Aβ or IAPP. However, a study investigating lipid membranes got different results (Seeliger et al., 2012). They found the aggregation kinetics of Aβ/IAPP mixtures was slower than that of hIAPP, but faster than that of Aβ. Hu et al. (2015) found the cross-seeding of Aβ/IAPP led to the retard of peptide aggregation at the nucleation stage due to structural incompatibility between different amyloid aggregates, and the acceleration at final fibrillation stage due to the formation of similar seed structures as templates for promoting cross-seeding. Another *in vitro* study found that the fibrils of amyloidogenic proteins, including IAPP, functioned as seeds in the Aβ aggregation, and the seeds accelerate the Aβ aggregation pathway. Also, E3, R5, H13, H14 and Q15 of Aβ are common binding regions between the Aβ monomer and the fibrillar seeds including IAPP (Ono et al., 2014). Andreetto et al. (2010) studied the cross- and self-interaction interface of Aβ and IAPP by using membrane-bound peptide arrays and fluorescence titration assays. They identified five

short peptide segments of Aβ and IAPP as hot regions of the Aβ-IAPP cross-interaction interface, including Aβ(27–32), Aβ(35–40), Aβ(19–22), IAPP(8–18) and IAPP(22–28), and these peptides also mediate the self-interaction of Aβ and IAPP. They suggested that hetero- and self-association of Aβ and IAPP most likely occur competitively. Overall, the *in vitro* studies investigated the seeding efficiency and the interaction regions of hetero- and homo-seeding, though some results are contradictory. However, amyloid fibrils *in vivo* are functionally different from fibrils grown *in vitro*. So, it is hard to draw biological conclusions from studies only *in vitro*.

In vivo Evidence

Moreno-Gonzalez et al. (2017) investigated whether IAPP could accelerate Aβ aggregation *in vitro* and *in vivo*. They found that the addition of pre-formed IAPP aggregates to Aβ40 monomers can accelerate the misfolding of Aβ compared with unseeded Aβ monomers *in vitro*, which is consistent with the former studies. Their *in vivo* study found that the transgenic animals expressing human IAPP and mutant APP showed increased Aβ burden in the hippocampus and cortex compared to AD transgenic mice or AD transgenic animals with type 1 diabetes. Additionally, IAPP colocalizes with amyloid plaques in the transgenic mice express hIAPP and mutant APP. Furthermore, injection of pancreatic IAPP aggregates into the APP-mutant mice resulted in more Aβ burden in the cortex and hippocampus and greater memory impairments than untreated animals. Based on these results, the team provided a hypothesis that IAPP aggregates can accelerate the transformation process of Aβ by recruiting the normal soluble protein into the growing aggregates, thereby accelerating or exacerbating the pathological features of AD.

Microbes

The microbiota is a newly discovered human organ that weighs about 1.5 kg, and contains approximately 90% of the cells of the human body, with a genetic repertoire that exceeds 100%–200% of the remaining organisms (Qin et al., 2010). The microbes inhabit different locations, including gut, skin, nose and vagina. The gut harbors the highest concentrations of microbiota so far and is the best-studied habitat (Scheperjans, 2016). In recent years, many studies found the association between gut microbes disorders and other disorders including diabetes, obesity, arthritis, allergy, cardiovascular and neurodegeneration diseases. The mechanisms through which gut bacteria influence central process include the neurotransmitters synthesized by gut bacteria, the activation of immune system, the metabolites, such as short-chain fatty acids and amyloid (Sherwin et al., 2018). Except for the microbiota, specific microbes including herpes simplex virus type1 (HSV1), *Chlamydia pneumoniae* and several types of spirochaete which were found in the aging human brain also play a role in the etiology of AD (De Chiara et al., 2012; Balin and Hudson, 2014; Itzhaki, 2014; Miklossy, 2015). In this review article, we will focus on the part of the activation of immune system in the initiation of Aβ.

The microbes of human microbiome can release large amounts of lipopolysaccharides (LPS), which might play a role in the production of proinflammatory cytokines related to the pathogenesis of AD (Pistollato et al., 2016). LPSs may modify gut homeostasis, gut inflammation, and gut permeability (Hufnagel et al., 2013). Additionally, the presence of bacterial LPS or endotoxin-mediated inflammation actively contributes to the potentiated fibrillogenesis of Aβ by stimulating fibril elongation (Asti and Gioglio, 2014) and attenuated amyloid clearance by down-regulating TREM2 (Zhao and Lukiw, 2015). Bacterial amyloid is considered as a pathogen-associated molecular pattern (PAMP) and induces activation of toll-like receptor-2 (TLR2) and other inflammatory mediators including NF-κB, as well as TLR1 and CD14 (Tukel et al., 2010; Nishimori et al., 2012). Besides, the activated inflammatory reaction caused by microbiome species, and their secretory products have shown to intensify the aggregation of amyloids into senile plaque lesions (Smith et al., 1996; Zhao et al., 2018).

Allen (2016) provided a novel hypothesis about the production of Aβ induced by spirochetes. They found spirochetes and innate immune system activity in the brains of AD patients. Additionally, they suggested that the innate immune system first responder TLR2 and its major pathway (MyD88) activates the secretases which generate Aβ. Interestingly, Aβ has been shown to be antimicrobial (Soscia et al., 2010). Therefore, they suggested that Aβ is generated for the purpose to rid the body of the spirochetal parasites, and the damages of tissue, as well as the neuronal circuits, are the adverse reactions. Kumar et al. (2016) gave a more detailed explanation of the antimicrobial role of Aβ. They found a rapid seeding and accelerated Aβ deposition after Salmonella Typhimurium bacteria infections in 5XFAD mice. And they showed that Aβ fibrils mediated adhesion inhibition and agglutination activities against Candida. This novel perspective deepens our understanding of the enigmatic role of Aβ in the etiology of AD.

Traumatic Brain Injury

Traumatic brain injury (TBI) is an universal health and socioeconomic problem. The risk of AD is increased in moderate and severe head injury for 2.3 and 4.5 times, respectively (Plassman et al., 2000). Besides, a growing number of epidemiological studies have considered TBI as one of the most potent risk factors for AD (Molgaard et al., 1990; O'Meara et al., 1997; Guo et al., 2000; Fleminger et al., 2003). However, two recent studies found that a history of TBI was not associated with AD or the Aβ deposition (Crane et al., 2016; Weiner et al., 2017). The conflicting result may due to the difference in severity and frequency of TBI, which may result in different neuropathologic outcomes.

Numerous studies have shown that TBI can trigger rapid and insidiously progressive AD-like pathological process, such as the production and accumulation of Aβ (Johnson et al., 2010). Up to 30% of patients who die from TBI have the Aβ plaques in their brain (Roberts et al., 1991, 1994). But the mechanism by which TBI induce Aβ accumulation is still obscure. The most common pathologies of TBI is diffuse axonal injury, which causes an accumulation of proteins in the axon, including APP (Gentleman et al., 1993; Gorrie et al., 2002).

Also, PS-1 and BACE1 were found in injured axons after TBI (Uryu et al., 2007; Chen et al., 2009). Furthermore, high APP production following TBI may increase β-secretase processing and Aβ genesis (Lou et al., 2017), due to the saturation of normal α-secretase processing pathway (Gentleman et al., 1993; Graham et al., 1996). TBI also induces Aβ genesis via oxidative-stress-mediated upregulation of BACE1 (Tamagno et al., 2005; Guglielmotto et al., 2009). TBI is accompanied by hypoperfusion, vascular dysfunction and ischemia (Ramos-Cejudo et al., 2018), which may play an important role in Aβ deposition. It has been shown that transient hypoxia elevated plasma Aβ42 levels (Gren et al., 2016); reduced blood flow activated β-secretase and γ-secretase (Pluta et al., 2013); metabolic acidosis after TBI could potentially contribute to Aβ accumulation due to the fact that Aβ is prone to aggregation in a pH-dependent manner (Acharya et al., 2016). S100A9-driven amyloid-neuroinflammatory cascade may be also involved in the accumulation of Aβ. S100A9 is an amyloidogenic protein associated with inflammation, which was found to form amyloid plaques itself in TBI (Wang et al., 2018) and AD (Shepherd et al., 2006). Recently, S100A9 was found abundant in TBI human brain tissue compared to Aβ and contributed to Aβ plaque formation (Wang et al., 2018). Another study also found S100A9 also co-aggregated with both Aβ40 and Aβ42 and promoted their amyloid deposition (Wang et al., 2014). How S100A9 interact with Aβ and whether aggregation of S100A9 could serve as seeds to accelerate aggregation of Aβ need a deep investigation.

Others

Beside processes mentioned above, many factors may influence the production and accumulation of Aβ. For example, dietary fats may affect cerebrovascular integrity and alter Aβ kinetics across the blood-brain barrier (Takechi et al., 2010). Sex hormones also have effects on Aβ pathology (Grimm et al., 2016). Women exhibit a greater vulnerability to AD (Mielke et al., 2014) and a more striking Aβ deposition compared to men (Corder et al., 2004). Additionally, olfactory impairment subjects have more Aβ accumulation than normal people (Vassilaki et al., 2017).

CONCLUSIONS

A wealth of studies supports the amyloid hypothesis that Aβ is the initiator of a complex network of pathologic changes in the brain. And many earlier events precede Aβ aggregation. The best way to eliminate the Aβ pathology is to stop it from taking hold in the first place. Although much has been learned, many important questions remain. How do the early events initiate Aβ aggregation? How can we prevent it? What is the target point? When should measures be taken? How to explain the pathogenesis of AD-like dementias without Aβ, and how to avoid it? The answers to these questions might bring us to find safe and effective treatments for AD.

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REVIEW
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The Role of APOE4 in Disrupting the Homeostatic Functions of Astrocytes and Microglia in Aging and Alzheimer's Disease

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APOE4 is the greatest genetic risk factor for late-onset Alzheimer's disease (AD), increasing the risk of developing the disease by 3-fold in the 14% of the population that are carriers. Despite 25 years of research, the exact mechanisms underlying how APOE4 contributes to AD pathogenesis remain incompletely defined. APOE in the brain is primarily expressed by astrocytes and microglia, cell types that are now widely appreciated to play key roles in the pathogenesis of AD; thus, a picture is emerging wherein APOE4 disrupts normal glial cell biology, intersecting with changes that occur during normal aging to ultimately cause neurodegeneration and cognitive dysfunction. This review article will summarize how APOE4 alters specific pathways in astrocytes and microglia in the context of AD and the aging brain. APOE itself, as a secreted lipoprotein without enzymatic activity, may prove challenging to directly target therapeutically in the classical sense. Therefore, a deeper understanding of the underlying pathways responsible for APOE4 toxicity is needed so that more tractable pathways and drug targets can be identified to reduce APOE4-mediated disease risk.

Keywords: APOE, Alzheimer's disease, astrocytes, microglia, aging

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INTRODUCTION

Alzheimer's disease (AD) is a devastating neurodegenerative disease of aging, the incidence of which is expected to increase exponentially as the proportion of the population over the age of 65 increases. Research in AD drug discovery has historically focused on the Amyloid Hypothesis, based primarily on findings from early-onset AD, which is caused by mutations in amyloid-β (Aβ) pathway proteins and which accounts for <2% of all AD cases. While the Amyloid Hypothesis predicts that enhanced production and diminished clearance of Aβ causes AD, therapeutics aimed at modulating Aβ levels have largely failed, although they have not yet been tested at presymptomatic stages of disease (Doig et al., 2017).

After aging, the ε4 allele of the *APOE* gene is the next greatest risk factor for AD, while the relatively rare ε2 allele confers AD protection (Corder et al., 1993; Saunders et al., 1993; Strittmatter et al., 1993). Although 25 years have passed since it was identified, there are still no approved drugs directly targeting APOE4, due partly to the inherent "undruggability" of lipoproteins. However, the atherosclerosis field has demonstrated that indirectly modulating the effect of lipoproteins can be a successful alternative strategy. For example, statins affect lipoprotein composition and disease risk by targeting a metabolic pathway (cholesterol synthesis); similarly, understanding the downstream pathways that mediate APOE4 disease risk might identify more tractable therapeutic targets for treating APOE4-mediated AD.

APOE in the brain is primarily expressed by astrocytes and microglia, and APOE4 expression alters the normal function of both of these glial cell types, potentially contributing to AD risk. Although the toxicity associated with APOE4 likely involves the impaired ability of APOE4-expressing glia to efficiently clear Aβ, it is also apparent that there are Aβ-independent effects on normal glial physiology. The role of APOE in mediating Aβ levels has been discussed in depth elsewhere (Ries and Sastre, 2016), and will only be briefly touched upon below. This review will instead focus on more recent findings that specifically describe the role of APOE in glial biology, in addition to and independent of Aβ modulation, particularly during aging, and will describe pathways in each glial cell type that may link APOE to disease pathogenesis.

Although astrocytes and microglia are the primary producers of APOE, whether an interaction between these cells exists in terms of APOE biology has not been carefully examined. Cross-talk between astrocytes and microglia in neurodegeneration is well-known (Jha et al., 2018); for example, astrocytes can secrete complement factor C3 in response to Aβ, which can then activate microglia *via* the C3a receptor (Lian et al., 2016). On the other hand, lipopolysaccharide-stimulated microglia can induce neurotoxic "A1" reactive astrocytes, as opposed to neurotrophic "A2" reactive astrocytes (Liddelow et al., 2017). The same group found that A1-type astrocytes are present in aging (Clarke et al., 2018) and AD brain (Liddelow et al., 2017), and that A1 astrocytes not only lose the neurotrophic capacity of A2 astrocytes, but also actively produce a neurotoxin to kill neurons and oligodendrocytes. Importantly, a recent study demonstrated that blocking this microglial-dependent induction of A1 astrocytes is protective in mouse models of Parkinson's disease (Yun et al., 2018). Whether blockade of such microglia/astrocyte cross-talk can help ameliorate neurodegeneration in humans and in AD has yet to be demonstrated. Furthermore, whether APOE is one such secreted factor that mediates interactions between astrocytes and microglia has not been reported, nor has a synergistic effect of APOE from each cell type been clearly defined. Even so, since both astrocytes and microglia express APOE, this review article will separately consider specific aspects of each cell type's normal physiology that might be impacted by APOE4 expression in aging and AD.

OVERVIEW OF APOE ISOFORMS

APOE is a lipoprotein that normally facilitates lipid transport between cells (Mahley, 1988). *APOE* transcription is activated by liver X receptor (LXR) and peroxisome proliferator-activated receptor γ (PPARγ), transcription factors that regulate lipid homeostasis and inflammation (Laffitte et al., 2001; Akiyama et al., 2002; Liang et al., 2004; Mandrekar-Colucci et al., 2012; Moutinho et al., 2019). In the lipid-rich brain, APOE is predominantly expressed by astrocytes and microglia, and perhaps in limited circumstances by neurons (Boyles et al., 1985; Pitas et al., 1987; Uchihara et al., 1995; Nakai et al., 1996; Xu et al., 1998, 2006).

The human *APOE* gene exists as three different alleles, ε2, ε3, and ε4, which are present at ∼7%, 79%, and ∼14%, respectively, in the entire population (Bertram et al., 2007), and which exhibit differences in lipid and receptor binding efficiency. The presence of one ε4 allele increases the risk of AD by threefold, while carriers with two ε4 alleles are eight times as likely to develop AD compared to those without any ε4 allele; and ε4 is associated with an earlier age of disease onset, from about 85 years without any ε4, to 75 years with one and 68 years with two ε4 alleles (Corder et al., 1993). These statistics make *APOE* ε4 the greatest known genetic risk factor for AD, more than any other gene to date. In contrast to the human gene, mouse *ApoE* exists as only one isoform, and the structure of the mouse APOE protein more closely matches human APOE3 (Raffai et al., 2001); targeted-replacement mice, in which the endogenous mouse *ApoE* gene has been replaced with either of the human *APOE* isoforms, have therefore been created to study differences in human APOE isoform function, and will be referred to throughout this review article (Sullivan et al., 1997; Knouff et al., 1999).

The three human APOE isoforms differ from one another in the protein sequence at amino acid positions 112 and 158 (**Figure 1**; Mahley, 1988; Raffai et al., 2001; Hatters et al., 2006). These single amino acid differences are enough to change the lipid and receptor binding ability of APOE (Weisgraber et al., 1982; Dong and Weisgraber, 1996; Gong et al., 2002). Specifically, R112 in APOE4 creates a domain interaction between the N-terminal receptor binding domain and the C-terminal lipid binding domain, preventing efficient binding to HDL compared to APOE2 and APOE3, with preferential binding to VLDL (Dong et al., 1994; Dong and Weisgraber, 1996). While APOE2 is protective against AD, the ε2 allele is also associated with hyperlipoproteinemia III, which is characterized by accumulated lipoproteins in the plasma and development of atherosclerosis (Giau et al., 2015). This is thought to be caused by impairment in the receptor binding region of APOE2, leading to delayed lipoprotein clearance and increased triglyceride and cholesterol levels (Havel and Kane, 1973; Weisgraber et al., 1982; Mahley and Rall, 2000). In the context of AD, APOE2 has been relatively understudied, although some research is ongoing (Wu and Zhao, 2016). It should be noted that APOE2 in many experimental settings is similar to APOE3 or performs qualitatively better (such as in amyloid clearance). For clarity, and because there is much less in the literature to explain the mechanism of action of APOE2, the present review will focus on different phenotypes conferred by APOE3 vs. APOE4.

APOE Isoforms and Amyloid Clearance

Both astrocytes and microglia clear Aβ (Paresce et al., 1996; Wyss-Coray et al., 2003; Ries and Sastre, 2016) and although there is some evidence that APOE4 may enhance Aβ production (Ye et al., 2005), it is widely thought that APOE4 confers AD risk through deficient Aβ clearance compared to APOE3 and APOE2 (Koistinaho et al., 2004; Deane et al., 2008; Simonovitch et al., 2016), although not necessarily *via* direct binding (Verghese et al., 2013). APOE isoforms differ not only in

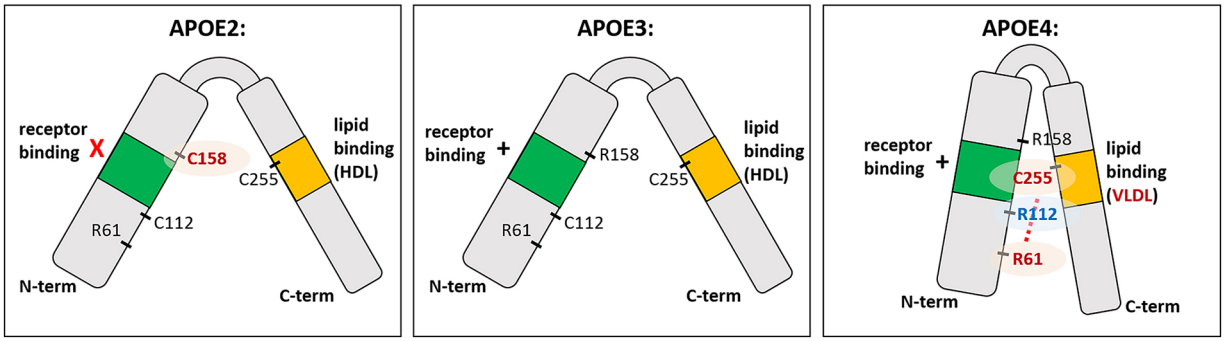

FIGURE 1 | The structure of APOE isoforms. APOE is a soluble secreted protein, with N-terminal and C-terminal domains linked by a central hinge region. The N-terminal domain contains the receptor binding domain (indicated in green), and the C-terminal domain contains the lipid binding region (indicated in orange). Each isoform differs from one another at amino acid position 112 and 158. Cysteine at position 158 (C158) in APOE2 is thought to cause deficient receptor binding, while arginine at position 112 (R112) in APOE4 changes the conformation of the entire domain such that R61 is exposed and interacts with C255 in the C-terminal domain (red dotted line). This "domain interaction" is thought to be the biophysical basis for differences in APOE4 function compared to the other isoforms; e.g., preference for VLDL over HDL. In APOE3 and APOE2, which have C112 instead of R112, the R61 is not exposed and there is no such domain interaction.

lipid binding ability, but also in affinity for specific APOE receptors (Ruiz et al., 2005; Holtzman et al., 2012). LRP1, a major receptor for APOE, mediates Aβ clearance in astrocytes and pericytes (Liu et al., 2017a; Ma et al., 2018), and astrocytes expressing APOE4 have reduced LRP1 surface expression, which could explain impaired amyloid clearance *in vivo* (Prasad and Rao, 2018). However, astrocytes also utilize other APOE receptors such as LDLR for Aβ clearance, but in an APOE-independent manner (Basak et al., 2012); furthermore, Aβ is cleared by transcytosis across the blood brain barrier, glymphatic and interstitial fluid bulk flow, and by extracellular degrading enzymes, highlighting the complexity around understanding how APOE4 contributes to amyloid accumulation.

APOE Isoforms and Tau Pathology

In addition to modulating Aβ, APOE also affects tau pathology, another hallmark of AD, in an isoform-specific manner. APOE4 worsens tau pathology in the P301S tau mouse model, and APOE4 genotype is associated with exacerbated neurodegeneration in human primary tauopathies (Shi et al., 2017). APOE4 status is associated with tau pathology particularly in instances when amyloid pathology is also present (Farfel et al., 2016). The relationship between APOE4 carrier status and CSF tau levels is more robustly correlated in women than in men (Hohman et al., 2018), suggesting a possible sex effect in APOE4-mediated toxicity. Neurons expressing P301S tau are less viable when co-cultured with APOE4-expressing glia compared to APOE2- or APOE3-expressing glia, while co-culture with APOE$^{-/-}$ glia leads to the greatest neuronal viability, supporting the idea that APOE4 represents a toxic gain-of-function (Shi et al., 2017). Higher CSF tau levels are associated with faster disease progression and reduced cortical plasticity in patients, but only in APOE4 carriers (Koch et al., 2017), further cementing a role for APOE4 in exacerbating tau pathology. Since some evidence suggests that APOE can be expressed by neurons under stress (Xu et al., 1998, 2006; Harris et al., 2004), it is possible that neuron-derived APOE4 directly mediates tau toxicity in neurons, but the above data suggests that glia-derived APOE4 is likely contributing as well.

Amyloid- and Tau-Independent Effects of APOE4: Glial Cell Biology

The role of APOE4 in neurological disease is certainly broader than the clearance or response to misfolded proteins, including Aβ and tau; for example, APOE receptors play diverse roles in brain physiology independent of Aβ (Holtzman et al., 2012) and APOE4 carriers may be susceptible to disorders that do not involve proteinopathy, such as chemotherapy-induced cognitive dysfunction (Mandelblatt et al., 2018; Speidell et al., 2019). In addition to the role of APOE4 derived from astrocytes and microglia, a growing body of literature also supports a role for APOE4 and pericytes at the blood brain barrier in neurovascular unit dysfunction and AD pathogenesis (Casey et al., 2015; Soto et al., 2015; Halliday et al., 2016; Ma et al., 2018). APOE has even been proposed to be proteolytically cleaved to form either cytotoxic or neuroprotective fragments, in a cell type- and isoform-specific manner (Brecht et al., 2004; Muñoz et al., 2018). Thus, the neurotoxicity conferred by APOE4 in AD may not be solely due to its effects on amyloid or tau pathology, but also to its effects on normal glial functions. How these processes fit into the current understanding of APOE function and neurodegeneration will be important for drug discovery efforts targeting APOE biology to treat AD.

Astrocytes play critical roles in brain lipid and energy metabolism, and both microglia and astrocytes have important immune functions in the brain. APOE4 expression in each of these cell types likely disrupts these pathways, ultimately leading to brain dysfunction in addition to any Aβ- and tau-mediated effects. The role of APOE4 and aging in each of these cell types and pathways will now be examined individually.

APOE AND ASTROCYTE BIOENERGETICS

The idea that APOE isoforms differentially mediate astrocyte bioenergetics has gained increasing support in recent years and implies that APOE4-expressing astrocytes have deficient lipid and glucose metabolism, impairing their ability to support energy-demanding neurons, particularly during aging. In the following sections, we will describe different aspects of lipid homeostasis and glucose metabolism in astrocytes, and how APOE may be involved in these processes.

Astrocytes and Lipid Homeostasis in the Aging and AD Brain

The most well-studied aspect of APOE biology in AD is lipid transport, which neurons rely upon for their proper function. Lipid homeostasis is clearly altered in AD: in his first description of the disease, Alois Alzheimer noted that "many glial cells show adipose saccules" (Alzheimer et al., 1995), and lipid accumulations are present in both human AD brain and in an AD mouse model (Hamilton et al., 2015), as well as in the aging mouse brain (Shimabukuro et al., 2016). Given that the brain is the most lipid-rich organ outside of adipose tissue (O'Brien and Sampson, 1965), it is therefore not surprising that lipoproteins, cholesterol and lipid homeostasis are critical for normal brain function, including neuronal repair, membrane remodeling, and plasticity (Mahley, 2016). For example, disrupting lipid homeostasis in mice by knocking out both the α and β isoforms of LXR, which are required for cholesterol and lipid efflux from astrocytes, leads to widespread abnormalities in the brain, including an age-dependent accumulation of lipid vacuoles in perivascular astrocytes (Wang et al., 2002). When SREBP2, a major positive regulator of cholesterol and lipid synthesis, is specifically knocked out in astrocytes, mice exhibit reduced brain weight and deficits in social behavior, learning and memory, and coordinated movement, as well as elevated glucose oxidation (Ferris et al., 2017). Interestingly, the neurons in these mice show elevated SREBP2, possibly to compensate for the lack of SREBP2 in astrocytes; yet this neuron-specific SREBP2 elevation was not enough to rescue the pathological changes associated with astrocyte-specific knock-out, underscoring the dependence of neurons on astrocytic lipids.

APOE4 from primary astrocytes is poorly lipidated compared to APOE3 (Gong et al., 2002); deficient lipid binding and transport by APOE4 might therefore be expected to result in the same type of widespread brain abnormalities described above, ultimately leading to increased risk for AD (**Figure 2**). But despite the poor lipid transport capabilities of APOE4 and the reliance of neurons on astrocyte-supplied lipid, APOE4 carriers have generally normal brain function throughout life. How then does aging uncover the deficits conferred by APOE4? The young brain may have mechanisms in place to cope with inefficient APOE4 lipid transport; but aging leads to decreased cholesterol synthesis in astrocytes (Boisvert et al., 2018), which, when combined with lower efflux from APOE4, could tip the balance and culminate in neuronal lipid deficits. Furthermore, Aβ inhibits SREBP2 in primary cultured cells from

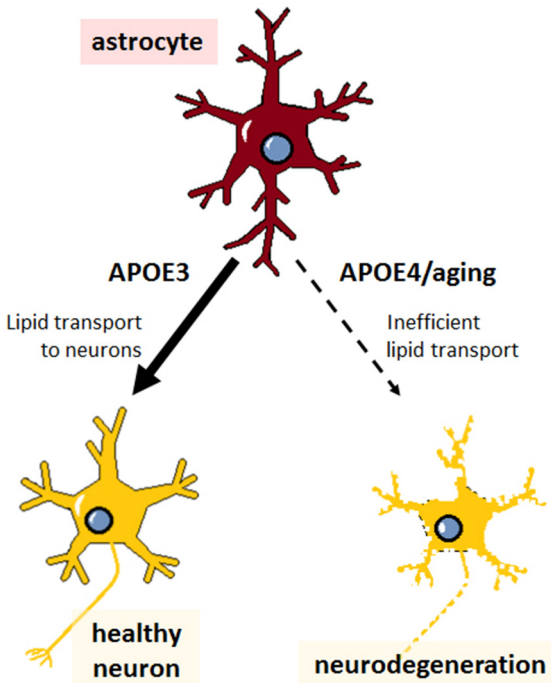

FIGURE 2 | Impaired lipid transport capacity of astrocytic APOE4 sensitizes neurons to degeneration during aging. Astrocytes (red) expressing APOE3 supply normal levels of cholesterol and other lipids to the cells of the brain, particularly to neurons (yellow), maintaining healthy neuronal function and cognition. Astrocytes expressing APOE4 are less inefficient at lipid transport, which, compounded with aging-associated lipid dysregulation, leads to neurodegeneration (neuron with rough edges) and is expected to predispose APOE4 carriers to Alzheimer's disease (AD).

mouse cortex (Mohamed et al., 2018), suggesting that amyloid deposition could make neurons even more dependent on astrocytic lipids, which would be lacking in ε4 carriers. Although cholesterol has been the most extensively studied, changes in other lipid classes are also observed in serum samples from AD patients, including sterols, sphingomyelin, phosphatidylcholine, glycerophosphoethanolamine, lysophosphatidylcholine, diacylglycerols, and triacylglycerols (Anand et al., 2017); therefore, APOE4 status could exacerbate other age-related changes in lipid homeostasis. While it is unclear whether these changes in lipid metabolism are a cause or an effect of AD, aging- and APOE-related perturbations may be expected to exacerbate amyloid pathology, and vice versa, culminating in widespread neurodegeneration.

Paradoxical Effects of APOE4 on Cholesterol Synthesis

Given that APOE4-containing lipoproteins are lipid-deficient, one might expect lipid secretion to be impaired. Surprisingly, human iPSC-derived astrocytes expressing APOE4 reportedly secrete *significantly more* cholesterol than their APOE3+ counterparts (Lin et al., 2018). Notably, this enhanced cholesterol secretion was accompanied by higher, not lower, intracellular cholesterol. Accumulated intracellular cholesterol is consistent

with the reduced ability of APOE4 to export cholesterol, which was confirmed in an independent study showing that APOE4 iPSC-derived astrocytes produce APOE-lipoprotein particles with less cholesterol than APOE3-expressing cells (Zhao et al., 2017a). But the increased cholesterol secretion is more difficult to explain; how could APOE4 promote both the intracellular accumulation and enhanced extracellular secretion of cholesterol *in vitro*? One explanation to unite these seemingly contradictory findings is that these cells are unable to properly sense that intracellular cholesterol levels are high. Normally, negative feedback loops ensure that cells laden with lipids reduce synthesis and uptake while increasing efflux—consistent with this signaling mechanism, SREBP2 was in fact downregulated in APOE4+ iPSC-derived astrocytes, as would be expected from cells with excessive lipids (Lin et al., 2018). APOE4 might therefore reduce the clearance of cholesterol *via* enzymatic oxidation. Consistent with this idea, APOE$^{-/-}$ mice have reduced 24-OH-, 7α, and 7β-hydroxycholesterol in their brains (Nunes et al., 2018). Furthermore, *APOE* mRNA levels are reduced in the APOE4+ iPSCs, and *APOE* is a major target gene of LXRs, which are activated by hydroxycholesterol. In contrast to the above finding, astrocytes from targeted-replacement mice expressing APOE4 were previously found to secrete less cholesterol than astrocytes from APOE3 mice (Gong et al., 2002; Riddell et al., 2008). While it is possible that species differences in cholesterol handling between mice and humans could explain these disparate findings (Dietschy and Turley, 2002), more research is needed to clarify exactly how APOE genotype affects astrocyte cholesterol metabolism.

APOE4 Disrupts Lipid Droplet Homeostasis

Recent observations indicate that APOE regulates intracellular lipid storage. A consequence of SREBP2 inhibition, as might occur during aging or amyloid deposition, is the reduction of autophagic lipid mobilization from structures known as lipid droplets (LDs; Seo et al., 2011; Kim et al., 2016). LDs are intracellular accumulations of neutral lipids and are central to cellular lipid homeostasis, particularly in astrocytes, where they play a dual role in managing lipids from neurons and in maintaining astrocytic energy demands. Elevated reactive oxygen species (ROS) in neurons induces lipid peroxidation and triggers subsequent efflux of lipids that accumulate as LDs in neighboring astrocytes, a process that is neuroprotective and dependent on APOE (**Figure 3**; Liu et al., 2015, 2017b). An increase in peroxidated lipids is associated with disrupted lipid homeostasis, decreased phosphatidylcholine synthesis, decreased mitochondrial metabolism, and ultimately cognitive decline (McDougall et al., 2017), and APOE mitigates this toxicity in neurons by transferring the burden of lipid accumulation and subsequent clearance to astrocytes. In a *Drosophila* model of neurodegeneration, APOE4 is a complete loss of function in terms of the neuroprotective formation of LDs in glial cells, leading to neuronal cell death (**Figure 3**;

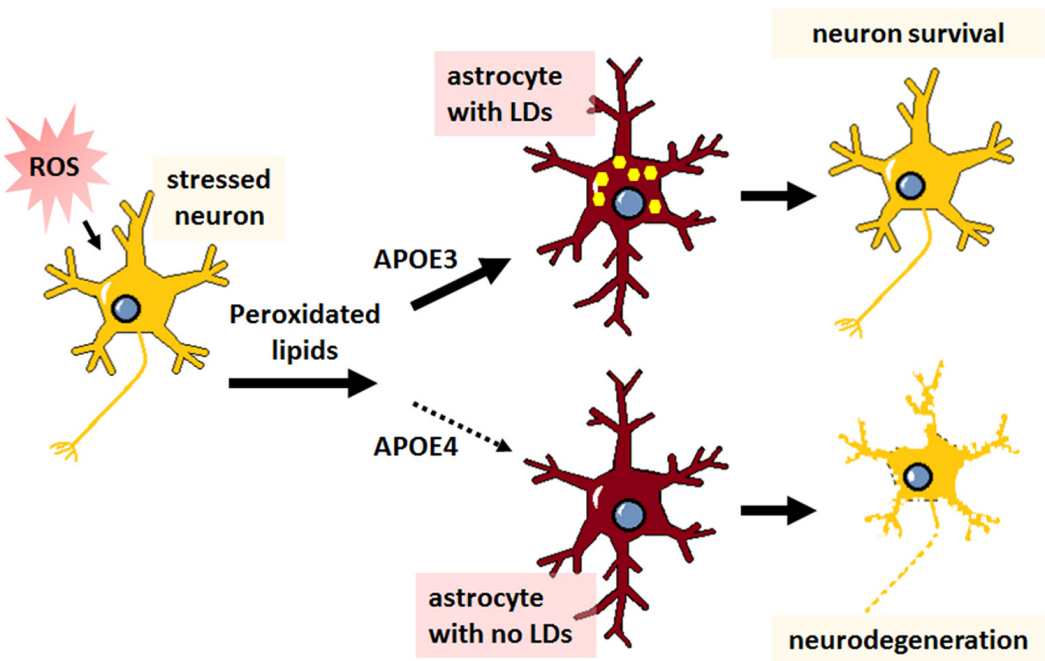

FIGURE 3 | The neuroprotective transfer of toxic lipids from neurons to astrocytes results in lipid droplet formation, which is abrogated in APOE4-expressing cells. Agents or stressors that induce reactive oxygen species (ROS) formation in neurons leads to increased levels of toxic peroxidated lipids. Neurons transfer these lipids to astrocytes *via* APOE. With APOE3 expression, this transfer results in the formation of lipid droplets (LDs; yellow dots) in astrocytes and neuroprotection. Conversely, APOE4 expression is thought to prevent the transfer of peroxidated lipids to astrocytes, resulting in no LD formation in astrocytes and subsequent neurodegeneration.

Liu et al., 2015, 2017b). These data indicate that APOE might play an important role not only in astrocyte-mediated synthesis and transfer of lipids to neurons, but in reverse as well, as an acceptor of neuronal-derived peroxidated lipids.

Although these studies show that APOE4 expression leads to a decrease in LD formation when neurons are the lipid donor and astrocytes are the recipient, APOE4 can also induce LDs in a cell autonomous manner. LD formation results from interactions between the endoplasmic reticulum (ER) and mitochondria, at structures known as mitochondria-associated ER membranes, and APOE may regulate LD formation by mediating ER-mitochondria communication at these sites (Tambini et al., 2016). Fibroblasts treated with APOE4 astrocyte conditioned medium (ACM) exhibit increased LDs, and blocking ER-mitochondria tethering returns lipid levels to normal (Tambini et al., 2016). These data indicate that either the APOE4 ACM contained a factor that signaled to the cells to induce LDs, or alternatively, that the APOE4 ACM is somehow nutrient-deprived compared to APOE3 media, since glucose deprivation also induces LD formation in astrocytes. Nutrient deprivation-induced LDs are used for β-oxidation of fatty acids to generate acetyl-CoA to meet cellular energy demands (Cabodevilla et al., 2013). Thus, it is possible that APOE regulation of LDs in astrocytes is context-dependent: nutrient deprivation induces formation of LDs and subsequent breakdown by autophagy to fulfill energy requirements, which APOE4 can stimulate; whereas increased neuronal oxidative stress leads to accumulation of toxic lipids, which are transferred to astrocytes, an activity that is lacking in APOE4 cells. In either case, APOE4-dependent deficiency in autophagy would also impair LD breakdown, causing toxic accumulation in either cell type (Simonovitch et al., 2016). Further study delineating the impact APOE4 has on LD homeostasis could identify points of therapeutic intervention.

Astrocyte Glucose Metabolism in the Aging Brain

The brain is a highly energy-demanding organ, and declines in brain glucose utilization and mitochondrial function during aging may interact with AD risk factors, including APOE, to negatively impact neuronal homeostasis. The data supporting this concept range from model organisms to epidemiology. For example, yeast genes that enhance or suppress Aβ toxicity exert their effect depending on the level of mitochondrial respiration (Treusch et al., 2011), suggesting that cellular energetics determines resiliency to amyloid. Energetics also impacts AD risk profile in humans: postmenopausal women characterized as having a poor metabolic profile, which includes elevated glucose and increased insulin resistance, exhibit worse cognitive performance compared to healthy metabolic subjects, and cognitive decline in this group is exacerbated by APOE4 carrier status (Karim et al., 2019). However, this relationship is likely complex; a study including both aged women and men found no difference in glucose levels in AD and APOE4 carriers vs. healthy and non-APOE4 carriers; there were marginal reductions in insulin and insulin resistance in APOE4 carriers, which was somewhat increased in individuals with AD (Morris et al., 2017).

To clarify the underlying relationship between APOE4 and energy homeostasis, APOE4 targeted-replacement mice have been studied. Aged (22 months) mice expressing APOE4 exhibit decreased insulin signaling in cortex and hippocampus (Zhao et al., 2017b) and middle-aged (6 months) female APOE4 mice are deficient in the uptake and utilization of glucose in the brain, with compromised respiratory capacity and decreased PPARγ signaling (Wu et al., 2018). In addition to downregulated PPARγ, another study found that insulin-degrading enzyme (IDE) was also downregulated in the hippocampus of the same aged APOE4 mice (Keeney et al., 2015). Reduction of PPARγ would be expected to trigger lipid dysregulation by decreasing lipid synthesis (as described above), as well as dampen anti-inflammatory signaling; and while lower levels of IDE would be expected to decrease Aβ clearance, lower IDE should also increase insulin and affect glucose and glycogen levels, perhaps leading over time to insulin resistance, although this would need to be determined experimentally. In the same study, aged mice expressing either APOE4 or APOE3 compared to the neuroprotective APOE2 were also found to have downregulated insulin signaling proteins IGF1, IRS1, and GLUT4 (Keeney et al., 2015), in agreement with the idea that aging itself causes deficient glucose metabolism independently of APOE genotype. As discussed above, energy deficiencies might be exacerbated in APOE4 carriers as lipid β-oxidation and lipid droplet autophagy become increasingly important for cell function.

Aerobic Glycolysis

Deficits in energy metabolism associated with APOE4 might also exacerbate aging-associated declines in aerobic glycolysis (Goyal et al., 2017; **Figure 4**). Aerobic glycolysis is the preferential conversion of glucose to lactate rather than pyruvate, even in the presence of oxygen, and is typically associated with cancer cells, although non-cancerous cells also engage in this process (Jones and Bianchi, 2015). In fact, astrocytes in mice are capable of surviving solely by aerobic glycolysis for at least as long as 1 year without any signs of pathology or neurodegeneration (Supplie et al., 2017). Certain brain regions tend to preferentially use aerobic glycolysis (Vaishnavi et al., 2010), and as the brain ages, there is a shift towards oxidative phosphorylation (OxPhos) to meet energy requirements (**Figure 4**; Goyal et al., 2017). This aging-related increased reliance on OxPhos has been proposed to lead to elevated ROS and peroxidated lipids (Harris et al., 2014), a situation likely made worse in APOE4 carriers, given the reduced ability of APOE4 to traffic neuronal peroxidated lipids to astrocytes for elimination. Thus, the switch to OxPhos could be an age-dependent trigger for APOE4 pathophysiology.

In line with the concept that aerobic glycolysis is beneficial or protective, and OxPhos is not, hiPSC-derived astrocytes from AD patients harboring the PSEN1 ΔE9 mutation are more oxidative than isogenic controls, with increased ROS production and decreased lactate secretion (Oksanen et al., 2017). On the other hand, PC12 and B12 cells that are resistant to

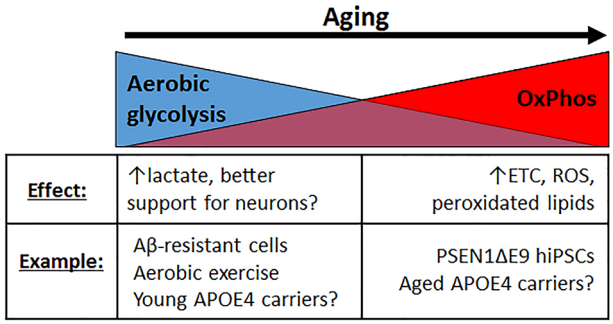

FIGURE 4 | Different modes of glucose metabolism are preferred in the young vs. aging brain. In the young brain, aerobic glycolysis is generally favored, resulting in increased lactate production, presumably by astrocytes, which should be supportive for increased neuronal activity. With aging, there is a shift toward oxidative phosphorylation (OxPhos) instead, resulting in increased electron transport chain (ETC) activity, increased ROS production, and more peroxidated lipids. Healthy cultured cells that are resistant to Aβ toxicity happen to exhibit a preference for aerobic glycolysis, and aerobic exercise, which is known to confer neuroprotection, elevates aerobic glycolysis. In contrast, cells from familial AD patients (PSEN1ΔE9) exhibit elevated OxPhos. While young APOE4 carriers may exhibit increased glycolytic activity, particularly in brain regions associated with AD [e.g., default mode network (DMN), entorhinal cortex], aged APOE4 carriers may exhibit elevated OxPhos instead, although more evidence to demonstrate whether and how such a metabolic shift occurs is warranted.

Aβ toxicity exhibit upregulated aerobic glycolysis (Newington et al., 2011). Furthermore, aerobic exercise, which improves cognitive scores in aging and AD patients (Panza et al., 2018), increases aerobic glycolysis and lactate production in the brain (Matsui et al., 2017). Interestingly, in the entorhinal cortex of APOE4 targeted-replacement mice, genes involved in OxPhos are upregulated, suggesting an APOE4-dependent increase in OxPhos and decrease in aerobic glycolysis and lactate (**Figure 4**; Nuriel et al., 2017b). Therapeutic strategies aimed at improving aerobic glycolysis may therefore help ameliorate APOE4-mediated toxicity.

Glycogen in Astrocytes

Despite its high energy demands, there are few energy stores in the brain compared to the rest of the body. In addition to storing lipids in the form of LDs, astrocytes are also the primary cell type in the brain to store glycogen. Astrocytic glycogen is important for maintaining healthy neurons and overall brain function, providing an energetic buffer during periods of low glucose availability (Bak et al., 2018). Primary astrocytes cultured in high (25 mM) vs. low glucose (5.5 mM) have elevated rates of glycolysis and glycogen content (Li et al., 2018). Elevated glycogen stores in co-cultured astrocytes are neuroprotective during glucose deprivation (Swanson and Choi, 1993). Significant evidence connects glycogen with memory formation: mice lacking glycogen synthase in the brain have impairments in learning- and memory-associated synaptic plasticity (Duran et al., 2013); glycogenolysis is important for memory consolidation (Gibbs et al., 2006); glycogen is a precursor to glutamate for learning (Gibbs et al., 2007); and glycogen content changes with early memory consolidation in 1-day-old chick (Hertz et al., 2003). Activated glycogen synthase kinase 3 has long been associated with the hallmarks of AD, including Aβ deposition, tau hyperphosphorylation, and brain inflammation, and would furthermore be expected to inhibit glycogen synthesis and thus decrease glycogen stores (Rayasam et al., 2009).

Energy metabolism in the brain may change during aging in a cell type-dependent manner. Inhibition of glycogen breakdown, termed "glycogenolysis," disrupts long-term potentiation in young, but not old, rat hippocampus (Drulis-Fajdasz et al., 2015). In a follow-up proteomics study, the same group found that, while glycogen phosphorylase (PYGB), the rate-limiting enzyme in glycogen degradation, is predominantly expressed in astrocytes in young animals, its distribution switches to being present in both neurons and astrocytes in old animals (Drulis-Fajdasz et al., 2018). As glycogen accumulation in neurons normally triggers apoptosis (Vilchez et al., 2007; Duran et al., 2012), the authors speculate that upregulation of PYGB in neurons may be a protective mechanism to keep neuronal glycogen stores low. However, total depletion of glycogen in neurons may not be desirable in all circumstances, as low levels of neuronal glycogen may be protective during hypoxia (Saez et al., 2014).

The importance of glycogen to enhanced memory is not necessarily ascribed to elevated pyruvate for mitochondrial OxPhos, since aged wild-type and adult APP/PS1 mice fed a diet supplemented with pyruvate still exhibit impairments in a passive avoidance task for fear memory, despite a preservation of glycogen stores and enhanced exploratory behavior (Koivisto et al., 2016). Rather than supplying pyruvate for OxPhos, glycogen may instead supply lactate to mediate its beneficial effects, as described in the following section.

Lactate, Glycogen and the Astrocyte-Neuron Lactate Shuttle Hypothesis

The Astrocyte-Neuron Lactate Shuttle (ANLS) hypothesis was first formulated in 1994 (Pellerin and Magistretti, 1994, 2012), and describes a process in which astrocytes metabolize glucose to export lactate for neurons during periods of high neuronal activity, during learning and memory, for example. The existence of an astrocyte-to-neuron transport of lactate would necessitate a lower basal concentration of lactate in neurons compared to astrocytes, and a recent study has indeed demonstrated such a gradient *in vivo*, using a genetically-encoded lactate sensor (Machler et al., 2016).

Despite findings in support of the ANLS hypothesis, there has been some disagreement in the field as to whether astrocytic lactate is really used by active neurons in the brain, if neurons are able to produce their own alternative energy substrates, or if astrocytes produce lactate in response to their own energetic demands (Dienel, 2012). For example, computer simulations of neuron/astrocyte energetics, based on fMRS data, support a model in which neurons readily metabolize glucose and export lactate, which is taken up by astrocytes, and not the other way around (Simpson et al., 2007; Mangia et al., 2009).

Although neurons are certainly capable of taking up glucose and secreting lactate themselves, there is compelling evidence that lactate secretion from astrocytes, derived from glycogen stores specifically, is important in contexts of neuronal high energy demand. For example, the transport of lactate specifically from astrocytes to neurons is necessary for long-term memory formation (Suzuki et al., 2011) and spatial working memory (Newman et al., 2011). Neuronal activity can upregulate astrocytic genes involved in lactate production and export (Hasel et al., 2017), ensuring that astrocytes are able to supply neurons with the necessary lactate during periods of intense energetic demands. Lactate derived from astrocytic glycogen can sustain neuronal activity in the absence of other forms of energy, and blocking the transfer of lactate from astrocytes to neurons in the absence of any other energy source leads to axonal/neuronal failure (Ransom and Fern, 1997; Wender et al., 2000; Brown et al., 2003, 2005; Suh et al., 2007; Walls et al., 2008). Blocking glycogen degradation or lactate transfer reduces glutamate release from neurons (Sickmann et al., 2009). Furthermore, exhaustive exercise decreases brain glycogen and elevates astrocyte-derived lactate (Matsui et al., 2017). While the original ANLS hypothesis may undergo revision and refinement, astrocytic glycogen-derived lactate certainly appears to be an important component of healthy neuronal function, particularly during times of nutrient deficiency.

Connecting APOE4 and Brain Energy Metabolism: Future Directions

How could aging glycogen metabolism interface with APOE4 genotype to exacerbate neurodegeneration? Young adult APOE4 carriers have altered expression of proteins involved in glucose metabolism in the posterior cingulate cortex (PCC), a central component of the DMN (Perkins et al., 2016). Subregions of the PCC are proposed to be involved in internally directed cognition, including memory retrieval and planning, as well as controlling attentional focus (Leech and Sharp, 2014). The DMN is highly metabolically active and is one of the earliest regions to deteriorate in AD and in normal aging (Leech and Sharp, 2014), and young APOE4 carriers exhibit increased activity in the DMN before any signs of disease (Filippini et al., 2009). In agreement with this increased activity, hiPSC-derived neurons from APOE4 patients are hyperactive (Lin et al., 2018), and APOE4 targeted-replacement mice exhibit a hyperactive entorhinal cortex compared to APOE3-expressing mice (Nuriel et al., 2017a). While young APOE4 carriers were found to express higher levels of glycolysis enzymes (GLUT1, GLUT3, HEX1, MCT2, SCOT, AACS) and complexes I, II, and IV of the electron transport chain (ETC), there were lower levels of MCT4, an important transporter for astrocytic lactate secretion (Perkins et al., 2016). Disruption of MCT4 impairs long-term memory, which is rescued by lactate injection, while memory impairment caused by disruption of the neuronal lactate transporter MCT2, is not rescued by lactate, strongly supporting the notion that astrocytic export of lactate is critical for long-term memory formation (Suzuki et al., 2011). Thus, the decreased MCT4 in young APOE4 carriers might be expected to cause a deficit in lactate secretion by astrocytes, despite higher glycolytic activity. Interestingly, the DMN is a region that relies on aerobic glycolysis in young, healthy brain (Vaishnavi et al., 2010), and so should be a region that relies on elevated lactate production to support neuronal activity; the increased neuronal activity and decreased capacity of astrocytes to keep up with such activity in APOE4 carriers might then be expected to burn out glycogen stores early, effectively accelerating an aging-associated metabolic phenotype reliant on OxPhos. Further work is necessary to determine whether this pathway could be induced by diet, exercise, or pharmacological intervention to preserve cognitive function in presymptomatic APOE4 carriers.

In summary, APOE performs a complex set of interrelated functions in astrocytes, ranging from its long-appreciated lipid transport function to regulation of lipid storage and utilization to cellular energetics. In the next section we will review emerging concepts around APOE function in the other major producer of APOE in the brain, microglia.

MICROGLIA-DERIVED APOE IN AGING AND AD

A large body of evidence implicates microglia in APOE-mediated AD pathogenesis, particularly in relation to aging. Microglial APOE production is strongly induced during injury and disease, including in AD (Olah et al., 2018; Ping et al., 2018; Rangaraju et al., 2018a). In 5XFAD transgenic mice, which harbor five different human familial AD-causing mutations and exhibit accelerated amyloid pathology (Oakley et al., 2006), microglial APOE mRNA is significantly increased (Wang et al., 2015). A similar increase in microglial APOE mRNA was also found in a separate but similar transgenic mouse model of accelerated amyloid pathology, APP/PS1 (Orre et al., 2014), as well as in aged (isolated from 24 month old mice) vs. younger (5 month old) mouse microglia (Hickman et al., 2013), and in the Ercc1 mutant mouse model of accelerated aging (Raj et al., 2014a; Holtman et al., 2015). The upregulation of APOE mRNA in these mouse models of AD and aging reflect concordant increases at the protein level and in human AD brain. A recent proteomics study of microglia isolated from 5XFAD mice identified APOE as one of the top upregulated proteins (Rangaraju et al., 2018a). Interestingly, immunohistochemical analysis in this same study indicated that the microglia with elevated APOE were those surrounding amyloid plaques, demonstrating that a distinct subset of microglia increase APOE expression, rather than all microglia. Furthermore, the aged mouse microglial proteome also shows an enrichment in APOE protein compared to non-aged mice (Rangaraju et al., 2018a). In agreement with these findings in mice, an analysis of frontal cortex human postmortem brain tissue found elevated APOE protein in AD patients vs. healthy controls (Ping et al., 2018). Another study performing a post-mortem human brain proteomics analysis also found APOE to be higher in the aged microglia (Olah et al., 2018). Thus aging alone, and not only disease pathogenesis, is sufficient to induce microglial APOE expression at both the mRNA and protein level.

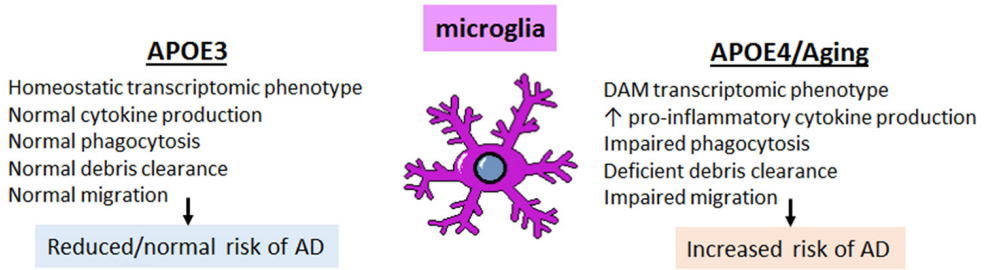

FIGURE 5 | The effect of APOE4 expression on microglia is cell autonomous and triggers a DAM, pro-inflammatory phenotype with impaired homeostatic functions. Microglia expressing APOE4 vs. APOE3 tend to exhibit a disease-associated microglia (DAM)-like phenotype; this includes increased pro-inflammatory cytokine production with impaired phagocytic ability, deficient clearance of debris (including amyloid plaques), and impaired migratory ability. These changes, compounded with the pro-inflammatory phenotype associated with normal aging, result in an increased risk of developing AD.

The expression of APOE in subsets of disease- and aging-associated microglia raises an important question: what role does APOE play in the microglial response to disease and aging, and how is this impacted by APOE4 genotype? Both mouse and human studies indicate that key microglial functions are affected by APOE genotype, including transcriptomic changes towards the disease-associated phenotype, the percent of microglia coverage around plaques, increased cytokine production, as well as chemotaxis, phagocytosis and, perhaps, synaptic pruning (**Figure 5**). Each of these functions are discussed in the following sections.

APOE and the Microglial Phenotype in AD

The transcriptional profile of microglia is altered in AD, switching from a homeostatic phenotype to a molecular profile often referred to as the disease-associated microglial (DAM) phenotype (Zhang et al., 2013; Keren-Shaul et al., 2017; Sarlus and Heneka, 2017; Rangaraju et al., 2018b). Genome-wide association studies (GWAS), including large-scale meta-analyses, have indicated that the majority of genetic variants conferring risk for late onset sporadic AD are immune-related and enriched in microglia, implicating DAM microglia in AD pathogenesis (Guerreiro et al., 2013; Lambert et al., 2013; Dos Santos et al., 2017; Huang et al., 2017). The affected genes include myeloid receptors *TREM2* and *CD33*, transcriptional regulators *SPI1* (Pu.1) and *MEF2C*, complement pathway (*CR1*), antigen presentation (*HLA-DRB5*), the *MS4A* family locus, and *ABCA7*, amongst several others (Lambert et al., 2013). Some single nucleotide polymorphisms (SNPs) are present in non-coding regions and alter expression of microglial genes (e.g., *SPI1*; *CD33*), whereas other SNPs result in a gain or loss of function in several microglial genes related to immune function (e.g., *TREM2*) (Raj et al., 2014b; Malik et al., 2015; Huang et al., 2017). A large-scale weighted gene coexpression network analysis (WGCNA) combined with pathological assessment of 1647 post-mortem brain tissues from late-onset AD patients and non-demented controls pointed to immune/microglial gene networks as having the most significant functional enrichment of all modules (Rangaraju et al., 2018b). Moreover, this microglial module was significantly associated with the greatest number of AD-relevant pathological traits, including the extent of brain atrophy, and represented immune pathways consisting of complement, Fc-receptors, major histocompatibility complex (MHC), cytokines/chemokines and toll-like receptors (Zhang et al., 2013). Since then, several groups have characterized this DAM phenotype/immune network, albeit with varying nomenclature (Gjoneska et al., 2015; Keren-Shaul et al., 2017; Rangaraju et al., 2018b), and attempts to identify key regulators of this transcriptomic phenotype have been underway (Gjoneska et al., 2015). A common theme on which these genetic risk factors converge is that they alter key microglia activities, including phagocytosis, cytokine production, and microglial encapsulation of amyloid plaques (**Figure 5**). Altogether, this work has repositioned the thinking in the field, emphasizing microglia as a potential source of attractive therapeutic targets for AD.

Interestingly, APOE is a key regulator of the microglial transcriptional signature, as demonstrated in post-mortem human brain studies, human cellular models as well as in AD mouse models and cultured microglia *in vitro* (Keren-Shaul et al., 2017; Krasemann et al., 2017; Pimenova et al., 2017; Lin et al., 2018; Olah et al., 2018). Studies performing single cell RNA sequencing of CD45+ microglia from 5XFAD mice, paired with *in situ* hybridization of DAM signature genes, indicate that both the morphology and molecular identity of microglia around plaques, as a population, are different from microglia distal to plaques (Keren-Shaul et al., 2017). These DAMs have increased APOE expression that is triggered following a downregulation in homeostatic genes such as CX3CR1 and P2Y12 (Keren-Shaul et al., 2017; Krasemann et al., 2017). Furthermore, APOE mediates the switch from homeostatic to the DAM phenotype; notably, knocking out APOE specifically in microglia in 5XFAD mice prevents the transition to the DAM phenotype and partially rescues neuronal cell death in an axotomized facial motor nucleus model (Krasemann et al., 2017). A full knockout of APOE conferred no additional protection over that of microglia-selective APOE deletion, highlighting the importance of microglia-specific APOE to this process (as opposed to astrocytic APOE, for example; Krasemann et al., 2017). However, an astrocyte-selective APOE model was not directly compared, so it remains possible that it is not the cellular source of APOE that matters, but

rather a reduction in total APOE levels that underlies this finding.

While elucidating the role of mouse APOE is informative, it is also critical to understand whether different phenotypes ensue with human APOE variants. Microglia isolated from aged vs. non-aged human postmortem brain for RNA sequencing analysis display an immune-enriched signature that is significantly associated with key traits, including APOE genotype (Olah et al., 2018). Although statistical significance was not reached for APOE4, the neuroprotective APOE2 was associated with a reduction in this aged microglial phenotype (Olah et al., 2018). In human cellular models, isogenic conversion of human iPSC-derived microglia from APOE3/E3 AD patients to APOE4/E4 is sufficient to transform the microglia transcriptome to a DAM-like phenotype (Lin et al., 2018). Notably, this APOE4 gene expression signature significantly overlapped with the transcriptional profile seen in human brain (Lin et al., 2018), in support of the notion that APOE4 may impact the DAM phenotype in human AD. A WGCNA transcriptomic analysis of brain from APOE3 or APOE4 targeted-replacement mice subjected to traumatic brain injury identified that the network most significantly associated with APOE genotype was the "innate immune response," which included complement activation; in this network, the genes were shifted toward increased expression along with APOE4 compared to APOE3 (Castranio et al., 2017), again supporting a model in which APOE4 confers a pro-inflammatory phenotype relative to APOE3.

APOE Regulation of Microglial Plaque Association

APOE immunoreactivity in human brain is enriched in congophilic, dense-core plaques (Navarro et al., 2003), as opposed to diffuse plaques, which are heterogeneous with respect to APOE immunoreactivity (Gearing et al., 1995). Notably, microglial activation around diffuse plaques is minimal (Maat-Schieman et al., 1994; Stalder et al., 1999; Mrak, 2012), begging the question as to whether the presence of APOE in plaques is the trigger that differentially activates microglia at specific plaque types, or whether the presence of APOE in the plaques is simply due to the upregulation of APOE upon transition from homeostatic microglia to DAMs (Ulrich et al., 2014; Krasemann et al., 2017).

APOE4-expressing immune cells are less efficient at plaque engulfment compared to APOE3-expressing cells. When GFP+ bone marrow cells from human *APOE3* or *APOE4* donor mice were transplanted into lethally-irradiated 5 month old *APPswe/PS1ΔE9* [i.e., bone marrow transplanted (BMT)-APP/PS1] mice, donor GFP+ macrophages are found in the brain 8 months later, with APOE3 exhibiting greater numbers of plaque-associated GFP+ Iba1+ cells (Yang et al., 2013). Interestingly, APOE4 was associated with reduced microglia coverage around Aβ plaques (Yang et al., 2013). Proper microglial encapsulation of plaques is thought to be protective, sequestering damage from surrounding cells, and decreased microglial coverage is associated with higher Aβ levels and increased neuronal dystrophy (Yeh et al., 2016). Indeed, the percentage of Aβ per area was significantly higher in the hippocampus and cortex of mice with APOE4 vs. APOE3 transplant (Yang et al., 2013). Furthermore, APOE4 BMT-APP/PS1 mice had significantly higher brain expression levels of the pro-inflammatory genes TNFα and macrophage migration inhibitory factor (MIF; which are upregulated in AD patients), lower levels of the anti-inflammatory gene IL-10, and impaired spatial working memory in the Barnes maze, compared with *APOE3* BMT-*APP/PS1* mice (Yang et al., 2013).

In another study using 5XFAD mice crossed to APOE3 or APOE4 targeted-replacement mice, mice expressing APOE4 exhibited significantly larger and more numerous amyloid plaques, as well as increased microglial dystrophy; but in contrast to the Yang et al. (2013) study, more microglia were found surrounding plaques in APOE4 vs. APOE3 and APOE2 (Rodriguez et al., 2014). It is difficult to distinguish whether the change in microglia phenotype in relation to plaque type is indirect, in response to worsened pathology or if it is also partly due to a cell autonomous effect of APOE4 on microglia, irrespective of plaque type. While these *in vivo* studies are informative, other recent studies indicate cell-intrinsic APOE4 effects on microglia. More specifically, human iPSC-derived microglia from APOE4 carriers have different morphology compared to isogenic APOE3 controls, and have a reduced capacity to phagocytose Aβ (Lin et al., 2018), in agreement with a change towards the DAM phenotype. Thus, APOE4 expression impairs the ability of microglia to efficiently clear amyloid pathology, although the precise mechanisms underlying microglial recruitment to specific amyloid plaques require further characterization.

APOE Genotype and Cytokine Production

An overwhelming body of evidence supports that the presence of APOE4, either recombinantly applied or endogenously expressed, confers an increase in pro-inflammatory cytokine production across rodent and human species, in blood, brain, and microglia. In support, rat primary glial cultures comprised of astrocytes and microglia produce higher levels of IL-1β when exposed to recombinant APOE4, purified from APOE-expressing HEK293 cell culture medium, than APOE3 (Guo et al., 2004). Cultured mouse microglia derived from APOE4 targeted-replacement mice have an activated morphology, produce higher levels of pro-inflammatory cytokines including TNFα, IL-6, and IL12p40, and nitric oxide (NO) along with lower levels of anti-inflammatory cytokines than their APOE3-derived counterpart when exposed to various pro-inflammatory mediators including LPS, IFNγ, or LPS+ IFNγ (Brown et al., 2002; Colton et al., 2005; Vitek et al., 2009). Notably, some of these effects (e.g., NO production) are APOE4 gene dosage-dependent (Vitek et al., 2009).

Similar to that seen in cultured microglia, APOE4 mice immune-challenged with a peripheral injection of LPS exhibit higher brain mRNA expression levels of TNFα and IL12p40 than in that from APOE3 TR mice (Vitek et al., 2009). A

similar increase in pro-inflammatory cytokines, namely TNFα and IL-6, in APOE4 mouse serum is seen following a peripheral injection with LPS compared to that in APOE3 mice (Lynch et al., 2003). Finally, when LPS is administered by intracerebroventricular injection, APOE4 mice have higher brain levels of IL-1β, IL-6, and TNFα than APOE3 mice (Zhu et al., 2012).

While this increase has been consistently observed by independent groups *in vivo* in APOE targeted-replacement mice in AD models (Tai et al., 2011), due to perhaps independent roles of APOE genotype on other aspects of the disease (e.g., Aβ plaque levels), it is not clear whether the increase in cytokines by APOE4 is due to the increase in pathology, or due to a direct effect of APOE4 on cytokine production, which could contribute to the increase in pathological changes. Cell culture experiments shed some light on the former, in that the effect of APOE4 seems to be a cell-autonomous effect on microglia as when stimulated in culture, they produce more pro-inflammatory cytokines such as IL-1β (Guo et al., 2004), which suggests the differential extent of pathology (e.g., amyloid plaque deposition) as not being the sole driver of the differential increase in cytokines due to APOE4 vs. APOE3 genotype. Since these APOE targeted-replacement mouse studies assess the effect of human APOE in a mouse context, it remains plausible that this toxic pro-inflammatory effect attributed to APOE4 could be specific to mouse; however, human data indicates otherwise and suggests this phenomenon is intrinsic to the human APOE isoform irrespective of species by which it is produced/acting upon. Indeed, over the past few years, studies using advanced human cellular models parallel the pro-inflammatory findings seen in mice (Lin et al., 2018). Further, human clinical data suggests something similar. In two Chinese populations with AD, APOE4 carriers, carrying either one or two copies, had elevated plasma levels of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β compared to that of APOE2 and APOE3 carriers (Fan et al., 2017). Also, APOE genotype modulates cytokine production in human peripheral blood when stimulated with pro-inflammatory mediators *ex vivo* as well as *in vivo*. More specifically, *ex vivo* stimulation of peripheral blood collected from healthy volunteers with TLR2 and TLR4 ligands demonstrated that TNFα, IL-1β, IL-6, IL-17, IFNγ, G-CSF, IL-8, MCP-1, MIP-1a, and IP-10 levels were robustly increased in that from APOE3/E4 compared to APOE3/E3 carriers (Gale et al., 2014). Similarly, healthy human subjects intravenously administered the TLR4 ligand LPS exhibited higher plasma TNFα levels in APOE3/E4 vs. E3/E3 (Gale et al., 2014). In recent years, more advanced human cellular models make the picture clearer and indicate the mouse findings are not species specific and extend to human microglia.

Are these effects good or bad? Notably, recent studies have been controversial as to whether the best therapeutic approach for AD with respect to targeting APOE would be to lower APOE levels or augment them. As in astrocytes, the effects of APOE4 in microglia are often confounded by reports that APOE4 production and/or protein stability is lower compared to APOE3 (Bertrand et al., 1995; Raffai et al., 2001; Glockner et al., 2002). Thus, it remains plausible that this inflammatory response could be due to a decrease in APOE levels, irrespective of genotype. APOE3 can dampen cytokine production, and removing APOE can lead to a more pro-inflammatory phenotype. So, would elevating APOE4 protein levels help ameliorate the pro-inflammatory phenotype, or worsen it? Microglial APOE is neuroprotective in rat microglia neuronal co-cultures (Polazzi et al., 2015) and this release of APOE and the resulting neuroprotective effect is lost when microglia are exposed to inflammatory stimuli, thus lowering APOE. Therefore, it is interesting to hypothesize that, in the context of AD, when microglia are exposed to pro-inflammatory stimuli, APOE synthesis and secretion is stunted (Saura et al., 2003; Polazzi et al., 2015), effectively decreasing any neuroprotective effects of the microglia. A study examining the effect of APOE genotype comparing WT neurons cultured with either APOE3 vs. APOE4 mouse-derived astrocytes or microglia found that only APOE4 microglia led to greater neurotoxicity (Maezawa et al., 2006). Interestingly, the greater toxicity of APOE4 correlated with higher pro-inflammatory cytokine levels (TNFα, IL-6, IL-1β). Finally, it should be noted that the APOE4 effects can be sex-specific in certain contexts (Colton et al., 2005).

APOE Effect on Phagocytosis, Synaptic Pruning, and Chemotaxis

While the effect of APOE genotype on synaptic pruning and phagocytosis has been not been studied in microglia, astrocytic phagocytosis has been evaluated using APOE2, APOE3, and APOE4 targeted-replacement mice crossed to mice expressing EGFP driven by the astrocyte-specific promoter *Aldh1l1* (Chung et al., 2016). Fluorophore-conjugated cholera toxin-β subunit (CTB-594) was used to label axonal projections of retinal ganglion cells and the dorsal lateral geniculate nucleus, an area with a high degree of synaptic pruning during development. In agreement with *in vitro* phagocytic assessments, astrocytes in APOE2 mice showed significantly enhanced phagocytic capacity compared with *APOE3*, whereas astrocytes in APOE4 mice demonstrated a significant decrease (Chung et al., 2016). Although this study focused on astrocytes, microglia are key players in synaptic pruning (Paolicelli et al., 2011; Schafer et al., 2012); thus, it would be informative to determine whether the effect of APOE genotype on synaptic pruning is cell type-specific or not. *In vitro*, ApoE$^{-/-}$ mouse-derived peritoneal macrophages demonstrated a decreased uptake of apoptotic cells, but no change in ability to uptake latex beads, compared to WT (Grainger et al., 2004). Given that APOE4 from human CSF was found to form smaller complexes than APOE2 and APOE3, it has been proposed that APOE4 may be deficient in lipid debris clearance, in accordance with phagocytic studies conducted on APOE4 human iPSC-derived microglia (Lin et al., 2018). Finally, microglial migration has also been found to be linked to APOE genotype (Cudaback et al., 2011). Mouse ApoE$^{-/-}$ microglia show reduced ATP- and C5a-triggered migration; likewise, in targeted-replacement mice, APOE2 and APOE4 have reduced ATP- and

C5a-triggered migration compared to APOE3 (Cudaback et al., 2011).

APOE and Other Microglial AD Risk Factors

Several studies have looked at potential interactions between APOE and TREM2, another genetic risk factor for AD (Guerreiro et al., 2013). APOE can bind TREM2 (Atagi et al., 2015; Yeh et al., 2016), and, either directly or indirectly, APOE can alter TREM2 signaling or function (Jendresen et al., 2017). Both APOE and TREM2 are implicated in key steps in the homeostatic to DAM phenotype (Krasemann et al., 2017). It is still unclear whether there is and to what extent there is an interaction in APOE and TREM2, genetically or functionally, warranting further investigation.

Other risk factors for AD have clear functional overlap with APOE. Of note, ABCA7, also expressed in microglia, has been associated with age of onset of AD in a similar manner as APOE. More specifically, the minor allele at rs3764650 in ABCA7 is associated with a delayed onset and shorter disease duration (Kim et al., 2006). While its function in regulating the homeostasis of phospholipids and cholesterol has been the most well studied function of ABCA7 in relation to APOE, it also plays a role in phagocytosis (Tomioka et al., 2017), which has been observed *in vitro* and *in vivo* and been reviewed more extensively elsewhere (Abe-Dohmae and Yokoyama, 2012; Aikawa et al., 2018).

Future Directions for Understanding Microglial APOE in Immunosenescence

Is microglial APOE upregulation in aging and AD helpful or harmful? Is it a compensatory mechanism, or does it contribute to accelerated aging and neurodegenerative disease pathogenesis? It is interesting to speculate that the two greatest risk factors for late-onset AD, aging and APOE, interact with respect to inflammation, with APOE4 promoting an enhanced inflammatory tone over the course of a lifetime (Olarte et al., 2006; Sando et al., 2008). Microglia undergo senescence with aging, a process termed immunosenescence (Costantini et al., 2018), consistent with a DAM phenotype, and accumulating evidence suggests that APOE4 genotype may aggravate this process to promote neuroinflammation and neurodegeneration in AD. The change in cellular source of APOE from predominantly astrocyte-derived, to astrocyte- and microglia-derived during disease or aging, raises questions as to whether the cellular source of APOE subserves differential functions. It should be noted however, that although APOE immunoreactivity has been demonstrated around plaques in post-mortem human AD brain, co-labeling of APOE with microglial markers has not been investigated. Thus, a careful evaluation of this is warranted given the recent advances in understanding APOE expression in AD models. Finally, although there are clearly centrally mediated and cell-autonomous effects of APOE4, several peripheral effects of APOE4 on immune cells have been observed, and as such, it is unclear as to what extent the peripheral component of APOE4 status has on the risk to AD.

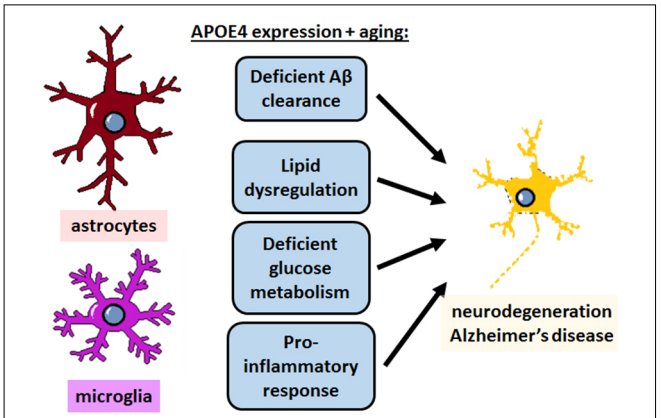

FIGURE 6 | APOE4 disrupts homeostatic pathways in astrocytes and microglia to cause neurodegeneration and AD. APOE4 expression and the normal aging process itself impair astrocyte and microglia physiology in specific pathways, which could theoretically be targeted to treat AD. In addition to deficient clearance of Aβ, emerging evidence specifically highlights lipid dysregulation and deficient glucose metabolism in astrocytes, and a neurodegenerative pro-inflammatory response in microglia, and to some extent in astrocytes as well. All of these pathways converge with similar deficits that occur in normal aging to ultimately lead to neurodegeneration.

CONCLUSION

The role of APOE4 in mediating AD risk is complex and multifactorial, involving a diverse array of cell types and functions that need to be taken into consideration for APOE-directed drug development. Studies from the last decade have made significant progress in defining what those functions are, and how aging might factor into the progression of APOE4-mediated AD. Cholesterol metabolism, LD formation and lipid transfer from neurons to glia, and glucose/glycogen/lactate metabolism from glia to neurons all appear to be important pathways in maintaining brain health, particularly during aging; and the pro-inflammatory nature of APOE4 and decreased phagocytic capacity of APOE4-expressing glia likely contributes to neurodegeneration as well (**Figure 6**). These pathways may yield viable therapeutic targets for treating AD, but the precise mechanisms and connections with APOE4 still remain poorly defined. It is also unclear how APOE4-mediated disrupted function in astrocytes and microglia separately could synergize to increase AD risk, warranting further investigation.

AUTHOR CONTRIBUTIONS

CF wrote the first draft of the manuscript. CF, MH and WR wrote sections of the manuscript. MM and CF created the figures. All authors contributed intellectually and to manuscript revision, and read and approved the submitted version.

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REVIEW
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High Cortisol and the Risk of Dementia and Alzheimer's Disease: A Review of the Literature

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Introduction: Cortisol effects on the brain are exerted through two distinct receptors, inducing complex and even opposite effects on the cerebral structures implicated in the various cognitive functions. High cortisol may also have deleterious effects on the brain structures and contribute to neurodegeneration, in particular Alzheimer's disease (AD), via different mechanisms.

Objective: To examine the interrelationships between cortisol, cognitive impairment and AD.

Methods: Review of the literature.

Results: Clinical studies found that elevated cortisol was associated with poorer overall cognitive functioning, as well as with poorer episodic memory, executive functioning, language, spatial memory, processing speed, and social cognition; while in animals, glucocorticoid administration resulted in cognitive impairment and abnormal behavior. In cognitively healthy subjects, higher cortisol levels have been associated with an increased risk of cognitive decline and AD. Subjects with dementia and Mild Cognitive Impairment (MCI) due to AD have been found to have higher CSF cortisol levels than cognitively healthy controls. Elevated CSF cortisol may also be associated with a more rapid cognitive decline in MCI due to AD. Elevated cortisol levels have been also found in delirium. High cortisol may mediate the impact of stressful life events, high neuroticism, depression, sleep disturbances, as well as cardiovascular risk factors on cognitive performance, neurodegeneration, and cognitive decline. High cortisol may also exert neurotoxic effects on the hippocampus, and promote oxidative stress and amyloid β peptide toxicity. Further possible underlying mechanisms include the interactions of cortisol with inflammatory mediators, neurotransmitters, and growth factors.

Conclusion: Elevated cortisol levels may exert detrimental effects on cognition and contribute to AD pathology. Further studies are needed to investigate cortisol-reducing and glucocorticoidreceptor modulating interventions to prevent cognitive decline.

Keywords: cognition, cortisol, memory, executive functions, dementia

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INTRODUCTION

Corticosteroids seem to be among the hormones with the most important effects on the brain function. Indeed, corticosteroids have been associated with effects on mood, stress, anxiety, sleep, appetite, as well as cognition (Lupien et al., 2007; Wolkowitz et al., 2009; Copinschi and Caufriez, 2013).

Once released from the adrenal cortex, cortisol, the main glucocorticoid in humans, easily crosses the blood–brain barrier, owing to its lipophilic character (Wolkowitz et al., 2009). Cortisol binds to specific intracellular receptors in the brain, in particular in regions implicated in cognitive functions (McEwen, 2007; Daskalakis et al., 2013; Vogel et al., 2016). Once activated, these receptors bind to " hormone response elements" in the DNA and regulate the transcription of target genes (Joels, 2006).

The resulting effects on cognition seem to be complex and involve several cognitive domains (Lupien et al., 2007; Lee C.M. et al., 2008; Tatomir et al., 2014; Geerlings et al., 2015; Vogel et al., 2016). Different levels of cortisol likely produce different and even sometimes opposite effects (de Kloet et al., 1999; Joels, 2006). While some of these effects are acute (Lupien and McEwen, 1997; Lupien et al., 2002; Meir Drexler and Wolf, 2016), some appear to be long-lasting and may even involve long-term changes in the brain structure (Geerlings et al., 2015).

Altered Hypothalamic-Pituitary-Adrenal (HPA) axis functioning, and in particular high cortisol levels in the elderly have been associated with an increased risk for dementia and Alzheimer's disease (AD) (Lupien et al., 1999; Rothman and Mattson, 2010; Ennis et al., 2017; Notarianni, 2017).

A better understanding of these interrelationships between cortisol, cognition and dementia may open the door to new prevention and therapeutic options involving the HPA axis. The effects of cortisol on emotional memory had already led to therapeutic trials of corticosteroids and corticosteroid receptor antagonists/modulators in AD (Pineau et al., 2016), as well as in treating or preventing post-traumatic stress disorder (PTSD) (Daskalakis et al., 2013), as well as in treating depression (Wolkowitz and Reus, 1999; Kling et al., 2009).

GLUCOCORTICOID RECEPTORS AND CORTISOL EFFECTS ON COGNITION

Cortisol exerts its effects on cognition through two types of receptors: type I (Mineralocorticoid Receptors, MRs) and type II (Glucocorticoid Receptors, GRs) (Joels, 2006; Daskalakis et al., 2013). Surprisingly, the MRs display 6 to 10 times higher affinity for glucocorticoids, mainly cortisol, than GRs (de Kloet et al., 1999; Joels, 2006).

These receptors are expressed differently throughout the brain. Indeed, the hippocampus, mainly implicated in episodic memory, expresses both MRs and GRs, whilst the prefrontal cortex, primarily responsible for executive functions, only expresses GRs (Lupien et al., 2007; McEwen, 2007). While MRs have been associated with positive/enhancing effects on the cognitive performance, GRs have, on the contrary, been linked to negative inhibitory effects. In this regard, it has been found that infusion of a GR antagonist, but not of MR antagonist, in the medial prefrontal cortex of a mouse blocked the deleterious effects of glucocorticoids on working memory (Barsegyan et al., 2010).

Cortisol effects on the hippocampus-related cognitive performance have often been described by the means of an inverted-U shape plot (**Figure 1**). Indeed, in the hippocampus, where both GRs and MRs are expressed, moderate levels of cortisol only activate the receptors with the higher affinity, i.e., MRs, leading to memory enhancement effects. As cortisol levels increase, this positive effect increases till MRs are saturated. Starting from this point, as cortisol levels rise, GRs are increasingly activated thus leading to increasingly detrimental effects on the memory. Distinctly, the effects of cortisol on executive functions are likely more linear. Since the prefrontal cortex region, mostly responsible for executive functions, only expresses GRs, higher levels of cortisol may lead to worsened executive functioning (**Figure 2**; Lupien et al., 2007; McEwen, 2007). The fact that both adrenal insufficiency and Cushing's disease have been associated with impaired declarative memory (Forget et al., 2016; Tiemensma et al., 2016) bolsters this biphasic effect hypothesis. Another argument is that the administration of the MR agonist fludrocortisone has been found to improve verbal and visuospatial memory performance in young as well as elderly healthy subjects (Hinkelmann et al., 2015); whereas the administration of hydrocortisone (mimicking the endogenous cortisol effects on both GRs and MRs) has been shown to enhance at lower doses yet impair at higher doses verbal memory retrieval in healthy subjects (Domes et al., 2005).

These effects that cortisol exerts on the brain structures involved in cognition are possibly mediated by modifications in responses to serotonin, in β-adrenergic receptor activation, in calcium influx, as well as in long-term potentiation (LTP), a process referring to a long-term strengthening of synaptic connections contributing to memory formation and consolidation (Joels, 2006; Lupien et al., 2007). Indeed, GR activation facilitates β-adrenergic signaling thus leading to the formation of adenosine 3′,5′-cyclic monophosphate (cAMP) and cAMP-dependent protein kinase (PKA). This pathway, once activated, is thought to inhibit the medial prefrontal cortex thus leading to an impairment in frontal functions, in particular working memory (McGaugh and Roozendaal, 2002; Barsegyan et al., 2010). Glucocorticoids have also been found to display certain effects on the hippocampus via actions on the serotoninergic system following the same biphasic pattern as described in **Figure 1**. Indeed, glucocorticoids can promote via MRs and, at the same time, inhibit via GRs the 5HT1A activation in hippocampal CA1 pyramidal cells (de Kloet et al., 2018).

Moreover, glucocorticoids have been reported to alter LTP in opposite directions depending on the MR/GR activation ratio: when the MR/GR activation ratio is high (central part of the inverted U-shaped plot, **Figure 1**), LTP is enhanced thus improving long-term memory consolidation. On the contrary, when the MR/GR ratio is low (extremes of the inverted U-shaped plot, **Figure 1**), LTP is suppressed thus worsening long-term memory consolidation (de Kloet et al., 1999; Lupien et al., 2007).

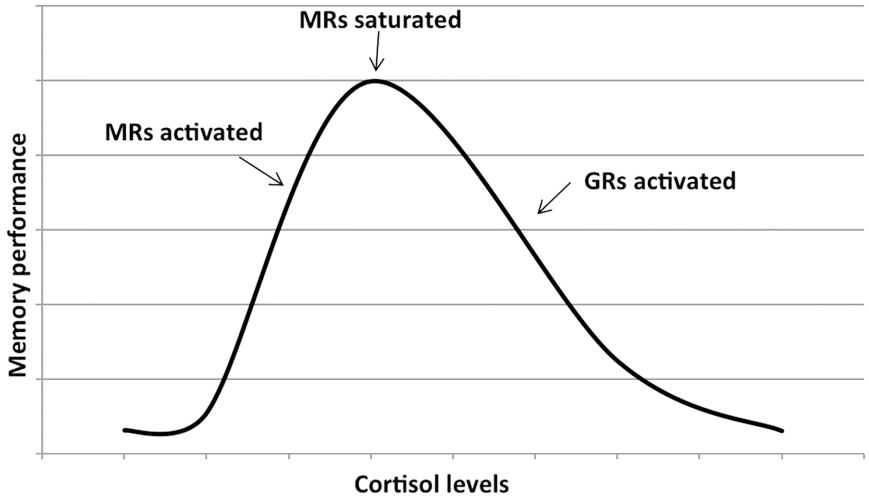

FIGURE 1 | Dose–response relationship between the memory performance and the cortisol levels. The first part of the plot shows that memory performance increases as cortisol levels increase (due to the activation of mineralocorticoid receptors or MRs). As soon as the MRs are saturated, further increase in cortisol levels activates the glucocorticoids receptors or GRs and memory performance decreases. Adapted with permission from Lupien et al. (2007).

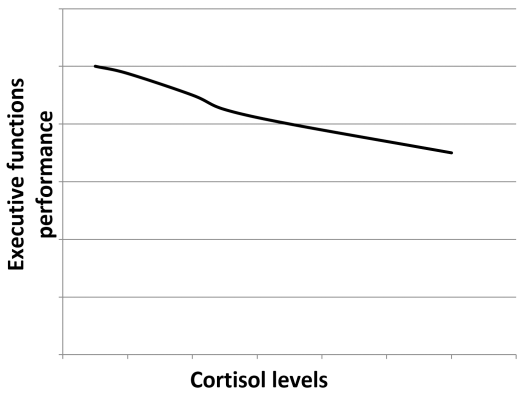

FIGURE 2 | Hypothetical dose–response relationship between the executive functions performance and the cortisol levels. As the prefrontal cortex only expresses GRs, the higher the cortisol levels, the poorer the executive functions performance.

In clinical studies, most studies examining the link between cortisol levels and global cognitive performance among non-demented older adults found that higher cortisol levels have been associated with poorer overall cognitive performance (Lupien et al., 2007; Lee B.K. et al., 2008; Ouanes et al., 2017a; Sang et al., 2018). Likewise, most (Beluche et al., 2010; Geerlings et al., 2015; Segerstrom et al., 2016; Ouanes et al., 2017a,b; Echouffo-Tcheugui et al., 2018), even though not all (Lee B.K. et al., 2008) studies exploring the relationship between episodic memory and cortisol levels have found an association between elevated cortisol and poorer episodic memory among older adults without dementia. These findings suggest that, even at levels that are within the normal range, cortisol can still activate GRs, and not just MRs. This also suggests that relatively small differences in cortisol levels can exhibit significant effects on memory performance. Studies exploring the relationship between cortisol levels and prefrontal cortex-mediated cognitive functions, mainly executive functions, processing speed and working memory, have found more discrepant results: while the expected negative association was reported in certain studies (Lee B.K. et al., 2008; Beluche et al., 2010; Geerlings et al., 2015), other studies failed to find such an association (Ouanes et al., 2017a,b; Echouffo-Tcheugui et al., 2018).

The differences in populations, assessment tools, as well as the likely effects of possible confounding factors including age, gender, educational level, as well as other neuroendocrine and psychological factors might explain these discrepancies.

CORTISOL AND CEREBRAL STRUCTURAL CHANGES

High cortisol has also been linked to decreased volume of several brain regions involved in cognitive functions. In fact, in a study by Geerlings et al. (2015) involving 4244 non-demented subjects, elevated evening cortisol was found to be associated with decreased volumes in all brain regions, in particular the gray matter (Geerlings et al., 2015). Similar findings have been reported in the dementia-free Framingham Heart Study participants: elevated cortisol was associated with decreased total brain volume, in particular decreased occipital and frontal gray matter volumes. In the same study, increased cortisol levels were associated with some microstructural changes, specifically in the corpus callosum and the posterior corona radiate (Echouffo-Tcheugui et al., 2018).

In addition, high levels of cortisol have been linked to hippocampal atrophy (Tatomir et al., 2014). This atrophy can be the consequence of the exposure to increased cortisol levels. Indeed, in Cushing's disease, the observed

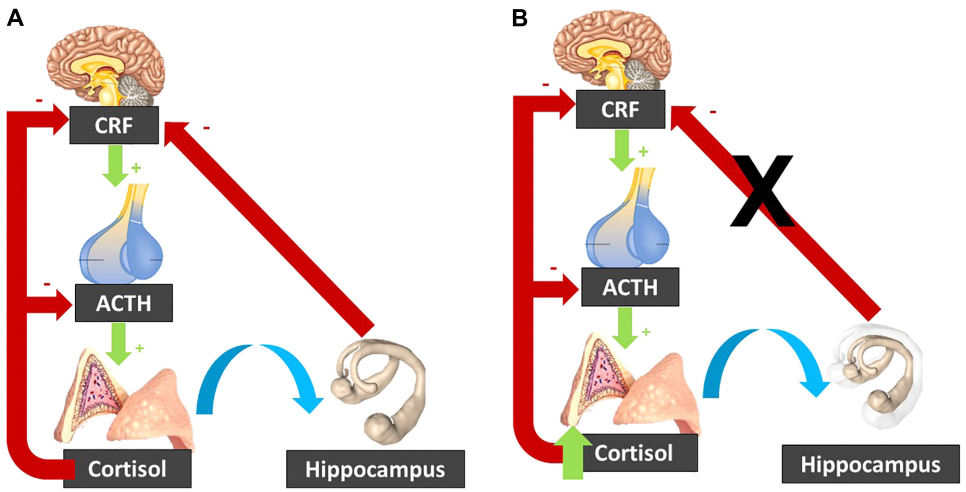

FIGURE 3 | (A) In normal circumstances, the CRF released by the hypothalamus activates ACTH release by the pituitary gland, which stimulates the adrenal glands to secrete cortisol. Cortisol inhibits its own secretion via a negative feedback loop. The hippocampus inhibits the hypothalamo-pituitary-adrenal axis. **(B)** When cortisol is elevated, it can induce hippocampal atrophy, which "lifts the brake" on the hypothalamo-pituitary-adrenal axis. The resulting cortisol increase induces further hippocampal atrophy, resulting in a vicious circle. CRF, corticotropin-releasing factor; ACTH, Adrenocorticotropic hormone.

hippocampal atrophy is reversed following treatment and normalization of the cortisol levels (Starkman et al., 1999). Yet, this atrophy can also be a cause of the elevated cortisol levels. Indeed, the hippocampus exerts an inhibitory effect on the HPA axis activity, and hence hippocampal atrophy might disinhibit the HPA axis leading to increased cortisol (Geerlings et al., 2015; **Figure 3**).

These effects on the hippocampal volume may be partly due to changes in brain-derived neurotrophic factor (BDNF) expression in the hippocampus (Suri and Vaidya, 2013). Similarly to their effects on cognitive performance, MR activation seems to increase whereas GR activation seems to decrease BDNF expression in the hippocampus (Kino et al., 2010; Suri and Vaidya, 2013).

In a study by Cox et al. (2015) elevated salivary cortisol levels at the start and at the end of a cognitive task appointment have been associated with a poorer white matter structure, i.e., greater white matter hyperintensity volume and/or elevated general factor of tract mean diffusivity. These findings suggest that aside from the "acute effects" of cortisol on cognition, chronically elevated cortisol levels likely bring about brain structural changes that may reflect long-term cognitive deficits.

HPA-AXIS DYSREGULATION, CORTISOL AND ALZHEIMER'S DISEASE PATHOLOGY, DISEASE RISK AND CLINICAL COURSE

Glucocorticoids have been reported to promote oxidative stress and to increase amyloid β (Aβ) peptide toxicity in cultured hippocampal neurons (Goodman et al., 1996). Besides, in a mouse model of AD, elevated cortisol has been linked to exacerbated Aβ peptide and tau pathology in the brain (Green et al., 2006).

In primates, year-long high-dose exposure to glucocorticoids was associated with decreased insulin-degrading enzyme levels, a candidate protease for the clearance of Aβ in the brain. At the same time, the Aβ1-42/Aβ1-40 ratio was increased indicating a relative shift toward increased production of the more brain toxic Aβ1-42 (Kulstad et al., 2005).

In a cross-sectional study examining the links between cardiovascular risk factors and Aβ brain burden as determined by Pittsburgh Compound B-positron emission tomography (PiB-PET), an association has been found between plasma cortisol and Aβ brain burden (Toledo et al., 2012).

Together, these findings suggest that increased cortisol may induce and/or exacerbate cerebral AD pathology by increasing Aβ brain burden, tau pathology as well as oxidative stress, which can all contribute to neurodegeneration. Effects on Aβ likely entail decreasing Aβ clearance as well as promoting the cleavage of Aβ into the most toxic compound (Aβ1-42) (Kulstad et al., 2005).

The aforementioned cerebral changes associated with elevated cortisol likely translate into findings of associations between increased cortisol levels and clinical features of AD, as shown in several clinical studies that reported increased cortisol levels in patients with clinical AD dementia (Dong and Csernansky, 2009; Popp et al., 2009; Ennis et al., 2017), and cortisol levels have even been found to correlate with the severity of the cognitive impairment (Pedersen et al., 2001; Zverova et al., 2013).

A prospective study by Ennis et al. (2017) S found that elevated cortisol (as measured by the urinary free cortisol/creatinine ratio) and elevated intra-subject cortisol variability (as measured by the within-person urinary free cortisol/creatinine ratio variability) were associated with a 1.31- and 1.38-times increase in AD risk. Furthermore, in cognitively healthy older adults with Aβ

positive PET imaging, high cortisol levels have been found to be associated with a faster decline in global cognition, in episodic memory, as well as in executive functioning, independently of age, sex, APOE genotype, or anxiety symptoms (Pietrzak et al., 2017). In a population based cohort study in 537 non-demented older adults (65 years or more at baseline), we found salivary cortisol day profiles to be not associated with faster cognitive decline over an average 5.3 years. However, preliminary analysis suggests that higher morning salivary cortisol measures may be associated with slight decline in global cognition (Albanese et al., 2018).

Higher plasma cortisol levels in patients with AD dementia have been associated with a more rapid cognitive decline in some studies (Pedersen et al., 2001; Huang et al., 2009). Similarly, Csernansky et al. (2006) found that high plasma cortisol levels were associated with faster cognitive decline in individuals with very mild or mild AD dementia.

In a cohort study from the Alzheimer Disease Neuroimaging Initiative (ADNI) investigating biomarkers able to predict progression from mild cognitive impairment (MCI) to AD within 1–6 years, plasma cortisol was one of the six biomarkers found to provide an accurate prediction (Lehallier et al., 2016).

Moreover, cortisol concentrations in the cerebrospinal fluid (CSF) have been found to be higher in subjects with dementia and MCI due to AD compared to control subjects (Popp et al., 2009, 2015). In MCI due to AD, high CSF cortisol was also predictive of a more rapid cognitive decline (Popp et al., 2015). Hence, elevated cortisol appears to contribute to exacerbate AD brain pathology, thereby contributing to the disease progression both pathologically and clinically. Cortisol levels appear to be increased at early stages of AD, and fasting plasma and CSF cortisol levels may even be pre-clinical markers (Notarianni, 2017).

These findings may be explained by increased Aβ neurotoxicity related to higher cortisol levels as well as neurodegeneration and functional impairment of the hippocampus occurring early in the course of the disease as both a consequence of exposure to high cortisol levels and a cause of HPA axis disinhibition, hence a vicious circle (Geerlings et al., 2015).

CORTISOL, COGNITION AND MEDIATING FACTORS

Certain factors may further explain the links between cortisol, cognitive impairment and dementia. Indeed, some of these factors may bring about HPA axis alterations that could affect cognition and the risk for dementia, in particular AD. These factors may include life events (Ouanes et al., 2017a), personality (Ouanes et al., 2017b; Tautvydaite et al., 2017; Terracciano et al., 2017), sleep disorders (Haba-Rubio et al., 2017), depression (Salvat-Pujol et al., 2017).

At the same time, some other factors such as metabolic syndrome, insulin resistance and effects on inflammation may mediate the effects of cortisol on cognition and brain structural changes (Kim and Feldman, 2015; Martocchia et al., 2016).

Cortisol, Cognition, Trauma and Life Events

Early trauma (of physical, sexual or emotional nature) has been linked to long-term cognitive deficits in adulthood (consisting in impaired spatial working memory and pattern recognition memory) in a study by Majer et al. (2010); however, this finding was not replicated in other studies (Saigh et al., 2006). Early stress has also been shown to be associated with structural and functional changes in brain regions involved in cognitive functions, including the frontal cortex as well as the hippocampus (Lupien et al., 2009).

Early trauma is also one of the most important established risk factors for PTSD. PTSD has been shown to be associated with an increased risk of dementia in both genders over an average of 8 years of follow-up (hazard ratio: 1.73[1.47, 2.02]) (Flatt et al., 2017). Nevertheless, Burri et al. (2013) found that the long-term cognitive deficits associated with PTSD were likely independent of earlier childhood adversity.

Aside from early trauma and PTSD, stressful life events have often been associated with HPA activation. Yet, some studies, conversely, showed decreased cortisol following stressful life events (Miller et al., 2007; Daskalakis et al., 2013). Results of the studies exploring the relationship between life events and cognition have been discrepant. On the one hand, several studies highlighted associations between stressful events and poorer subsequent cognitive performance, in particular in memory and executive functions (Xavier et al., 2002; VonDras et al., 2005; van Gelder et al., 2006; Lupien et al., 2007) above and beyond the impact of depression (Comijs et al., 2011). Importantly, stressful events have been also associated with an elevated risk of late-life dementia (Johansson et al., 2010) and late-life cerebral atrophy, and white matter lesions (Johansson et al., 2012). On the other hand, other studies failed to find any association between stressful life events and cognitive performance in the elderly (Ward et al., 2007; Fountoulakis et al., 2011; Sundstrom et al., 2014) and some even showed a possible improvement in cognition following certain stressful events (Deeg et al., 2005).

In a study exploring the mediation hypothesis between cortisol, life events and cognition in 796 non-demented subjects aged at least 65 we found elevated salivary cortisol levels to be linked to poorer cognitive performance, but this association was not related to life events (Ouanes et al., 2017a).

These discrepancies regarding the relationships between life events and cortisol on the one hand, and life events and cognition on the other hand, may be explained by different life events displaying different effects on cortisol and thus on cognition (Ouanes et al., 2017a).

Cortisol, Cognition and Personality

High neuroticism is the personality trait most consistently often associated with high cortisol (Bridges and Jones, 1968; van Eck et al., 1996; Miller et al., 1999, 2016; Portella et al., 2005; Yoshino et al., 2005; Gerritsen et al., 2009; Nater et al., 2010; Garcia-Banda et al., 2014). However, other studies found no association (Adler et al., 1997; Schommer et al., 1999; Ferguson,

2008), or even an opposite link (Ballenger et al., 1983; LeBlanc and Ducharme, 2005).

Higher neuroticism has also been reported to be cross-sectionally linked to lower cognitive performance above and beyond the effects of depression (Jorm et al., 1993; Boyle et al., 2010), especially to poorer episodic memory (Jorm et al., 1993; Meier et al., 2002; Klaming et al., 2016). In addition, high neuroticism scores have been found in association with elevated risk of AD (Terracciano et al., 2014).

A few studies examined the relationship between the other personality traits and cognitive performance and risk of dementia. Lower pre-morbid conscientiousness, agreeableness, openness and extraversion have been associated, although not consistently, with lower cognitive performance and higher risk for AD (Terracciano et al., 2014, 2017; Tautvydaite et al., 2017). In a cohort of memory clinic patients and cognitively healthy elderly volunteers we found lower extraversion and openness to correlate with CSF markers of AD pathology: tau, ptau-181, tau/Aβ1–42, and ptau-181/Aβ1–42 ratios, but not with the Aβ1–42 level (Tautvydaite et al., 2017).

In a population-based cohort study examining the interrelationships between cortisol, cognition and personality traits, salivary cortisol did not seem to mediate the link between personality traits and cognitive deficits (Ouanes et al., 2017b).

Besides methodological differences, these observed discrepancies may be due to the impact of depression and/or anxiety which has been controlled for in a few studies but not in others, but also to the difficulties (in cross-sectional studies, mainly) to disentangle pre-morbid personality traits from the personality modifications accompanying the cognitive decline.

Cortisol, Cognition and Sleep Disorders

Cognitive impairment has been associated with more time spent in stage N1 (first step of non-rapid eye movement sleep) and less in stage N3 (third step of non-rapid eye movement sleep) and in REM sleep, lower sleep efficiency, and more wake after sleep onset, as well as more severe sleep disordered breathing (as evidenced by higher apnea/hypopnea index or AHI, and higher oxygen desaturation index or ODI) (Haba-Rubio et al., 2017).

In a study of the same research group, involving 456 elderly non-demented subjects, obstructive sleep apnea (OSA) has been found to be linked to cognitive impairment, but the relationship did not appear to be mediated by diurnal cortisol levels (Haba-Rubio et al., 2018).

In other studies, OSA has been associated with increased nocturnal plasma cortisol levels (Chopra et al., 2017). Edwards et al. (2014) found that higher night-time cortisol was associated with worse cognitive performance, mainly affecting memory, above and beyond the apnea severity in a sample of patients with OSA.

Taken together, the results of these studies highlight links between cognitive performance and sleep disorders on the one hand, and between cortisol levels and cognitive functioning on the other hand, but do not provide evidence to support that cortisol may actually mediate the relationship between sleep disorders and cognitive impairment.

Cortisol, Cognition and Depression

Depression has been associated, on the one hand with HPA axis hyperactivity and impaired negative feedback (Anacker et al., 2011), and on the other hand with cognitive deficits involving attention, episodic memory and executive functions (Salvat-Pujol et al., 2017). Depression has also been tied to late-life dementia, in particular with vascular and AD dementia (Brunnstrom et al., 2013). This association is not just a mere comorbidity, as late-life depression may also be a risk factor for both AD and vascular dementia (Diniz et al., 2013; Herbert and Lucassen, 2016). In the AGES-Reykjavik population-based study (Geerlings et al., 2017), both current major depressive disorder and high evening cortisol levels were associated with an higher risk of incident AD and non-AD dementia, but cortisol did not seem to be a major factor explaining the relation between depression and risk of dementia.

Some of the observed cognitive deficits in verbal and visual memory and executive functions may remain present even after the depressive symptoms fully remitted (Herrera-Guzman et al., 2010; Rock et al., 2014; Salvat-Pujol et al., 2017). Likewise, the HPA axis abnormalities associated with depression may persist even after remission (Lok et al., 2012; Salvat-Pujol et al., 2017), possibly constituting trait rather than state markers for depression, even though this remains a matter of debate (Zverova et al., 2013; Salvat-Pujol et al., 2017).

Remission status in depression did not moderate the association between cognitive performance and the Dexamethasone suppression test ratio or the cortisol awakening response (CAR), defined by the increase in cortisol secretion after awakening (Fries et al., 2009). However, remission appeared to moderate the association between cortisol slope defined by the difference between maximal and minimal cortisol levels during the nyctemera, and certain cognitive tasks assessing processing speed and executive function (Salvat-Pujol et al., 2017). HPA axis alteration in depression may inhibit neurogenesis, partly through reducing BDNF which is involved in hippocampal neurogenesis, thus possibly explaining one of the mechanisms by which depression may be a risk factor for AD (Herbert and Lucassen, 2016).

Delirium is common in AD, and it is associated with more rapid clinical disease progression (Popp, 2013). Depression symptoms and cognitive impairment have been independently associated with higher risk of developing delirium. In a yet-to be-published study by the same team, increased cortisol levels have been observed in patients with delirium suggesting HPA axis dysregulation to be involved in the pathophysiology of delirium. In a cohort of elderly patients undergoing elective cardiac surgery, pre-operative geriatric depression scale scores were found to predict post-operative delirium. However, pre-operative morning plasma cortisol levels were not associated with post-operative delirium in this study.

Whether and how HPA axis dysregulation and increased cortisol levels may contribute to the magnitude of cognitive and non-cognitive symptoms in AD, needs further investigation. In a study in patients with AD dementia, plasma cortisol levels have been shown to reflect the degree of cognitive deficits in AD dementia rather than the severity of the comorbid depression (Zverova et al., 2013).

Altogether, these data suggest that, mostly, cognitive deficits linked to increased cortisol and HPA axis dysregulation cannot be entirely explained by a co-occurring depressive disorder. AD pathology may exacerbate HPA axis dysregulation which may contribute to the manifestation of depressive symptoms and to the severity of cognitive impairment and increase the risk of other non-cognitive syndromes, including delirium. Even though depression-associated HPA axis dysregulation may predispose to and/or exacerbate the course of AD (Herbert and Lucassen, 2016), studies suggest a link between HPA axis dysregulation and AD itself above and beyond depression.

Cortisol, Cognition and the Metabolic Syndrome

Elevated cortisol levels have been tied to insulin resistance and metabolic syndrome, which in turn, have been associated with both AD and vascular dementia. (Kim and Feldman, 2015; Martocchia et al., 2016). Hence, elevated cortisol may lead, through its metabolic syndrome-associated effects on glucose, blood pressure and lipids, to an increased cardiovascular risk (Lattanzi and Silvestrini, 2017). Indeed, higher cortisol has been associated with a higher number of carotid plaques (Hamer et al., 2010). The resulting vascular lesions in the brain may directly induce cognitive disturbances, but can also contribute to the neurodegeneration observed in AD (Attems and Jellinger, 2014). Moreover, insulin resistance itself may negatively influence the amyloid cascade (Stefanelli et al., 2014).

At the same time, AD-associated hypercortisolemia, present at very early stages, may also induce pre-diabetes. The resulting increased insulin secretion can further exacerbate the hypercortisolemia, thus possibly negatively affecting the course of AD (Notarianni, 2017).

Cortisol, Cognition and Inflammation

While cortisol is generally known to exert broad anti-inflammatory effects, high cortisol levels may activate NACHT, LRR and PYD domains-containing protein 1 (NLRP-1) inflammasome in hippocampal neurons, thus promoting neuroinflammation and thereby neuronal injury (Zhang et al., 2017).

Moreover, certain cytokines, in particular IL-1-Beta and IL-6, which are also known to be involved in the pathophysiology of AD, can activate the HPA axis (Besedovsky and del Rey, 2000). The resulting increased cortisol can reinforce the toxic effects on the hippocampus exerted by the pro-inflammatory cytokines (Sudheimer et al., 2014), thus contributing to the pathophysiology of AD.

CORTISOL AND POTENTIAL PREVENTIVE AND THERAPEUTIC INTERVENTIONS FOR ALZHEIMER'S DISEASE:

Since increased cortisol has been associated with both AD pathology and more rapid clinical disease progression, and since most detrimental effects of cortisol are likely exerted via GRs, therapeutic interventions targeting the GRs have been investigated. Indeed, the GR antagonist mifepristone has been shown to decrease both Aβ and tau load in the brain as well as to improve the pathologically induced cognitive impairments in a triple-transgenic (3xTg AD) mouse model of AD (Baglietto-Vargas et al., 2013). In a similar way, mifepristone has been shown to reduce the hippocampal Aβ levels and rescue the cognitive deficits induced by early life stress in APP/PS1 transgenic mice (Lesuis et al., 2018). As these pathological processes start years or even decades before the onset of the first symptoms, cortisol lowering or cortisol effects modulating interventions in midlife may slow down the development of amyloid pathology and neurodegeneration, and prevent cognitive decline in later life (Lante et al., 2015; Lesuis et al., 2018). Such interventions could prove useful, in particular in subjects at risk for developing clinical AD (Pietrzak et al., 2017) and prone to stress, and HPA-axis dysregulation. Prevention trials with focus on cortisol or HPA-axis in human subjects with normal cognition have not been reported so far, however.

One randomized controlled trial in a small sample of patients with mild to moderate AD dementia showed improvement of cognitive performance in memory tasks, but the premature termination did not allow any firm conclusions regarding efficacy (Pomara et al., 2002). Other trials using mifepristone that were initiated were terminated without being published, indicating that these trials were not completed, or yielded negative results (O'Banion, 2013).

Also, several non-pharmacological intervention in subjects with MCI (Baker et al., 2010) or dementia (Woods et al., 2009; Schaub et al., 2018) have shown cortisol lowering effects.

However, there has been some loss of interest in GR antagonists because of their side effects due to GRs being ubiquitous, especially as more selective molecules, namely GR modulators have been developed (Canet et al., 2018).

Glucocorticoid Receptor modulators have been shown to normalize basal glucocorticoid plasma levels, decrease hippocampal Aβ peptide deposition, inhibit neuroinflammation, and apoptotic processes, and improve cognitive performance in a mouse model of AD (Pineau et al., 2016).

Another potential mechanism by which cortisol effects can be reduced pharmacologically is the inhibition of cortisol synthesis, one of the key enzymes being the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Currently, a phase II trial of

an 11β-HSD1 inhibitor (UE2343) as a potential treatment for AD is being conducted (Webster et al., 2017).

STRENGTHS AND LIMITATIONS

This narrative review provides a concise overview of the different molecular, cellular, and clinical (including diagnostic, prognostic, and therapeutic) aspects of the interrelationships between cortisol, cognition, dementia, and AD. However, it does not cover all possible facets of these complex relationships. We focused on the most important and the most clinically relevant aspects of the topic, rather going in depth into one particular aspect of the topic.

CONCLUSION

There is a growing body of evidence that increased cortisol may be deleterious for the late-life cognitive performance, and may be associated with an increased risk for cognitive decline and dementia, in particular dementia due to AD. In patients with AD, the increased cortisol at preclinical and early clinical stages is associated with a poorer prognosis and a more rapid cognitive decline. Increased cortisol may represent a pathophysiological mediator between stressful life events, personality, mood, and sleep, and may increase both the risk of AD and the extent of symptoms at clinical stages of the disease. Yet, the exact underlying mediating factors are not fully understood. Direct deleterious cortisol effects on the hippocampus and on the prefrontal cortex are likely, but also cortisol links with metabolic syndrome and neuroinflammation; and HPA axis disinhibition due to neurodegeneration are other possible mechanisms that may explain the association of cortisol with late-life cognitive impairment and AD.

Further studies are needed to confirm the value of cortisol levels as a possible preclinical marker associated with higher risk, and/or as a prognostic parameter in subjects with clinical AD. Future research may also bring in new HPA-based interventions for the prevention and/or management of symptoms, and of the clinical progression of AD.

AUTHOR CONTRIBUTIONS

SO participated in literature review and in writing the first draft of the manuscript. JP participated in literature review and revised the article.

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frontiers in Aging Neuroscience

REVIEW
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The Influence of Genetic Factors and Cognitive Reserve on Structural and Functional Resting-State Brain Networks in Aging and Alzheimer's Disease

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Magnetic resonance imaging (MRI) offers significant insight into the complex organization of neural networks within the human brain. Using resting-state functional MRI data, topological maps can be created to visualize changes in brain activity, as well as to represent and assess the structural and functional connections between different brain regions. Crucially, Alzheimer's disease (AD) is associated with progressive loss in this connectivity, which is particularly evident within the default mode network. In this paper, we review the recent literature on how factors that are associated with risk of dementia may influence the organization of the brain network structures. In particular, we focus on cognitive reserve and the common genetic polymorphisms of *APOE* and *BDNF* Val66Met.

Keywords: fMRI, Alzheimer's disease, default mode network, cognitive reserve, BDNF, APOE

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INTRODUCTION

Recently, it was estimated that more than 47 million elderly people are affected by dementia globally (Alzheimer's Disease International, 2009; Prince et al., 2016) and that an additional 131 million people will develop this health-challenging syndrome by 2050 (Prince et al., 2016). Alzheimer's disease (AD), a progressive condition causing behavioral changes, memory loss, and decline in learning capacity (Anand et al., 2014), is the most common cause of dementia worldwide (Hardy, 1997). Most cases of AD occur in individuals over the age of 75, but, relatively younger individuals, including those carrying certain genetic mutations (Loy et al., 2014), may develop the disease before 65 years of age (Alzheimer's Association, 2015).

Knowledge of the brain changes that occur in AD has increased remarkably from the late 20th century due to extensive research on a range of related neurodegenerative processes. Particular progress has been made with regard to what has been termed the pathological 'hallmarks' of AD – the presence of amyloid plaques and neurofibrillary tangles (NFTs) – which detrimentally affect axons, dendrites, and synapses (Vickers et al., 2000, 2016). Plaques are the result of accumulations of an abnormal form of the beta amyloid (Aβ) protein in the brain. NFTs are formed by the aggregation of aberrant tau protein (Vickers et al., 2000; Savva et al., 2009) and are more directly related to the

death of neurons (Jacobs et al., 2012). Within the cerebral cortex, the earliest plaques are usually found in the neocortex, whilst initial formation of tangles occurs in medial temporal lobe (MTL) structures, such as the entorhinal cortex and hippocampus (Price and Morris, 1999). The MTL is a very important region responsible for memory formation and long-term memory (Squire and Zola-Morgan, 1991). Throughout the cerebral cortex, neurons that provide long corticocortical connections are the most prone to NFT-induced deterioration (Morrison and Hof, 1997), which may then underlie the pattern of synaptic loss seen in AD. Entorhinal-hippocampal circuits are compromised early in AD, followed by the gradual disconnection of the MTL, and then the loss of connectivity between association neocortices (Morrison and Hof, 1997). This pattern of progressive and degenerative pathology may underlie the deterioration of certain cognitive functions during aging, leading eventually to frank AD. The early pathological accumulation of Aβ has been linked to cognitive impairment and could also affect functional connectivity between spatially distant brain regions (Delbeuck et al., 2003). A summary table of studies examining functional connectivity and Aβ in healthy aging and AD can be found in **Table 1**. Neuroimaging is a vital component of international research collaborations (Hendrix et al., 2015) and has been used to investigate mechanisms of interrupted structural and functional connectivity underlying the course of AD (Dennis and Thompson, 2014). A better understanding of how the pathological changes in AD affect the organization of brain networks, or how these networks may respond or adapt to accumulating pathology, might offer further insights into the potential scope of functional resilience. The term resilience is described as the capability of a tissue to be resistant to damage (Cosco et al., 2017). In this respect, factors such as education and lifestyle could increase resilience by heightened connectional redundancy and/or preserving functional connections in the brain, and may ultimately delay the clinical expression of AD pathology. Indeed, studies investigating the association of education and cognitive decline in AD have found that more highly educated individuals are able to tolerate more neuropathology before the clinical expression of AD (Bennett et al., 2003), potentially because education moderates the relationship between brain pathological load and cognitive impairments (Brayne et al., 2010; Valenzuela et al., 2011), as well as functional connections (Marques et al., 2016).

Studies have shown that functional connectivity is damaged or interrupted in AD (Stam et al., 2006, 2008), and, conversely, investigating the impact of AD on structural and functional networks may also provide more accurate information regarding brain connectivity and how brain regions communicate with each other (Sheline and Raichle, 2013). This review focuses on the methods with which brain connectivity is analyzed, the changes in structural and functional networks found in AD, and the role of cognitive reserve and specific genetic factors in partially determining functional brain connectivity. In this regard, potential changes in functional connectivity and resistance to pathology will involve both non-modifiable and modifiable factors that will impact on how brain systems respond to accumulating pathological burden. Hence, we discuss features of structural and functional brain networks in relation to genetic biomarkers and environmental factors linked to AD risk, progression and resilience.

METHODS TO ANALYZE CONNECTIVITY

Neuroimaging techniques (**Figure 1**), such as magnetic resonance imaging (MRI), have long been used to investigate anatomical connections, detect pathological alterations, and monitor the progression of neurodegenerative diseases, including AD (**Figure 1A**). MRI involves the generation of a strong static magnetic field to create images and to map fluctuation signals related to brain activity (Heeger and Ress, 2002). MRI also allows the quantification of brain atrophy, which can be used to distinguish normal brain aging from AD (Frankó et al., 2013). For example, a recent study found that MRI and cognitive testing in cognitively healthy individuals are useful tools for predicting the development of AD, particularly when investigating the progress from healthy cognition to the appearance of mild cognitive impairments (MCIs) after 5 years (Albert et al., 2018). The delayed presence of clinical symptoms makes it challenging to diagnose individuals in preclinical stages. Therefore, animal models could provide an opportunity to identify biomarkers of early disease (Sabbagh et al., 2013), which include insights from neuroimaging, such as gray and white matter alterations measured by diffusion tensor imaging (DTI; Weston et al., 2015).

Diffusion tensor imaging is an MRI-based neuroimaging method that measures the diffusion of water molecules, enabling the assessment of the fiber-tract structures of white matter (Jones et al., 2013; Teipel et al., 2016). This technique allows the strengths and differences of white matter tract connections in specific population groups to be compared (Jones et al., 2013) before a reduction of cognition is evident (López-Gil et al., 2014), for example between older individuals with and without AD (**Figure 1C**). Other structural imaging parameters that are currently used to gain further insight into the integrity of the brain over the life include intracranial volume and the presence and number of white matter hyper-intensities (Bartrés-Faz and Arenaza-Urquijo, 2011).

Functional MRI (fMRI) permits simultaneous monitoring of the activity of different brain regions while a subject is at rest or performing a task (Binder et al., 1999). In fMRI, oxygen in blood is measured through blood-oxygen-level-dependent (BOLD) signals (Ogawa et al., 1990; Heeger and Ress, 2002). Specifically, the underlying premise is that more oxygen is required for greater neuronal activity, thereby creating a signal that can be detected using fMRI (**Figure 1B**). Thus, it is possible to measure changes in oxygen concentration, cerebral blood flow (CBF) and volume (CBV) that are delayed by 1–2 s after MRI excitation. This is referred to as the hemodynamic response (Buxton et al., 2004). If the BOLD signal from different areas of the brain show similar and synchronized activity, it is assumed that these regions communicate with each other and transfer information, which is defined as functional connectivity (Raichle, 1998). Functional

TABLE 1 | Studies examining functional connectivity and amyloid-beta in healthy aging and Alzheimer's disease.

Study	Samples	Imaging measures	Main findings
Fischer et al. (2015)	CN preclinical AD (*n* = 12), Age-matched controls (*n* = 31)	DTI using tractography, measuring fludeoxyglucose-PET	CN preclinical AD (with Aβ positivity) exhibited similar white matter network changes to clinical AD as compared to controls; for instance, CN preclinical AD had more shorter paths and reduced global efficiency compared to controls.
Grandjean et al. (2014)	Transgenic mice (*n* = 38) Wild-type mice (*n* = 36)	Structural MRI, Rs-fMRI DTI	The progression of functional connectivity was disrupted in somatosensory and motor cortex in ArcAβ transgenic mice compared to wild-type mice. This decrease was noticeable even before amyloidosis in transgenic mice.
Mormino et al. (2011)	CN older (*n* = 44), AD (*n*=22)	Structural MRI, Rs-fMRI, PIB-PET imaging	Increased Aβ in CN older individuals was associated with decreased default mode network functional connectivity in multiple posteromedial regions suggesting that the accumulation of Aβ and related brain changes occurs before overt cognitive impairment.
Sheline et al. (2010b)	35 AD, 68 CN older PIB− (*n* = 24) PiB+ (*n*=20)	Structural MRI, Rs-fMRI, and Dynamic PET scan	CN people with Aβ deposition exhibited impairments in functional connectivity, particularly default mode network disruptions.
Bero et al. (2012)	Young APP/PS1 transgenic mice (*n* = 7) Old APP/PS1 transgenic mice (*n* = 7) Young wild type mice (*n* = 13) Old wild type mice (*n* = 10)	Functional connectivity optical intrinsic signal imaging	Aβ accumulation was related to decreased functional connectivity in older APP/PS1 mice compared to young APP/PS1 mice and wild-type mice. Brain regions that had more Aβ showed the most conspicuous age-related decreases in connectivity.
Hedden et al. (2009)	38 CN older adults, PIB− (*n* = 17), PiB+ (*n* = 21)	Structural MRI, fMRI, Dynamic PET	Functional connectivity was disrupted in CN older adults with Aβ positivity. Connectivity impairments related to Aβ deposition were evident between the hippocampus and posterior cingulate (default mode network regions) and associated with memory deficit.
Drzezga et al. (2011)	CN PiB− (*n*=12) CN PiB+ (*n* = 12) MCI PiB+ (*n* = 13)	Structural MRI, Rs-fMRI, fluorodeoxyglucose-PET, PiB-PET	MCI with Aβ burden exhibited hypometabolism, decrease of neuronal activity and disruption of functional connectivity in posterior brain regions (precuneus/posterior cingulate) compared to CN older adults.
Lim et al. (2013)	165 CN PIB− (*n* = 116) PiB+ (*n* = 49) BDNF Met carriers (*n* = 58) BDNF Val/Val (*n* = 107) APOE e4 (*n*=70)	Structural MRI, PET PiB imaging, Neuropsychological assessments at baseline, 18 and 36 months	BDNF Met carriers with Aβ burden positivity demonstrated an accelerated decline in memory function as well as a reduction of hippocampal volume compared to BDNF Val homozygotes.
Franzmeier et al. (2017b)	CN Aβ+ (*n* = 24) amnestic MCI Aβ (*n* = 44)	Structural MRI, Rs-fMRI, FDG-PET	Individuals with amnestic MCI with Aβ positivity and more years of education demonstrated attenuation of precuneus hypometabolism and relatively increased global frontal cortex functional connectivity.

AD Alzheimer's disease, Aβ Amyloid-beta, APP/PS1 Amyloid precursor protein presenilin, APOE Apolipoprotein E, BDNF Brain-derived neurotrophic factor, CN Cognitively normal, DTI Diffusion tensor imaging, FDG Fluodeoxyglucose, MCI Mild cognitive impairment, MRI Magnetic resonance imaging, PET Positron-Emissions-Tomography, PiB Pittsburgh Compound B, Rs-fMRI Resting-state functional magnetic resonance imaging.

connections, defined as temporal correlations between spatially distant cortical brain regions, are revealed through fluctuations in low-frequency portions of BOLD signals (Ogawa et al., 1990). With age, functional connectivity networks gradually decrease (Dennis and Thompson, 2014), which may be important for understanding early AD or the series of brain changes that make the older brain more or less susceptible to additional disease processes.

Resting-state fMRI is an increasingly frequent method employed to study differences between various cohorts and involves the investigation of the activity of the brain while the individual is at rest and not performing a task. Resting-state fMRI can be used to determine how different brain regions operate and process information in functional space. Additional advantages are that resting-state fMRI is less demanding on the individual and easier to apply than task-related fMRI (Sheline and Raichle, 2013). The individual is instructed to not fall asleep while keeping their eyes closed in a lying position.

There are a variety of approaches for analyzing resting-state fMRI. For instance, seed-based analysis (Beckmann et al., 2005) investigates the BOLD signals between the selected region of interest (seed region) and the rest of the brain (Biswal et al., 1995). In AD, the precuneus has showed decreased functional connectivity to other brain regions, such as the left hippocampus, left parahippocampus, anterior cingulate cortex and gyrus rectus, as compared to non-dementia controls (Sheline and Raichle, 2013). The investigation of simultaneous neuronal connections across the brain is called independent component analysis (ICA),

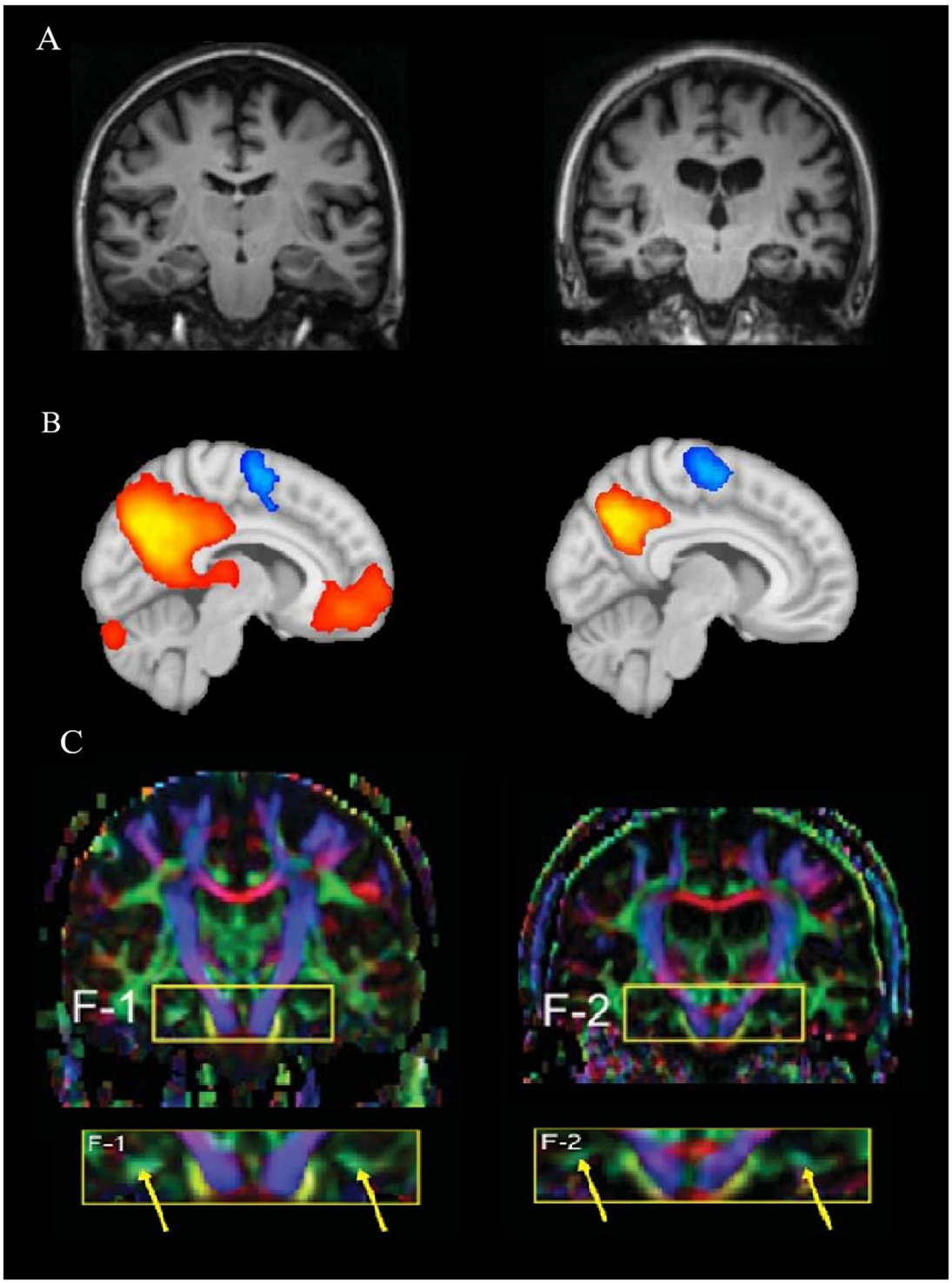

FIGURE 1 | Differences among the imaging techniques, MRI, fMRI, and DTI. **(A)** A structural MRI comparison between a healthy human brain (left) compared to pathological changes in Alzheimer's disease (AD, right; Oishi et al., 2011). **(B)** A functional MRI representing brain activation of a resting-state network in a healthy brain (left) compared to a hypothetical AD brain activation (right). The representation of the connectivity map shows how brain activity decreases with pathology within the default mode network (DMN); red/orange represents higher connectivity, while blue represents inversely correlated activity. **(C)** A comparison between a cognitively healthy woman (72 years old, left) and a woman with AD (70 years old; Oishi et al., 2011). The yellow arrows show the different color strength of the cingulum hippocampal area after DTI analysis. **(A,C)** Reprinted from Oishi et al. (2011) with permission from IOS Press. The publication is available at IOS Press through https://content.iospress.com/articles/journal-of-alzheimers-disease/jad0007.

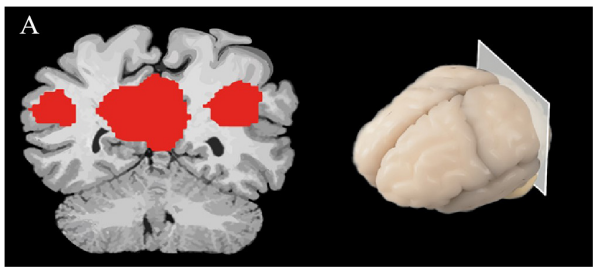

FIGURE 2 | A spatial map of a brain slice is represented, demonstrating brain activity in the DMN; red represents regions that are most active while the individual is at rest.

and is a wholly data-driven form of analysis (Beckmann et al., 2005) (**Figure 2**). Using ICA-based analysis, Greicius et al. (2004) reported a decline of resting-state functional connectivity between hippocampus and posterior cingulate cortex (PCC) in the AD group compared to healthy older individuals.

Another technique used to examine resting-state functional connectivity is graph analysis, which employs a way of specifically visualizing the complex interactions in the brain (Mijalkov et al., 2017). Using graph theory, functional connectivity is represented as a series of 'nodes' (voxels) and 'edges' (correlated activity between nodes) (Watts and Strogatz, 1998; Stam et al., 2007). It has been predicted that small-world networks in human fMRI studies with low-frequency oscillation might reveal connectivity of the brain structure. A specific focus of this form of analysis in network organization is the average minimum number of edges that must be traversed between any two nodes in a brain network, referred to as 'effective path length.' The characteristics of small-world networks are clustering coefficient, high integration and their typical feature is shorter effective path length (Travers and Milgram, 1967; Rubinov and Sporns, 2010; Kaminski and Blinowska, 2018). Cluster coefficient is described as a measurement of nodes that are locally interconnected (Kaminski and Blinowska, 2018). This approach is particularly useful when measuring and comparing differences in structural and functional connectivity (Bullmore and Sporns, 2009), and could be used to advance our understanding of the pathology of neurodegenerative diseases (**Figure 3A**). A further advantage of graph theory analysis is that it makes no assumptions about how close any two nodes are in space.

CHANGES IN STRUCTURAL AND FUNCTIONAL CONNECTIVITY IN AD

Structural Connectivity

In AD, the loss of connections between neurons can result in other structural alterations, such as atrophy, hypometabolism, and NFT accumulation (Zhang et al., 2009). Significant atrophy in AD, identified through MRI, occurs in the posterior hippocampus and the temporal and parietal cortices, which are three of the structures that are involved in the default mode network (DMN; Greicius et al., 2003). The default mode is a network in the brain that is activated when individuals are not engaged in a task, but are spontaneously thinking of past or future events (Buckner et al., 2008). The DMN is a highly interconnected set of cortical regions that demonstrate substantial correlated activity, particularly when the attentional network is inactive (Shulman et al., 1997; Buckner et al., 2008).

Diffusion tensor imaging studies investigating white matter changes in individuals with AD have demonstrated that the disease causes a deterioration of white matter fiber bundles in the MTL (Zhang et al., 2007), which may be present years before overt episodic memory deficits (Sexton et al., 2010), impaired executive function (Reijmer et al., 2014), and other symptoms of cognitive impairment (Zhang et al., 2007; Fischer et al., 2015). Similarly, in an animal model, López-Gil et al. (2014) reported neuronal differences in structural networks of chronically hypertensive rats before the manifestation of disrupted executive functioning occurred, which may provide insights into early stages of dementia. Moreover, Grandjean et al. (2014) discovered reduced fractional anisotropy values in transgenic mice with cerebral Aβ. In cognitively healthy individuals with elevated Aβ in the brain, potentially the pathological correlate of early AD, structural changes appear similar to individuals with MCI in terms of the topology of structural network connectivity (Fischer et al., 2015). Interestingly, these individuals with high brain Aβ load despite no overt cognitive symptomatology, demonstrated increased shortest path length in white matter networks in the absence of major neurodegenerative features such as atrophy or reduction of cortical glucose (Fischer et al., 2015).

Finally, the structural networks (or nodes) of individuals with AD who possessed fewer connections (or edges) were more susceptible to global disruption of white matter tracts than individuals with more connections (Daianu et al., 2015). In addition, a rat transgenic model bearing mutant human amyloid precursor protein (APP) and presenilin genes also demonstrated a reduction of local and global efficiency, as well as less clustering as compared to non-transgenic rats (Muñoz-Moreno et al., 2018). Moreover, Muñoz-Moreno et al. (2018) found alterations in the right medial PFC in these transgenic rats, while in human studies, the right medial frontal cortical areas in AD indicated a decline in nodal efficiency compared to healthy controls (Lo et al., 2010). In summary, changes in structural connectivity could be useful in predicting the degradation of white matter bundles, as well as the strength of functional connectivity networks (Greicius et al., 2009).

Functional Connectivity

Performance within many domains of cognitive function decreases slowly with age, but, importantly, higher cognitive performance has been correlated with increased functional connectivity in older adults (Arenaza-Urquijo et al., 2013). Compared to animal studies, transgenic AD rat models require longer cognitive training to achieve the same performance as non-transgenic rats. Although the structural network was changed, these alteration did not result in functional network differences proposing associations between the capability to learn and the reorganization of functional networks in the

brain (Muñoz-Moreno et al., 2018). Nevertheless, a gradual decrease in functional connectivity among the hippocampus and medial prefrontal cortex (PFC) is expected with age (Damoiseaux et al., 2016).

It has been proposed that disconnection of functional networks in the brain, such as those observed in AD, could serve as critical markers for the presence of early stages of neurodegenerative diseases, particularly with regard to the abnormal accumulation of Aβ in the brain (Stam et al., 2007; Zhang and Raichle, 2010). Resting-state studies have reported a decrease in functional connectivity in healthy older individuals with Aβ burden in the posteromedial regions, ventral medial PFC, right angular gyrus, and the left middle and superior frontal gyri (Mormino et al., 2011), as well as between the precuneus and left hippocampus, parahippocampus, anterior cingulate, gyrus rectus and dorsal cingulate (Sheline et al., 2010a,b). Early accumulation of Aβ in older healthy individuals, particularly in the precuneus, has been suggested to result in impairment in hippocampal function (Sheline et al., 2010a,b). In contrast, Mormino et al. (2011) reported that DMN connectivity responds in a varied manner to the presence of higher Aβ deposition in older non-demented people with Aβ accumulation. Specifically, the authors found that there was increased connectivity in regions of the right dorsal PFC, left anterior medial PFC and left temporal cortices, as well as decreased DMN connectivity in several posteromedial regions, the ventral medial PFC, right angular gyrus, and the left frontal gyri (Mormino et al., 2011). Disruption within the DMN has also been found in healthy older individuals with high amyloid burden (Hedden et al., 2009). Interestingly, these healthy individuals (n = 38) exhibited the same amount of Aβ burden compared to half of the individuals with MCI (n = 46) and all individuals with AD (n = 35).

Such associations have also been investigated in animal models. Bero et al. (2012) demonstrated an aging-related reduction of bilateral functional connectivity in the retrosplenial cortex in wild-type mice, which could be a pre-existing biomarker for neural dysfunction due to its significant association with memory performance (Corcoran et al., 2011). Interestingly, in transgenic AD mouse model involving cortical amyloidosis, it has been shown that an age-related decrease in functional connectivity in specific brain regions is more severe in the presence of higher Aβ deposition (Bero et al., 2012). Grandjean et al. (2014) also reported reduced functional connectivity in transgenic mice, however, this reduction appeared in the early months before the accumulation of Aβ in the somatosensory and motor cortex.

A study investigating whole-brain connectivity found abnormalities in cortical hubs of the temporo-parietal cortex and precuneus/PCC in healthy mild cognitive impaired subjects with Aβ burden (Drzezga et al., 2011). In general, greater atrophy has been related to less brain connectivity (Hoffstaedter et al., 2015), but not all studies have found support for this association. For example, a study by Gili et al. (2011), reported that functional connectivity decline was not related to the amount of gray matter atrophy in the PCC in individual with MCI.

Disconnection between functional networks could be an essential biomarker for AD. For instance, individuals with AD exhibit disruption of functional connectivity between the inferior lateral temporal cortex (ITC), precuneus, right thalamus and the PCC (Zhang et al., 2009), between the left hippocampus and PCC (Sorg et al., 2007), as well as between the right hippocampus and the right and left cuneus, precuneus, and right ITC (Wang et al., 2006). This pattern of disconnection is likely associated with impairments in memory, processing speed and executive function (Damoiseaux et al., 2016). Another proposed early biomarker for AD could lie in the disruptions that have been identified within the visual cortices, specifically the impairments in connectivity between the PCC and the dorsal and ventral visual pathways (Zhang et al., 2009). These changes have been suggested to lead to deteriorating visual function in AD (Zhang et al., 2009).

Small-world network analysis in AD has shown longer path length in the central, temporal, and frontal brain regions as compared to age-matched, non-demented individuals (Stam et al., 2007). Decreased local connectedness within networks, also called clustering, has also been reported in individuals with AD, and correlated with lower cognitive performance (Stam et al., 2007). This finding led Stam et al. (2007) to speculate that individuals in the early stages of AD may show relatively diminished topology of small-world networks. A recent study found support for this notion by demonstrating that individuals with MCI and AD had a longer characteristic path length compared to healthy controls (Mijalkov et al., 2017). Moreover, AD appeared to be associated with a greater number of edges connecting to a node regionally, as well as increases and decreases in the efficiency of local nodes when compared to the controls (Mijalkov et al., 2017). To understand these differences in network topology, it is necessary to account for genetic variations that might affect the organization of the brain and which may also be linked to neurodegeneration in AD (**Figure 3A**).

ROLE OF GENETIC FACTORS RELATED TO AD IN FUNCTIONAL CONNECTIVITY

Apolipoprotein E (APOE)

The inheritance of gene-related factors such as apolipoprotein E (*APOE*), in particular the *APOE* ε4 allele, is associated with an increased risk of AD (Mahley et al., 2006, This genetic polymorphism is associated with increased Aβ deposition in the brain (Mahley et al., 2009; Morris et al., 2010; Sheline et al., 2010a), possibly influencing brain functional connectivity (Mahley et al., 2009), as well as affecting cognitive functioning in older age (Wisdom et al., 2011).

Resting-state fMRI studies have reported diverging associations of *APOE* polymorphisms and functional connectivity in healthy individuals that may relate to the age of the sample groups (Goveas et al., 2013; Wu et al., 2016). For example, *APOE* ε4 alleles have been associated with both increased and decreased DMN functional connectivity in cognitively healthy individuals (Fleisher et al., 2009). Comparing non-demented middle-aged (50–65 years) individuals carrying the *APOE*ε4 with non-carriers, ε4 carriers showed elevated functional connectivity in the middle frontal gyrus, whilst non-ε4 carriers had greater functional connectivity in the right medial

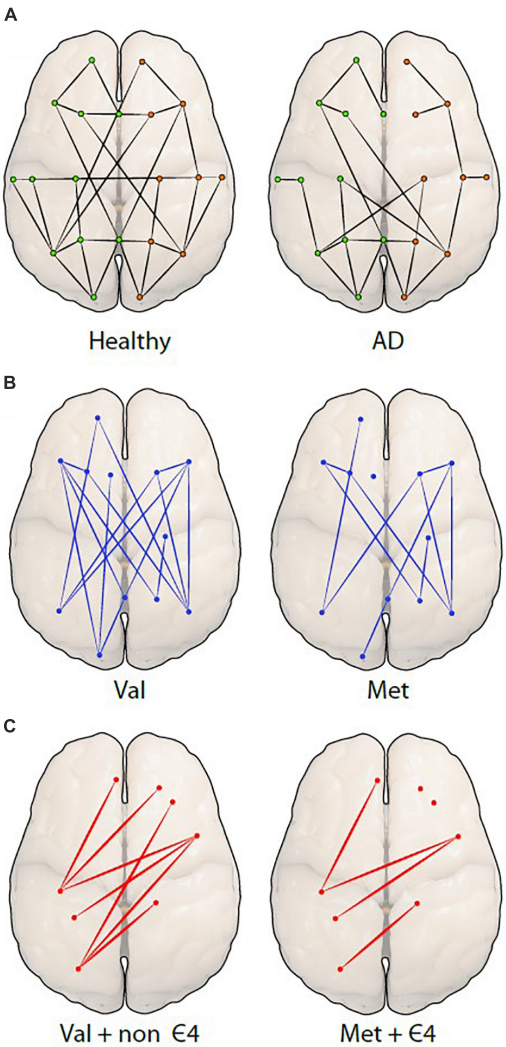

FIGURE 3 | Differences among network organizations are shown using a graph theoretical approach. **(A)** A graph of a healthy person (left) is compared to a person with Alzheimer's disease (AD; right), showing fewer connections (edges) between the spatially distant brain regions (nodes or dots) in AD. The green (left) and orange (right) dots represent hemispheres. The next two figures are hypothetical figures of the *BDNF* Val66Met polymorphism **(B)**, in which the connections are noticeably decreased in Met carriers. The last figure represents the carriage of both **(C)**, *BDNF* and *APOE* displaying a distinct reduction of edges and nodes in individuals.

frontal gyrus (Wu et al., 2016). Conversely, Goveas et al. (2013), demonstrated decreased functional connectivity within the DMN in cognitively healthy *APOE* ε4 carriers (44–65 years of age) in the bilateral dorsomedial PFC, superior frontal gyri, and in the left hippocampus, as well as increased functional connectivity in the left lentiform nucleus and bilateral caudate. Additionally, a decrease in interhemispheric functional connectivity within the DMN was found in healthy elderly *APOE*ε4 carriers (65–80 years of age; Lu et al., 2017). Notably, most of these regions are also affected in AD, which emphasizes the significance of the involvement of the DMN in the preclinical phase of AD (Sheline et al., 2010a). More recently, Zheng et al. (2018) investigated functional connectivity in young adults who were APPs/presenilin-1/2 mutation carriers or *APOE*ε4 positive carriers relative to adults without these AD-linked genetic factors (18–35 years). Interestingly, greater functional connectivity was observed in both the *APOE*ε4 carriers and in the APP/presenilin-1/2 group as compared to healthy controls. This increased connectivity was found between the left hippocampus and the bilateral medial PFC/precuneus. Only *APOE*ε4 carriers displayed increased connectivity between the right hippocampus and the left middle temporal gyrus. Here, the authors have suggested that the 'beneficial' effect of *APOE* ε4 in functional connectivity in younger individuals may be due to mechanisms of compensation of cognitive disruptions, which may be detrimental as the individual ages.

Due to inconsistencies in published evidence, it is important to consider how *APOE* polymorphisms may be associated with other measures of functional connectivity. Studies that have investigated *APOE* effects in small-world networks have reported higher susceptibility of fewer functional hubs and reduced centrality in healthy older ε4 carriers compared to non-ε4 carriers (Seo et al., 2013). Regional cerebral glucose metabolism, clustering of whole-brain functional networks, and path length have all been reported to be decreased in ε4 carriers (Seo et al., 2013). However, in a study with a greater sample size of 147 cognitively normal individuals, more clustering and longer path lengths were identified in ε4 carriers when compared to non-carriers (Goryawala et al., 2015). Non-demented ε4-carriers also had more long-distance connections in the parietal and temporal lobes, whilst non-ε4 carriers exhibited more short-distance connections in the parietal and occipital lobe. Healthy older individuals with the ε4 allele also had less short-distance connections in the frontal lobe connections, while both groups showed more long-distance connections in the frontal lobe (Goryawala et al., 2015). In summary, this study found the brain networks of those carrying *APOE*ε4 to be organized into an abnormal structure when compared to non-carriers, with fewer connections in the frontal lobe and more structural long length connections, which could partially explain the negative *APOE* ε4 cognitive phenotype.

Brain-Derived Neurotrophic Factor (BDNF)

Another genetic factor related to AD is the *BDNF* gene (Brown et al., 2014). The BDNF protein belongs to the family of nerve growth factors, which affect neurogenesis (Erickson et al., 2010) as well as long-term potentiation (LTP) and activity-dependent synaptic plasticity (Egan et al., 2003). Post-mortem studies of AD have shown that BDNF protein levels are decreased in the hippocampus, entorhinal cortex, temporal, frontal, and parietal cortex when compared to cognitively intact age-matched controls (Connor et al., 1997; Garzon et al., 2002). Lower BDNF levels may be related to volume loss in the hippocampus (Erickson et al., 2010), but this may be secondary to other pathological changes that occur in AD (Buchman et al., 2016). BDNF concentration is highly variable between individuals and is relative to physiological state; for example, after physical exercise,

peripheral blood BDNF concentration is increased (Dinoff et al., 2016). A recent review supported this finding by reporting increased neurogenesis and plasticity in the hippocampus in rats and mice after treadmill exercise, which led to improved short- and long-term memory functions (Jahangiri et al., 2018).

A common single nucleotide polymorphism in the *BDNF* gene, specifically a valine-to-methionine substitution at codon 66 (Val66Met), has an influence on LTP as well as activity-dependent BDNF secretion (Egan et al., 2003). *BDNF* Val66Met has been associated with cognitive performance as well as with AD brain morphology. In particular, the *BDNF* Met gene carriers (aged 60 and older), which were in preclinical stages of AD, demonstrated reduced memory function and smaller hippocampal and temporal lobe volume as compared to Val homozygotes (Lim et al., 2013; Brown et al., 2014). Authors also observed that more physical exercise was related to larger hippocampal and temporal lobe volumes in Val homozygotes but not in Met carriers (Brown et al., 2014). Notably, in Met carriers, physical activity was linked to reduced volumes of the temporal lobe, which is likely due to more apoptotic alterations (Brown et al., 2014). Likewise, Egan et al. (2003) demonstrated that the *BDNF* Met allele is related to qualitative changes of the hippocampus, which might cause insufficient memory functioning. Studies have proposed that there might be a relationship between Aβ and *BDNF* Val66Met, in which the *BDNF* polymorphism might mediate the effects on Aβ neurotoxicity on the brain (Fahnestock, 2011). Lim et al. (2013) reported not only a faster rate of atrophy in hippocampal volume, but also a faster decline in episodic memory performance in *BDNF* Met carriers who had a high Aβ load over a 36-month period compared to healthy individuals with *BDNF* Met but low levels of Aβ. Relative to Val homozygotes with a low Aβ load, Val homozygotes with a high Aβ load also experienced reduced cognitive performance, indicating that being a Val homozygote would not necessary protect against cognitive decline (Lim et al., 2013).

In older adults with late-onset depression, *BDNF* Met carriage was associated with reduced resting-state functional connectivity between the bilateral hippocampus and cerebellum (Yin et al., 2015). *BDNF* Met carriers with late-onset depression also had reduced strong (positive) functional connectivity between the hippocampus and the temporal cortex; however, there was also evidence of increased anti-correlated (negative) functional connectivity between the hippocampus and the dorsal anterior cingulate cortex, dorsal-lateral PFC, and angular gyrus (Yin et al., 2015). Similarly, Wang et al. (2014) observed elevated functional connectivity between the dorsal lateral PFC and the anterior insula in cognitively healthy *BDNF* Met carriers. Finally, Park et al. (2017) investigated the influence of *BDNF* Val66Met polymorphism on structural networks of middle-aged healthy individuals. The authors targeted nodes and edges in their analysis and simulated manipulation of the white matter networks. They demonstrated that Val homozygotes were more robust and resistant to gray matter damage compared to Met carriers (Park et al., 2017). Studies of white matter networks determined that *BDNF* Met carriers were more susceptible to node disruptions than Val homozygotes (Park et al., 2017).

The interaction of the *BDNF* Met and *APOE*ε4 polymorphisms was investigated by Gomar et al. (2016) in healthy older adults, as well as in individuals with MCI and AD. Here, the authors found that *BDNF* Met alleles were associated with poorer cognitive performance, predominantly in memory and semantic fluency. In support, Ward et al. (2014) found decreased performance in episodic memory function in *BDNF* Met carriers, however, only in combination with carriage of the *APOE* ε4 allele, the latter perhaps representing a cumulative effect of carriage of both risk alleles. This cumulative effect may be influencing the functional brain networks and reduce connections between different brain regions. *BDNF* Met carriers may have fewer connections compared to *BDNF* Val homozygotes (**Figure 3B**) and *APOE* ε4/*BDNF* Met carriers may have even fewer connections compared to non ε4/*BDNF* Val homozygote carriers, which may decrease connectivity (**Figure 3C**).

In a separate study, *BDNF* Met/*APOE*ε4 carriers with high brain Aβ levels demonstrated a faster rate of decline over a 54-month period in verbal and visual episodic memory and language processing when compared to *BDNF* Met/non-*APOE* ε4 carriers (Lim et al., 2015). In comparison, *BDNF Val*/ε4 carriers with a high Aβ burden demonstrated a relatively mild reduction in cognitive functioning. In *BDNF* Met/*APOE*ε4 carriers with high Aβ load, memory deficits are detectable after 3 years, whereas it takes 10 years in *APOE*ε4-/*BDNF* Val homozygotes with a high Aβ load to reach the same clinical threshold (Lim et al., 2015). A recent meta-analysis investigated the relationship between *APOE* and *BDNF Val66Met* and concluded that there were more women with AD carrying the *BDNF Met* polymorphism (Zhao et al., 2018). However, no significant relationships between *APOE*ε4 carriers and *BDNF* Met carriers were identified in the overall analysis that included both men and women with AD.

APOE and *BDNF* polymorphisms may interact with each other and possibly influence functional connectivity. *BDNF* Met carriers with the *APOE* ε4 allele exhibited decreased brain activation in the MTL (Kauppi et al., 2014). Atrophy, particularly in the entorhinal cortex, and acceleration of AD pathology, has been linked to poor compensation mechanisms of the brain in individuals with *BDNF* Met carrying the *APOE*ε4 (Gomar et al., 2016). Ward et al. (2015b) investigated the effect of *BDNF* and *APOE* on cognitive function and cognitive reserve, the latter which is a theoretical construct where neural networks compensate for lost neurons and connections (Stern, 2002). The authors observed that the *BDNF* Val66Met polymorphism, but not *APOE* variants, moderated the relationship between executive function and cognitive reserve, in which exposure to a more cognitively enriched environment was associated with better executive functioning in Val homozygotes but not in Met carriers (Ward et al., 2015a). In another study, Ward et al. (2017) investigated the same healthy older adult sample and found that differences in executive functioning between cognitive reserve tertile groups became smaller over time in *BDNF* Val homozygotes, but cognitive reserve-related differences became more pronounced in *BDNF* Met carriers. An explanation for these results is that cognitive reserve could have varying cognitive effects depending on the *BDNF* Val66Met polymorphism (Ward

et al., 2017). Altogether, experimental studies indicate that the *BDNF* polymorphism influences key neurobiological processes associated with development and activity-dependent learning (Egan et al., 2003).

COGNITIVE RESERVE AND BRAIN CONNECTIVITY

It is possible that common variation in the *BDNF* gene may result in differences in the development and maintenance of structural and functional networks throughout the life course, which ultimately may be associated with either better or worse brain resilience to neurodegenerative disease processes, such as in AD. Given the role of *BDNF* in development and adult brain plasticity, it is also possible that this gene variation may have an influence on the construction of patterns of connectivity that underlie resistance to pathology, perhaps related to the theoretical construct of cognitive reserve (Stern, 2002, 2006), in which neurons are compensating for impaired and lost neurons.

Stern (2002, 2009) proposed two different kinds of reserve in relation to a brain challenged by insult and/or neurodegeneration. Brain or neural reserve, which is often referred to as the 'passive' model of reserve, focuses on anatomical brain structures, especially brain size and the number and architecture of neurons and synapses (Katzman, 1993). This model, later revised by Satz (1993), proposed that individuals with higher synaptic count, dendritic branching and larger brain volume should be able to withstand the loss of more neurons without functional consequence, providing compensation for the pathological changes of AD (Stern, 2009). The brain reserve model suggests that most of its capacity is established in the early years of life, usually by the age of five (Reiss et al., 1996). Nevertheless, investigations have demonstrated that brain reserve may be modifiable. For example, the brains of adult monkeys are able to form and renew cells throughout life (Eriksson et al., 1998), and human brains have also been proposed to have neurogenic capacity, particularly in the dentate gyrus (Kempermann et al., 2015).

The 'active' model of reserve is often referred to as 'cognitive reserve,' which is a hypothetical construct that relates to the functional resilience of the brain against accumulating pathological changes (Stern et al., 1999). According to the theory of cognitive reserve, brains with more complex neural networks have a higher level of inbuilt redundancy, which are subsequently able to compensate for degenerative or lost neurons (Stern, 2002, 2006). Factors such as lifetime experience, educational and occupational attainment, and socioeconomic status are posited to play a significant role in the development of cognitive reserve (Stern, 2009, 2012). For example, individuals with AD and higher cognitive reserve (education levels) had greater DMN connectivity compared to individuals with AD and lower education levels (Bozzali et al., 2015). Bastin et al. (2012) on the other hand, determined that there was more cerebral pathology and reduced activity of metabolism in the temporoparietal cortex in healthy individuals with higher education. Furthermore, although Brayne et al. (2010) found that the amount of accumulation of pathological burden in the brain was not affected by the number of years of education that an individual had completed, higher levels of educational attainment was found to be associated with a lower risk of demonstrating dementia on the background of the burden of pathology.

Lifelong engagement in cognitively stimulating activities may reduce the risk of developing dementia by 40% (Scarmeas and Stern, 2003; Valenzuela et al., 2011). In support, Jahangiri et al. (2018) noted that exercise was associated with improved memory function, as well as reduced risk of developing neurodegenerative disease in different animal models. In human studies, Larsson et al. (2017) reported that individuals with higher educational attainment had a lower risk of developing AD. Similarly, in healthy participants (50–79 years), education later in life (university study for at least 12 months) was positively associated with cognitive reserve (as estimated by current psychological assessment scores) compared to those who did not complete any further university education (Lenehan et al., 2016). Associations between education and age are evident particularly in the attention and speed processing domains (Perry et al., 2017). In line with these findings, Summers et al. (2017) found that 92.5% of individuals 50 years and older who had attended university for at least 12 months showed increased cognitive performance in domains that may be a proxy for cognitive reserve.

Stern (2009) hypothesized that individuals with AD who have higher cognitive reserve possess more flexible neural networks and will retain a higher level of cognitive performance with an increasing neuropathological load. This notion of neural flexibility could potentially be demonstrated in re-organizable functional networks of the brain observed in cognitively healthy individuals (Bosch et al., 2010). In this study of healthy older individuals, higher cognitive reserve was associated with increased brain activity in the DMN, but it was also associated with decreased brain activity in regions associated with speech comprehension. In contrast, in individuals with MCI or AD, decreased activation in the DMN and more activation in language processing in subjects was associated with higher cognitive reserve (Bosch et al., 2010).

Education and cognitive reserve have a positive effect on functional connectivity networks (Marques et al., 2016) and cognitive functioning (Bozzali et al., 2015). There is evidence that high cognitive reserve levels were related to working memory, while age had a negative effect on cognition (Ward et al., 2015a). High cognitive reserve has been associated with greater functional connectivity in healthy elderly individuals (Marques et al., 2016). Arenaza-Urquijo et al. (2013) examined a cognitively healthy older population (60–80 years) and described better brain metabolism, higher gray matter volume as well as enhanced functional connectivity in individuals who had more years of early-life formal education. In particular, the authors found higher functional connectivity in regions such as the anterior cingulate cortex, right hippocampus, right PCC, left inferior frontal lobe and left angular gyrus in people with those with more education.

Marques et al. (2015) likewise examined the relationship between education and functional connectivity and found that individuals with more education had larger networks.

These enlarged networks were connected to all lobes in each hemisphere and influenced functional connections in a positive way, which was predicted to moderate the effects of age on brain connectivity (Marques et al., 2015). Moreover, Marques et al. (2016) investigated whether sex and the number of years of education [used as demographic characteristics (DEM)], in 120 healthy older individuals influenced functional networks in the brain. The authors demonstrated that the DEM had a positive effect locally (in the neighborhood areas), on the strength of nodes, efficiency and on clustering coefficient, exhibiting greater communication within the networks of the occipital and parietal lobe areas. There was also a relationship found between the DEM and network transitivity indicating that individuals with more education use different neural processing (Marques et al., 2016). Network transitivity is defined as the connection between two nodes that are linked to each other via an edge in a network.

In addition, Marques et al. (2016) examined how cognitive reserve measured by educational attainment affected functional connectivity in resting state fMRI. They demonstrated that larger networks with more functional connections in the brain were related to higher cognitive reserve. Greater local efficiency and higher local clustering in the cuneus, as well as in the areas of the superior and middle occipital lobe were related to higher levels of cognitive reserve (Marques et al., 2016). The inferior temporal gyrus is predicted to have a significant role for cognitive reserve, because of its betweenness centrality and nodal strength, which demonstrated a positive correlation with cognitive reserve. The fraction of all shortest paths in the network that pass through a given node is called betweenness centrality (Rubinov and Sporns, 2010). The inferior temporal gyrus is a significant hub responsible for recognition and visualization of words and numbers (Grotheer et al., 2016), which are important functions involved in cognitive reserve networks (Marques et al., 2016). Finally, global efficiency, which is "a measure of functional integration" (Marques et al., 2016), was greater in individuals displaying higher cognitive reserve compared to individuals with lesser cognitive reserve.

Colangeli et al. (2016) conducted a meta-analysis of whether functional brain networks were associated with cognitive reserve in healthy older adults, as well as in amnestic MCI (aMCI) and AD. Findings in all subgroups showed greater functional brain activation in the anterior cingulate in the left hemisphere while performing a cognitively stimulating task (e.g., recognition memory task). However, the cognitively healthy older adult group demonstrated greater activation in several brain regions as compared to the aMCI and AD groups. These activated brain regions included the left anterior cingulate and left precuneus, the right cingulate gyrus, and the superior frontal gyrus of the dorsolateral PFC, all of which are susceptible to degenerative changes in individuals diagnosed with AD and aMCI (Colangeli et al., 2016).

Bozzali et al. (2015) investigated whether cognitive reserve modifies resting-state functional connectivity in healthy, aMCI, and AD individuals (mean age 74.6 years). Functional connectivity was associated with the cognitive reserve proxy, education, within the DMN. Higher functional connectivity within the PCC was associated with higher education in individuals with AD, in which education possibly initiated mechanism of compensation. Education may also have led to brain plasticity and supported the PCC from atrophying. Some of the aMCI group exhibited similar connectivity strength, however, there was no strong functional connectivity found in the healthy group (Bozzali et al., 2015).

Franzmeier et al. (2016) also demonstrated that higher global functional connectivity was present in individuals with MCI with relatively higher levels of education. Individuals with more years of education and prodromal AD were able to compensate for fluorodeoxyglucose (FDG)-PET hypometabolism in the precuneus and had greater connectivity in the left frontal lobe, as well as better performance in memory (Franzmeier et al., 2017a,b). Moreover, Franzmeier et al. (2017b) demonstrated that individuals with MCI who had higher educational attainment and high Aβ levels had a more global left frontal cortex connectivity when controlled for age and sex, whereas, in healthy individuals, global left frontal cortex connectivity was not related with metabolism in the precuneus. Negative connectivity between the left lateral frontal cortex and the DMN was also found in people with MCI who had achieved higher education (Franzmeier et al., 2017a). Perry et al. (2017) demonstrated a positive correlation between years of education and cognitive functioning (e.g., visuospatial, executive function, language) but a weak relationship between education and brain networks, especially when the brain already showed evidence of age-related changes in healthy individuals. The greatest impact in age-related alterations later in life was found in the sensorimotor networks, especially those underlying processing speed and attention (Perry et al., 2017).

In summary, education early in life and other life-long cognitively stimulating activities could be possible protectors against neurodegenerative diseases, and might bolster cognitive reserve later in life (Ward et al., 2015b).

CONCLUSION

The brain is a large set of complex networks that are connected structurally and functionally. Different areas of the brain share and communicate information in functional space, creating networks. These networks can be adversely or positively influenced by various genetic and environmental factors. For instance, studies reported that *APOE* ε4 was associated with decreased functional connectivity (Lu et al., 2017) and longer path length in functional networks (Goryawala et al., 2015). However, there was also decreased path length (Seo et al., 2013) and increased functional connectivity found in healthy *APOE* ε4 carriers (Wu et al., 2016). Similarly, healthy older *BDNF* Met carriers were associated with reduced functional connectivity, while Val homozygotes showed a more robust network in the brain structure (Park et al., 2017). Cognitive activities and environmental enrichment have favorable effects on *BDNF* Val homozygotes, and over time also on *BDNF* Met carriers (Ward et al., 2017), which possibly may promote maintaining healthy cognitive functioning and reduce the detrimental effects progressing age. In general, studies provided evidence that education

and cognitive reserve are associated with an increase of functional connectivity in the brain networks (Marques et al., 2016). This could potentially affect brain networks in a positive way and may mitigate and protect against cognitive impairments later in life, and hopefully delay or even prevent the onset of AD (Prince et al., 2013). Future studies should investigate whether cognitive reserve and environmental enrichment work as compensatory mechanisms to influence and alter the networks of more susceptible genetic polymorphisms to AD, such as *APOE* ε4 and *BDNF* Met carriers. Education later in life increases cognitive reserve and could provide more resistance and resilience to brain pathology. Overall, these findings indicate that the functional networks of the brain are influenced by a combination of genetic and environmental factors. An improved understanding of these relationships is vital in order to fully grasp how neurodegenerative changes affect brain function, but also to determine how cognitive resilience to neurodegenerative changes may be promoted.

AUTHOR CONTRIBUTIONS

The manuscript of this review was prepared, formalized, and developed by MP. AK and DW assisted with refinement. JV contributed to the development and refinement of the manuscript.

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Pathological Changes in Microvascular Morphology, Density, Size and Responses Following Comorbid Cerebral Injury

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Aberrations in brain microcirculation and the associated increase in blood-brain-barrier (BBB) permeability in addition to neuroinflammation and Aβ deposition observed in Alzheimer's disease (AD) and ischemia have gained considerable attention recently. However, the role of microvascular homeostasis as a pathogenic substrate to disturbed microperfusion as well as an overlapping etiologic mechanism between AD and ischemia has not been thoroughly explored. In this study, we employ temporal histopathology of cerebral vasculature in a rat model of β-amyloid (Aβ) toxicity and endothelin-1 induced-ischemia (ET1) to investigate the panorama of cerebral pathology and the protein expression on d1, d7, and d28 post-injury. The combination of Aβ and ET1 pathological states leads to an alteration in microvascular anatomy, texture, diameter, density, and protein expression, in addition to disturbed vessel-matrix-connections, inter-compartmental water exchange and basement membrane profile within the lesion epicenter localized in the striatum of Aβ+ET1 brains compared to Aβ and ET1 rats. We conclude that the neural microvascular network, in addition to the neural tissue, is not only sensitive to structural deterioration but also serves as an underlying vascular etiology between ischemia and AD pathologies. Such investigation can provide prospects to appreciate the interrelationships between structure and responses of cerebral microvasculature and to provide a venue for vascular remodeling as a new treatment strategy.

Keywords: beta-amyloid, ischemia, microvessels, basement membrane, vascular anatomy

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INTRODUCTION

In general, the microvascular bed of the brain is considered functionally adynamic and less sensitive to the morphological injuries than the neurons they serve and their supportive cells (del Zoppo et al., 2000), due to the comparative resistance of brain endothelium to hypoxia, to a certain extent (Tagaya et al., 1997). However, cerebral microvessels [includes the capillaries and their afferent

Abbreviations: ABC, avidin-biotin complex; AD, Alzheimer's disease; APP, amyloid precursor protein; AQP4, aquaporin4; Aβ, β-amyloid; BBB, blood-brain barrier; DAB, 3,3′-diaminobenzidine tetrahydrochloride; ET1, endothelin-1; ICV, Intracerebroventricular; MMP-9, matrix metalloproteinase9; PBS, phosphate-buffered saline; βDG, β-dystroglycan.

(arterioles) – efferent (venules) connections] are either the target or consequence of numerous insults such as focal ischemia (del Zoppo et al., 2000), cerebral amyloid angiopathy (Ujiie et al., 2003), vascular dementia (Parnetti et al., 1994), hypertensive angiopathy (Baumbach and Heistad, 1989), autoimmune vasculitides, hyperglycemia, and inflammatory disorders (Paul et al., 2007). Chronic hydrocephalus, in particular, is associated with reduced cerebral blood flow and direct compression of fibers, blood vessels or brain tissue [*reviewed in* Owler and Pickard (2001)].

Consequential footprints of vascular defects are BBB breakdown, leakage of blood-borne molecules, swelling of astrocytic endfeet, interruption of inter-compartmental fluid exchange, disruption of basement membrane (BM), vessel-matrix-connections (Bailey et al., 2004), and disturbed capillary physiology (Paul et al., 2007; Brown and Thore, 2011) that leads to altered brain microcirculation [*reviewed in* Amtul and Hepburn (2014)]. Vascular functional defects have been described in AD transgenic animal models (Ujiie et al., 2003) and patients (Farrall and Wardlaw, 2009). For instance, vaccination against β-amyloid (Aβ); the hallmark of the AD, has been shown to reverse BBB pathology (Dickstein, 2006). Similarly, BBB breakdown due to the dissolution of primary endothelial cell permeability barrier and resulting hypoperfusion is also an antecedent event to cerebral ischemia (Hawkins, 2005). Intriguingly, there is a great majority of patients with first-ever stroke, who developed post-stroke dementia (Pendlebury and Rothwell, 2009), establishing that ischemic and AD pathogeneses interact with each other.

In this regard, we have demonstrated some very key pathological changes in the combined animal model of Aβ toxicity and ischemia, such as lateral-ventricle enlargement, neuroinflammation, Aβ deposition, altered insulin signaling/BBB, hippocampal injury, and impairment in learning and memory (Amtul and Hepburn, 2014; Amtul et al., 2011a,b, 2014, 2018a,c; Yang et al., 2014; Amtul, 2016, 2018). Using sequential computed tomography (CT) imaging we have also established altered microperfusion and associated elevated BBB disruption in the comorbid model of Aβ toxicity and ischemia (Yang et al., 2014; Amtul et al., 2018d). Surprisingly little is known about the effect of comorbid injury on the anatomical alteration of cerebrovasculature, topographic distribution, density, diameter and their role in maintaining cerebral water homeostasis and vessel-matrix-connections. Due to the serious consequences; damage to cerebral vasculature homeostasis and thus function must be considered a critical contributing component in the development of neurological disorders. Moreover, analyzing the vascular anatomical defects is also essential for adequate interpretation of brain imaging data to take us one step closer to develop remedies to restore vascular architecture, which may emerge as a new therapeutic target. Therefore, our working hypothesis is that the alteration in microvascular anatomy, texture, diameter, density, protein expression, and function serve as the interrelated pathogenic and etiologic link between AD and ischemia. Therefore, in the present study, we studied the anatomy of striatal microvasculature and the associated effects on water exchange and vascular-matrix connections by investigating various vascular markers at different time points in a comorbid rat model of Aβ toxicity and ischemia.

MATERIALS AND METHODS

Study Design and Animal Treatment

All of the animal experiments were conducted in full compliance with the guidelines and approval of the Animal Care and Use Committee of the Western University (approval ID: 2008-113). All possible steps were taken to reduce the number of animals and their discomfort level. Two- to three-month old male Albino Wistar rats (Charles River Canada) weighing 250 to 310 g at the beginning of the experiment were used. Animals were divided into four groups for three different (d1, d7, and d28) timelines (n = 4 for each group). For stereotactic surgery, the animals were positioned into David Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, United States) and anesthetized using 1.8% isoflurane. Body temperatures were maintained at 37°C throughout the surgery with the help of a heated blanket. To insert 30 gauge Hamilton needle, three burr holes were drilled in the parietal bone of each rat. All stereotaxic coordinates were determined using Paxinos and Watson Atlas (Paxinos and Watson, 2005).

Induction of Striatal Cerebral Ischemia

The rat model of ischemia (group 1; ET1) receiving 60 pmol/3 μL injection of endothelin-1 (ET1; Sigma-Aldrich, Oakville, ON, Canada) into the right striatum (anterior/posterior +0.5 mm, medial/lateral −3.0 mm relative to bregma, and dorsoventral −5.0 mm below dura) has been described in detail elsewhere (Amtul et al., 2014, 2018a,b,c).

Induction of β-Amyloid Toxicity

The rat model of β-amyloid toxicity (group 2; Aβ) receiving bilateral ICV injections of oligomeric 50 nM Aβ25–35/10 μL was modeled (anterior/posterior: −0.8 mm, mediolateral: ± 1.4 mm relative to bregma, and dorsoventral: −4.0 mm below dura) as described in detail elsewhere (Amtul et al., 2014, 2018a,b,c).

Induction of Comorbidity and Control Procedure

Third group of rats received both bilateral ICV Aβ25–35 and unilateral striatal ET1 injections (group 3; Aβ+ET1) as described (Amtul et al., 2014, 2018a,b,c). The control rats (group 4; Control) received saline injections in identical locations.

Post-surgery Treatment

After stitching the skin incisions, all rats were administered a subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic, RB Pharmaceuticals Ltd., Berkshire, United Kingdom) and an intramuscular injection of 1 mg/20 μl enrofloxacin antibiotic (Baytril, Bayer Inc., Canada). Twenty four hours (d1), 7 days (d7), and 28 days (d28) following surgery, corresponding rats were euthanized by an intraperitoneal pentobarbital overdose (Pentobarbital Sodique, Ceva Santé Animale, Cambridge, ON,

Canada) followed by a transaortic perfusion with heparin containing phosphate buffered saline (PBS) and thereafter by 4% paraformaldehyde (pH 7.4).

Tissue Preparation

The brains were harvested, postfixed in 4% paraformaldehyde, and cryopreserved in 30% cold (4°C) sucrose solution for 36 h. Next, the entire rat brains were serially sliced into 35 μm thick coronal cryosections.

Immunohistochemistry

Immunoperoxidase protocol of avidin-biotin-peroxidase complex (ABC) was carried out as described (Hsu et al., 1981) to stain the free-floating cryosections with the following antibodies: Matrix metalloproteinase-9 (MMP-9; 1:1000, Millipore, AB19016), β-dystroglycan (βDG; 1:200, Leica B-DG-CE-S); βDG is an extracellular matrix adhesion protein abundant in astrocytic endfeet, co-localized with AQP4 and laminin and is needed for precise astrocytic anchoring around the cerebral vasculature (Zaccaria et al., 2001; Milner et al., 2008). SMI71 (Covance, 1:2000), an explicit endothelial barrier antigen (EBA) restricted to the luminal surfaces of all mature endothelium of the vasculature with intact BBB. BM-Laminin (1:1000, Sigma, L 9393) staining was used as an indicator of the BM rupturing, while neurons also contain laminin like molecules. For astrocytic antigen, glial fibrillary acidic protein (GFAP, 1:1000, Sigma, G3893) and binary water-channel protein, aquaporin4 (AQP4, 1:1000, Chemicon, AB2218) were employed. Fluorophore-conjugated donkey anti-rabbit FITC (Sc-2090, 1:500) and donkey anti-mouse TR, (Sc-2785, 1:500) were prepared in 0.3% Triton X-100 containing 0.1 M PBS were used for fluorescence staining. Serums, biotinylated antibodies, and ABC reagent were purchased from the Vectastain Elite ABC Kit (Vector Laboratories, Inc., Burlingame, CA, United States).

Analyses

Histological sections of brain were analyzed under light and fluorescence microscope. Leica Digital Camera DC 300 (Leica Microsystems Ltd., Heerbrugg, Switzerland) connected to a Leitz Diaplan microscope was used to take the photomicrographs. Cells and blood vessels were identified and counted, blindly, on the basis of positive labeling in the region of interest (ROI) on six non-neighboring slices with 210 μm distance between the sections using systematic random sampling method.

The Region of Interest Determination

The region of positive laminin staining on consecutive slices in the right hemisphere (ipsilateral to the striatal ischemia) was noted as the location of the signal. This signal was limited to the striatal region between 1.6 to −0.92 mm anterior to posterior, relative to bregma. All histological analyses were performed in this region of the sections. The center of the ROI with dense laminin staining may alternatively be termed as lesion epicenter or lesion core, while the ROI surrounding the epicenter is termed as penumbra throughout the study. Six microscopic fields-of-view (each covering an area of about 0.50 mm^2) in the ROI at 10× to 40× magnification with 0.25 to 0.65 numerical aperture were analyzed.

Lumen Diameter Measurement

The diameters of lumen were determined using the ImageJ's (National Institute of Health, version 1.48, United States) diameterJ plug-in analysis tool. Pixels in images with known scale bars were converted into micrometer in Image J. Next, a line selection was drawn across the structure of interest to measure the diameters.

Vessel/Cell Density Measurement

For vascular/cellular density both manual as well as cell counter plugin was used to count the number of stained cells, vessels, layered vessels, split vessels, vessels with fibrosis and vessels with greater sizes. The results were presented as the number of vessels or cells per millimeter square of the ROI. Effect of MMP-9 on vascular fibrosis was quantified by calculating the ratio of the MMP-9 expression relative to the number of vessels displaying the fibrosis and expressed as negative log of n.

AQP4 Polarization Measurement

AQP4 polarization is characterized as its dense, concentrated and localized expression within the astrocytic endfeet, ensheathing the BBB interface of cerebral microvessels, related to the astrocytic soma. Likewise, the loss of localized expression of AQP4 from perivascular endfeet processes and shifting toward astrocytic soma and coarse processes is referred to as its depolarization. AQP4 polarization was measured by counting the number of vessels expressing the AQP4 at their perivascular astrocytic BBB interface within each field of view. The relative values for AQP4 polarization on d7 and d28 were expressed as a percentage of the AQP4 polarization on d1 by normalizing it to 100 percent.

Fluid-Filled Spaces Measurement

Fluid-filled spaces in the ROI were determined by drawing them on the computer screen with the help of image J quantification plugin analysis tools. The fluid-filled space on each section was normalized to the corresponding striatal volume, and thereafter to hemispheric swelling by dividing the contralateral hemisphere volume to the ipsilateral hemispheric volumes, multiplying by fluid-filled spaces. The relative area of fluid-filled spaces on d7 and d28 was calculated as the percentage volume of the fluid-filled spaces on d1 by normalizing it to 100 on d1.

Statistical Analyses

All values were displayed as a mean ± standard error of the mean (S.E.M.). All measurements were analyzed by using one-way ANOVA followed by *post hoc* Dunnett's tests. Except for inter-group comparisons at different time points, a *t*-test was used, and log values, two-way ANOVA was used. The significance level was $p \leq 0.05$.

RESULTS

This is the first comprehensive histopathologic study of blood vessels pathology after the comorbid cerebral injury. These data suggest that the development of vascular pathology is a global phenomenon that affects the vascular dilation, lumen diameter, basement membrane integrity as well as the fluid homeostasis across the injured region after comorbid ischemia and amyloid toxicity.

Dilated Blood Vessels and Lumen Diameter

In control rats, laminin antibody only detected neurons, and in Aβ rats a few faint, isolated microvessels at each time point (**Figure 1A**). This is consistent with the literature that shows that in PFA-perfused tissues the laminin antibody does not stain BM-laminin but only neurons (Hagg et al., 1989). Conversely, a significantly robust network of capillaries containing BM-laminin is usually promptly evident after Aβ+ET1 or ET1 injections in the lesion epicenter due to the damage to BM and enhanced antibody penetration resulting in the robust staining of laminin protein. Alongside an intermittent yet widespread increase in the diameter of microvessels (square boxes) within the lesion core of Aβ+ET1 than ET1 rats (p = 0.054) was readily detected immediately after the injury compared to the control (p = 0.0005) and Aβ (p = 0.0006) rats (**Figures 1B,D**). Numerous thinning out laminin-positive pyknotic neurons were also obvious in the lesion core of ET1 and Aβ+ET1 rats on d1 (**Figure 1B**). Dilated microvessels in ET1 and Aβ+ET1 rats with larger lumen diameter (**Figures 1C,E**) also demonstrate punctate SMI71 staining of the luminal surface of the endothelial cells indicating BBB break down in those microvessels (**Figure 1C**).

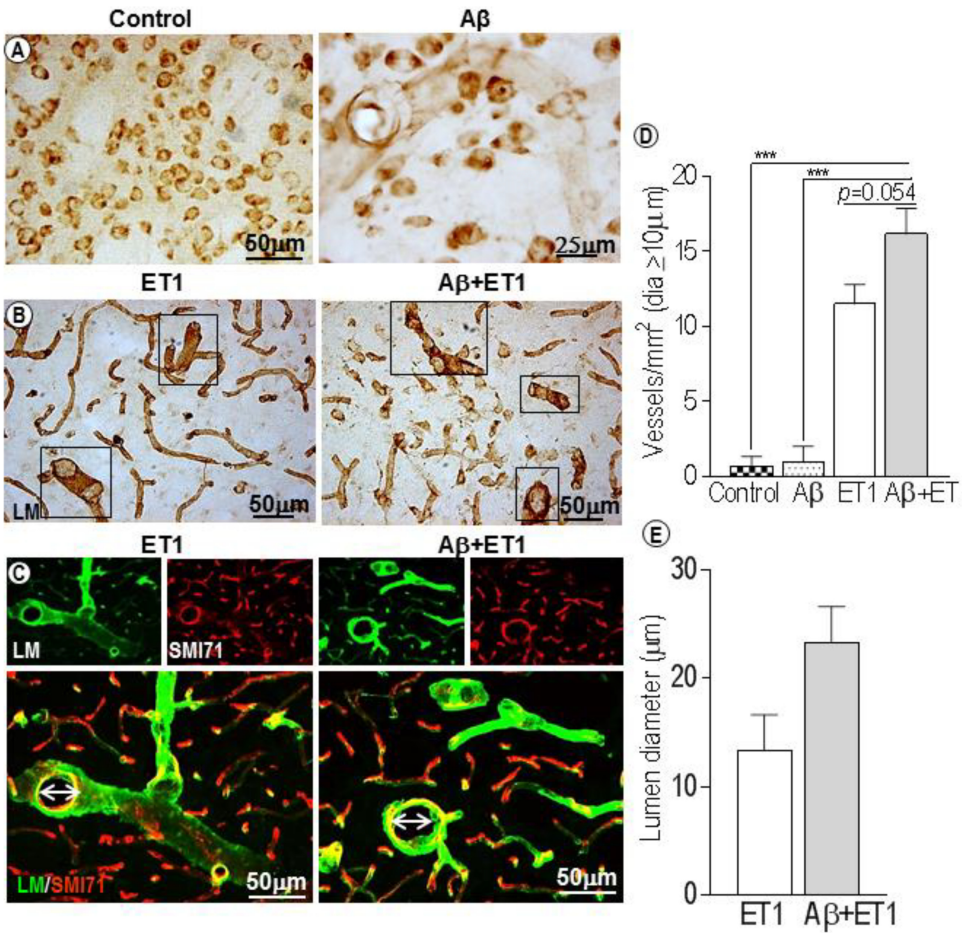

FIGURE 1 | Dilated blood vessels and lumen diameter: Control and Aβ rats show BM-laminin positive neurons on d1. Aβ rats also show some isolated, inconsistent laminin stained microvessels **(A)**. Several dilated microvessels and numerous declining typical pyknotic neuronal cells can be seen in the ROI of ET1 and Aβ+ET1 rats on d1 **(B)**. Fluorescent staining of BM-laminin (green) indicates lumen diameters on d1 in the ROI or lesion core of ipsilateral striatum of ET1 and Aβ+ET1 rats. Punctate SMI71 staining (red) of endothelial cells can also be seen in the background. **(C)**. The plots show quantitative analyses of laminin-stained microvessels with equal or greater than 10 μm diameter in the ipsilateral striatum of control, Aβ, ET1, and Aβ+ET1 rats **(D)**, and lumen diameter of dilated microvessels in ET1 and Aβ+ET1 rats **(E)** on d1, ***$p < 0.001$.

Basement Membrane Profile and Disruption

Basement membrane pathology including splitting, dissolution and rupturing was more prominent in ET1 rats compared to layering out tunica intima in Aβ+ET1 rats, that represents an earlier step to splitting (**Figures 2A,B,D,E**). On d28, laminin meshwork with degraded BM can be seen throughout the lesion core with more pronounced splitting around digested microvessels in ET1 rats and distinct layering in Aβ+ET1 rats (**Figures 2C–E**). Additionally, ET1 rats on d7 showed the splitting of BM-laminin with irregular SMI expression. In contrast, Aβ+ET1 rats showed layering of BM with relatively regular BBB (**Figure 2B**). Images on both fluorescent and 0.05% DAB staining were included to provide readers better clarity and resolution that might have been missed by the one procedure or another.

MMP-9 Expression and Microvascular Fibrosis

In control and Aβ rats, MMP-9 appeared to stain a large number of neuronal cells, as obvious from their structure and distribution pattern (**Figure 3A**). While in ET1 and Aβ+ET1 rats on d1, MMP-9 stained striatal cells that appeared to be the reminiscent of microvascular endothelium, representing the disturbed vascular-matrix connection, with more in ET1 and less in Aβ+ET1 rats (**Figure 3B**). On d7, in the penumbra-core interface MMP-9 started appearing as astrocytes (**Figure 3C**). On d28 MMP-9 staining showed complete loss of endothelial profile observed on d1, and strongly indicated an expression of astrocytes in the lesion core with significantly more in ET1 brains than Aβ+ET1 ($p < 0.05$) rats (**Figure 3D**). Besides, the capillary structure in the lesion core started getting severely compromised (**Figure 3E**). The percent fibrosis of vessels in Aβ+ET1 rats was strongly correlated with an MMP-9 increase ($p = 0.033$) in the lesion core, suggesting that fibrosis was a critical process in the penumbra of Aβ+ET1 rats. The images themselves contain sufficient detail to clearly display capillary structures, allowing the visualization of principal BM layer and the vasculature. More detailed visualization of capillary atrophy intercalated by a dense BM network included string, fragmented and tortuous structures (circle). An uneven thickening on the abluminal surface of capillaries, duplication, branching (three arrows), and microvascular fibrosis of BM (dotted line), was common in the lesion core of treated rats, in general.

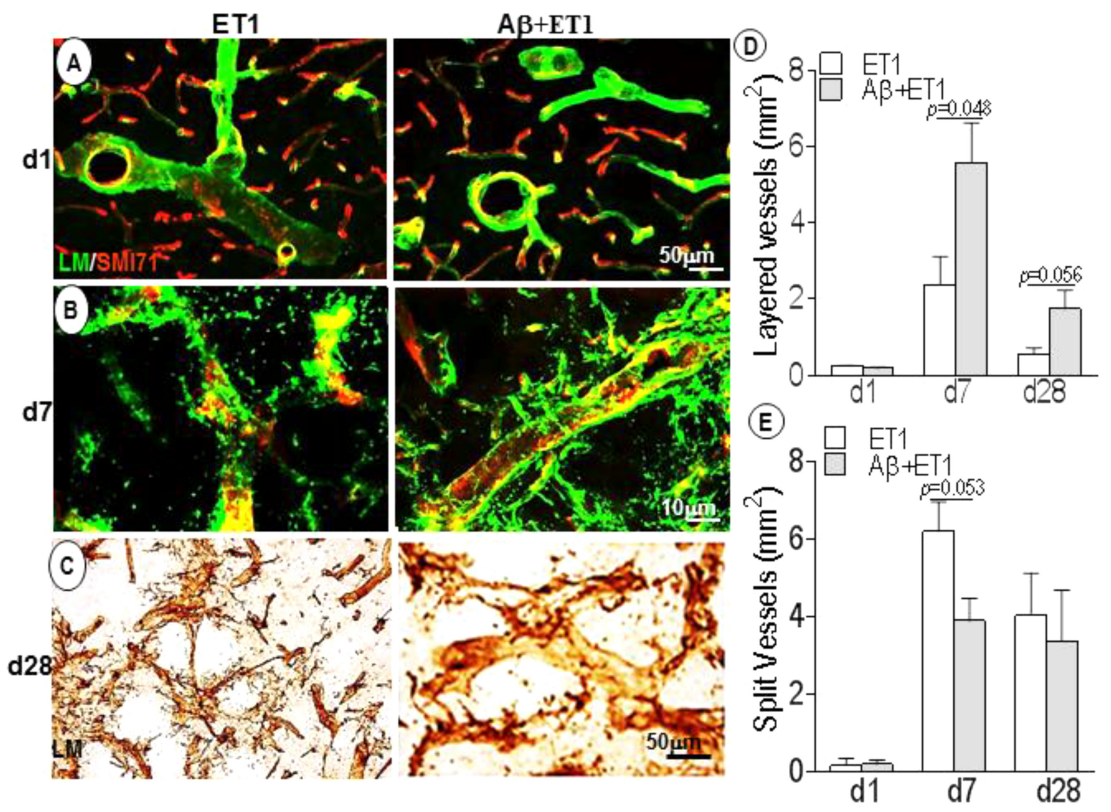

FIGURE 2 | Basement membrane profile and disruption: Fluorescent images indicate an increase in BM-laminin immunoreactivity (green) in the lesion core localized to the ipsilateral striatum of ET1 and Aβ+ET1 rats from d1 **(A)** to d28 **(C)**. On d7, the BM starts splitting out in ET1 rats and layering out in Aβ+ET1 rats. A punctate and faint SMI71 staining indicative of disrupted BBB is also observed in microvessels with greater BM-laminin leakage, dissolution, and destabilization **(B)**. On d28, splitting reaches to its peak in ET1 rats while layering started fading out in Aβ+ET1 rats **(C)**. The plots show quantitative analyses of laminin-stained microvessels showing layering **(D)** and splitting **(E)** in the ipsilateral striatum of ET1 and Aβ+ET1 rats on d1, d7, and d28.

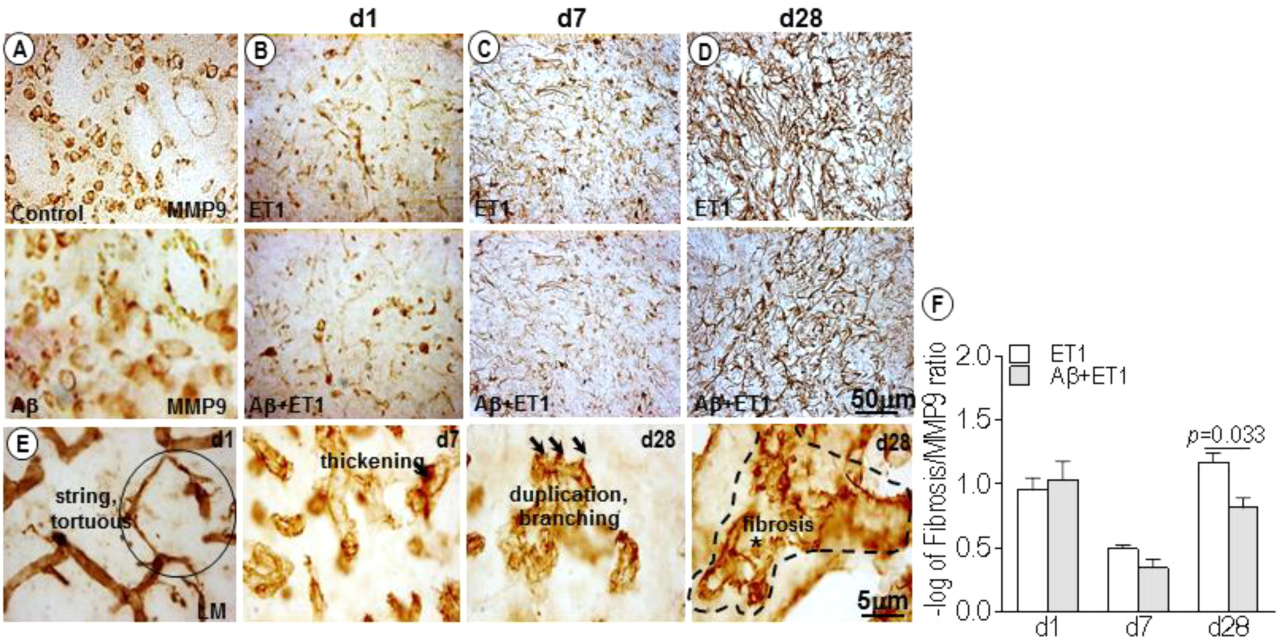

FIGURE 3 | MMP-9 expression and microvascular fibrosis: DAB staining shows expression pattern of MMP-9 in the lesion core of the ipsilateral striatum of control, Aβ **(A)** ET1 and Aβ+ET1 rats from d1 to d28 **(B–D)**. In control and Aβ rats MMP-9 exclusively stain neuronal cells throughout the course of study **(A)**. On d1, MMP-9 is mainly expressed by the neuronal (pyknotic) cells and occasionally by the endothelial cells in the lesion core of ET1 and Aβ+ET1 rats **(B)**. On d7, MMP-9 staining still shows neuronal profile and appearance of astrocytic morphology **(C)**. On d28, MMP-9 acquires massive astrocytes appearance **(D)**. High-resolution images of BM-laminin staining show different stages of capillary wall pathology from d1 to d28 in Aβ+ET1 rats, such as stringed and tortuous structures (circle), local irregular thickening on the abluminal surface of capillaries, duplication, branching (three arrows), and fibrosis of BM (dotted line) **(E)**. The plot shows –log of the ratio of the MMP-9 expression relative to the number of vessels displaying fibrosis **(F)** in the ipsilateral striatum of ET1 and Aβ+ET1 rats on d1, d7, and d28.

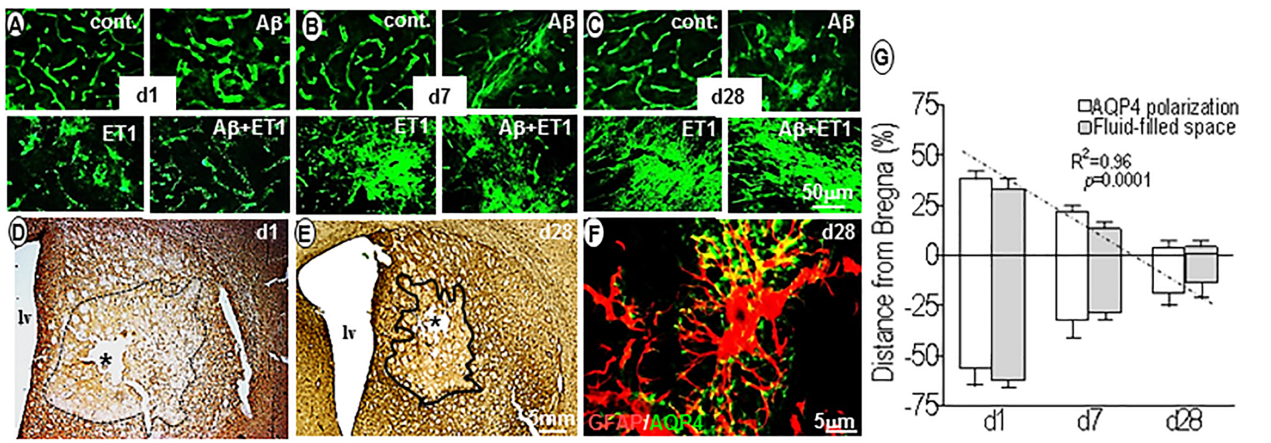

FIGURE 4 | Aquaporin4 polarization vs. fluid homeostasis: Fluorescent images show the expression pattern of AQP4 immunostaining in the ROI of control, Aβ, ET1, and Aβ+ET1 rats from d1 to d28 **(A–C)**. The punctate AQP4 expression on d1 in ET1 and Aβ+ET1 rats **(A)** starting to depolarize on d7 **(B)** and intensely depolarized on d28 **(C)**. While control rats show smooth, regular AQP4 staining throughout, and Aβ rats show an increase in AQP4 haziness from d1 to d28 **(A–C)**. Low-resolution images show the topographical distribution of fluid-filled spaces by astrocytes **(D)** and βDG **(E)** immunostaining in the penumbra and lesion core; ROI of Aβ+ET1 rat on d1 **(D)** and d28 **(E)**. The epicenter of the lesion core also shows a prominent necrotic lesion (asterisk) **(D,E)**, lv; lateral ventricles. High-resolution image shows depolarization of AQP4 expression from GFAP positive astrocytic end-feet to the astrocytic soma **(F)**. The plot shows a quantitative decrease in AQP4 polarization and fluid-filled spaces in an anterior (+) to posterior (−) manner from the bregma (0), in the ipsilateral striatum of ET1 and Aβ+ET1 rats from d1 to d28 **(G)**.

AQP4 Polarization vs. Fluid Homeostasis

Within 24 h, AQP4 staining was highly polarized around endothelial cells of microvessels in control (p = 0.002) and Aβ (p = 0.001) brains compared to the ET1 and Aβ+ET1 brains. On the contrary, AQP4 staining appeared punctate in the lesion core of ET1 and Aβ+ET1 rats. While the neuropil space in between the AQP4 stained vessels was missing any AQP4 staining in Aβ+ET1 rats, in ET1 rats it exhibited haziness indicative of relocation of AQP4 protein (**Figure 4A**). On d7, the depolarization of AQP4 became more distinct in ET1 and Aβ+ET1 rats and a hazy fluorescence appeared to extend into the neighboring neuropil compared to control and Aβ rats (**Figure 4B**). On d28, an increased AQP4 immunoreactivity was accompanied by the re-distribution of polarized AQP4 expression from astrocytic endfeet to the soma with more in ET1 rats than Aβ+ET1 rats (**Figure 4C**). A subset of microvessels in Aβ rats showed AQP4 haziness and depolarization on d28; however, it did not reach statistical significance (**Figure 4C**). Low-resolution images indicated the topographical immunostaining of GFAP (**Figure 4D**), and βDG positive (**Figure 4E**) astrocytes in the ipsilateral striatum of Aβ+ET1 rat brain on d28. On d1, both ET1 and Aβ+ET1 rats had approximately a100% increase in fluid-filled spaces in the ROI compared to control and Aβ rats, consistent with the vasogenic edema formation (**Figures 4D,G**). While, on d28 with the increase in AQP4 depolarization, the size of these fluid-filled spaces got shrunk to one-third of their original size from d1 (R = 0.96, p = 0.0001) (**Figure 4E**). The dark line showed the division between core and penumbra, where a palisade layer is formed by GFAP positive astrocytes. While βDG expression on astrocytic end feet demonstrates an attempt by the astrocytes to restore vascular-matrix connections (**Figures 4D,E**). The high-resolution image showed co-labeling of AQP4 and GFAP staining to demonstrate AQP4 depolarization from astrocytic foot processes to soma in the fluid-filled space in an Aβ+ET1 rat on d28, in an attempt to increase the inter-compartmental water exchange from fluid-filled spaces to the bloodstreams (**Figure 4F**). Co-expressed GFAP and AQP4 staining were not used for any quantitative analyses.

DISCUSSION

In this study, we demonstrate that the comorbid occurrence of ischemia and Aβ toxicity provoked substantial dynamic, rapid and highly coordinated changes in the vascular anatomy and physiology in the striatal lesion core and penumbra of Aβ+ET1 rats. The present study also showed a correlation between vascular structure deterioration and the deficits in microvascular responses, such as inter-compartmental water exchange and vessel-matrix-connections of cerebral capillaries after comorbid injury.

Immediately after ET1 and/or Aβ+ET1 injections, the compression of small capillaries (not arterioles and venules) and a drop in cerebral perfusion, due to the potent vasoconstrictive effects of ET1, led to the dilation of precapillary resistance vessels in order to maintain cerebral perfusion. This was observed by the homogenous distribution of certain vessels with increased diameter throughout the penumbra in Aβ+ET1 rats compared to ET1 rats on d1. This shifted the calculated average size of vessels to the considerably larger values in Aβ+ET1 rats. Whereas the changes in the microvessels structure in ET1 rats accompanied by a decrease in the outer diameters of the vessel lumen. These alterations perhaps referring to some hyperplasia or hypertrophy of the vessel wall in these rats, significantly impacted vascular function (Baumbach and Heistad, 1989). This also referred to a different pattern of remodeling in Aβ+ET1 rats compared to ET1 rats alone (**Figure 1**).

Following flow restoration in the constricted vessels, the downstream capillary network endured the impact (del Zoppo et al., 2000). For instance, in injured rats once maximum vasodilation was reached, autoregulation failed and progressive continued compression of small capillaries (Liebeskind, 2003) finally resulted in the opening of tight junction proteins of endothelial cells and breakdown of BBB permeability, as observed by a punctate EBA or SMI71 staining in ET1 and Aβ+ET1 rats on d7 (**Figure 2B**).

A breakdown of BBB or permeability barrier was accompanied by significant alterations in microvessel structure in these rats. As, spatially endothelial cells are in a perfect position to sense minor changes in cerebral perfusion, thus shear stress. These pathologic changes seemed to first affect the intima, media, and adventitia layers of microvessels. Immediately after injury, these layers started separating from the vessels, however, more robustly in ET1 rats compared to the Aβ+ET1 rats. As, ET1 rats demonstrated splitting, which is usually followed by a vascular layering of tunica intima; that comprised of endothelial cells and the contiguous BM (Stratman et al., 2009), starting from d7 (**Figure 2B**). Possibly, our endpoint timing of euthanization couldn't catch the window of vascular layering in ET1 rats. Splitting, in turn, led to multiple tears within the microvascular layers and BM-endothelium of ET1 rats by d28 (**Figure 2C**).

This study also highlights vascular fibrosis or vascular BM thickening as a pathologic phenomenon associated with ischemia and comorbid injury. In Aβ+ET1 brains, BM sufferd the most dramatic and pronounced damage and emerged as a central target of vascular pathological changes (Hawkes et al., 2011; **Figure 3**). Vascular fibrosis; linked with many pathological processes and clinical conditions (Harvey et al., 2016) is characterized by reduced compliance, such as lumen diameter, increased vascular collagen, reduced elasticity (Selvin et al., 2010), and associated excessive deposition/remodeling of extracellular matrix (ECM) (Iwazu et al., 2011). Increased expression and activation of matrix metalloproteinases (MMP) is characterized as one of the molecular mechanisms underlying ECM remodeling and vascular fibrosis (Epstein et al., 1994). Accordingly, many of the partially digested microvessels that still remained in the striatal lesion core were associated with MMP-9 positive staining in ET1 and Aβ+ET1 rats on d1 (**Figure 3B**) indicating the diminishing or residual vessel-matrix-connections. By d28 complex interplay between ischemia, Aβ toxicity and astrocytic expression of MMP-9 (Zhao et al., 2006; Wang and Hatton, 2009; **Figure 3D**), resulted in accelerated vascular remodeling (as observed by elevated astrocytic expression) in ET1 rats and fibrosis (as accompanied by lowered astrocytic expression) in

Aβ+ET1 rats. This again confirms a different pattern of remodeling in ET1 and Aβ+ET1 rats, as mentioned above. In addition, the presence of cerebral microvessels with string, tortuous structures (**Figure 3E**) and reduced capillary density in Aβ+ET1 rats may be responsible for the increased resistance of microvessels and resulting moderate hemodynamic obstruction in these rats by d28, reported earlier (Yang et al., 2014).

The very peculiar set of idiosyncratic variations was noticed in the polarized expression of end feet pool of AQP4 from d1 to d28 (**Figure 4**). Within 24 h, punctate, granulated, polarized expression of AQP4 channel protein, enclosing the cerebral endothelial layers in the lesion core demonstrated re-localization or redistribution from the end feet membranes to the parenchymal soma membranes of the astrocyte body starting from d7 (Wolburg-Buchholz et al., 2009). Perhaps this is to aid in astroglial migration toward fluid-filled spaces in forming glial scar in the lesion core, necessary for filopodia formation (Saadoun, 2005; Badaut et al., 2007), as well as to help in intra-astroglial water accumulation or cytotoxic edema in an effort to clear up fluid-filled spaces formed in the lesion core of ET1 and Aβ+ET1 rats on d1 (**Figures 4D,E**). On d28, the reduction in fluid-filled spaces in injured rats when AQP4 depolarization was at its peak, confirmed its role as the principle bidirectional water transporting channel of astrocytes (Nagelhus et al., 2004) and thus in fluid clearance from the brain into the bloodstream.

Currently, it is uncertain if Aβ rats will express significant degeneration in vascular physiology and markers in a four-week time frame, as Aβ toxicity alone was not detrimental enough. Low numbers of animals per group (n = 4) might be a contributing limiting factor. However, in the given time, striatal microvessels in the Aβ rats showed vascular degeneration and BBB disruption compared with controls, although non-significantly. Further studies with extended time intervals will likely provide an enhanced appreciation of the structural links of vascular injury with vascular responses in these rats.

In summary, we propose that the anatomical or morphological alterations in the cerebral network of microvessels after injury could be used effectively to describe the neuropathology of the injured brain. For example, the impaired BBB restoration after Aβ+ET1 toxicity reported earlier (Amtul et al., 2018d) might be the direct cause of the altered anatomy of the cerebral vasculature in these rats. Lastly, longitudinal magnetic resonance evaluations can aid to correlate imaging of vessel size and microvascular morphology to the fate of neural tissue to further improve the tailoring of the treatments. Such an investigation could lead to a new rationale to induce vascular remodeling by designing therapeutics for demented patients.

AUTHOR CONTRIBUTIONS

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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Direct Measurements of Abdominal Visceral Fat and Cognitive Impairment in Late Life: Findings From an Autopsy Study

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Background: The relationship between cognitive impairment and abdominal visceral is controversial. Moreover, all studies so far used imaging studies to evaluate visceral fat and this association has not been described yet using autopsy material, which allows the direct quantification of abdominal fat. We aimed to investigate the association between direct measurements of abdominal visceral fat and cognitive impairment in an autopsy study.

Methods: In this cross-sectional study, we collected information on sociodemographics, cardiovascular risk factors, and cognitive status from subjects aged 50 or older at time of death in a general autopsy service in Brazil. Abdominal visceral fat was obtained *in natura* by the dissection of perirenal, mesenteric, omental, and mesocolon fat. The associations of total abdominal visceral fat with cognitive impairment [clinical dementia rating (CDR) score $\geq$0.5] and CDR-sum of boxes (CDR-SB) were evaluated using logistic regression and negative binomial regression models, respectively. All analyses were adjusted for height, age, sex, education, hypertension, diabetes mellitus, stroke, smoking, alcohol use, and physical inactivity. In addition, we compared the discrimination of visceral fat, body mass index (BMI), and waist circumference (WC) measurements in predicting cognitive impairment.

Results: We evaluated 234 participants (mean age = 71.2 $\pm$ 12.9 years old, 59% male). Abdominal visceral fat was inversely associated with cognitive impairment (OR = 0.46, CI = 0.30; 0.70, $p < 0.0001$) and with CDR-SB scores ($\beta = -0.85$, 95% CI = -1.28; -0.43, $p < 0.0001$). When we compared the area under the ROC curve (AUC),

visceral fat (AUC = 0.754), BMI (AUC = 0.729), and WC (AUC = 0.720) showed similar discrimination in predicting cognitive impairment (p = 0.38).

Conclusion: In an autopsy study, larger amount of directly measured abdominal visceral fat was associated with lower odds of cognitive impairment in older adults.

Keywords: aging, autopsy, obesity, dementia, abdominal fat

INTRODUCTION

Dementia is a common disease among the older population, affecting around 50 million people worldwide with projections indicating that dementia will affect 152 million by 2050 (Alzheimer's Disease International, 2010; WHO, 2017). It is also a main cause of disability and dependency among older people (Alzheimer's Disease International, 2010; WHO, 2017). Similarly, obesity prevalence had almost tripled in the last 40 years, leading to an increased risk for cardiovascular diseases (Bastien et al., 2014; Ebbert et al., 2014; Mandviwala et al., 2016; WHO, 2018). Results on the relationship between obesity and dementia are conflicting. Being overweight or obese in midlife was associated with higher risk of dementia in later life (Whitmer et al., 2005; Hassing et al., 2009; Pedditizi et al., 2016). However, the association of late-life obesity and dementia in unclear with studies showing a reverse (Buchman et al., 2005; Dahl et al., 2008; West and Haan, 2009; Cronk et al., 2010; Power et al., 2011; Pedditizi et al., 2016), a positive (Gustafson et al., 2003), and also no association (Luchsinger et al., 2007) between obesity and dementia in late-life.

Although most of the previous studies have evaluated adiposity using body mass index (BMI) as an assessment of obesity, this anthropometric measurement may not the best marker of adiposity because it cannot distinguish between fat and lean mass, nor between visceral abdominal fat and subcutaneous abdominal fat (Cereda et al., 2007). The validity of BMI as a measure of adiposity is especially problematic in older adults, who experience changes in body composition, such as decrease in muscle mass and bone mineralization, and increase in body fat (Zamboni et al., 1997; Noel and Reddy, 2005). Other measurements of adiposity, such as waist circumference (WC), waist-to-hip ratio (WHR), and abdominal visceral fat may be more appropriate in this age group (Hassing et al., 2009). In mid-life, some anthropometric measurements such as skinfold thickness (Whitmer et al., 2005) and sagittal abdominal diameter (Whitmer et al., 2008) were associated with higher risk of dementia in late life. On the other hand, prior studies have shown a positive (West and Haan, 2009) or no association of WC and cognitive impairment with dementia in late-life (Luchsinger et al., 2007; Yoon et al., 2012). Inverse associations of WHR with dementia and hippocampal volume were reported (Power et al., 2011), as well as a positive association with white matter hyperintensities (Jagust et al., 2005).

Few studies investigated the association between abdominal visceral fat and cognitive impairment. These studies used imaging methods to evaluate the amount of visceral adipose tissue (Kamogawa et al., 2010; Yoon et al., 2012; Spauwen et al., 2017). Prior studies showed no association between abdominal visceral fat area (Kamogawa et al., 2010; Spauwen et al., 2017) with cognitive impairment, while another found a positive association between visceral adipose tissue and poor cognitive performance, but only in participants younger than 70 years (Yoon et al., 2012). Autopsy studies can generate more reliable data due to the possibility of direct measurements of visceral fat surrounding different abdominal organs, as well as the total amount of visceral adiposity (van der Kooy and Seidell, 1993). However, to our knowledge, the association between cognitive impairment and abdominal visceral fat has not been investigated using autopsy material. Therefore, we aimed to evaluate this association in a large population-based autopsy study.

MATERIALS AND METHODS

Participants

This cross-sectional study was conducted at the São Paulo Autopsy Service (SPAS) from University of São Paulo. Data were collected from October 2011 to April 2014. The SPAS performs autopsy in individuals who died from non-traumatic causes of death with unclear diagnosis during life in São Paulo, Brazil (Grinberg et al., 2007). This study was approved by the institutional review board, and the informant who agreed to participate in the study signed a written informed consent.

We included individuals aged 50 years or older at time of death, who had an informant with at least weekly contact with the deceased in the last 6 months prior to death. Exclusion criteria were inability to obtain reliable data from the informant, post-mortem interval >24 h, weight loss of 10% or more in the last 6 months prior to death, signs of autolysis according to Crossley criteria (Crossley, 1974), and retained material by the pathologist.

Sociodemographic and Clinical Data

Information about sociodemographic data (age, sex, race, marital status, and education), frequency of contact of the informant with the deceased, and cardiovascular risk factors (current smoking and alcohol use, physical inactivity, hypertension, diabetes mellitus, and stroke) were obtained with the informant using a semi-structured interview. The cause of death and the post-mortem interval were collected from the autopsy report. Weight and height were measured with the deceased without clothes in the supine position. BMI was calculated by dividing

the weight in kilogram by the squared height in meters. WC was measured with an inelastic tape in the region of the umbilicus (Nishizawa et al., 2016).

Cognitive Assessment

We evaluated the cognitive impairment using the Clinical Dementia Rating (CDR) scale (Hughes et al., 1982; Morris et al., 1997), which contains questions regarding six areas involved in cognition (memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care). We used only the informant part of the CDR scale due to study design. Individuals were classified into five groups: no impairment (CDR = 0); questionable dementia (CDR = 0.5); mild dementia (CDR = 1); moderate dementia (CDR = 2); and severe dementia (CDR = 3). Individuals with CDR = 0 were considered with normal cognition, and those with CDR $\geq$ 0.5 were considered with cognitive impairment (Suemoto et al., 2017). We also used the sum of the boxes for each domain for the CDR sum of boxes (CDR-SB) score, which ranged from 0 to 18 (O'Bryant et al., 2008).

Evaluation of Abdominal Visceral Fat

The abdominal visceral fat was obtained by the *in natura* dissection of perirenal, mesenteric, omental, and mesocolon fat, and weighed using a calibrated electronic scale (Toledo Brazil® model 3,400/05). The weight values were expressed in grams (g). To avoid measurement error, we were careful to calibrate the scale before each use. Subsequently, the values of all fat deposits were summed to obtain a measure of the total abdominal visceral fat and expressed in kilograms (kg; Nishizawa et al., 2016).

Assessment of Other Adiposity Measurements

We measured the deceased's weight in kg using a calibrated electronic scale, and the height in centimeters (cm) using a stadiometer. Both measurements were performed with the individual in supine position and without any clothes. Then, we calculated the BMI in kg/m^2. We measured the WC in the umbilicus region. The abdominal subcutaneous tissue thickness (ASTT) was measured at the abdominal midline, 4 cm above the umbilicus. WC and ASTT were in cm, using an inelastic tape (Nishizawa et al., 2016).

Statistical Analysis

A sample size of 200 participants was estimated using a power of 80%, an alpha level of 5%, and a medium effect size of 0.3 for the correlation between abdominal visceral fat and cognitive impairment in two-tailed tests (Cohen, 1992). We used mean and standard deviation (SD) for quantitative variables, or absolute and relative frequency for categorical variables to describe the sample characteristics. Sociodemographic and cardiovascular risk factors were compared among individuals with and without cognitive impairment, using unpaired *t*-test for continuous variables, and chi-square test for categorical ones.

The dependent variables were cognitive impairment evaluated by a binary variable (CDR categorized into 0 and $\geq$0.5) and by a continuous variable (CDR-SB); and the independent variable was the amount of abdominal visceral fat (continuous variable). As a sensitivity analysis, we also investigated the association between visceral fat and dementia (CDR $\geq$1), excluding those with CDR = 0.5. To evaluate the association between abdominal visceral fat and cognitive impairment, we used logistic regression. The association between abdominal visceral fat and CDR-SB was evaluated using negative binomial regression. Both analyses were adjusted for height, age, sex, education, hypertension, diabetes mellitus, stroke, smoking, alcohol use, and physical inactivity. Based on the fact that the association between obesity and CDR-SB could be different according to age groups (Yoon et al., 2012), we also included an interaction term between age and visceral fat. Finally, we calculated measures of abdominal visceral fat accuracy and compared the discrimination of visceral fat, BMI, WC, and ASST measurements in predicting cognitive impairment using the area under the receiver operating characteristic curves (AUC). We then compared the AUC using the nonparametric methods described by DeLong et al. (1988). The alpha level was set at 0.05 in two-tailed tests. We used Stata 12 (StataCorp., College Station, TX, USA) to perform the statistical analyses.

RESULTS

During the study period, 1,647 subjects were eligible to participate in this study. Two-hundred and thirty-four subjects met the eligibility criteria for this study (**Figure 1**). Included and excluded subjects had similar age (Included: 71.16 $\pm$ 12.98; excluded: 70.02 $\pm$ 12.20; $p = 0.19$), and sex distribution (Included: 58.5% men; excluded: 58.2% men; $p = 0.93$). Thus, the study sample had similar demographic characteristics to the source population. Compared to participants with normal cognition, the 59 participants (25%) with cognitive impairment were older and mostly female. In addition, participants with cognitive impairment had more stroke, were more physically inactive, had lower values of BMI, and lower amount of abdominal visceral fat (**Table 1**).

We observed that an increase in abdominal visceral fat was associated with lower scores in the CDR-SB after adjustment for

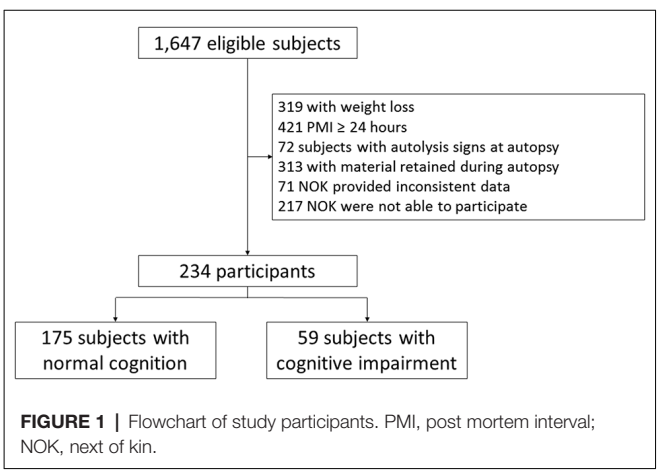

FIGURE 1 | Flowchart of study participants. PMI, post mortem interval; NOK, next of kin.

TABLE 1 | Sample characteristics according to cognitive impairment status (n = 234).

Variables	Total	CDR = 0 (n = 175)	CDR ≥ 0.5 (n = 59)	p
Age (years), mean (SD)*	71.2 (12.9)	68.3 (11.8)	79 (12.8)	<0.0001
Male, n (%)†	137 (58.5)	112 (64.0)	25 (42.4)	0.004
White, n (%)†	147 (62.8)	110 (62.9)	37 (62.7)	0.98
Married, n (%)†	114 (48.7)	94 (53.7)	20 (33.9)	0.01
Education (years), mean (SD)*	5.1 (3.8)	5.5 (3.9)	3.7 (3.1)	0.002
Daily contact of the informant with the deceased, n (%)†	194 (82.9)	145 (82.9)	49 (83.0)	0.97
Cardiovascular cause of death, n (%)†	173 (73.9)	141 (80.6)	32 (54.2)	<0.0001
Hypertension, n (%)†	172 (76.8)	128 (77.6)	44 (74.6)	0.64
Diabetes mellitus, n (%)†	72 (32.1)	54 (32.7)	18 (30.5)	0.76
Stroke, n (%)†	35 (15.6)	17 (10.2)	18 (30.5)	<0.0001
Current smoking, n (%)†	65 (28.0)	58 (33.3)	7 (11.9)	0.01
Current alcohol use, n (%)†	74 (31.9)	67 (38.5)	7 (12.1)	0.001
Physical inactivity, n (%)†	158 (67.5)	106 (60.6)	52 (88.1)	<0.0001
BMI (kg/m^2), mean (SD)*	23.8 (5.9)	25.0 (5.4)	20.2 (5.8)	<0.0001
WC (cm), mean (SD)*	89.8 (15.5)	93.0 (14.0)	80.3 (16.0)	<0.0001
ASTT (cm), mean (SD)*	2.4 (1.3)	2.7 (1.2)	1.8 (1.1)	<0.0001
Abdominal visceral fat (kg), mean (SD)*	1.9 (1.3)	2.2 (1.3)	1.2 (1.1)	<0.0001

Unpaired t-test; †chi-square test. SD, standard deviation; BMI, body mass index; WC, waist circumference; ASTT, abdominal subcutaneous tissue thickness.

possible confounding factors (β = −0.79, 95% CI = −1.02; −0.57, p < 0.0001; **Table 2**). Similarly, we also found that the increase in abdominal visceral fat was associated with 54% fewer odds of cognitive impairment (OR = 0.46, CI = 0.30; 0.71, p < 0.0001). Sensitivity analysis confirmed that a larger amount of visceral fat was associated with lower odds of dementia (**Table 3**).

Additionally, we observed an interaction between abdominal visceral fat and age on the association between CDR-SB and visceral fat (β = 0.018, 95% CI = 0.004; 0.031, p = 0.01; **Figure 2**), suggesting that smaller amounts of abdominal visceral fat were associated with higher CDR-SB scores in older individuals compared to younger ones. Regarding visceral fat accuracy to detect cognitive impairment, the best cutoff of abdominal visceral fat according to the Youden index was 1.23 with a sensitivity of 66% and specificity of 77% (**Table 4**). Higher visceral fat levels had also a good negative predictive value for cognitive impairment. Visceral fat (AUC = 0.754, 95% CI = 0.676–0.831), BMI (AUC = 0.729, 95% CI = 0.646–0.812), WC (AUC = 0.720, 95% CI = 0.636–0.803), and ASTT (AUC = 0.692, 95% CI = 0.612–0.773) showed good discrimination in predicting cognitive impairment with similar AUC values (p = 0.52; **Figure 3**).

TABLE 2 | Association between abdominal visceral fat and CDR-SB (n = 234).

Model	Coefficient (95% CI)	p*
I	−0.72 (−1.08; −0.36)	<0.0001
II	−0.86 (−1.25; −0.46)	<0.0001
III	−0.85 (−1.28; −0.43)	<0.0001

*CDR-SB, clinical dementia rating sum of boxes; CI, confidence interval. *Negative binomial regression. Model I: adjusted for height. Model II: adjusted for height, age, sex, and education. Model III: adjusted for height, age, sex, education, diabetes mellitus, hypertension, stroke, current smoking status, current alcohol use, and physical inactivity.*

TABLE 3 | Odds ratio for association of abdominal visceral fat with cognitive impairment (CDR ≥0.5) and dementia (CDR ≥1).

Model	Cognitive Impairment (n = 234) OR (95% CI)	Dementia (n = 226) OR (95% CI)
I	0.44 (0.30–0.64)	0.32 (0.20–0.52)
II	0.42 (0.28–0.64)	0.30 (0.18–0.50)
III	0.46 (0.30–0.71)	0.31 (0.18–0.55)

OR, odds ratio; 95% CI, confidence interval; p < 0.0001 for all analyses. Model I: logistic regression model adjusted for height. Model II: logistic regression model adjusted for height, age, sex and education. Model III: logistic regression model adjusted for height, age, sex, education, diabetes mellitus, hypertension, stroke, current smoking status, current alcohol use, and physical inactivity.

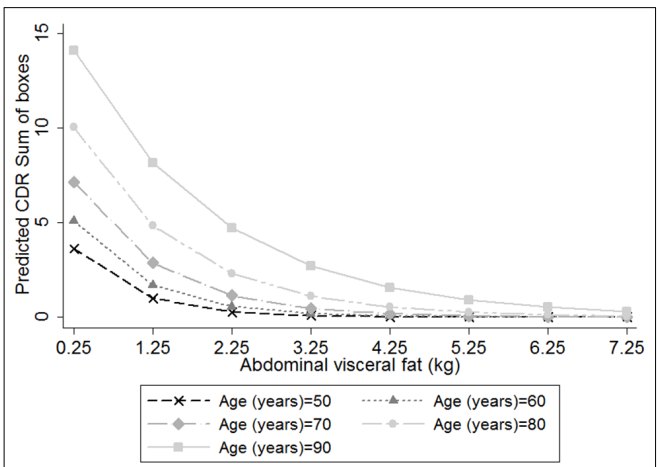

FIGURE 2 | Predicted clinical dementia rating (CDR)-sum of boxes (CDR-SB) scores, according to the amount of abdominal visceral fat in individuals with different ages, considering the inclusion of an interaction term between visceral fat and age on the association between visceral fat and CDR-SB. We used negative binomial regression adjusted for height, age, sex, education, diabetes mellitus, hypertension, stroke, current smoking status, current alcohol use and physical inactivity. Age = 50 years old (cross marker); Age = 60 years old (triangle marker); Age = 70 years old (diamond marker); Age = 80 years old (circle marker); and Age = 90 years old (square marker).

DISCUSSION

We found that a larger amount of visceral fat was associated with lower odds of cognitive impairment and lower scores in

TABLE 4 | Accuracy of abdominal visceral fat in predicting cognitive impairment (n = 234).

Area under the ROC curve (95% CI)	0.751 (0.672–0.830)
Youden Index	1.23
Sensitivity	0.661
Specificity	0.771
Positive Predictive Value	0.487
Negative Predictive Value	0.865
Positive Likelihood Ratio	2.886
Negative Likelihood Ratio	0.440
Diagnostic Odds (95% CI)	6.090 (5.451–6.729)

ROC, receiver operating characteristic; CI, confidence interval.

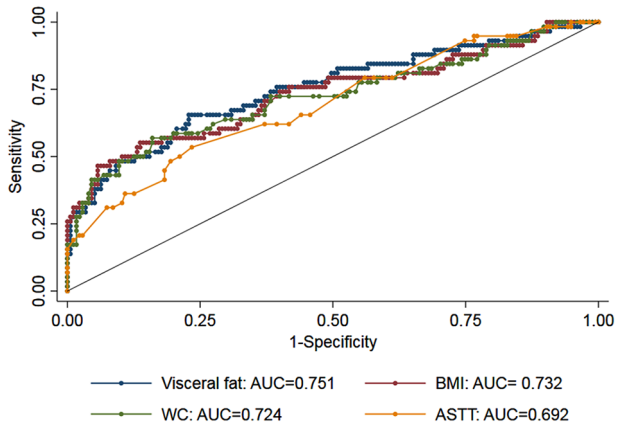

FIGURE 3 | Receiver operating characteristic (ROC) curves for measurements of visceral fat (blue marker), body mass index (BMI: red marker), waist circumference (WC: green marker), and abdominal subcutaneous tissue thickness (ASTT: yellow). The area under the ROC curves (AUC) for the three measurements showed similar discrimination (p = 0.52).

the CDR-SB in a population-based autopsy study. Moreover, we also found an interaction between age and visceral fat on the association between visceral fat and CDR-SB scores that suggests that this inverse association was more severe in older participants. Anthropometric measurements, ASTT and autopsy-measured visceral fat showed similar discriminations for cognitive impairment.

Several studies have demonstrated that overweight or obese individuals in midlife were at a higher risk of cognitive impairment in late life (Whitmer et al., 2005; Hassing et al., 2009; Pedditizi et al., 2016). On the other hand, if adiposity and dementia were analyzed in late-life, the results are conflicting. Our results with direct measurements of abdominal visceral fat are in line with some prior studies that generally support an inverse association between obesity and cognitive impairment (Nourhashémi et al., 2003; Buchman et al., 2005; Dahl et al., 2008; West and Haan, 2009; Cronk et al., 2010; Power et al., 2011; Pedditizi et al., 2016). For example, a significant decrease in BMI (>10%) in late-life was related to a 118% greater risk of developing dementia in the following 3 years (Atti et al., 2008). Another study did not find an association between BMI and late-life dementia (Luchsinger et al., 2007), while higher BMI was related to a higher risk of cognitive impairment, especially in certain groups, as women (Gustafson et al., 2003) and those younger than 70 years (Yoon et al., 2012).

While we observed an inverse association between abdominal visceral fat and cognitive impairment using autopsy material, previous imaging studies found no association between abdominal visceral fat area and cognitive impairment (Kamogawa et al., 2010; Spauwen et al., 2017). On the other hand, a positive association between visceral adipose tissue and poor cognitive performance, but only in participants younger than 70 years (Yoon et al., 2012). Another study among 184 older adults without cognitive impairment evaluated the visceral fat and the brain structure using magnetic resonance imaging (Isaac et al., 2011). Visceral fat accumulation was associated with worse performance on memory and attention tests, and lower hippocampal volume (Isaac et al., 2011). In 1,570 older adults, visceral fat and peripheral fat mass measured by the bioelectrical impedance were related to a higher risk of severe cognitive impairment (Papachristou et al., 2015). Finally, total fat mass was evaluated using dual-energy x-ray absorptiometry, and visceral fat by computed tomography in 3,054 elderly individuals. Higher levels of adiposity were associated with worsening cognition only in men (Kanaya et al., 2009).

Although imaging studies are the most similar to ours since they measured the visceral fat, the quantification of abdominal visceral fat by autopsy is the "gold standard" because it allows the direct measurement of the visceral fat surrounding different abdominal organs (van der Kooy and Seidell, 1993). Indeed, the different findings in imaging studies could be related to the indirect measurement of visceral fat. In addition, some important differences in the sample composition need to be noted. Previous studies were performed in high-income countries (Kanaya et al., 2009; Kamogawa et al., 2010; Isaac et al., 2011; Yoon et al., 2012; Spauwen et al., 2017), while this study was performed in a low-middle income country from Latin America with great ethnic diversity and lower levels of education. Although direct measurements of visceral fat are the most accurate measurement of adiposity, we found that visceral fat, BMI, WC, and ASTT had similar discrimination to predict cognitive impairment in our sample. This finding suggests that both BMI and WC, which are accessible anthropometric measures, could be used to evaluate adiposity and the risk of cognitive impairment.

An explanation for our findings is reverse causation. A prior study showed that participants with lower BMI at baseline had lower memory scores after 10 years. Inversely, participants with lower memory scores in baseline presented a decline in BMI in the following decade. They also demonstrated that preclinical dementia may influence the body composition by weight loss even in individuals in their late 50s (Suemoto et al., 2015). Since weight loss may begin upto 20 years prior to the onset of the dementia (Knopman et al., 2007), maybe these results may be reflecting the preclinical phase of dementia. In a longitudinal study of 8-years of follow-up, lower BMI increased dementia risk in older people. However, when the dementia

cases diagnosed at 1–3 years of follow-up were excluded from analysis, this relationship was not significant, suggesting that lower BMI is probably not a risk factor, but an early clinical sign of the dementia (Nourhashémi et al., 2003). In the same way, a previous autopsy study showed that the higher levels of AD neuropathology were associated with lower BMI in both participants with and without dementia. It suggests that AD pathology may contribute to weight loss even in the preclinical phase of dementia (Buchman et al., 2006). In another study, individuals with early AD had less lean mass compared with non-demented individuals (Burns et al., 2010), as well as lower weight and BMI.

Additionally, our findings of lower visceral fat with higher odds of cognitive impairment was expected since cognition and abdominal visceral fat were evaluated at the same time, and our sample was mainly of older adults. The significant interaction between age and visceral fat on the association between visceral fat and CDR-SB scores found in this study also shows that lower BMI was associated with higher cognitive impairment in older participants than in younger ones. In addition, our findings may be consequent to malnourishment associated with overt clinical dementia. Dementia may interfere with the imbalance between energy intake and energy expenditure through different mechanisms, such as damage to appetite control, forgetting to eat, refusal to eat, increased energy expenditure, apraxia, communication problems in relation to the desire of eating, impaired decision-making ability, and decreased interest in food due to apathy (Aziz et al., 2008; Droogsma et al., 2015).

Another possible explanation is that obesity is, in fact, protective against cognitive impairment due to the excess of leptin, a hormone involved in obesity pathogenesis and also related to memory and learning (Fewlass et al., 2004; Farr et al., 2006). A prior study showed that higher leptin levels were associated with greater BMI and total percent body fat. Individuals with higher levels of leptin had nearly 50% less cognitive decline compared with those with lower leptin levels (Holden et al., 2009). Additional longitudinal studies with long follow-up periods (e.g., 30 years or more) followed by autopsy, with direct measurements of visceral abdominal fat and leptin levels are necessary to clarify this association.

Based on our findings as well as on results from prior studies, we need to emphasize two points. First, we found that lower amounts of directly measured visceral fat were associated with higher odds of cognitive impairment in older participants. Therefore, it is important to evaluate carefully the cognitive function in older adults, who present with weight loss without apparent cause, because this symptom can be due to preclinical or mild dementia. Second, we found that anthropometrics measurements that are easy to obtain (BMI and WC) had similar accuracy in predicting cognitive impairment to visceral fat, which is more costly to measure since it requires an imaging exam, as computed tomography, magnetic resonance imaging or bioelectrical impedance. Thus, in settings of scarce resources as in lower middle-income countries, anthropometric measurements could be used to predict cognitive impairment. Future research should investigate the predictive value of investigating patients with weight loss for cognitive impairment early diagnosis.

Our study should be considered regarding the study limitations. This is a cross-sectional study, which limits our ability to draw causal inferences of the adiposity effect on cognitive impairment. Moreover, we did not have cognitive evaluation prior to participants' death. To overcome this important limitation, we only included informants, who had at least weekly contact with the deceased. In addition, the evaluation with the informant was validated in clinical settings showing good accuracy for the diagnosis of cognitive impairment (Ferretti et al., 2010). In addition, information on the association between directly measured visceral fat and neuropathological lesions is not currently available. Finally, we did not have information on laboratory exams, medications, and family history. Multiple longitudinal cognitive evaluation and neuropathological information will be important to clarify the association between visceral fat and cognitive impairment in future autopsy studies. On the other hand, we should also consider the study strengths. We measured visceral fat directly in autopsy material, being possible to anatomically separate all fats and obtain the total amount of visceral fat. Additionally, our sample is from a low-middle income country with an ethnically diverse background and with low educational levels. In addition, all participants had short post-mortem intervals, which contributed to the quality of the samples. We also compared the discrimination for cognitive impairment of direct measurements of abdominal visceral with anthropometric measurements (BMI and WC) and with measurements of subcutaneous tissue obtained during the autopsy. Finally, we also excluded participants that had lost weight recently to limit the role of reverse causation. In conclusion, larger amounts of visceral fat directly measured directly in autopsy material was associated with lower odds of cognitive impairment in older adults from a lower middle-income country.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of University of São Paulo Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Comissao de Etica em Pesquisa from University of São Paulo.

AUTHOR CONTRIBUTIONS

AN, AC and CS conducted the data analysis and drafted the initial manuscript. AN, AC, DF-I and CS helped with results interpretation and gave critical comments for the manuscript. RN, WJ-F and CP secured funding for data collection. All authors read and approved the final manuscript.

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OPINION
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Infection-Induced Systemic Inflammation Is a Potential Driver of Alzheimer's Disease Progression

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The cases that are categorized as familial Alzheimer's disease (AD) account for 5% of the total AD cases, whereas sporadic cases account for 95% (Masters et al., 2015; Baker et al., 2018). Among the different risk factors underlying sporadic cases of AD, infection might play a role in late-onset AD. Over the past three decades, infectious agents such as bacteria, viruses, fungi, and protozoa have been reported to trigger the development of AD (Sochocka et al., 2017). The infection hypothesis is not a recent idea; the involvement of microorganisms in AD progression was proposed by Aloysius Alzheimer (Fulop et al., 2018). In the 1990s, three laboratories from different countries associated the infection with the etiology of AD. Elderly patients infected with herpes simplex virus (HSV)-1 developed toxic accumulation of amyloid β (Aβ) and phosphorylated (p)-tau protein in the brain (Itzhaki et al., 2016). In autopsy cases with histopathologically confirmed AD, spirochetes were found in blood, cerebrospinal fluid, and brain tissue (Miklossy, 1993). A third study by Balin et al. reports that *Chlamydia pneumoniae* was present in post-mortem brain samples from patients with AD (Balin et al., 1998). In another study, systemic infection by *C. pneumoniae*, a Gram-negative bacterium, was associated with a 5-fold increase in AD occurrence, and in many AD patients, elevated anti-*C. pneumoniae* titers in blood have also been reported (Balin et al., 2008). The result from an association study of 128 AD patients and 135 healthy controls provides evidence of infectious burden, comprising viruses, and bacteria, that is associated with AD (odds ratio ∼4) (Bu et al., 2015). A national representative survey of US residents involving 1,194 patients with 1,520 hospitalizations for infection with severe sepsis revealed that sepsis survivors were independently associated with substantial and persistent new cognitive impairment and functional disability (Iwashyna et al., 2010). All of these studies support the notion that infectious etiology might be a causative factor for the inflammatory pathway associated with AD progression.

The accumulation of misfolded Aβ in the brain has been proposed to be the critical triggering event in a complex pathophysiological cascade that leads to AD pathology. The additional physiological role of Aβ as an antimicrobial agent in *in vitro* and *in vivo* models has been shown by Robert Moir and Rudolph Tanzi (Soscia et al., 2010). In both rodent and nematode models, the authors reported the antimicrobial properties of the Aβ peptide. Transgenic mice expressing the human mutant form of APP were infected with *Salmonella enterica*; the nematode *Caenorhabditis elegans* expressing the human $A\beta_{42}$ peptide were infected with *Candida albicans*. The mice and *C. elegans* expressing the Aβ peptide survived longer than did the control group without Aβ expression after infection. In another Aβ-overexpressing mouse model, *S. typhimurium* injection in the brain resulted in the induction of Aβ amyloid deposits with an extended survival rate. These studies

also suggested that Aβ oligomerization, which is considered a pathological development in the context of neurodegeneration, may be a necessary step to potentiate the antimicrobial activity of the peptide (Kumar et al., 2016). These results raised some important questions about the association between AD and microbial infection. The authors also unveiled the mechanism by which Aβ elicits its antimicrobial property. Aβ binds to a microbe and entraps it by forming amyloid fibrils. The presence of microbes serves as an efficient surface for nucleation of amyloid aggregates, thereby raising the possibility of amyloid deposition (Golde, 2016) (**Figure 1**). Thus, brain infection in a mouse model of AD triggered formation of Aβ plaques earlier than they usually developed. The above reports on neuroinflammation-mediated neurodegeneration and the role of Aβ as an antimicrobial agent have impelled the emanation of the "antimicrobial protection hypothesis" (Moir et al., 2018) in addition to different hypotheses concerning development of AD, including the cholinergic hypothesis, amyloid hypothesis, tau hypothesis and inflammatory hypothesis (Du et al., 2018). Even so, the findings raise the question of how the protective function of Aβ fails. The possible answer is microglial dysfunction; accumulation of biologically active peptides following an infection might have not been effectively cleared by microglia in the brain of patients with AD (Stilling and Cryan, 2016) (**Figure 1**). Additionally, Aβ accumulation in the brain may act as an early toxic event in the pathogenesis of AD. The Aβ monomers, soluble and probably nontoxic, would aggregate into different complex assemblies, including soluble oligomers and protofibrils, with various degrees of toxicity. That may spread throughout the brain, and eventually developed into insoluble amyloid fibrils further assembled into amyloid plaques, which are one of the characteristic histological lesions on AD brains. In the context of AD, the biological significance of Aβ conformational states is important as the different types of assemblies might differentially influence the development of neurodegenerative stages (Miklossy, 2011; Tycko, 2015; Chen et al., 2017). Hence, it would be extremely important to gain knowledge on Aβ conformational changes following infection that potentially affect the central nervous system (CNS).

Recently, the results from three different groups of investigators demonstrated that sepsis, a life-threatening acute organ dysfunction due to a dysregulated host immune response after infection, induces systemic inflammation that exacerbates the accumulation of Aβ and triggers AD progression. A study by Gasparotto et al. reported that sepsis induction in a cecal ligation and perforation model escalated the levels of Aβ, p-tau protein and receptor for advanced glycation end products (RAGE) markers with simultaneous cognitive impairment in wild-type rats. The increase in AD markers was accompanied by activation of microglia and astrocytes (Gasparotto et al., 2018). Another study by Wang et al. demonstrated that the induction of sepsis in a lipopolysaccharide (LPS) endotoxemia model upregulated the levels of soluble monomeric Aβ (1–42) and p-tau. The levels of the inflammatory markers, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) and cortical microglial density, increased after systemic injection of LPS (Wang et al., 2018). The third study by Ehler et al. demonstrated staining of β-amyloid precursor protein (APP) in the post septic rat brain after experimental sepsis induction by fecal peritonitis, and demonstrated staining for β-APP in the postmortem septic brain (Ehler et al., 2017). Together, all of these reports suggest that inflammation is a cardinal component of the pathophysiology of sepsis. Thus, the role of inflammation might be associated with the long-term cognitive impairment observed in sepsis survivors.

A compromised blood-brain barrier (BBB) is one of the consequences after bacterial and viral infections, which leads to diffuse cerebral dysfunction after the systemic inflammatory response, with or without direct CNS infection (Cain et al., 2017; Al-Obaidi and Desa, 2018). Increased BBB permeability drives significant alteration in consciousness, facilitating the storm of pro-inflammatory cytokines in the CNS that leads to brain dysfunction. Infection-induced systemic inflammation provokes microbiome dysbiosis in response to pathogenic microorganisms and/or as a result of altered immune function. Altered immune function after infection acutely exacerbates the peripheral load of cytokines. The systemic inflammation-induced BBB breach escalates the transportation of a number of pro- and anti-inflammatory cytokines and chemokines to the brain, including TNF-α, IL-1β, transforming growth factor beta (TGF-β), and monocyte chemoattractant protein 1 (MCP1) (Semmler et al., 2008). An increased level of the systemic inflammatory marker TNF-α was demonstrated to be associated with an increase in cognitive decline in AD patients (Holmes et al., 2009). Recent reports demonstrate that in a *Drosophila* model, *Enterobacteriaceae* family infection exacerbates the progression of AD by promoting immune hemocyte migration to the brain (Wu et al., 2017). Additionally, polymicrobial infection-induced RAGE accumulation facilitates the transport of the Aβ peptide across the BBB and increases the central Aβ load (Gasparotto et al., 2018) (**Figure 1**). Therefore, endothelial activation followed by BBB alteration modulates the transport of potential neurotoxic components from the peripheral circulation to the cerebral compartment, which facilitates the neuroinflammatory cascade of AD.

Recent evidence from both preclinical and clinical studies suggests the activation of microglia after CNS infection by viruses, bacteria, fungi and parasites (Rock et al., 2004; Ashraf et al., 2018). Microglia, an indicator of brain inflammation, have multiple facets for neuroinflammation, including cytotoxicity, repair, regeneration, and immunosuppression, due to their ability to acquire diverse activation states or phenotypes (Chhor et al., 2013). During infection, microglia express immunoreceptors (IRs), which are capable of recognizing foreign molecules and triggering innate immune responses. Pattern-recognition receptors (PRRs), one of the examples of IRs, are the central components of the innate immune system that recognize danger signals, such as invading bacteria, and initiate the immune response. PRRs recognize conserved pathogen molecular structures, commonly known as pathogen-associated molecular patterns (PAMPs), and intracellular molecules released from damaged host cells, collectively known as damage-associated molecular patterns (DAMPs) (Linnartz and Neumann, 2013). The PRRs that trigger amyloidosis include TLRs, RAGE,

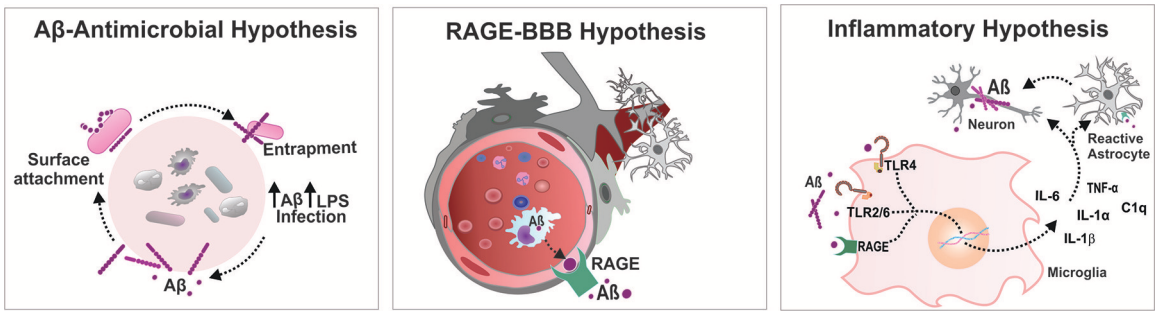

FIGURE 1 | Infectious etiology triggers AD progression. Aβ-Antimicrobial hypothesis: Aβ binds to a microbe and entraps it by forming amyloid fibrils, thereby raising the possibility of amyloid deposition. RAGE-BBB hypothesis: RAGE acts as an important transporter via regulating influx of circulating Aβ into brain. Inflammatory hypothesis: Systemic inflammation increases the BBB permeability and activates microglia cells triggering Aβ deposition in the brain. Aβ, amyloid beta; BBB, blood brain barrier; IL, interleukin; LPS, lipopolysaccharide; RAGE, receptor for advanced glycation end products; TLR, toll-like receptor; TNF, tumor necrosis factor.

cluster of differentiation (CD)14, and purine receptors (P2X7). The biologically active Aβ binds to these receptors and upregulates the Aβ load in the CNS. A recent systematic review and meta-analysis concluded that inhibition of RAGE, a danger signal that triggers the inflammatory response, improves outcomes after systemic inflammation in animal models (Zhao et al., 2018). Intriguingly, the study by Keren-Shaul et al. identified an unexpected population of microglia called disease-associated microglia (DAM) using single-cell RNA sequencing technology and demonstrated its significance relevant to AD pathology (Keren-Shaul et al., 2017). A recent report revealed that pro-inflammatory microglia secrete IL-1α, TNF, and C1q, and these cytokines are sufficient to activate astrocytes termed A1 reactive astrocytes. The A1-reactive astrocytes produce complement components that release toxic factors that, in turn, damage neurons, and oligodendrocytes, thereby contributing to the cognitive decline (Clarke et al., 2018). To understand how infection induces brain dysfunction, deep insights into brain-immune cross talk are required, which can be achieved by identifying the role of DAM and reactive astrocytes after infection. Together, all these findings support the "inflammation hypothesis of AD" that seems more relevant to the development of the sporadic form of the disease than to the familial form (Krstic and Knuesel, 2013) (**Figure 1**).

Inflammation is a complex biological response of the immune system to harmful stimuli caused by chemical, physical, and biological factors. Although not only triggered by infection, inflammation secondary to infection plays a key role in the etiopathogenesis of AD progression (Ashraf et al., 2018). Infection-induced systemic inflammation is characterized by acute or chronic activation of a dysregulated host immune response, and the signals are not only restricted locally but also have potential systemic effects (Thorburn et al., 2018). C-reactive protein (CRP) is an important component of the innate immune system that is also used as a biomarker of inflammation (Kuo et al., 2005). The levels of this acute-phase reactant are elevated in bacterial and viral infections (Hu et al., 2017; Vasileva and Badawi, 2019). Many population-based prospective studies have suggested the association of CRP levels with the development of cognitive decline, especially AD (Duong et al., 1998; McGeer et al., 2000).

During the past decade, several studies have documented the possible contribution of peripheral infection and the role of peripheral immune activation in the progression of AD pathology (Kamer et al., 2008; Cao and Zheng, 2018; Choi et al., 2019). Infiltrating peripheral myeloid cells participate in Aβ clearance, as well as in replacing ablated microglia, to adopt a microglia-like phenotype in the brain with limited phagocytic capacity (Cao and Zheng, 2018). A recent study demonstrated that oral *Porphyromonas gingivalis* infection in a rodent model exacerbated the production $A\beta_{1-42}$. The same pathogen was also identified in AD patients brain (Dominy et al., 2019). Thus, the prominent molecular and cellular changes in the periphery might have significant role in AD progression (Abbayya et al., 2015).

Nevertheless, the Aβ clearance after an infection remains a largely unexplored area. Knowing the fact that infection followed by systemic inflammation is sometimes accompanied by organ dysfunction, liver and kidney dysfunction need to be considered (Fujishima, 2016). However, the liver and kidney are the primary organs involved in the elimination of peripheral Aβ peptide. Thus, the major question remains: what is the fate of Aβ after infection? To answer this question, it would be necessary to gain a deeper insight into the post infection pathway of Aβ clearance.

Systemic inflammation induced by different infectious etiologies supports the amyloid hypothesis, inflammatory hypothesis, and antimicrobial hypothesis of AD. Thus, the accumulated knowledge, views and hypotheses from recent findings explains the infectious origin as one of the risk factors of AD progression. Although the molecular cascade that links systemic inflammation and neuroinflammation is still enigmatic, the possible modules that occur after infection, which lead to long-term impairment and brain dysfunction that ultimately trigger AD pathology, may include the following: Invading microorganisms escalate the peripheral Aβ load, a necessary step to neutralize and eliminate the pathogen from the peripheral environment. The peripherally produced Aβ and cytokines enter the CNS as systemic inflammation is able to increase BBB permeability. An increase in RAGE expression

during systemic inflammation also facilitates the transport of Aβ to the central compartment. Finally, the entry of foreign substances triggers brain-immune system crosstalk, which in turn leads to activation of microglia/ astrocytes and local production of inflammatory mediators and reactive species (**Figure 1**). Further comprehension of these mechanisms with newer insights is warranted to develop a strategy for the potential advancement of therapeutics for infection-induced AD progression.

AUTHOR CONTRIBUTIONS

VG wrote the manuscript and proof the manuscript. FM, FP, and FD-P critically reviewed the manuscript. TB devised the main conceptual ideas and proof outline and designed the figure.

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frontiers in Aging Neuroscience

REVIEW
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Modifiable Risk Factors for Alzheimer's Disease

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Since first described in the early 1900s, Alzheimer's disease (AD) has risen exponentially in prevalence and concern. Research still drives to understand the etiology and pathogenesis of this disease and what risk factors can attribute to AD. With a majority of AD cases being of sporadic origin, the increasing exponential growth of an aged population and a lack of treatment, it is imperative to discover an easy accessible preventative method for AD. Some risk factors can increase the propensity of AD such as aging, sex, and genetics. Moreover, there are also modifiable risk factors—in terms of treatable medical conditions and lifestyle choices—that play a role in developing AD. These risk factors have their own biological mechanisms that may contribute to AD etiology and pathological consequences. In this review article, we will discuss modifiable risk factors and discuss the current literature of how each of these factors interplay into AD development and progression and if strategically analyzed and treated, could aid in protection against this neurodegenerative disease.

Keywords: Alzheimer's disease, risk factors, comorbidities, vascular disease, traumatic brain injury, epilepsy, depression, lifestyle

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INTRODUCTION

Alzheimer's dementia is an age-related neurodegenerative disease characterized by several neuropathological markers including extracellular amyloid-β (Aβ) plaques, intracellular neurofibrillary tangles (NFTs), inflammation, synaptic impairment, and neuronal loss that leads to cognitive impairment (Querfurth and LaFerla, 2010). A multitude of studies has shown strong evidence for the concept that the misfolding, aggregation and brain accumulation of protein aggregates are a triggering event in the pathogenesis of Alzheimer's disease (AD) and responsible for the subsequent pathological alterations that lead to the clinical disease (Moreno-Gonzalez and Soto, 2011). Due to the progressive aging of the population, the number of people affected by AD in the United States is predicted to reach 14 million by the year 2050 (Mebane-Sims and Alzheimer's Association, 2009). Nowadays, there is not a definitive cure for this disease. Treatment and daily care of AD patients are considered costly in emotional and economical aspects. Available drugs used to treat AD are expensive, and they focus only to alleviate symptoms that are invariably fatal. The etiology of sporadic AD—more than 95% of cases—is not completely understood. The lack of knowledge about sporadic AD etiology makes it very difficult to prevent its onset and detect risk factors. Although the avoidance or prevention of modifiable risk factors may not have full impact in the future development of the disease, a recent study determined that good lifestyle habits and management of comorbidities may lead to a lower risk of dementia (Baumgart et al., 2015).

Therefore, identifying potential risk factors may facilitate a reduction in the burden of people affected by AD. In this review article, we will discuss proposed modifiable risk factors for AD, focusing on their effect on protein aggregation and deposition, and whether their prevention and control may have a direct impact in the potential to develop this dementia.

COMORBIDITIES

Vascular Diseases

The cerebrovascular network and the neurovascular control mechanisms have a pivotal role in maintaining the activity and integrity of the brain by assuring constant blood flow (Iadecola, 2004). In this process, the neurovascular unit—a specialized entity of neurons, astrocytes and vascular endothelial cells—has a crucial function. Alterations in this vascular system contribute to a reduction in global cerebral perfusion leading to brain dysfunction and cognitive impairment (Iadecola, 2013), thereby introducing the concept of the vascular hypothesis of AD. In this hypothesis, vascular pathologies promote the neuropathologic hallmarks of AD (de la Torre, 2018). Indeed, vascular risk factors are critically involved in the progression of dementia leading the conversion from mild cognitive impairment (MCI) to AD (Luchsinger et al., 2005; Li et al., 2011). Less than 10% of demented individuals develop only vascular dementia (Brenowitz et al., 2017). There is a growing body of evidence that supports the idea of vascular factors as contributors of the pathological mechanisms of AD. Epidemiologically, different risk factors for vascular diseases have been shown as significant risk factors for AD (Panpalli Ates et al., 2016). It has been suggested that the link between cerebrovascular disease (CVD) and AD is even much more important than the influence of aging (Love and Miners, 2016). Some of these common risk factors shared between CVD and AD are hypertension, diabetes, atrial fibrillation, atherosclerosis, hypercholesterolemia, and apolipoprotein E (ApoE) genotype (Vijayan and Reddy, 2016).

A history of prehypertension and hypertension in midlife or late in life increases the risk of developing dementia and enhances the neuropathology of AD (Dickstein et al., 2010; Gottesman et al., 2017a). The Honolulu-Asia study revealed that hypertensive patients showed an abundance of amyloid plaques and NFTs in the brain and atrophied hippocampus (Launer et al., 2000). Furthermore, high blood pressure promotes atherosclerosis in cerebral arteries, blocking the cerebral blood supply (Ninomiya et al., 2011), leading to lacunar or cortical infarcts, and, ultimately, cognitive impairment (Dickstein et al., 2010). Angiotensin-converting enzyme (ACE), that regulates blood pressure, is able to degrade Aβ (Hemming and Selkoe, 2005) and the use of anti-hypertensive medications, such as ACE inhibitors, to reduce the risk to develop AD may in fact lead to an opposite effect than desired. However, ACE inhibitors have been proven to not increase amyloid burden *in vivo* (Hemming et al., 2007). In humans, ACE inhibitors do not have a beneficial effect in cognitive impairment either (Peters et al., 2008), but other antihypertensive treatments may still be helpful to reduce the risk of AD. Hence, hypertension could be established as one of the strongest risk factors for AD. On the other hand, it has been proposed that hypertension could be induced by the action of Aβ before dementia onset—being responsible for high blood pressure and cerebrovascular impairment (Petrovitch et al., 2000). Therefore, hypertension could be just a result of Aβ accumulation rather than a risk or a combination of both.

Hypertension is the main risk factor for stroke, a phenomenon that deprives the supply of blood flow to the brain. In fact, the severity of stroke is higher in diabetic patients, which increases the rate of death (Air and Kissela, 2007). Clinical history of stroke is associated with a prevalence of dementia, denoted as post-stroke dementia (Pendlebury and Rothwell, 2009), doubling the risk of developing AD in the elderly (Sun et al., 2006). Among single or multiple stroke patients, post-stroke dementia is a common outcome. Mechanistically, there are several processes that potentially link AD and stroke. It has been proposed that stroke could promote Aβ production, hamper Aβ clearance, and/or aggravate synaptic and neuronal loss already triggered by Aβ and tau pathology (Sun et al., 2008; Garcia-Alloza et al., 2011; Hongpaisan et al., 2011).

Heart disease (atrial fibrillation, arrhythmias, or cardiac arrest) causes a reduction in cerebral perfusion, leading to nerve cell damage (Kwok et al., 2011), brain dysfunction, and cognitive decline (Alosco et al., 2013). Atrial fibrillation is known as another risk factor for stroke, increasing the prevalence of AD and dementia (Ott et al., 1997; Kilander et al., 1998). The association between heart failure and cognitive impairment is supported by the induction of brain hypoxia and neuronal loss after a hypoperfusion event (Muqtadar et al., 2012). In addition, an elevation in Aβ42 serum levels has been reported following a cardiac arrest episode, which would also contribute to AD neuropathology (Zetterberg et al., 2011). Overall, cardiovascular diseases seem to induce a lack of perfusion/oxygenation in the brain, leading to cognitive impairment and dementia mediated by an increase in Aβ levels due to different mechanisms. Although already existing Aβ aggregates can also induce cerebral perfusion impairment, a history of hypertension, stroke or heart disease can be considered a risk factor to develop AD.

The increased risk of developing AD dementia is also associated with atherosclerosis, a common vessel disorder in the elderly. AD patients show atherosclerosis in the circle of Willis (cerebral arterial circle at the base of the brain) much more severe and more frequently than healthy age-matched controls (Roher et al., 2003), and hypertension can have a role in promoting this intracranial atherosclerosis. This intracranial atherosclerosis reduces the brain blood perfusion and is linked to an increase in neuritic plaque burden and higher Braak stage in AD patients (Beach et al., 2007). Cholesterol has been consistently associated to AD. High levels of cholesterol have been linked to increased Aβ levels and greater cognitive impairment and progression in AD. Cholesterol seems to impair Aβ degradation and promote its production (Barbero-Camps et al., 2018). In fact, the use of statins, a cholesterol-lowering medication, such as simvastatin, has shown to lower the risk of AD diagnosis particularly in women (Zissimopoulos et al., 2017)

even in ApoE homozygotes (Geifman et al., 2017) and levels of phospho-tau in the cerebrospinal fluid (CSF; Li G. et al., 2017). The proposed mechanism is the direct interaction of statins and Aβ protofibrils (Shakour et al., 2019), inhibition of apoptosis (Hu et al., 2018). Therefore, hypercholesterolemia has been suggested to be a high-risk factor for AD and cholesterol-lowering medication should be considered as a preventive therapy for dementia.

Cerebral amyloid angiopathy (CAA) is a condition where Aβ deposits accumulate within the walls of the meningeal and intracerebral arteries, arterioles, and very rarely, veins and capillaries. This engenders a thickening of vessels walls and constriction of vascular lumen thereby promoting potential micro-aneurysms. This pathology increases the risk to develop hemorrhages, ischemic lesions, and encephalopathies, resulting in profound cerebral damage that contributes to neurodegeneration and cognitive dysfunction (Ellis et al., 1996; Haglund et al., 2006). CAA is associated to a more rapid cognitive decline in both demented and non-demented persons (Pfeifer et al., 2002). Certainly, CAA has a close association with AD and additive effects on the risk of developing dementia through AD pathology. The diagnosis of probable AD is related to the presence of CAA. In fact, CAA is highly prevalent in AD patients, being present in about 80%–90% of AD patients (Arvanitakis et al., 2011). Moreover, the role of hypertension has a significant additional causal factor that contributes to the progression of CAA-related vasculopathies.

Other CVDs have been described in the aging and AD brain supporting that cerebrovascular dysfunction contributes to neurodegeneration, cognitive dysfunction, and lowers the threshold for developing AD dementia. These cerebrovascular pathologies are cortical infarcts, lacunes, hemorrhages, microbleeds, intracranial small vessel atherosclerosis-arteriosclerosis, and blood brain barrier (BBB) dysfunction (Toledo et al., 2013). Generally, a higher number and the extent of cortical infarcts is directly associated with a higher risk of dementia. Extensive CAA and cerebral small vessel disease have also been proposed to contribute to neurodegeneration in AD (Toledo et al., 2013). Likewise, numerous microbleeds contribute to cognitive function decline and severe white matter lesions that lead to a 4-fold increased risk of developing MCI (Benedictus et al., 2015) that can eventually lead to AD development.

Regarding the effect of CVD on protein misfolding and deposition, there is a greater tendency of amyloid accumulation in patients with vascular risk factors (Langbaum et al., 2012; Gottesman et al., 2017b). Vascular insufficiency results in hypoperfusion and hypoxia that activate the amyloid precursor protein (APP) cleavage enzyme β-secretase (Xu et al., 2007) and facilitates a robust deposition of fibrillar amyloid. Therefore, Aβ not only promotes cerebrovascular dysregulation increasing the brain susceptibility to ischemia but also ischemia upregulates Aβ cleavage and its accumulation. On the other hand, the main Aβ clearance mechanisms are altered and damaged under the presence of vascular dysfunction contributing to parenchymal and vascular accumulation of Aβ (Garcia-Alloza et al., 2011). Tau hyperphosphorylation and NFTs are also associated with vascular risk and the synergistic effect of elevated Aβ burden (Vemuri et al., 2017; Rabin et al., 2019). It has been also described that increased plasma levels of Aβ are linked to vascular disease both in the brain (white matter lesions and microbleeds) and in the periphery (hypertension, diabetes and ischemic heart disease; Janelidze et al., 2016). It should be noted that the contributions of vascular dysfunction occur at the early stages of AD pathophysiology and may represent a casual pathway towards dementia, facilitating an earlier diagnosis of AD. Furthermore, the vascular component is a promising target to decrease the risk of dementia and the neuropathological progression of AD. Nonetheless, further studies are required to elucidate the mechanisms underlying vascular pathologies as they relate to AD and dementia. This, along with the development of precise vascular biomarkers will be fundamental to discover new ways to prevent and treat AD and related dementias. Therefore, the improvement in the vascular health and the control of vascular risk factors may reduce the risk of developing vascular pathologies that trigger AD neuropathology.

Type 2 Diabetes

Diabetes is estimated to affect over 30.3 million people with over 7.2 million undiagnosed, and 90%–95% of these cases are delineated as type 2 diabetes (T2D; Centers for Disease Control and Prevention, 2017). T2D is a complex metabolic disorder that is characterized prominently by hyperinsulinemia, insulin resistance, glucose metabolism impairments, and, ultimately, pancreatic β-cell destruction. In T2D, pancreatic β-cells secrete excessive insulin in response to insulin resistance causing hyperinsulinemia, while allowing blood glucose levels (BGLs) to be maintained. As this continues over time, it begins to burden the β-cells, leading to insulin insufficiency and finally causing T2D. T2D and AD have a strong epidemiological link—so substantial that some researchers define AD as type 3 diabetes. T2D is proposed to increase the risk of AD and dementia from 1.3 up to 5.5 times and the Rotterdam study in the 1990s described T2D having double the risk for AD and dementia (Ott et al., 1999; Li et al., 2015). T2D patients are at ∼60% greater risk for the development of dementia compared with individuals without diabetes (Chatterjee et al., 2016). Additional evidence of a systematic analysis concluded that T2D is convincingly a major risk factor for AD and vascular dementia (Bellou et al., 2017). There are simultaneous influences within each of these diseases that also adjoin T2D and AD, such as progressing age, diet, body mass index (BMI) and obesity, and sedentary lifestyle. In fact, adiposity, being overweight, or obese is a chief cause for insulin resistance (Luchsinger and Gustafson, 2009). There is a strong epidemiological link for T2D and AD and this may be due to their shared pathological mechanisms (Baglietto-Vargas et al., 2016).

Hyperinsulinemia has been associated with AD risk as indicated by such studies as the Honoulu-Asia Aging study (Luchsinger and Gustafson, 2009). Other than regulating the peripheral metabolism, insulin has insulin receptors expressed throughout the central nervous system (CNS). The function of brain insulin receptors is not clearly understood. It is known to regulate circuit function and plasticity by controlling synapse density and plays a role in the cholinergic system. Impairments

in insulin receptors and hyperinsulinemia have been associated with aging and AD. A decreased level in insulin receptors and their sensitivity in AD patients compared to middle-aged controls and expression and metabolism of Aβ and tau are also affected (Frölich et al., 1998; Sims-Robinson et al., 2010). Moreover, irregular insulin levels can disrupt the cholinergic system, which is also compromised in AD, as insulin aids in stimulating choline acetyltransferase (ChAT; Rivera et al., 2005). Insulin degrading enzyme (IDE) is vital for the degradation of insulin and Aβ. Thus, hyperinsulinemia can lead to a competition of insulin and Aβ for IDE, thereby increasing amyloid levels in the brain. Loss-of-function mutations of IDE in rodents exhibit glucose intolerance and accruement of Aβ aggregates, whereas, IDE action revealed opposite results (Shen et al., 2006). Downstream insulin signaling pathways are also affected. Pathways that are known to be involved in AD pathogenesis such as mitogen-activated protein kinase (MAPK), protein kinase B (Akt), and glycogen synthase kinase-3β (GSK-3β) are altered due to insulin dysregulation. MAPK expression is correlated with Aβ production and NFTs and is increased in AD patients. Under insulin resistance, Akt signaling can inhibit GSK-3β, which dephosphorylates and activates glycogen synthase in glycogenesis and ultimately resulting in the hyperphosphorylation of tau. Accordingly, the cognitive impairment that is noted in both T2D and AD could be intervened by insulin administration. Tied to insulin issues is the dysfunction of glucose metabolism. The brain is estimated to consume 20% of energy stored in the body, and neurons depend on a steady peripheral transport of glucose through the BBB facilitated by glucose transporters (GLUTs), especially GLUT-1 and -3. Deficiency in GLUT-1 and -3 is reported in AD brains, and this decrease correlated to the decrease in O-GlcNAcylation, hyperphosphorylation of tau, and to the density of NFTs (Liu et al., 2008, 2009). Indeed, imaging techniques such as Fluorodeoxyglucose (FDG)-PET imaging are able to detect glucose metabolism alterations in AD related to anatomical areas associated with pathology and preceding cognitive impairments (Ballard et al., 2011; Nordberg, 2015).

Both AD and T2D are diseases related to protein misfolding and aggregation (Soto, 2003; Moreno-Gonzalez and Soto, 2011; Morales et al., 2013). In T2D, the aggregation of an amyloidogenic protein called islet amyloid polypeptide (IAPP) or amylin is seen in up to 96% of T2D patients in pancreatic β-cells (Clark et al., 1995; Westermark, 2011). IAPP is a hydrophobic hormone co-secreted with insulin into blood circulation at 1:100 ratio. It contributes to glycemic control by slowing down gastric emptying and inhibiting digestive secretion and other pancreatic hormones. Although it is not clear that IAPP is a cause or an effect of T2D, amyloidogenic IAPP aggregates are toxic and have been proposed to destroy β-cells and facilitate the progression of the disease (Westermark, 2011; Abedini et al., 2015). In fact, genetically modified rodent models expressing human IAPP demonstrate aggressive diabetic-like phenotype, such as insulin impairments and hyperglycemia, with IAPP deposits and β-cell loss (Clark et al., 1995; Janson et al., 1996). Interestingly, IAPP has been discovered in AD brains, whereas Aβ and tau have been found in T2D pancreas; in addition, these proteins were seen to co-localize in brain and pancreas (Miklossy et al., 2010; Valente et al., 2010; Moreno-Gonzalez et al., 2017). Moreover, T2D patients display increased amounts of NFTs and Aβ in the hippocampus (Miklossy et al., 2010). In AD patients, there is an extensive prevalence of IAPP compared to non-AD (Janson et al., 2004). Brain of T2D/AD patients show an augmented number of cortical Aβ plaques and tau-positive cells compared to affected AD brains suggesting that T2D/AD patients have a more severe pathology with much more rapid progression (Bretherton-Watt and Bloom, 1991). Therefore, these amyloidogenic proteins may interact directly. IAPP [8–18] and IAPP [22–28] sequences were noted hot regions for IAPP and Aβ40 interaction (Kapurniotu et al., 2010). One proposed mechanism is the cross-seeding of Aβ and IAPP when oligomers composed by one protein seed the aggregation of a different protein by a seeding-nucleation process (Morales et al., 2013; Jucker and Walker, 2015; Moreno-Gonzalez et al., 2017). Many studies reveal a successful heterologous seeding aggregation when both proteins are present *in vitro* (Berhanu et al., 2013; Yan et al., 2014; Moreno-Gonzalez et al., 2017). Double transgenic mice overexpressing both Aβ in brain and IAPP in pancreas show increased Aβ burden compared to controls (Moreno-Gonzalez et al., 2017). IAPP pancreatic homogenate injected into an AD mouse model provided a potent seeding effect by accelerating AD pathology and impairing memory (Moreno-Gonzalez et al., 2017). Independently of the mechanism of action, T2D can be considered one of the main risk factors for AD.

Traumatic Brain Injury

Recent research has posited that traumatic brain injury (TBI) is a robust factor that leads to the advancement of AD or dementia. The severity of TBI is quantified by the Glascow Coma Scale (GCS). Severe TBI (sTBI) represents head injuries that result in permanent or an extended period of unconsciousness, amnesia, or death following a head injury with a GCS of 3–8. Moderate TBI involves a period of unconsciousness or amnesia from 30 min $\geq$24 h with a GCS of 9–12. Mild TBI (mTBI) is recognized as head injuries that cause a brief state of altered consciousness resulting in $\leq$30 min of unconsciousness, though most mTBIs do not result in a loss of consciousness. mTBI represents a majority of reported cases and has been linked to AD pathology (Edwards et al., 2017). The harsher the TBI the greater risk of developing AD (Graves et al., 1990; Guo et al., 2007). World War II veterans that had a TBI event had an increased risk of developing AD (Plassman et al., 2000). After a TBI event, dementia diagnosis was found to be strongest within the first year (4–6 times) but maintained significance up to 30 years (Nordström and Nordström, 2018). In a large cohort study, the overall risk of dementia in individuals with a history of TBI was 24% higher than those without a history of TBI. A sTBI increased AD risk by 35% and a single mTBI or concussion increased the risk by 17%. The risk of dementia was increased with the number of TBI events—33% higher for two or three TBIs, 61% higher for four TBIs, and 183% higher for five or more TBIs (Fann et al., 2018), demonstrating the link between TBI severity

and the number of events and dementia. TBI begins with an instant, irreversible initial blow or impact causing direct damage to the surrounding neuronal and astroglial cells and vasculature. Primary insult can either be a focal injury or diffuse axonal injury (DAI). The impact can trigger a rapid necrosis due to the mechanical damage, edema, increased intracranial pressure, and ischemia. This will secondarily lead to neuronal excitotoxicity, mitochondrial dysfunction, oxidative stress, inflammation, synaptic dysfunction, axonal degeneration, neuronal death, and, ultimately, instigating cognitive and behavioral impairments (Gentleman et al., 2004; Breunig et al., 2013). Therefore, TBI shares many of the molecular mechanisms observed in AD. Following TBI, multiple cell death pathways get activated leading to synapsis reduction and finally neuronal loss as loss of total brain volume in the hippocampus, cortex and other medial temporal lobe structures, and elevation in ventricular volume has been described (Blennow et al., 2016). This neurodegenerative process initiated by TBI may trigger the development of memory problems that may then convert into AD.

Numerous *in vivo* studies demonstrate Aβ and tau pathophysiology along with other pathological consequences alike to AD following TBI. Aβ plaques are reported in up to 30% of post-mortem TBI patients. Aβ can accumulate rapidly following TBI, reported as early as 2–4 h (Graham et al., 1996). Plaque formation is described as diffuse plaques seen in early-stage AD and seen in all ages, even children, but determined to be more robust in elderly affected individuals (Johnson et al., 2012). Post-mortem TBI brains show greater Aβ density compared to age-matched controls years following injury (Johnson et al., 2012). Notably, Aβ42 is seen to be the major type following a TBI event in brain and CSF (Breunig et al., 2013). Interestingly, Aβ can accumulate in the white matter following TBI, unlike AD where it is more prominent in gray matter. It is described that the key constituents that generate Aβ (APP, β-secretase, and γ-secretase) anomalously colocalize at swollen and disconnected axonal bulb sites, producing and releasing Aβ into the brain parenchyma (Chen et al., 2009). Repetitive mTBI is associated to other tauopathies, especially to chronic traumatic encephalopathy (CTE). NFT pathology was found in 8 out of 27 post-mortem TBI brains (Smith et al., 2003). In fact, widespread NFTs are present in up to a third of patients following survival of a year or more from a single TBI (Johnson et al., 2012). Tau levels in CSF from TBI patients expressed over a 1,000-fold increase compared to various neurological-diseased and non-diseased individuals (Zemlan et al., 1999). Alterations of multiple protein kinases and phosphatases, neurofilament proteins, APP, BACE1, and even aggregated α-synuclein have been also found after TBI (Uryu et al., 2007). Therefore, TBI induces disease processes that may accelerate the formation and aggregation of misfolded proteins, possibly through axonal damage, and perhaps building upon the pathogenesis of AD.

Epilepsy

Epilepsy can be defined as a neurological disorder where there is a continual and spontaneous propensity to have seizure activity as convulsions or non-convulsions due to abnormal neural firing and networks. Genes, developmental mechanisms, injury insult, and neuronal plasticity are thought to play chief roles in epilepsy (Scharfman, 2007). The convoluted functional neuronal network imbalances in epilepsy can ultimately result in neuropathological changes, brain atrophy, and cognitive decline (Friedman et al., 2012). It is not known if epileptic seizures are a cause or an effect of AD, but certainly, they both share mutual molecular and cellular mechanisms (Bazil, 2003). MCI and early-stage AD patients with epileptic activity show earlier onset of AD and accelerated rate of cognitive decline (Vossel et al., 2013, 2016; Cretin et al., 2016). On the other hand, multiple studies indicate that AD patients have an elevated risk of developing seizures or epilepsy (Vossel et al., 2016). Indeed, the pervasiveness of seizures is about 7–8 fold higher in individuals with AD than individuals without dementia (Amatniek et al., 2006). Additional studies posit that the younger the age of dementia onset is correlated to increased seizure risk, as well as the severity or stage of AD, has a parallel relationship with seizure (Horvath et al., 2016). Seizures are commonly described in cases of familial AD and strongly related to Down syndrome cases. Thus, epileptic seizures could be an early event in AD progression or an integral part of AD severity. However, more studies are needed as a systemic database meta-analysis between dementia and epilepsy concluded significant gaps of knowledge in epidemiology between the two disorders with insufficient data to pool an overall incidence rate.

Central neural circuits maintain a mean firing rate related to constant, spontaneous neuronal activity, which is dependent on intrinsic circuit excitability, and their synaptic properties and functional connectome. Firing instability and limited synapse flexibility at early AD stages trigger a vicious cycle and dysregulation of an integrated homeostatic network (Frere and Slutsky, 2018). Firing rates also can be dependent on the balance of excitation and inhibition ratio, and its imbalance could play a role in AD and epilepsy. Hyperexcitability can be triggered by the mismanagement of glutamate levels and Ca^{2+} homeostasis in the brain. Glial cells could also play a role in hyperexcitability and seizures as induced seizures are shown to excite astrocytes directly by stimulating the release of glial glutamate (Ding et al., 2007). Phase-coupling of oscillations in the brain is central for normal brain function. Gamma oscillations (30–150 Hz) are known to increase locally for sensory processing and memory encoding, while other oscillations would be reduced accordingly, such as alpha, beta, and theta. AD patients exhibit reductions in gamma power oscillations. Pharmacological inhibition of gamma oscillations leads to augmented epileptic activity in experimental animals (Maheshwari et al., 2016). Gamma oscillations can be increased by social interactions and mental stimulation; therefore, these activities have been suggested as a preventative measure in AD development. Antiepileptic treatments, such as levetiracetam, reverse neural network impairments and behavior (Sanchez et al., 2012), decrease brain dysrhythmia (Das et al., 2018), and improves cognition in animal models and MCI patients (Bakker et al., 2012).

A plethora of AD transgenic animal models reveals stochastic epileptiform. In general, Aβ is thought to induce neuronal hyperexcitability by differentially attacking excitatory and

inhibitory neurons. Aβ can affect nACh, N-Methyl-D-aspartate (NMDA), and AMPA receptors, and calbindin pathways (Corbett et al., 2017). In APP23xPS45 mice, neuronal hyperexcitability occurs before any Aβ plaque deposition. Inhibition of oligomeric Aβ restores neural activity while inoculation of oligomeric Aβ in wild-type mice induces hyperexcitability (Busche et al., 2008). AD Tg mice also reveal cortical hyperactivity near amyloid plaques due to decreased GABAergic inhibition. APP/PS1 mice have early-impaired GABAergic interneurons in the hippocampus and entorhinal cortex (Ramos et al., 2006; Moreno-Gonzalez et al., 2009; Baglietto-Vargas et al., 2010). Administration of Aβ suppresses gamma oscillations *in vivo* and *in vitro* (Mucke and Selkoe, 2012). The chronic presence of Aβ and hyperexcitability effect could have an indirect effect by exhausting inhibitory neurons resulting in their deterioration. On the contrary, vulnerable neural networks produced by epileptogenic episodes could aid in the triggering of Aβ plaques. Chronic neural stimulation promotes amyloid deposition and elicits epileptic activity in an AD mouse model (Yamamoto et al., 2015), as well as increasing firing rate has been noted to surge Aβ production (Kamenetz et al., 2003). Tau protein could play a much larger role in neuronal activity and, therefore, epileptiform activity than previously thought. Epileptic patients present elevated levels of total tau in CSF (Monti et al., 2015). In fact, pharmacologically induced epilepsy in 3xTg-AD mice leads to elevated hyperphosphorylated tau levels in the dentate granule cells and mossy fibers (Yan et al., 2012), indicating the effect of epileptic activity in tau misfolding and aggregation. Moreover, synaptic activity can stimulate the release of tau and spreading of tau pathology, induce tau phosphorylation, and relocate tau to the dendritic spines (Khan et al., 2014; Frandemiche et al., 2014; Wu et al., 2016). On the other hand, reduction of tau protein levels prevents cognitive decline, synaptic impairment, and spontaneous epileptiform activity in several APP mouse models (Roberson et al., 2011; de Calignon et al., 2012). A152T tau transgenic mice present abnormal brain oscillations (Das et al., 2018), suggesting a mutual effect of epileptic activity and tau aggregation. Nevertheless, future studies are needed to elucidate epileptiform activity in the etiology and progression of AD; however, current research dictates a strong relationship between epilepsy and AD.

Depression

Depression, also termed major depressive disorder, is a serious medical illness with a wide range of mental health issues that affects about 300 million people worldwide (World Health Organization, 2017). This disease is characterized by feelings of sadness and loss of interest in ordinary things. A common triad of symptoms seen include anhedonia, low energy or fatigue, and a low or depressed mood. Depression is a common symptom seen in people suffering from AD (Drevets and Rubin, 1989; Lyketsos et al., 1996). There is a debate whether depression is a risk factor for developing AD, rather than just a symptom. Recently, several clinical studies bolstered the idea of depressive symptoms as a crucial risk factor for cognitive decline and AD. It has been shown that the age of onset for AD is expedited in MCI patients with a history of depression (de Oliveira et al., 2015). In fact, there is a strong association between depression and AD onset (Barnes et al., 2012; Steenland et al., 2012). In addition, a less studied area between depression and AD is whether the age of onset of depression could lead to a different pathology of AD. Early-life depression (ELD) is characterized as onset before the age of 60 as opposite to late-life depression (LLD), so there is interest to determine how the age of onset of depression would influence the progression of AD. It remains to be elucidated if depression onset, whether ELD or LLD, could influence the progression of AD or even engender disparate pathology in AD. Large-scale prospective studies proposed that LLD, but not early or mid-life, increases the risk of AD (Barnes et al., 2012). These findings were confirmed by a recent meta-analysis study suggesting LLD increases the risk of AD incidence by 1.65-folds (Diniz et al., 2013). Moreover, a recent study reported elevated Aβ deposition in patients with a lifetime history of major depression (Li P. et al., 2017). Additionally, individuals with MCI plus coexistent depressive symptoms have an elevated Aβ load and a higher risk of faster conversion to AD compared to non-depressed MCI patients (Hebert et al., 2013).

Neurotransmitters like dopamine and serotonin play a crucial role in both the development of depression and in AD pathology (Chen et al., 1996; Jacobsen et al., 2012). Serotonin helps regulate mood, social behavior, and memory, whereas, dopamine, functions in motor control and reward-motivated behavior. Therefore, these two neurotransmitters could be key in the conversion of depression into AD. In AD mice, dopaminergic neuronal loss in the midbrain leads to memory impairment (Nobili et al., 2017), whereas restoration of dopamine release improves cognitive dysfunction (Guzman-Ramos et al., 2012). In depression, there is a decrease in dopamine production leading to a loss of reward-motivated and low levels of serotonin have been associated with depressive behavior since serotonin regulates mood and social behavior. Additionally, selective serotonin reuptake inhibitors or SSRIs reduce brain Aβ levels by increasing serotonin levels in the brain (Nelson et al., 2007).

Many factors can lead to the onset of depression, but the most studied cause is stress (Monroe et al., 2007; Ross et al., 2018). Stress works by activating the hypothalamus-pituitary-adrenal (HPA) axis leading to the release of glucocorticoid hormones from the adrenal cortex—cortisol in humans and corticosterone in rodents (Caruso et al., 2018). Rising levels of cortisol can negatively affect the HPA axis and refrain it from maintaining its sensitivity and regulating the stress response. Increased cortisol levels have been seen in biological fluids of patients affected by AD (Hatzinger et al., 1995; Rasmuson et al., 2001; Curto et al., 2017). Stress increases the production of Aβ and enhances the formation of amyloid plaques by increasing corticotropin-releasing factor release, which leads to an increase in neuronal activity, stimulating the production of Aβ, and demonstrating a link between depression and AD development (Dong and Csernansky, 2009). In addition, stress induces neuronal loss in the hippocampus, an area known to be one of the earliest regions affected by AD neuropathology, by increasing glucocorticoid release and

decreasing neurotrophic factors (Kumamaru et al., 2008). Oral administration of corticosterone leads to morphological changes in the hippocampal region of rats, adding to the idea that stress triggers directed neurodegeneration (Magariños et al., 1998). Recently, patients suffering from LLD presented a faster hippocampal atrophy rate than those with ELD, eluding that LLD, and not ELD, leads to a higher risk of developing AD. Therefore, there may be a different progression of the disease depending on the age of depression onset (Taylor et al., 2014), and LLD could be considered a risk factor for AD development. The effect of taking antidepressants on AD pathology and development before the onset of the disease as a preventive therapy in LLD population remains to be determined.

LIFESTYLE

Physical Activity

Under normal circumstances, an elderly individual without dementia diagnosis will exhibit hippocampal volume shrinkage of 1%–2% each year (Erickson et al., 2011). Hippocampal shrinkage may be reversed by a moderate-intensity exercise training. A 1-year aerobic exercise intervention was effective at increasing hippocampal volume by 2% and offsetting normal decline associated with aging (Erickson et al., 2011) and individuals with life-long exercise routine reveal larger brain volume and improved executive function than inactive older adults (Tseng et al., 2013). However, the increase in volume was selective since it only influenced the anterior hippocampus including the dentate gyrus, in which cell proliferation occurs. Clinical studies indicate that physical activity may be neuroprotective by preserving cognition and maintaining the brain neuroplasticity (Kramer et al., 1999; Winter et al., 2007). In addition to prevention, exercise has shown to have a favorable outcome on improving cognitive symptoms. AD patients performing a moderate exercise program for a year exhibited a slower decline in the capability to achieve activities of daily living and amelioration on the physical impairment (Rolland et al., 2007; Pitkälä et al., 2013). Some other studies have also found that aerobic exercise is able to improve memory performance and cognitive function in aging, MCI, and AD patients (Baker et al., 2010; Vidoni et al., 2012; Morris et al., 2017). Although all these studies indicate that exercise may be effective in reducing the clinical symptoms observed in AD patients, there are no studies reporting its effect on amyloid deposition and how physical activity may prevent from developing AD in at risk population. In this direction, several studies have intended to investigate the beneficial effects of exercise on cognitive function and amyloid deposition in AD models. Streptozotocin-induced mice where placed on treadmill exercise daily for 30 min for a month. Afterwards, rats showed a decline in amyloidogenesis and tauopathy as well as a suppression of neuroinflammation and oxidative stress leading to selective anti-inflammatory microglia activation and pro-inflammatory microglia inhibition, hippocampal neuroprotection, and overall cognitive preservation (Lu et al., 2017). In transgenic animal models of AD, exercise leads to amelioration of behavior impairment, reduction of Aβ deposition, lager hippocampal volume and decreased apoptosis (Adlard et al., 2005; Nichol et al., 2007; Um et al., 2008; Liu et al., 2013), especially in animals under a voluntary exercise routine (Yuede et al., 2009). Enhanced cognitive function was also observed in ApoEε4 mouse models (Nichol et al., 2009). Likewise, Tau transgenic animals subjected to forced or voluntary treadmill exercise for several months show reduced levels of total tau, ptau and insoluble tau, although it had no neuroprotective effects (Leem et al., 2009; Belarbi et al., 2011; Ohia-Nwoko et al., 2014). Likewise, exercise decreases BACE (secretase beta-site APP cleaving enzyme-1) activity and APP levels compared to sedentary rats (Alkadhi and Dao, 2018). Regarding neuroprotection, both treadmill and swimming exercise are able to decrease caspase-3 expression and revers the Bax to Bcl-2 ratio observed in AD (Jin et al., 2014; Baek and Kim, 2016). Treadmill exercise also increases sirtuin-1 (SIRT-1), a modulator of neuronal survival, in a transgenic AD mouse (Koo et al., 2017) and enhances spatial memory in AD mice through upregulation of c-Fos, an indicator of neuronal activity expressed after depolarization (Jee et al., 2008).

Brain-derived neurotrophic factor (BDNF) serves as a mediator in neurogenesis as well as dendritic expansion, playing a vital role in memory formation. Acute exercise increases BDNF production in the brain (Neeper et al., 1995; Ferris et al., 2007). Modifications in BDNF serum correlate with changes in the hippocampal volume in MCI and Borba et al. (2016). AD models exposed to treadmill exercise show promotion of cell proliferation and amelioration of memory impairment observed by an increase in BDNF and TrkB levels (Liu et al., 2011; Kim et al., 2014; Sim, 2014). BDNF also enhances non-amyloidogenic APP processing by activating α-secretase and, therefore, reducing the amount of toxic Aβ peptides after voluntary exercise (Nigam et al., 2017). A recent report suggests that additional stimulation of adult hippocampal neurogenesis and increase in BDNF levels is necessary to induce the cognitive beneficial effect of exercise (Choi et al., 2018). Irisin is a myokine that is also released by physical exercise (Wrann et al., 2013). FNDC5/irisin prevents the binding of Aβ oligomers to neurons reducing its toxicity *in vitro* whereas irisin knockout mice present a deterioration in long-term potentiation and memory. In AD brains, irisin levels are reduced positing this myokine as a mediator of the beneficial effects of exercise in preventing or reducing the deleterious effects of AD pathology (Lourenco et al., 2019). Therefore, recent reports suggest that physical activity has a positive effect on synaptic plasticity, hippocampal shrinkage, and memory formation in animal models and, moreover, it can decrease the load of amyloid aggregates. Studies performed in AD patients indicate that exercise ameliorate some of the AD-related clinical symptoms and helps to decrease the progression of the disease. It still remains unknown whether exercise could diminish the risk to develop AD although studies performed in MCI patients may shed light to its potential benefits for prevention.

Sleep Disturbance

The sleep-wake cycle refers to a 24-h daily sleep pattern, typically consisting of 16 h of being awake and 8 h of sleep. This

cycle, controlled by the body's circadian rhythm and sleep homeostasis, is important to many brain functions and plays a role in removing toxins from the brain that have accumulated throughout the day. A sleep cycle consists of stages N1, N2, and N3 non-rapid eye movement (NREM) sleep followed by REM sleep. During REM sleep, the brain is highly active as it is being rewired and is considered the most important part of the sleep-wake cycle. With aging, the sleep pattern is altered by a reduction in sleeping time and REM sleep. Sleep-wake cycle disturbances, including increased daytime sleep, reduced nocturnal sleep, and sleep fragmentation, are a common feature seen in AD patients (Bonanni et al., 2005; Moran et al., 2005). It is well established that in AD patients electroencephalograms are characteristic of increases in N1 and N2 NREM sleep and REM latency, and decreases in REM sleep, leading to an overall decrease in sleep duration (Loewenstein et al., 1982). Recent studies indicate that prolonged sleep duration could be indicative of at risk population (Westwood et al., 2017) and, in fact, NREM characteristics may provide evidence of an already deteriorated cognitive condition (Taillard et al., 2019). Due to the association between aging, cognition and sleep disorders, it has been proposed that sleep disturbances may lead to an increased risk for AD development (Roh et al., 2012). In fact, disorders in the sleep pattern have been related to an increased risk to develop cognitive deficiency, including MCI and dementia (Diem et al., 2016). On the other hand, deposition of Aβ seems to deteriorate sleep efficiency (time in bed spent asleep), especially during the preclinical stage (Ju et al., 2013).

An increase in the amount of light throughout the sleep-wake cycle leads to an increase in insoluble tau and memory impairment since continuous light input suppresses the production of the hormone melatonin that regulates the sleep-wake cycle (Di Meco et al., 2014). It has been shown that sleep increases the rate of Aβ clearance in the brain through the glymphatic system (Xie et al., 2013). In fact, the interstitial concentration of Aβ was higher in awake humans when compared to sleeping ones, indicating that wakefulness is associated with an increased production of Aβ (Bateman et al., 2006). The glymphatic system is able to clear waste products through convective bulk flow of interstitial fluid (ISF) that is facilitated by astrocytic aquaporin 4 (AQP4) water channels. Moreover, removal of these AQP4 channels led to reduced clearance of Aβ by 65%, suggesting the importance of these channels in removing unwanted waste from the brain (Iliff et al., 2012). During sleep, there is an increase in the interstitial space, which leads to an augmentation in the exchange between CSF and ISF, facilitating Aβ clearance. In fact, just one night of acute sleep deprivation increases the levels of Aβ in the brain, independently of ApoE genotype, indicating the direct effect of sleep in AD pathology (Shokri-Kojori et al., 2018). Additionally, sleep also led to a decrease in ISF tau levels, shown by ∼90% increase during wakefulness and ∼100% increase during sleep deprivation (Holth et al., 2019). Moreover, these results were also seen in the CSF of patients who were sleep deprived, leading to a 50% increase in CSF tau levels. Changes in sleep precede the onset of cognitive symptoms seen in AD patients, and sleep-wake cycle disturbances are proposed as one of the earliest symptoms seen in AD. Thus, disruptions in the sleep-wake cycle could be considered a risk factor for AD and early management of sleep-wake cycle disturbances could prevent or slow the subsequent pathology and later onset of AD. Several strategies can be considered to modulate the effect of sleep disturbances in dementia risk, from sleep drugs to physical exercise (McCleery et al., 2016; Law et al., 2019). Orexin is a neuropeptide that regulates wakefulness and is implicated in various sleep disorders, such as narcolepsy and cataplexy. Treatment to effectively block orexin receptors mitigated brain ISF levels of Aβ that were elevated due to wakefulness or sleep deprivation in AD mice (Kang et al., 2009; Roh et al., 2015).

Diet

Compiling evidence suggests that a Mediterranean diet (MeDi) or Mediterranean-Dietary Approaches to Stop Hypertension diet (MIND) reduce the risk of developing MCI or AD (Morris et al., 2015). MeDi is highly popular on its preventive effects on AD (Scarmeas et al., 2007). A MeDi consists of a low intake of saturated fatty acids, such as meat and poultry; a low-to-moderate consumption of dairy products, such as cheese and yogurt; a moderate amount of alcohol, such as wine; and a high intake of vegetables, legumes, fruits, cereals, fish and unsaturated fatty acids. Studies performed in Spain, France, North America, and recently in Australia, demonstrated that the higher the adherence to a MeDi lowered the risk to contracting diseases associated as risk factors for AD (Tangney et al., 2011; Gardener et al., 2012) and protects against cognitive decline in elderly population, specifically episodic memory and global cognition (Trichopoulou et al., 2015; Valls-Pedret et al., 2015; Loughrey et al., 2017), indicating that MeDi may reduce Alzheimer's risk. A 3-year brain imaging study evaluated the effects of a low to a high adherence MeDi on AD biomarkers in 30–60-year-old cognitive normal participants. The study concluded that a higher MeDi adherence provided an average of 1.5–3.5 years of protection against AD as well as a lower adherence highlighted important AD biomarkers (Berti et al., 2018). MeDi has been shown to reduce oxidative stress by decreasing intracellular reactive oxidative species, apoptosis, and cells containing telomere shortening. Elderly patients adhering highly to MeDi demonstrated longer telomere length and high telomerase activity (Boccardi et al., 2013), and high intake of vegetables is also directly associated with longer telomere length (Gu et al., 2015). Several studies point out that the polyphenols found in the characteristic olive oil in the MeDi regimen are the main active components to prevent AD (Omar et al., 2018). Oleuropein aglycone, present in extra virgin olive oil, induces autophagy, decreases the amount of aggregated proteins, decreases inflammation, and improves cognitive function seen in AD (Grossi et al., 2013; Cordero et al., 2018). Hydrocytyrosol, another olive oil product, has antioxidant and anti-inflammatory properties. In APP/PS1 mice, hydrocytyrosol administration reduces mitochondrial oxidative stress, neuronal inflammation and apoptosis (Peng et al., 2016).

On the contrary, a high-fat diet (HFD) raises the risk of developing obesity, leading to increased chances to develop diabetes and, therefore, promoting the development of cognitive deficits, and perhaps AD. A HFD consists of a regimen where the majority of the calories ingested come from a fat source rather than carbohydrates or protein. AD transgenic mice fed using HFD for 4 months presented significant memory impairment compared with AD mice eating regular diet (Sah et al., 2017). There were no differences in the levels of Aβ or ptau suggesting that HFD induces cognitive impairments in an amyloid-independent pathway. In fact, HFD has shown to accelerate age-associated cognitive decline by decreasing BDNF levels, inducing oxidative stress, and generating a loss in synaptic plasticity (Thériault et al., 2016).

As already mentioned, insulin resistance, impaired glucose metabolism, and T2D are well known risk factors for AD. These conditions can be developed following a diet of high sugars, carbohydrates and glycemic loads. High-glycemic diet includes: high-sugar beverages and foods, white pasta and rice, French fries and baked potatoes, cereals with added sugar, and sundried fruit. A high-glycemic regimen correlates with an increment in Aβ accumulation before AD manifestation (Taylor et al., 2017). Consumption of fish oil and an omega-3 fatty acid-rich diet decreases plasma arachidonic acid/docosahexaenoic acid (AA/DHA) ratio levels. MCI and AD patients carrying ApoEε4 present increased AA/DHA ratio compared to carriers who did not present any cognitive deficiencies (Abdullah et al., 2017). DHA demonstrates a pleiotropic effect by balancing cell signal pathways, synaptic plasticity, and the enzymatic processing of Aβ (Davinelli et al., 2012). Fish oil/omega-3 fatty acid was correlated with a decline in AA/DHA levels and even in AD-ApoEε4 patients (Abdullah et al., 2017), indicating that omega-3 supplementation could be considered as an intervention against the risk of acquired AD, especially in ApoEε4 carriers. Whole food diet (WFD) consists of freeze-dried fish, fruits, and vegetables. AD mice fed with the WFD were highly impaired in spatial memory compared to controls and produced an elevated neuroinflammatory response (Parrott et al., 2015).

A large body of literature suggests that a balanced diet, full of fruits, vegetables, and lean meat and fish along with low sugar and high good fat content may be beneficial against cognitive impairment and, therefore, decrease the chances to develop AD. However, recent studies have found no significant association between dietary patterns, including MeDi, and risk for dementia (Haring et al., 2016; Akbaraly et al., 2019), indicating that adherence to healthy dietary patterns may not be enough to reduce the risk to develop age-related cognitive impairment and dementia.

Smoking

Worldwide, approximately 1 billion people use tobacco products in the form of cigarettes, and annually, there are at least 6 million global deaths caused by tobacco-smoking related diseases (World Health Organization, 2013). Today, smoking-related incidence has expanded from including CVD and stroke to now including neurocognitive abnormalities (Swan and Lessov-Schlaggar, 2007; Durazzo et al., 2010). Smoking leads to cognitive impairment and decline shown by faster declines in verbal memory and slower visual search speeds (Richards et al., 2003). Additionally, cognitive decline in smokers is directly proportional to the number of packs they smoke per day (Kalmijn et al., 2002). Indeed, it is known that smoking has negative effects on cardiovascular diseases which, as mentioned, are risk factors of AD, stressing out the deleterious importance of smoking in promoting dementia.

Historically, smoking has been considered a preventative measure from developing AD as many have stated that nicotine improves short-term cognitive performances and inhibits amyloid formation (Brenner et al., 1993; Lee, 1994). Actually, nicotine has been proven to reduce APP secretion (Lahiri et al., 2002), inhibit Aβ aggregation (Dickerson and Janda, 2002), and reduce Aβ load in AD transgenic mice independently from inflammation (Nordberg et al., 2002; Hellström-Lindahl et al., 2004). However, more recent studies have questioned this evidence and indicate that smoking increases the chance to develop dementia and cognitive decline (Ott et al., 1998; Anstey et al., 2007; Reitz et al., 2007). Confirming this negative effect, nicotine exacerbates tau phosphorylation in experimental animals (Oddo et al., 2005). In addition, exposure to cigarette smoke exacerbates Alzheimer's-like pathology by increasing amyloid deposition, inducing tau hyperphosphorylation, and exacerbating the inflammatory response in a smoke-consumption concentration dependent manner (Moreno-Gonzalez et al., 2013). Still some studies indicate that when epidemiological data is adjusted for competing risk of death without dementia, smoking seems not to be associated with dementia development (Abner et al., 2018), and, surprisingly, ApoEε4 carrier smokers are at a lower risk of developing dementia than smokers without this allele (Reitz et al., 2007). The mechanism by which smoking may lead to an increased risk in AD development is uncertain, and further studies need to be conducted for potential mechanisms responsible for a possible increased risk of AD development.

Alcohol

Alcohol consumption is considered a major risk factor for many health problems. Heavy drinking is defined as: consuming more than four drinks a day (or 14 drinks a week) for males and consuming more than three drinks a day (or seven drinks a week) for females (Rehm, 2011). Knowing that alcohol negatively affects cognitive and motor functions, there is no surprise that heavy drinking has been associated with an increased risk of AD, whereas mild to moderate alcohol intake has been associated with a lower risk (Heymann et al., 2016). The extent to which alcohol affects AD pathology is still debated today as some believe that alcohol is protective of AD development (Luchsinger et al., 2004), while others believe the contrary (Piazza-Gardner et al., 2013), the latter being the strongest current of opinion. Heavy alcohol consumption leads to a decline in cognitive performance similar to that observed in AD (Weissenborn and Duka, 2003). Loss of cholinergic neurons observed in AD patients has also been reported in individuals exposed to ethanol consumption (Fernandez and Savage, 2017;

Vetreno and Crews, 2018) as well as hippocampal atrophy (Topiwala et al., 2017), linking heavy alcohol consumption with cognitive impairment that may eventually trigger AD development. In fact, a combination of both smoking and drinking can have a more impactful effect of AD incidence than just one of those habits (Zhou et al., 2014). Some recent reports indicate that alcohol use is not associated with prodromal AD or disease progression (Heffernan et al., 2016; Bos et al., 2017). In addition to the potential link to trigger dementia, alcohol abstinence after AD diagnosis seems to ameliorate the cognitive damage initially observed (Toda et al., 2013), suggesting the alcohol consumption can, not just increase the risk of AD, but also worsen the progression of the disease in heavy intake conditions (Heymann et al., 2016). A potential mechanism proposed for alcohol to induce AD is by decreasing glymphathic function (Lundgaard et al., 2018). The glymphatic system plays an important part in removing brain waste, including Aβ. Since alcohol decreases glymphatic function, heavy drinking could induce Aβ accumulation by reducing its clearance triggering the cognitive abnormalities that are seen in alcohol use and AD.

On the other hand, alcoholic beverages such as wine—particularly red—contain polyphenols including morin, quercetin, resveratrol, and tannins, that are able to inhibit amyloid aggregation and can have other beneficial effects including reduction of oxidative stress, inflammation, and balance of protein homeostasis (Dhouafli et al., 2018). In fact, moderate drinking (1–2 drinks/day) has been proposed to be protective against AD by reducing amyloid burden, decreasing mortality, and reducing the risk of dementia (Russo et al., 2003; Deng et al., 2006; Wang et al., 2006), being low doses of wine the most recommended to reduce the risk of dementia (Xu et al., 2017). In AD animal models, low doses of ethanol decrease Aβ-mediated synaptic toxicity by direct interaction with Aβ peptide (Muñoz et al., 2015). This may be an indication that the amount (dinks/day), length of consumption, period of consumption (early or late life), and type of alcoholic beverages (fermented or distilled drinks) should be taken into consideration to determine the effect of alcohol consumption to protect or induce AD dementia.

CONCLUDING REMARKS

Despite over a century since discovering AD, there is no cure that can halt, slow down, or reverse the progression of this neurodegenerative disease. Regardless of the tremendous effort that the scientific field has done to find effective treatments for AD, promising candidates fail when tested in AD patients (Cummings et al., 2007; Raschetti et al., 2007; Extance, 2010; van Dyck, 2018). Most of the recent clinical trials that attempt treating AD focus on inhibiting the main known culprits of AD. The central targets are amyloid production and aggregation, largely Aβ by immune therapy and pharmacological enzyme inhibition (BACE inhibitors); the use of NSAIDs to reduce inflammation; and even stem cell therapy to fight against neurodegeneration. However, all these attempts have failed probably because the therapeutic intervention was done in an already very advanced pathology or because the treatment is directed to the wrong target (Mehta et al., 2017). It could also well be that the proposed approach is targeting only one of the players of this multifactorial disease. Therefore, an alternative strategy to fight against AD could be the prevention of the known modifiable risk factors and related mechanisms for the disease. This includes proper management of comorbidities associated such as vascular diseases (hypertension, CVD, stroke, ischemia), diabetes, epilepsy, brain injuries, and depression as well as modification of lifestyle and avoidance of deleterious habits. Here, we have reviewed many of them including: physical activity, sleep, diet, and use of tobacco and alcohol, and how by different mechanisms, these factors are able to reduce amyloid deposition and ameliorate cognitive impairment. Recent studies have estimated that intervention of several modifiable risk factors could prevent up to 35% of dementia cases (Livingston et al., 2017). Management of diet, exercise, and vascular risk in at-risk elderly population can, in fact, prevent cognitive functioning deterioration (Kivipelto et al., 2013; Ngandu et al., 2015; Soininen et al., 2017). However, some reports suggest that changes in these habits should be done early in life since modifying later life lifestyle factors may not decrease the conversion of MCI to AD dementia (Reijs et al., 2017) although it may ameliorate the course of the disease. Most of the studies compiled in this review evaluate the effect of risk factors when the AD-associated pathological changes are already present, but very few analyze the potential of those factors in preventing the onset of the disease, rather than the further development. Furthermore, most of the risk factors analyzed here can be considered both the cause and effect of AD. If they are a cause or risk factor, preclinical intervention may prevent the onset and development of AD, but if these factors are in fact an effect or a symptom, their treatment will still slow down the progression of the disease. Hence intervention of potential risks is highly recommended for either prevention or even amelioration of clinical symptoms of AD since most of these actions will also benefit general health status. Therefore, and while an effective treatment(s) is developed to treat AD, the most reasonable approach is to prevent AD onset by managing multiple risk factors way before any clinical symptom is observed.

AUTHOR CONTRIBUTIONS

GEd, NG, GEs and OC drafted the article. IM-G provided a critical revision and generated the last version of the manuscript.

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Liver Dysfunction as a Novel Player in Alzheimer's Progression: Looking Outside the Brain

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Alzheimer's disease (AD) afflicts an estimated 20 million people worldwide and is the fourth-leading cause of death in the developed world. The most common cause of dementia in older individuals, AD is characterized by neuropathologies including synaptic and neuronal degeneration, amyloid plaques, and neurofibrillary tangles (NTFs). Amyloid plaques are primarily composed of amyloid-beta peptide (Aβ), which accumulates in the brains of patients with AD. Further, small aggregates termed Aβ oligomers are implicated in the synaptic loss and neuronal degeneration underlying early cognitive impairments. Whether Aβ accumulates in part because of dysregulated clearance from the brain remains unclear. The flow of substances (e.g., nutrients, drugs, toxins) in and out of the brain is mediated by the blood-brain-barrier (BBB). The BBB exhibits impairment in AD patients and animal models. The effect of BBB impairment on Aβ, and whether BBB function is affected by non-neurological pathologies that impair peripheral clearance requires further investigation. In particular, impaired peripheral clearance is a feature of nonalcoholic fatty liver disease (NAFLD), a spectrum of liver disorders characterized by accumulation of fat in the liver accompanied by varying degrees of inflammation and hepatocyte injury. NAFLD has reached epidemic proportions, with an estimated prevalence between 20% and 30% of the general population. This chronic condition may influence AD pathogenesis. This review article summarizes the current state of the literature linking NAFLD and AD, highlighting the role of the major Aβ efflux and clearance protein, the LRP-1 receptor, which is abundantly expressed in liver, brain, and vasculature.

Keywords: amyloid beta, NAFLD, LRP-1, BBB, Alzheimer's

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AMYLOID BETA ROLE IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) belongs to a large group of neurodegenerative diseases characterized by the pathophysiological brain changes related to the accumulation of misfolded proteins. Specifically, extracellular peptide variants of the amyloid-β (Aβ) accumulate in the form of amyloid plaques or senile plaques, and the intracellular accumulation

of neurofibrillary tangles (NTFs) composed by phosphorylated Tau protein (pTau; Bloom, 2014; Héraud et al., 2014; He et al., 2018).

Both are reported to underlie progressive synaptic dysfunction in the AD brain, loss of dendritic spines, and neuronal death (Serrano-Pozo et al., 2011; Busche et al., 2019). Although AD was first described 100 years ago, its precise etiology remains unknown. Efforts to better understand AD have resulted in multiple hypotheses to explain events in AD pathogenesis, for example, the amyloid cascade theory that describes the imbalance between Aβ production and clearance (Selkoe and Hardy, 2016). Here, we provide an overview of the etiology of AD, and the principal concepts that support the critical role of the brain-blood barrier (BBB) and liver in AD development and progression.

In neurons under physiological conditions, Aβ is secreted to maintain normal synaptic function, morphology, and plasticity (Wang et al., 2012; Gouras et al., 2015; Klevanski et al., 2015). Aβ is a by-product generated from cleavage of the amyloid protein precursor (APP). APP plays an important physiological role in regulating γ-aminobutyric acid type B receptor ($GABA_BR$) and modulating synaptic transmission and plasticity (Chen et al., 2017; Doshina et al., 2017; Rice et al., 2019). In primary cortical neurons, APP modulates frequency and amplitude of calcium oscillations essential for synaptic transmission (Octave et al., 2013). A mouse model deficient for APP demonstrated that APP is necessary for the synapsis and maintenance of dendritic integrity in the hippocampus (Tyan et al., 2012). Likewise, hippocampal neurons in culture derived from APP knockout mice showed APP is critical for synaptogenesis and dendritic and axonal growth process and regulates substrate adhesion (Southam et al., 2019).

On the other hand, in the amyloidogenic (i.e., disease-causing) pathway, APP is cleaved by β- and γ-secretase to generate Aβ, which accumulates as senile plaques (Hardy and Selkoe, 2002; Konietzko, 2011). AD-related plaques are associated with high levels of soluble oligomeric forms of Aβ (AβOs; Esparza et al., 2013). AβOs comprise soluble dimers and trimers of low molecular weight and soluble oligomeric forms of 12–14 monomers (Mroczko et al., 2018). In addition, these oligomers have been identified as the toxic conformers of Aβ plaques in AD (Jin et al., 2011; Verma et al., 2015). AβOs can diffuse across synaptic membranes (Hong et al., 2014) and trigger a cascade of injurious events in neurons, causing synaptic failure and memory loss (Morris et al., 2014; Brito-Moreira et al., 2017). Moreover, AβOs are associated with dystrophic neurites, reactive astrocytes, and aberrant activation of glutamatergic neurotransmission; the consequence of these changes is neuronal death by excessive neuronal influx of sodium and calcium (Ziegler-Waldkirch and Meyer-Luehmann, 2018). Postsynaptic protein disruption (Lésne et al., 2013) and hippocampal synaptic plasticity impairment by AβOs contributes to memory loss (Müller-Schiffmann et al., 2016). Intracellular AβOs are detectable in cholinergic neurons, suggesting that they play a critical role in cholinergic deficiency (Baker-Nigh et al., 2015). These devastating events not only lead to memory loss and learning impairment in AD patients, but also affect the capacities of reasoning, abstraction, and language (Duyckaerts et al., 2009).

BLOOD-BRAIN BARRIER BREAKDOWN AND ROLE OF LRP-1 IN ALZHEIMER'S DISEASE

The blood-brain barrier (BBB) is a specialized structure that supports brain function. This structure supports the brain by regulating electrolyte flux, cerebral blood flow (CBF) and efficient oxygen and metabolite delivery, and restricting entry of potentially toxic and even some therapeutic agents into the brain (Provias and Jeynes, 2014; Andreone et al., 2015; Di Marco et al., 2015). BBB function is mediated by neurovascular units (NVU) comprising neurons, glial cells, pericytes, and brain endothelial cells, which maintain homeostasis of the cerebral microenvironment (Armulik et al., 2011). Brain endothelial cells are an important component mediating the flow between brain and blood by cell-to-cell communications called tight junctions and adherent junctions; these junctions connect cell networks (Deli et al., 2005; Van de Haar et al., 2015) and regulate the paracellular permeability of substances across the BBB (Bowman and Quinn, 2008; Viggars et al., 2011; Kook et al., 2013; Chow and Gu, 2015; Ulrich et al., 2015). Tight junctions proteins ZO-1, Occludin and CLN-5 are key to maintaining BBB integrity (Jiao et al., 2011). ZO-1 joins tight junctions with the actin cytoskeleton, working as accessory proteins (Xiao et al., 2017). Occludin and CLN-5 are transmembrane tight junction proteins involved in signal transduction of cytokines (Haseloff et al., 2015). The high expression of these proteins on brain endothelial cells regulates the transport of essential molecules through the BBB, such as the free and rapid diffusion of oxygen and carbon dioxide (Lin et al., 2015; Pardridge, 2015). Hydrophobic molecules permeate the BBB faster and more easily than hydrophilic molecules, while molecules that are larger than 180 KDa or water-soluble do not penetrate the BBB (Kroll and Neuwelt, 1998; Zlokovic, 2005; Masserini, 2013). For example, the BBB restricts the passage of albumin and immunoglobulins, high-molecular-weight proteins from the peripheral blood circulation (Xiao and Gan, 2013).

Another important component of brain endothelial cells is a complex and specific transport-receptor protein system that also contributes to BBB permeability (Zlokovic, 2011). The luminal side of the BBB contains transporters for specific classes of nutrients, such as glucose and vitamins, and receptors for peptides, proteins, and hormones. These mediators facilitate transport across the BBB from circulating blood into the brain (Deane and Zlokovic, 2007; Simpson et al., 2007). In contrast, the transport system of the abluminal side of the BBB eliminates neurotoxic molecules and metabolic waste (Begley and Brightman, 2003).

Dysfunction of the BBB, therefore, could result in altered permeability. Indeed, age-dependent BBB breakdown at the hippocampus is associated with mild cognitive impairment and correlates with pericytes injury. This finding suggests that the cerebrovascular integrity loss that begins at the hippocampus

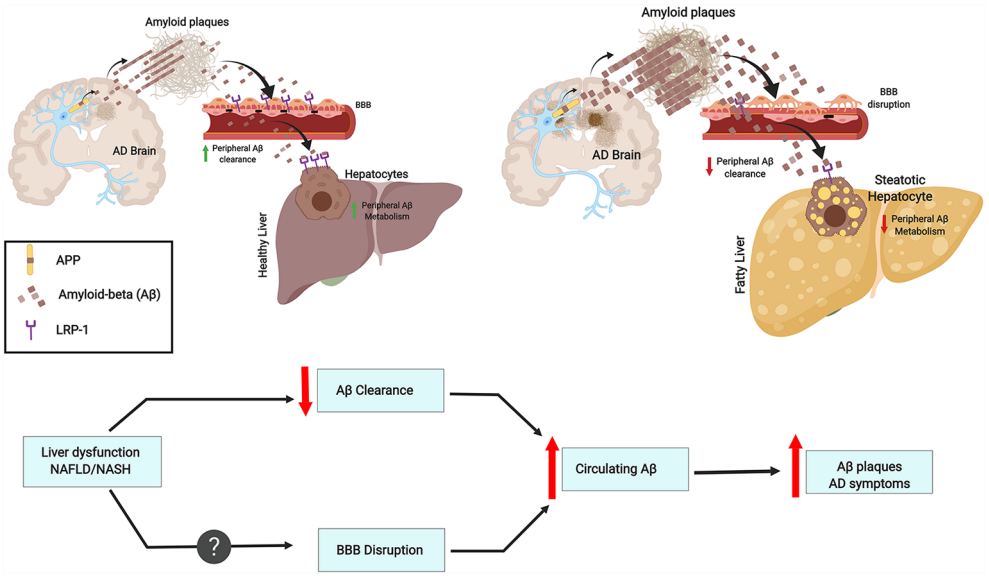

FIGURE 1 | Chronic liver diseases may increase amyloid burden and Alzheimer's pathology. This contribution results from an imbalance in peripheral amyloid-β (Aβ) clearance as a result of decreased LRP1 levels, general liver dysfunction, and chronic inflammation. These features may worsen blood-brain-barrier (BBB) impairment and contribute to a vicious cycle. As an example, the figure depicts fatty liver disease as a chronic liver condition.

may contribute to early stages of dementia associated with AD (Montagne et al., 2015). Similarly, early cognitive dysfunction has been associated with capillary damage and BBB breakdown in older adults (Nation et al., 2019).

This breakdown of BBB function may be related to alterations in specific components of the BBB structure. Low-density lipoprotein receptor-related protein 1 (LRP-1) is a membrane receptor that mediates the cellular internalization of multiple ligands. Further, LRP-1 regulates several tight junction proteins in endothelial cells of the BBB (Zhao et al., 2016). Functional LRP-1 is expressed in liver sinusoidal endothelial cells (LECs), highly specialized scavenger cells, and LRP-1 expression contributes to the rapid removal of its blood ligands (Øie et al., 2011). Cell surface LRP-1 and circulating sLRP-1 are needed for brain and systemic clearance of Aβ; however, in AD, both cell surface LRP-1 and circulating sLRP-1 concentrations are dramatically reduced (Sagare et al., 2012). Importantly, these alterations may begin as early as two decades before the manifestation of cognitive impairment symptoms (Beason-Held et al., 2013; Jack et al., 2013; De Strooper, 2014). Clearance of Aβ may also be affected by other pathologies, however.

CLEARANCE OF Aβ AT THE PERIPHERY: ROLE OF THE LIVER

Peripheral organs, including the kidney and the liver, play an essential role in the clearance of circulating Aβ. Elimination of Aβ from the circulation may contribute to AD progression, by helping to displace the dynamic equilibrium from Aβ deposited in the senile plaques toward soluble Aβ. This hypothesis is supported by evidence that peritoneal dialysis reduces the circulating levels of Aβ in humans and diminishes AD features in an animal model (Jin et al., 2017). Insufficient clearance of brain Aβ also contributes to the progression of sporadic AD (Wang et al., 2006). As brain Aβ equilibrates with Aβ in plasma, peripheral clearance of Aβ provides a potential approach to facilitate efflux of Aβ from the brain (Liu et al., 2015). Peripheral organs and tissues are key in clearing brain-derived Aβ under physiological conditions (Xiang et al., 2015).

The liver has many functions, one of which is metabolic detoxification. When the liver is under constant injury, as is found in metabolic diseases, it exhibits decreased detoxification capacity. Indeed, the expression of metabolic enzymes decreases in conditions such as obesity, diabetes, and cirrhosis (Rolle et al., 2018). Hepatocytes can act directly on circulating Aβ, promoting its clearance by degradation or through bile excretion. Further, Aβ uptake from circulation can be mediated through LRP-1, which is highly expressed in hepatocytes (Kanekiyo and Bu, 2014). Interestingly, liver dysfunction is accompanied by low LRP-1 hepatic expression and high levels of circulating Aβ. This correlation suggests that Aβ clearance decreases due to low hepatic LRP-1 activity (Wang et al., 2017; see **Figure 1**).

AD pathophysiology has not been evaluated from a hepatic point of view; yet, the evidence points to a critical role for liver in AD pathogenesis. Aβ levels found in liver samples from AD patients are lower when compared to neurologically healthy controls, raising the possibility that the liver is not properly eliminating circulating Aβ (Roher et al., 2009). This observation is supported by studies where insulin promotes LRP-1 translocation to the cell membrane in hepatocytes, favoring Aβ clearance (Tamaki et al., 2007). The stimulation of LRP-1-mediated liver uptake improves cognitive impairment and decreases Aβ aggregation in the brain in AD transgenic mice (Sehgal et al., 2012).

NAFLD/NASH AFFECTS Aβ CLEARANCE

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver disorders characterized by excessive fat deposition in hepatocytes from individuals who drink little or no alcohol. NAFLD is an umbrella term for several subtypes ranging from isolated hepatic steatosis, or fatty liver, to nonalcoholic steatohepatitis (NASH). NASH is defined by the presence of fatty changes with inflammation and several degrees of hepatocellular injury or fibrosis. Thus, NASH is the aggressive form of NAFLD and can progress to advanced fibrosis and cirrhosis.

NAFLD/NASH is the leading cause of chronic liver disease worldwide and has reached epidemic proportions. Interestingly, most of the deaths in NAFLD patients are not restricted to liver-related morbidity or mortality; rather, cardiovascular disease (CVD) and cancer predominate (Armstrong et al., 2014). Therefore, the presence of fatty liver is not a benign pathology as was historically considered by most clinicians. Indeed, extensive evidence in recent years shows that NAFLD also increases the risk of end-stage liver disease, hepatocellular carcinoma (HCC), liver-related mortality, and all-cause mortality. These observations prompted the idea that NAFLD/NASH, either independently or concomitantly with other metabolic risk factors, determines or even drives extra-hepatic diseases such as CVD, chronic kidney disease, colorectal cancer, endocrine disorders like type 2 diabetes mellitus, osteoporosis, and, indeed, AD. Recent studies have linked insulin-resistance (the key pathophysiological feature of NAFLD) to several of the neurodegenerative mechanisms of AD including oxidative stress, mitochondrial dysfunction, and inflammation, via dysregulated insulin/IGF-1 signaling with attendant impairments in signal transduction and gene expression (de la Monte and Tong, 2014; de la Monte, 2017; Kim et al., 2016).

A network clustering analysis conducted by Karbalaei et al. (2018) indicated that there are 189 genes shared between NAFLD and AD. Further, three main groups of pathways are candidates for contributing to both AD and NAFLD: carbohydrate metabolism, long fatty acid metabolism, and IL-17 signaling pathways (Karbalaei et al., 2018). This suggests that diabetes and obesity might be considered as a risk factor for AD and NAFLD.

One study showed that NAFLD promotes AD in mice (Kim et al., 2016). This study evaluated whether NAFLD induction, through a dietary approach (high-fat diet), promotes the development of AD signs. Brains of HFD-fed mice showed increased levels of neuro-inflammation, characterized by higher levels of cytokines, toll-like receptors, and microgliosis. These features were accompanied by increased plaque formation in a transgenic mouse model of AD. In addition, intense and frequent signs of cerebral amyloid angiopathy (CAA)—a condition characterized by the Aβ deposition in the media and adventitia of small and mid-sized arteries—were observed in mice fed with HFD.

An abnormal lipid metabolism is linked with increased risk for AD development, and the liver plays a crucial role since is the main peripheral organ responsible for lipid metabolism (Fukumoto et al., 2002; Hooijmans and Kiliaan, 2008). Aβ is able to bind Apolipoprotein E (ApoE) and can be cleared from the brain together with cholesterol (Mahley, 1988). Interestingly, ApoE is a ligand of LRP-1 and both are genetically associated with AD and plasma Aβ levels (Kang et al., 2000). This link is intriguing since LRP-1 is suggested to facilitate Aβ clearance from the brain across the BBB (Deane et al., 2004; Sagare et al., 2012; see **Figure 1**).

LIVER INFLAMMATION AND Aβ LEVELS

Hepatitis B is a liver infection that can become chronic and severe. Interestingly, Hepatitis B Virus (HBV) carriers have significantly higher plasma Aβ levels than non-carriers. Moreover, HBV carrier status is associated with plasma Aβ levels (Jin et al., 2017). Overall infectious burden including cytomegalovirus (CMV), herpes simplex virus type 1 (HSV-1), *Borrelia burgdorferi*, *Chlamydophila pneumoniae* and *Helicobacter pylori* was found to significantly contribute to AD pathogenesis (Bu et al., 2015). However, currently, no epidemiological study has been designed to understand the association between HBV infection and the risk for AD. The effect of chronic inflammation on Aβ clearance is lesser than the effects of HBV infection or liver dysfunction (Liu et al., 2013). Further, although plasma concentrations of cytokines IL-1β and IL-6 are significantly increased in cirrhosis patients and plasma IL-6 levels are correlated with Aβ40 levels (a 40 amino acid proteolytic product of APP cleavage that has gained attention as a biomarker correlating with AD), no association is observed by linear regression between IL-6 and Aβ40 levels. On the other hand, the ratio of AST/ALT, which is an indicator of liver functional impairment (Giannini et al., 1999), is significantly associated with circulating Aβ40 levels (Wang et al., 2017). Furthermore, hepatic dysfunction may lead to a plethora of systemic changes. Approximately 95% of Aβ in the blood is bound to serum albumin (Stanyon and Viles, 2012). The serum albumin pool represents an important reservoir for peripheral clearance of Aβ. Thus, a diminution in blood albumin in cirrhotic patients might contribute to the increase in plasma Aβ levels (see **Figure 1**).

CONCLUDING REMARKS

AD is a degenerative condition that will afflict an increasing number of people as the global population ages. Unfortunately, current treatments have only transient or modest effects. This article reviews evidence that supports the involvement of liver diseases, a growing health concern, in AD pathogenesis. The liver is the major player in the clearance of Aβ at the periphery, and an impairment of this clearance may shift the delicate Aβ equilibrium toward brain accumulation.

As to the possible role that the liver plays in brain-derived Aβ clearance, the impaired clearance of serum Aβ might contribute to the high Aβ levels in NAFLD patients. This effect is likely due to an intensification of the BBB disruption and drop in LRP-1

levels, the major receptor for Aβ efflux and important effector of clearance.

It is possible that hepatic malfunction contributes to AD in a plethora of non-excluding pathways, including: (i) the failure to maintain Aβ homeostasis at the periphery; (ii) acting as a source of pro-inflammatory cytokines when chronic inflammation follows different types of injury (like virus infection, drug-induced injury, and metabolic diseases); and (iii) through metabolic impairment.

AUTHOR CONTRIBUTIONS

LE wrote and edited the manuscript. PA participated in manuscript writing. DC wrote the manuscript and designed figures. JA participated in manuscript writing and editing.

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A Prospective Study on the Association Between Grip Strength and Cognitive Function Among Middle-Aged and Elderly Chinese Participants

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Objective: To study the efficacy of grip strength (GS) as a predictor of cognitive function in a large, nationwide sample of Chinese participants aged 45 years and above.

Methods: We used data from three waves (W1, W2, and W3) fielded by the China Health and Retirement Longitudinal Study (CHARLS). Cognitive function was tested biennially and calculated using two categories: episodic memory and mental intactness. Demographics, health behaviors, and medical conditions were considered potential confounders. Using multivariate linear regression models (MLRMs), we examined the association between baseline GS (measure in W1) and cognitive function in W3. Using a generalized estimating equation (GEE), we examined baseline GS as a predictor of cognitive function change.

Results: Total 9,333 individuals (53.2% women), with a mean baseline episodic memory score of 6.5, mean baseline mental intactness score of 7.2, and aged over 45 years (mean age = 58.6), were selected. The mean follow-up time was 4.0 years (range: 3.3–5.0 years). Using MLRMs and comparing the lowest GS score with the highest baseline GS score, we observed a significant correlation with a higher global cognitive function in both women ($\beta = 1.061$, $p < 0.001$) and men ($\beta = 1.233$, $p < 0.001$). After adjusting baseline global cognition, the highest GS level was still statistically significant in both women ($\beta = 0.543$, $p < 0.05$) and men ($\beta = 0.742$, $p < 0.001$). GEE suggested that the participants in the highest GS quartile had better cognitive performance over time in both women ($\beta = 0.116$, $p = 0.030$) and men ($\beta = 0.143$, $p = 0.008$) than those in the lowest quartile.

Conclusion: Higher baseline level of GS was significantly related to better cognitive function and slowed the rate of its decline. Thus, it is an independent predictor of better cognitive status in middle-aged and elderly Chinese.

Keywords: aging, cognitive function, grip strength, prospective study, predictor

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INTRODUCTION

Cognitive disorders (CDs), also known as neurocognitive disorders (NCDs), are a category of mental health disorders that primarily affect cognitive abilities such as learning, memory, perception, and problem solving. NCDs include delirium and mild to major NCD (previously known as dementia) (Simpson, 2014), which contribute to the disability and decreased life-span, considerably affecting quality of life in the elderly (Murray et al., 2013). Currently there are no cures for these diseases, thus, identifying predictive clinical signs of cognitive decline and dementia is imperative for the implementation of an adapted care. However, the complex association between physical performance and cognitive function might provide an insight into the possible therapeutic and prophylactic measures in these diseases (Amieva et al., 2005). Previously, grip strength (GS) has been represented as a predictive factor for Alzheimer's disease (AD) (Rijk et al., 2015), considering that cognitive impairments, AD and other common neurodegenerative diseases, are preceded by a "silent" clinical period that can last longer than a decade. Identifying such "soft" physical signs associated with the progressive decline of cognitive function has important implications in the early intervention for these illnesses.

Several studies aimed to assess the associations between GS and cognitive decline or dementia; some (Camargo et al., 2016; Praetorius Björk et al., 2016; Veronese et al., 2016; Hooghiemstra et al., 2017), but not all (Atkinson et al., 2010), reported a positive relationship. It is evident that poor GS is associated with a greater risk of dementia. Furthermore, a small number of studies have suggested that higher GS at baseline is a protective factor in preventing the development of AD (Rijk et al., 2015). However, their cross-sectional and longitudinal association have not been fully investigated, and thus, remain unclear in China. Therefore, we aimed to examine the predictive accuracy of baseline GS levels for cognitive function as well as its slow decline over time in a large, population-based sample derived from the "China Health and Retirement Longitudinal Study (CHARLS)."

MATERIALS AND METHODS

Study Sample

The China Health and Retirement Longitudinal Study is a nationwide longitudinal survey conducted by the National School of Development at Peking University in China on people above 45 years of age. The data of CHARLS is publicly accessible. Researchers could apply for the data by signing a data usage agreement online and providing his/her basic information. Details of the survey protocol and implementation involved in the CHARLS have previously been described (Zhao et al., 2014). The survey included three waves covering 150 county-level units distributed in 28 provinces of China. The baseline (W1) survey was conducted in 2011–2012 on 17,708 participants with a high response rate. But only 78.9% of them did physical performance measures (Zhao et al., 2014), reducing the sample to 13,965 individuals. Compared to the baseline sample ($n = 17,705$), this subsample were, on average, significantly older ($p = 0.006$), with a higher proportion of females ($p = 0.043$), people with married status ($p < 0.001$) and people less educated ($p < 0.001$) (**Supplementary Table S1**). Of the 13,965 individuals, 13,204 individuals with baseline GS was included (269 individuals were excluded because they were less than 45 years old, 203 individuals were excluded for memory-related diseases at baseline, 284 individuals were excluded for stroke history at baseline and 5 outliers were identified for GS at W1). The third wave (W3) survey successfully re-interviewed 10,641 of these individuals in 2015–2016, and 2,563 (19.4%) were lost to follow-up. All surveys, including the questionnaire, laboratory measurements, and physical function were administered by well-trained clinicians in a face-to-face setting. Here, 9,333 individuals who underwent the three wave surveys were included (407 individuals had missing value for GS at W1, 901 individuals did not complete the cognitive test at W1, W2, or W3). There were no significant demographic characteristics (gender and educational attainment), heath status (other than hearing problems), health behavior differences between the baseline participants 13,965 and the third wave 9,333. Compared to the baseline sample, 9,333 individuals were significantly younger ($p < 0.001$), with higher proportion of married status ($p < 0.001$), a lower proportion of hearing problems ($p < 0.001$) and better average cognitive ($p < 0.001$) and GS ($p < 0.001$) scores (**Table 1**). Study diagram and exclusion criteria were listed in the **Figure 1**.

Cognitive Function

Cognitive performance, in CHARLS, was calculated using two categories: episodic memory and mental intactness. Each respondent was asked to immediately repeat as many Chinese nouns as possible, from a list read to him/her (immediate word recall) and to recall the same 5 min later (delayed recall) (Wang et al., 2017). Episodic memory was defined as the summation of immediate and delayed recall scores ranging from 0 to 20. Mental intactness scores, which included numerical ability, time orientation, and picture drawing, were obtained from the following set of questions: serial sevens, temporal orientation (date, month, year, day of week, and season), and intersecting pentagon copying test. Answers to these questions were accumulated into a score named mental intactness ranging from 0 to 11. The global cognitive function was the summation of the episodic memory and mental intactness scores. Baseline cognition scores were calculated at W1.

Grip Strength

Grip strength (kilogram) was estimated through the dynamometer (WCS-100, Nantong, China). Individuals needed to squeeze the handles as long and as tightly as possible or until the needle stopped rising. Individuals also needed to be in a standing position with their arms hanging naturally at their sides. Additional measurements were recorded for each hand, while alternating the sides, giving a total of two readings for each side. The best of the four GS measurements was used in statistical analyses. We conducted the analysis separately for men and women to identify gender differences in muscle strength (Metter et al., 1997; Gallagher and Heymsfield, 1998; Baumgartner et al., 1999). GS scores were divided into quartiles,

TABLE 1 | Demographic characteristics of the samples.

	Wave 3 (*N* = 9,333)	Baseline sample (*N* = 13,965)	*p*-value
Follow-up time(years), mean ± SD	4.0 ± 0.1(3.3–5.0)		
Age(years), mean	58.6 ± 8.7	59.3 ± 10.0	**<0.001**
Gender (%)			0.920
Male	4365(46.8)	6522(46.7)	
Female	4968(53.2)	7443(53.3)	
Marital status (married) (%)	7908(84.7)	11488(82.3)	**<0.001**
Educational attainment (%)			0.946
≤primary school	6456(69.2)	9666(69.2)	
>primary school	2877(30.8)	4299(30.8)	
Baseline cognition, mean ± SD			
Global cognition	13.7 ± 5.7	13.1 ± 6.2	**<0.001**
Episodic memory	6.5 ± 3.7	6.2 ± 3.9	**<0.001**
Mental intactness	7.2 ± 3.1	6.9 ± 3.3	**0.003**
Health status			
Hypertension (%)	2116(22.7)	3370(24.1)	0.010
Fall-related injuries (%)	1471(15.8)	2241(16.1)	0.559
Hip fracture (%)	146(1.6)	224(1.6)	0.812
Dyslipidemia (%)	794(8.5)	1241(8.9)	0.315
Diabetes or high blood sugar (%)	498(5.3)	796(5.7)	0.248
Cancer or malignant tumor (%)	83(0.9)	141(1.0)	0.356
Heart problems (%)	1023(11.0)	1629(11.7)	0.097
Near-vision impairment (%)	2178(23.3)	3218(23.0)	0.603
Far-vision impairment (%)	2015(21.6)	3107(22.3)	0.234
Hearing problems (%)	1168(12.5)	2067(14.8)	**<0.001**
Depressive symptoms (CES-D), mean ± SD	19.7 ± 4.9	19.5 ± 5.6	0.627
Health behaviors			
Smoking (%)	3633(38.9)	5481(39.3)	0.622
Drinking (%)	2356(25.2)	3406(24.4)	0.139
Body mass index (kg/m^2)			0.056
Thin(<18.5)	581(6.2)	964(6.9)	
Normal(18.5–24)	4938(52.9)	7448(53.3)	
Overweight(≥24)	3814(40.8)	5553(39.8)	
Grip strength (kg), mean ± SD	33.0 ± 10.2	32.3 ± 10.5	**<0.001**
Male	39.7 ± 8.9	38.9 ± 9.46	
Female	27.1 ± 7.3	26.6 ± 7.6	
Grip strength (kg) quartiles			
Male, n (%), mean ± SD			
Q1(≤34 kg)	1140(26.6), 28.8 ± 4.8		
Q2(34–40 kg)	1250(28.7), 37.5 ± 1.9		
Q3(40–45.2 kg)	880(19.7), 42.9 ± 1.5		
Q4(>45.2 kg)	1095(25.0), 51.0 ± 4.6		
Female, n (%), mean ± SD			
Q1(≤22.5 kg)	1281(25.1), 18.3 ± 3.7		
Q2(22.5–27 kg)	1282(26.9), 25.1 ± 1.2		
Q3(27–31 kg)	1173(23.5), 29.3 ± 1.1		
Q4(>31 kg)	1232(24.5), 36.2 ± 4.9		

SD, standard deviation.

independently, for both the sexes. We categorized the GS scores of ≤34.0 kg, 34.0–40.0 kg, 40.0–45.2 kg, and >45.2 kg as Q1, Q2, Q3, and Q4, respectively, for men. Similarly, for women GS scores of ≤22.5 kg, 22.5–27.0 kg, 27.0–31.0 kg, and >31.0 kg were categorized as Q1, Q2, Q3, and Q4, respectively.

Potential Confounders

We also included other covariates such as the following: age, follow-up time, educational attainment, smoking, drinking, body mass index (BMI), hypertension, fall-related injuries, hip fracture, dyslipidemia, diabetes or high blood sugar, cancer or

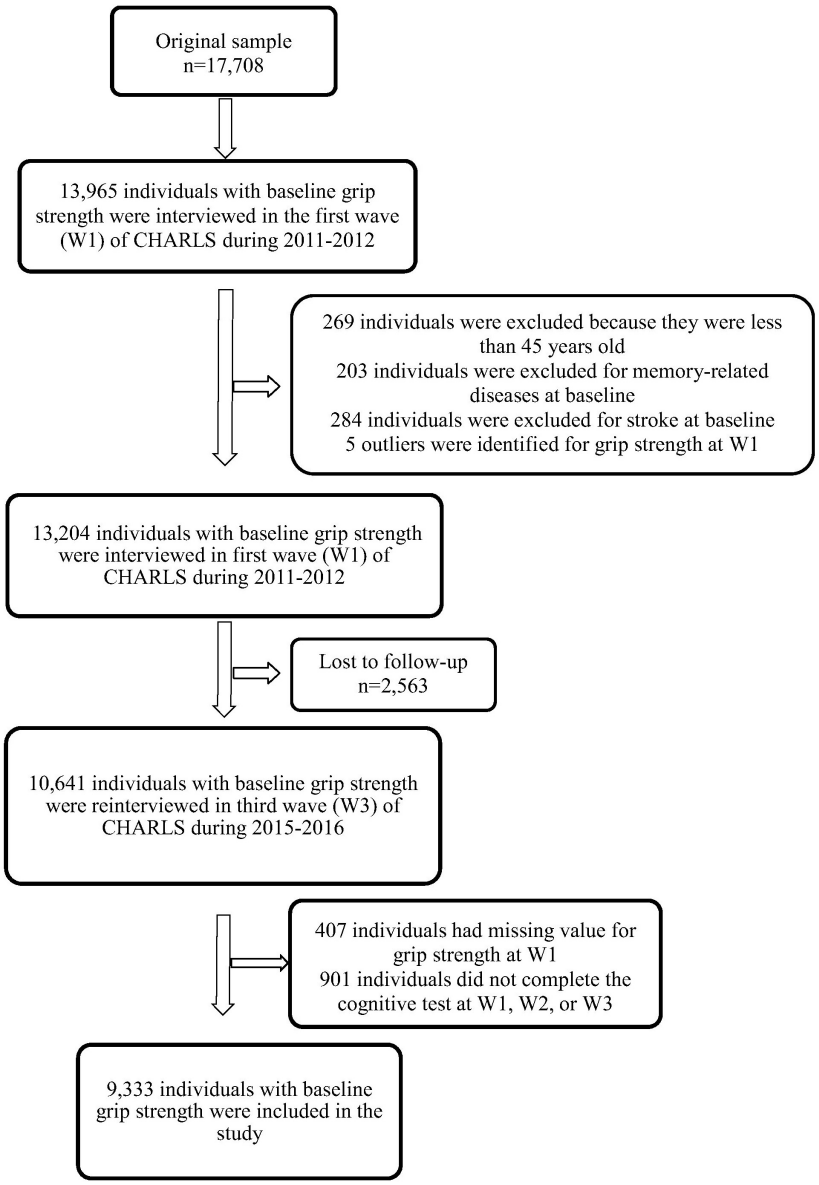

FIGURE 1 | Study diagram.

malignant tumor, heart problems, stroke, near and far-vision impairment, hearing problems, memory-related diseases, and depressive symptoms. Educational attainment was categorized as either "lower" or "higher" than primary school. Smoking and drinking habits were classified as either "never" or "current." Depressive symptoms were assessed using the 10-item Center for Epidemiologic Studies Short Depression Scale (CES-D 10). Others were dichotomized as either "no" or "yes."

Statistical Analysis

First, descriptive statistics were used to show the characteristics of the study sample. The *t*-test/Mann–Whitney *U* test and chi-square test were used for comparison of baseline characteristics between two samples. The linear correlations between baseline GS and cognitive function in W3 were estimated using multivariate linear regression models (MLRMs) with potential confounders. Generalized estimating equation (GEE) was used to examine the predictive capability of baseline GS for changes in cognitive function over a period of 4 years. MLRMs was used to analyze the cross-sectional association using two models. We adjusted for age, education, marital status, health status, health behaviors, and BMI in model 1. Model 2 was further adjusted as model 1 with baseline cognition. GEE was used to analyze longitudinal association using three models. In model 1, the analysis was adjusted for baseline global cognition, age, follow-up time, education, marital status, BMI. Model 2 was adjusted as model 1 with further adjustment for GS. Model 3 was adjusted as model 2 with further adjustment for health status,

health behaviors, and BMI. We chose GEE because it extends the generalized linear model to allow further analysis of longitudinal data. Secondly, because parameter estimation in GEE models remained relatively stable, it allowed us greater flexibility in modeling the effects of time on our results (Zeger and Liang, 1986; Zeger et al., 1988). All data were analyzed using STATA version 13 (StataCorp LP, College Station, TX, United States). The level of significance was set at $p < 0.05$.

RESULTS

Table 1 presents the baseline characteristics of the sample. A total of 9,333 individuals (4,365 men and 4,968 women) were included in the current study after excluding those who did not complete the necessary measurements at W1 or W3 and who were under 45 years of age at W1 (**Figure 1**). The mean participant age was 58.6 years [standard deviation (SD) = 8.7 years], 53.2% of the participants were women, and 84.7% were married. With regards to educational attainment, 30.8% attended primary school or above. Near-vision impairment (23.3%), hypertension (22.7%), far-vision impairment (26.1%), fall-related injuries (15.8%), hearing problems (12.5%), and heart problems (11.0%) were the most common medical conditions. The mean of follow-up time was 4.0 years (SD = 0.1 years), ranging from 3.3 to 5.0 years. Baseline GS ranged from 6 to 73 kg/m^2 for men (mean = 39.7 kg/m^2, SD = 8.9 kg/m^2), and from 2 to 100 kg/m^2 for women (mean = 27.1 kg/m^2, SD = 7.3 kg/m^2). The mean baseline global cognition score, episodic memory and mental intactness were 13.7 (SD = 5.7), 6.5 (SD = 3.7), and 7.2 (SD = 3.1), respectively.

Table 2 shows the relationship between the baseline GS level and baseline cognitive function through MLRMs. The higher GS significantly associated with better cognition in wave 1. After adjusting for potential confounders in female, referenced to the lowest GS level, the third quartile was the most highly associated with global cognition (β = 1.442, $p < 0.001$). Alternatively, for men, the highest GS level was the most highly related to global cognitive function (β = 1.388, $p < 0.001$).

Table 3 shows the relationship between the baseline GS level and the follow-up cognitive function in MLRMs. After adjusting for potential confounders in women, referenced to the lowest GS level, the third quartile was associated with better global cognition (β = 1.112, $p < 0.001$), and the highest GS level was associated with higher global cognitive function (β = 1.061, $p < 0.001$). In model 2, baseline global cognition was also included as an independent variable. The second quartile of GS level and the global cognitive function did not demonstrate statistically significant association. The third quartile was associated with better global cognition (β = 0.509, $p < 0.05$) and the highest GS level showed statistical significance (β = 0.543, $p < 0.05$). Alternatively, for men, the highest GS level was related to highest global cognitive function (β = 1.233, $p < 0.001$). In model 2, the second and third quartiles level of GS did not show statistical significance. However, positive correlations between the highest GS level and better cognitive measures were observed (β = 0.742, $p < 0.001$). **Supplementary Figures S1, S2** show the plotted estimated average global cognition in W3 across the baseline GS and their 95% confidence interval for both women and men before and after adjusting for baseline global cognition.

Table 4 summarizes the results from the GEE for GS quartiles as a predictor of cognition over a period of 4 years in a population of middle-aged and elderly individuals. The rate of decline in global cognition was 0.06 points every year. The fourth quartile of GS was associated with higher cognitive function in 4 years after adjusting for age, follow-up time, civil status, educational attainment, BMI, and baseline global cognition in model 1. The interaction between GS quartile and follow-up time (GS-by-time) was estimated in model 2. There were significant associations between individuals with the highest GS, indicating that people in highest GS quartile showed a

TABLE 2 | Association between baseline grip strength and baseline cognition by multivariate linear regression.

Sex	Independent variable	Global cognition β(SE)
Female	Q1(≤22.5 kg)	Ref.
	Q2(22.5–27 kg)	0.733(0.198)***
	Q3(27–31 kg)	1.442(0.209)***
	Q4(>31 kg)	1.239(0.216)***
Male	Q1(≤34 kg)	Ref.
	Q2(34–40 kg)	0.900(0.194)***
	Q3(40–45.2 kg)	1.155(0.221)***
	Q4(>45.2 kg)	1.388(0.223)***

$p < 0.001$. Ref.: reference, β: beta coefficient, SE: standard error.

TABLE 3 | Association between baseline grip strength and follow-up cognition by multivariate linear regression.

Sex	Independent variable	Global cognition β(SE)
Female	Model 1	
	Q1(≤22.5 kg)	Ref.
	Q2(22.5–27 kg)	0.628(0.193)**
	Q3(27–31 kg)	1.112(0.204)***
	Q4(>31 kg)	1.061(0.210)***
	Model 2	
	Q1(≤22.5 kg)	Ref.
	Q2(22.5–27 kg)	0.321(0.174)
	Q3(27–31 kg)	0.509(0.185)*
	Q4(>31 kg)	0.543(0.191)*
Male	Model 1	
	Q1(≤34 kg)	Ref.
	Q2(34–40 kg)	0.735(0.193)***
	Q3(40–45.2 kg)	0.809(0.220)***
	Q4(>45.2 kg)	1.233(0.222)***
	Model 2	
	Q1(≤34 kg)	Ref.
	Q2(34–40 kg)	0.417(0.181)
	Q3(40–45.2 kg)	0.400(0.206)
	Q4(>45.2 kg)	0.742(0.209)***

*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$. Ref.: reference, β: beta coefficient, SE: standard error.*

TABLE 4 | Longitudinal global cognition by baseline grip strength among middle-aged and elderly Chinese participants: generalized estimating equation (N = 9,333).

Sex	Independent variable	Model 1 β(SE)	Model 2 β(SE)	Model 3 β(SE)
Female	GS (kg) quartiles			
	Q1(≤22.3 kg)	Ref.	Ref.	Ref.
	Q2(22.3–27 kg)	0.342(0.104)**	0.222(0.107)*	0.201(0.110)
	Q3(27–31 kg)	0.256(0.111)*	0.156(0.112)	0.116(0.114)
	Q4(>31 kg)	0.464(0.113)***	0.087(0.115)	0.051(0.119)
	GS(kg) quartiles × time			
	Q1 × follow-up time		Ref.	Ref.
	Q2 × follow-up time		0.040(0.051)	0.038(0.052)
	Q3 × follow-up time		0.033(0.552)	0.030(0.054)
	Q4 × follow-up time		0.125(0.052)*	0.116(0.053)*
Male	GS (kg) quartiles			
	Q1(≤34 kg)	Ref.	Ref.	Ref.
	Q2(34–40 kg)	0.245(0.105)*	0.096(0.120)	0.083(0.122)
	Q3(40–45 kg)	0.367(0.114)**	0.226(0.131)*	0.230(0.135)
	Q4(>45 kg)	0.553(0.116)***	0.131(0.128)	0.075(0.131)
	GS (kg) quartiles × time			
	Q1 × follow-up time		Ref.	Ref.
	Q2 × follow-up time		0.050(0.055)	0.040(0.055)
	Q3 × follow-up time		0.035(0.058)	0.037(0.059)
	Q4 × follow-up time		0.140(0.053)*	0.143(0.054)*

*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$. Ref.: reference, β: beta coefficient, SE: standard error.*

significantly lower rate of decline in global cognition over time compared to those in the lowest quartile. The participants in the fourth quartile, compared to those in the first quartile, had a parameter estimate of 0.125 points per year (SE = 0.052; $p < 0.05$). In model 3, after adjusting for all covariates, the correlation between GS-by-time interaction (fourth quartile) and cognitive function over 4 years remained statistically significant (β = 0.116 with SE = 0.053, p = 0.030). Similar results (β = 0.143 with SE = 0.054, p = 0.008) were also observed for participants in fourth quartile compared to those in first quartile with regards to men. Other factors, such as older age and hearing problems, were associated with a decline in global cognition score in women. However, factors such as far-vision impairment besides older age were associated with poor performance in global cognition for men. Higher educational attainment and marital status were associated with better cognitive function in both the sexes. **Figure 2** depicted predictive margins from the GEE in women and men on different models, focusing on the variance in the predicted slopes.

DISCUSSION

We examined the longitudinal relationship between GS and cognitive performance in 9,333 middle-aged and elderly Chinese participants. The analysis identified that higher baseline GS level was associated with better cognitive function with ageing and lower rates of decline in cognitive performance over a period of 4 years in both men and women. Even after adjusting for the relevant, potentially confounding independent variables, the two parameters showed significant association.

Our findings are similar to those of previous studies, which demonstrated that GS could predict cognition over time (Stijntjes et al., 2016; Jeong and Kim, 2018; Wang et al., 2019), for instance, Veronese et al. (2016) found that lower handgrip strength could predict incident cognitive decline in a population of 1,249 elderly community dwellers over a period of 4.4 years. However, there were studies that reported results contrary to our findings, for example, a 6-year follow-up study by Atkinson et al. (2010) revealed that there was no significant association between physical performance (such as gait, balance, and GS) and cognitive changes in 1,793 elderly women. There are several reasons for the differences in the reported results, one of which could be that our study included a significantly larger sample size and demonstrated a better study design.

Mechanistically, our findings are in accordance with the most notable hypotheses known as the "common cause hypothesis," which demonstrates that cognition and muscle strength may share the same brain regions and networks (Christensen et al., 2001). Several researchers have drawn similar conclusions by observing the association between gait and cognitive function (Demnitz et al., 2016; Kueper et al., 2017). Furthermore, they also introduced Motoric Cognitive Risk (MCR) syndrome based on these associations, which can be used to identify people at risk of dementia in the population (Ayers and Verghese, 2016). This form of bounded rationality provides a reasonably straightforward way to implement the concept that simple motor tests or physical functions could be studied as biomarkers for identifying patients at a higher risk of cognitive impairment and dementia. However, there is still no direct imaging evidence to prove the rationality of this

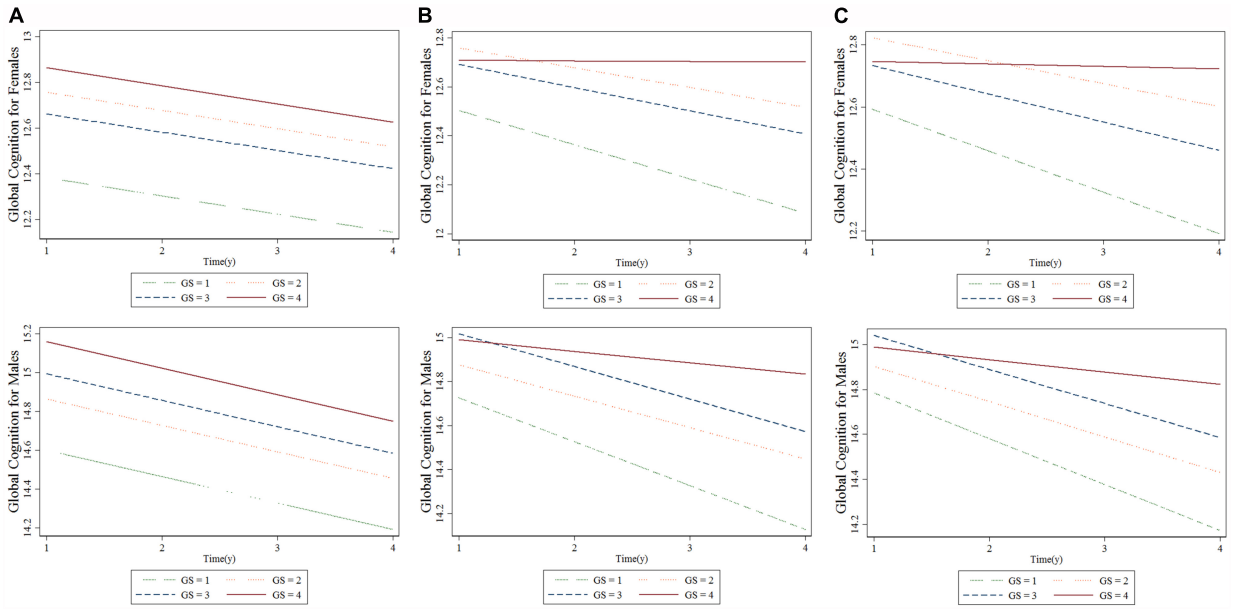

FIGURE 2 | Longitudinal association between baseline grip strength (GS) and cognitive changes over time for females and males: generalized estimating equation. **(A)** Model 1, **(B)** Model 2, and **(C)** Model 3. "GS = 1" is the baseline GS first quartile, "GS = 2" is the baseline GS second quartile, "GS = 3" is the baseline GS third quartile, "GS = 4" is the baseline GS fourth quartile.

theory. Although it could be speculated from some studies (Rosano and Snitz, 2018) that brain areas between motor coordination and cognitive function have an overlap, we would need a significantly intuitive research design to prove and refine this theory.

Recognition of early risk factors for CDs has paramount practical significance, particularly if the predictors were in the form of easily developed indicators. Training programs that improve balance and GS might also help to either prevent or slow cognitive decline in the elderly, particularly in those with reduced muscle strength. Lower grip strength, poor balance, and gait might be crucial identification markers for patients who require exercise programs. A number of randomized controlled trials reveal that exercise programs in elderly adults can improve both their physical and cognitive functions (Kim, 2011; Yoon et al., 2016). However, other studies show contradicting results (Emery and Gatz, 1990). This is an important area that requires further exploration.

One of the advantages of this prospective study was that it has a large number of subjects, hence drawing significantly reasonable conclusions. Last but not least, this study used a longitudinal design to confirm that GS predicts changes in cognition over a relatively long follow-up time in Chinese population. However, this study had several limitations. Firstly, the cognitive domains studied were relatively limited and we could not evaluate the specific cognitive domains. Secondly, 19.4% of the original participants were lost to follow-up and 12.3% of the re-interviewed subjects at W3 were not included in the study due to incomplete baseline GS test or incomplete cognitive test at W1, W2 or W3. Otherwise, their inclusion may have influenced the association between GS and cognitive function as determined in this study. Thirdly, other confounding factors, such as healthy diet and physical activity, may influence the results that could not be accounted for this time. The future study will include additional related factors, such as gait speed, balance and other physical measurements to verify the present conclusion.

CONCLUSION

This study suggests that higher GS in middle-aged and elderly adults predicted better global cognition over 4 years, unaffected by confounding factors. We need further research to understand the possible underlying mechanisms that may affect muscle strength and cognitive decline. A better understanding of the association between muscle strength and cognition may help us in the early identification of age-related cognitive decline and in order to find participants who could benefit from training programs.

DATA AVAILABILITY

The data used in this manuscript are from the China Health and Retirement Longitudinal Study (CHARLS). We applied the permission for the date access (http://charls.pku.edu.cn/zh-CN) and got the access to use it. Prof. Yaohui Zhao (National School of Development of Peking University), John Strauss (University of Southern California), and Gonghuan Yang (Chinese Center

for Disease Control and Prevention) are the principle investigator of the CHARLS, and they make the data available online for academic use freely.

ETHICS STATEMENT

Each participant included in this study signed a written informed consent form before taking the survey. Ethics approval for the data collection in the CHARLS was obtained from the Biomedical Ethics Review Committee of Peking University (IRB00001052-11015).

AUTHOR CONTRIBUTIONS

YL and CL designed the study. YL, XC, NG, and BY acquired the data. YL performed the statistical analysis, assisted by JW and CL. YL and CL drafted the manuscript. XC, NG, BY, and JW reviewed the manuscript. All authors approved the final version for submission.

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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.2019.00250/full#supplementary-material

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Benzodiazepines and Related Drugs as a Risk Factor in Alzheimer's Disease Dementia

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Benzodiazepines (BZDs) and Z-drugs are compounds widely prescribed in medical practice due to their anxiolytic, hypnotic, and muscle relaxant properties. Yet, their chronic use is associated with cases of abuse, dependence, and relapse in many patients. Furthermore, elderly people are susceptible to alterations in pharmacodynamics, pharmacokinetics as well as to drug interaction due to polypharmacy. These situations increase the risk for the appearance of cognitive affectations and the development of pathologies like Alzheimer's disease (AD). In the present work, there is a summary of some clinical studies that have evaluated the effect of BZDs and Z-drugs in the adult population with and without AD, focusing on the relationship between their use and the loss of cognitive function. Additionally, there is an assessment of preclinical studies focused on finding molecular proof on the pathways by which these drugs could be involved in AD pathogenesis. Moreover, available data (1990–2019) on BZD and Z-drug use among elderly patients, with and without AD, was compiled in this work. Finally, the relationship between the use of BZD and Z-drugs for the treatment of insomnia and the appearance of AD biomarkers was analyzed. Results pointed to a vicious circle that would worsen the condition of patients over time. Likewise, it put into relevance the need for close monitoring of those patients using BZDs that also suffer from AD. Consequently, future studies should focus on optimizing strategies for insomnia treatment in the elderly by using other substances like melatonin agonists, which is described to have a much more significant safety profile.

Keywords: benzodiazepines, Alzheimer's disease, dementia, cognition, risk factors

INTRODUCTION

Sleep disturbances have been reported to increase Amyloid Beta (Aβ) levels in the cerebrospinal fluid of healthy subjects, contributing to the advancement of neurodegeneration and the appearance of mild cognitive impairment (MCI) (Lopez et al., 1999; Virta et al., 2007; Modabbernia et al., 2011; Consensus and Statements, 2014; Di Meco et al., 2014; Benedict et al., 2015; Gage et al., 2015; Chen et al., 2016; Gaugler et al., 2016; Kincheski et al., 2017; La Frenais et al., 2017; Livingston et al., 2017; Atkin et al., 2018; Burke et al., 2018). At preclinical level, it has been described that sleep deprivation in 3xTg mice acts as a chronic stressor, favoring the decrease of Cyclic adenosine monophosphate (cAMP) response element binding (CREB) and affecting synaptic plasticity and cognitive functions (Di Meco et al., 2014) (**Figure 1**). It has been described that sleep restriction increases susceptibility to Amyloid beta (Aβ)-induced memory impairment in mice (Kincheski et al., 2017), accompanied by increased plasma levels of corticosterone, just like higher levels of brain pro-inflammatory cytokines [tumor necrosis factor alfa (TNFα), interleukin 1 beta (IL1-β) and IL-6], which contributed to memory impairment and synapse damage. Consequently, sleep alterations have become major risk factors for the development of sporadic pathologies like AD and need to be properly managed by drugs that will restore balanced physiological sleep periods (Kincheski et al., 2017; Hennawy et al., 2019).

Benzodiazepines (BZDs) and their analogous Z-drugs are psychotropic drugs commonly used in medical practice against anxiety, nervousness, convulsive states, depression, and psychosis. They also act as skeletal muscle relaxants and hypnotics for the treatment of short-term acute insomnia (Dolder et al., 2007). On a molecular level, BZDs and Z-drugs facilitate the inhibitory activity of the neurotransmitter gamma-aminobutyric acid (GABA) on its receptor (Duke et al., 2018), favoring the flow of chlorine ions through the ionotropic channel bound to the receptor and producing the hyperpolarization of neuronal membranes (Sigel and Ernst, 2003). The $GABA_A$ receptor is an ionotropic receptor composed of five protein subunits that mediate different behavioral and pharmacological responses (Mehdi, 2012; Duke et al., 2018). The α1 subunit of the $GABA_A$ receptor is thought to be responsible for sedative effects, while the α2 and α3 subunits exert anxiolytic and antidepressant activities. Finally, the α5 subunit is involved in the control of cognitive functions such as memory and learning (Rissman et al., 2007; Savić et al., 2010).

From a pharmacokinetic point of view, BZDs and related drugs are divided into three groups according to their half-life. It can either be long (over 24 h), intermediate (between 6 and 24 h), or short (<6 h). Usually, short and intermediate-acting BZDs are prescribed for insomnia, while longer-acting BZDs are reserved for anxiety, but their effects can vary with age and liver metabolic capacity. Old age is associated with a decrease of oxidative metabolism, causing an extension on drug half-life due to changes in pharmacokinetics and pharmacodynamics (Taipale et al., 2015; Hessmann et al., 2018). In fact, the prolonged use of these drugs (over 2 months) in advanced age has shown to produce serious side effects, causing tolerance and dependence,

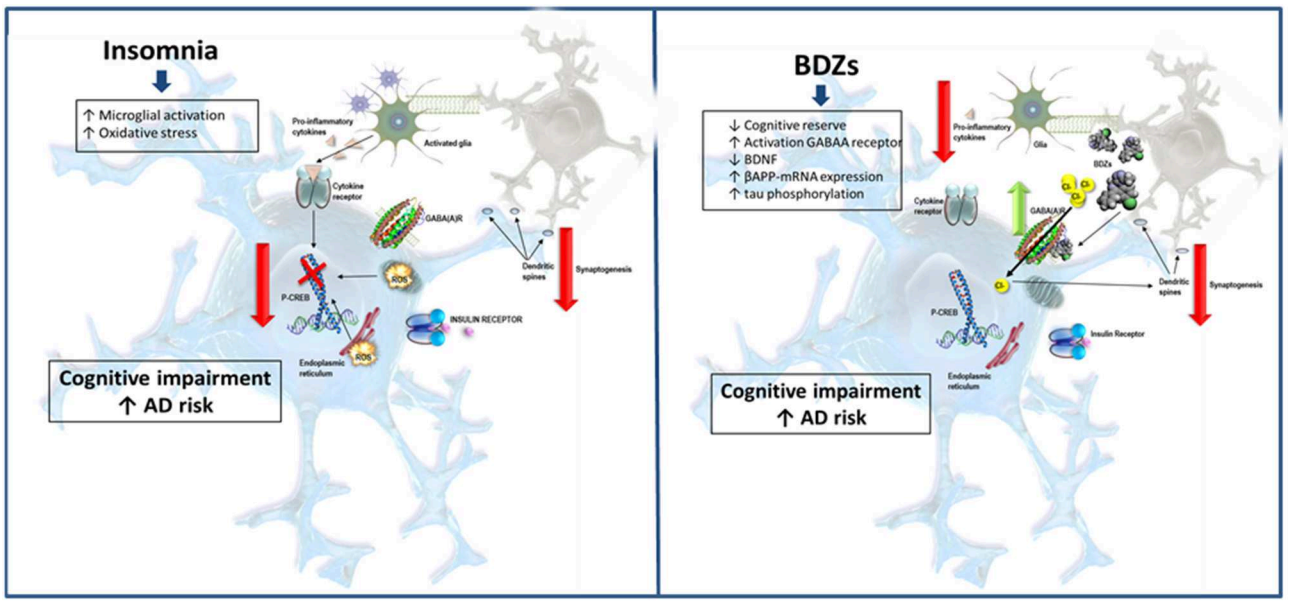

FIGURE 1 | Schematic representation of potential pathways by which insomnia and benzodiazepines could increase AD risk. Insomnia is a CNS stressor, which induces microglial activation and oxidative stress. Likewise, oxidative stress may be involved in cognitive impairment by decreasing phosphorylation levels of p-CREB and altering dendritic spines and synapses. Moreover, sleep disturbances prevent clearing toxic metabolites such as β-amyloid. These lead to an increased production of inflammatory cytokines and the formation of Aβ-plaques. In turn, BZDs activate $GABA_A$ receptors, thus interfering excitatory synapses and decreasing cognitive reserve. Moreover, these drugs have been shown to decrease BDNF as well as increase β-amyloid precursor protein (APP) mRNA levels and tau phosphorylation. All these mechanisms could increase the risk of cognitive impairment through neuroinflammation, decrease synaptic plasticity and brain insulin signaling as well as accumulation of Aβ plaques and neurofibrillary tangles.

increased risk of falls and fractures as well as an impairment of cognitive processes (Pharmd et al., 2003; Obradovi et al., 2005; Stewart, 2005; Rissman et al., 2007; Savić et al., 2010; Rosenberg et al., 2012; Makaron et al., 2013; Biétry et al., 2017; Nørgaard et al., 2017; Duke et al., 2018; Picton and Pharm, 2018; Underlien et al., 2018; Scott and Aricescu, 2019).

The effects of BZDs and other hypnotic drugs on cognition in elderly patients are intensive areas of research nowadays (Carlisle, 2017). In a recent study, Kurlawala et al. reported a case of a 76-year-old male who presented onset of short-term memory loss after a 3 year treatment with a BZD (Kurlawala et al., 2018). Magnetic resonance imaging studies revealed that cognitive loss could be a result of atrophy in the hippocampus and cortex (Barker et al., 2004; Hessmann et al., 2018; Kurlawala et al., 2018; Picton and Pharm, 2018). Studies performed by the groups of Glass et al. and Kripke et al. reported that the adverse effects of hypnotics outweigh the benefit they achieve in the population older than 60 years, going so far as to increase mortality risk in some patients (Glass et al., 2005; Kripke et al., 2012; Hammond and Esclapez, 2015). Consequently, these results have led the National Institutes of Health and the Beers Criteria of the American Geriatric Society to list molecules, such as eszopiclone, zolpidem, and zaleplon, as "potentially inappropriate medications" (Consensus and Statements, 2014; Investigations, 2015; Taipale et al., 2015; Wennberg et al., 2018). It was determined that BZDs should be avoided or should only be prescribed for short and specific situations (Letter, 2019; Walsh and Roth). However, BZDs and Z-drugs are still widely inappropriately prescribed in sleepless patients (Gunja, 2013; Pariente et al., 2016; Nørgaard et al., 2017; Richardson et al., 2018).

Luckily, alternatives to BDZs exist to treat situations of sleep deprivation. Sateia et al. published a clinical practice guideline for the treatment of insomnia in the Journal of Clinical Sleep Medicine (Sateia et al., 2017a,b). In their manuscript, the authors suggest ramelteon for sleep-onset insomnia. This drug selectively binds to the melatonin receptors, avoiding dependence and other important side effects associated with BZD long-term treatment. In the same paper, the authors also recommend Z-drugs or BZD hypnotics for sleep maintenance in insomnia (Sateia et al., 2017a). In 2018, the U.S. Food and Drug Administration (FDA) approved several non-BZD compounds for the treatment of insomnia (Richardson et al., 2018). These include antidepressants with anxiolytic or sedative action, such as escitalopram, doxepin, trimipramine, and amitriptyline as well as heterocyclic drugs like trazodone and mirtazapine (Gunja, 2013; Richardson et al., 2018).

The objective of this article was to review and discuss published material on the risk of BZD and Z-drug use and their role in the appearance of cognitive loss in cases of AD.

THE POTENTIAL MOLECULAR MECHANISMS INVOLVED IN BENZODIAZEPINES AND RELATED DRUG INDUCED COGNITIVE IMPAIRMENT

Although the molecular mechanisms by which BZDs and psychotropic drugs could induce cognitive impairment are uncertain, several hypotheses have been suggested (Gage et al., 2015). These mechanisms are summarized in **Figure 1**.

One hypothesis states that elderly long-term consumers of BZDs show limited cognitive reserve capacity. This concept refers to the ability to tolerate age-related and disease-related changes in the brain due to the existence of strong and redundant synaptic connections in the brain. This mechanism would allow resisting longer neurodegenerative pathologies without developing clear cognitive clinical symptoms or memory loss (Stern, 2012). Since BZDs and Z-drugs are positive $GABA_A$ receptor modulators, they decrease brain activation and reduce synaptic plasticity, affecting the patient's ability to create a new memory. Thus, BZDs interfere with the function of excitatory synapses, which is required for memory. In addition, the loss of social networks in aging people could act as an additional factor that may also affect cognitive function. Likewise, BZD treatment for sleep disturbances in aging could limit the capacity to create social communication networks and precipitate the development of dementia (Wan et al., 2004; Pariente et al., 2016; Mohamad et al., 2019).

The composition of $GABA_A$ receptors could also be involved in cognitive alterations related to hypnotic drugs. It has been reported that the binding of BZDs to the $\alpha 5GABA_A$ subunit, which is almost exclusively found in the hippocampus and deep layers of the cortex, impairs memory for contextual information in monkeys (Wan et al., 2004; Mohamad et al., 2019). Of note, Zolpidem did not impair the performance of a task based on visual cues which could be explained by its affinity for the $\alpha 1GABA_A$, instead of $\alpha 5GABA_A$ (Mohamad et al., 2019). Moreover, it has been reported that the memory-impairing effects of BZDs are not blocked by the α1GABAA-selective BZD antagonist β-carboline-3-carboxylate-3-butyl-ester, whereas the $\alpha 5GABA_A$ antagonist XLi-093 blocked the effects of triazolam but not zolpidem (Caraiscos et al., 2004; Mohamad et al., 2019). These findings suggest a specific role of $\alpha 5GABA_A$ receptor in BZD-related cognitive impairment. Furthermore, recent reports suggest that the modulation of extrasynaptic tonic inhibition of α5GABAA in the CA1 hippocampus and cerebral cortex could improve and regulate memory processes in the hippocampus. Therefore, negative modulation of $\alpha 5GABA_A$ could be a suitable target for the development of potential therapies against cognitive dysfunction in neurological diseases (Caraiscos et al., 2004). Contrarily, Joksimović et al. demonstrated that $\alpha 1GABA_A$ subunit receptor activation may affect the spatial learning performance in rodents (Joksimović et al., 2013). They also reported that $\alpha 1GABA_A$ subunit is involved in anterograde amnesia, sedation, motor incapacitation, and anticonvulsant BZD effects.

On another front, the "GABAergic deafferentation hypothesis of brain aging" introduced by Marczynski is based on the fact that administration of diazepam to rats causes a diminution of glucose utilization in the brain (Marczynski, 1995, 1998). Since a decrease in cellular adenosine triphosphate (ATP) levels is a feature of the aging process and AD, it would be logical to consider a potential metabolic influence of BZDs–GABA receptor complex in the brain, predisposing to AD in aging (Marczynski, 1995). Regarding this, the administration of flumazemil, an antagonist of BZDs, increases glucose utilization in rodents (Marczynski,

1998). Thus, a potential mechanism explaining the deleterious effects of BZDs on cognitive processes could be the depolarization and the depressive action of BZD agonists leading to brain energy metabolism deficit. In addition, BZD agonists may inhibit the effects of paracrine-autocrine neurotrophin family. Indeed, it is well-known that nerve growth factor (NGF)-related proteins participate in the regulation of survival, growth, and maintenance of neurons. In this sense, Zhao et al. reported that mice under long-term diazepam administration showed behavior alterations and reduced hippocampal synaptophysin and brain-derived neurotrophic factor (BDNF) levels (Zhao et al., 2012). This is a matter of importance since BDNF influences the functional aspects of synaptic transmission by (i) enhancing the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the postsynaptic membrane, (ii) enhancing Long term potentiation (LTP), and (iii) reducing $GABA_A$ receptor expression and decreasing inhibitory GABA-ergic neuro-transmission in the hippocampus (Jovanovic, 2004). Through this mechanism, BZDs could inhibit the axonal transport in both directions, increase the formation of neurofibrillary tangles, and also induce the βAPP-mRNA gene expression, thus increasing the risk of AD. Furthermore, the glutamate levels could also be affected by destabilizing neuronal Ca^{2+} homeostasis, and neurons could be more sensitized to the effects of glutamate (Jovanovic, 2004).

Recently, an interesting study of Whittington and collaborators reported that midazolam increased tau phosphorylation in C57BL/6 mice (Whittington et al., 2019). Consequently, the authors suggested that the effects of the most frequently used BZDs on tau phosphorylation should be evaluated in deep since they could be strongly involved in the increase of AD risk. Indeed, pathogenic forms of tau, including soluble tau oligomers, can promote neuronal dysfunction and cognitive loss by several mechanisms at the early stages of disease (Forner et al., 2017; Tracy and Gan, 2018). Likewise, Marciniak and collaborators reported that tau protein could be involved in the regulation of brain insulin signaling, which plays a fundamental role in the cognitive process (Marciniak et al., 2017). They demonstrated that the alteration of insulin signaling in preclinical models of AD could occur through alterations of tau.

It is also well-known that apolipoprotein E (APOE) 4 allele is a risk factor of AD (Stonnington et al., 2009). The presence of this allele is associated with an increased Aβ accumulation as well as with an increase in cognitive decline and disease development when compared to other APOE allelic variants. In this respect, Pomara and collaborators reported an increased sensitivity to the cognitive adverse effects of acute doses of lorazepam in elderly carriers of the APOE4 allele (Pomara et al., 2011). Thus, it seems that APOE4 could also be a risk for psychotropic drug-mediated cognitive decline. Likewise, the same group suggested that subjects who carry the very long Translocase of Outer Mitochondrial Membrane 40 Homolog (TOMM40) Poly-T Length and do not possess the ϵ4 allele might also be at increased risk for BZD-related cognitive loss. Therefore, the influence of APO E genotype on hypnotics as risk factors for AD seems relevant, and APOE4 genotyping could be useful in guiding physicians about avoiding BZD prescription in at risk patients. Finally, Stonnington and collaborators reported that acute 2 mg dose of lorazepam given to middle aged (50–65 years) cognitive normal adults caused higher decline in verbal episodic memory and visuospatial memory/executive function in ε4 carriers compared to non-carriers (Stonnington et al., 2009).

However, Aβ might also have indirect effects on the inhibitory GABAergic transmission as a result of the dynamic GABAergic balance modulation of the other two excitatory systems (cholinergic and glutamatergic neurotransmission). Interestingly, it has recently been suggested that the imbalance between excitatory and inhibitory systems underlies the synaptic dysfunction caused by Aβ (Rissman et al., 2007).

CLINICAL STUDIES

Between November 2018 and February 2019, we performed a literature review on clinical studies linked to the research topic in three acknowledged databases: Web of Science, Scopus, and PubMed. The collocated keywords were as follows: Alzheimer's disease AND benzodiazepines, Benzodiazepines AND cognitive impairment, Benzodiazepines AND cognitive decline, hypnotics AND cognitive decline, Z-drugs AND cognitive impairment, hypnotics AND Alzheimer's disease. The keywords were combined and integrated in the database and journal searches. The terms used were searched using AND to combine the keywords listed and using OR to remove search duplication where possible. References of retrieved articles that the authors' searches may have missed or could have been ignored were also assessed.

All these studies were fully investigated and considered under the following inclusion criteria:

- All articles had to be published studies conducted on human subjects up to February 2019.
- All articles had to be written in English.
- The primary outcome had to be focused on cognitive decline and Alzheimer's disease.

Case–Control Studies

Data from studies reviewed in this section can be found in **Table 1**.

In a nested case–control study, Lagnaoui et al. concluded that there was a small increased risk of dementia after the administration of $GABA_A$ activators (Lagnaoui et al., 2002). Notwithstanding, the authors acknowledged that the effect of exposure to other non-evaluated drugs with possible Central Nervous System (CNS) effects, such as antipsychotics, could have biased the results. In another study, the same group evaluated the prevalence of BZD use in AD patients for 3 months, raising awareness about the use of BZDs in elderly AD patients (Lagnaoui et al., 2002). The same authors conducted a case–control study with data from a large representative cohort of Canadian older women in order to examine the association between BZDs and AD. They found a non-significant tendency toward an association between former use of BZDs and increased risk of cognitive decline. Perhaps the low number

TABLE 1 | Overview of selected case–control studies exploring the effect of BZDs and Z-drugs on the delay of cognitive decline in the elderly and Alzheimer's disease patients.

References	Objective	Intervention	Number of subjects	Main outcome measures	Findings
Lagnaoui et al. (2002)	To investigate link between BZD and dementia in a large representative cohort of French community dwelling population. Data from PAQUID (Personnes Agées Quid) Research Program in Bordeaux.	1989–1997	150 cases and 3,519 controls. Age ≥ 65. Data from the UK-based Clinical Practice Research Datalink	Cognitive impairment was evaluated using the Mini-Mental Status Evaluation (MMSE) and CT scanner. Diagnosis was based on Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) and NINCDS-ADRDA	BZD consumption constitutes a risk factor for dementia in the elderly.
Wu et al. (2009)	To explore the association between long-term BZD use and the risk of dementia. Nested case–control study (Taiwan)	1997–2004	4,626 control subjects, and 779 dementia patients treated with hypnotics. Age ≥ 45.	Cumulative dose DDD of sedative-hypnotics and average days, per year.	Long-term use of hypnotic-sedative drugs increases AD risk.
Wu et al. (2011)	To explore if BZDs discontinuation affects the risk of dementia. Nested case–control study (Taiwan)	1997–2007	8,434 patients with dementia and 16.706 control subjects. Age ≥45.	BZD discontinuation.	The risk of AD increases with BDZs, but it decreases with BZDs discontinuation.
Billioti de Gage et al. (2012)	To evaluate the association between use of BZDs and dementia.	1987–1989	1,063 community dwelling people. Age ≥ 65.	Dementia evaluated based on the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised (DSM-III-R).	The use of BDZs was associated with increased risk of dementia.
Billioti de Gage et al. (2014)	To evaluate the association between former BZD use and the risk of AD and to investigate the potential dose–effect relation (Canada)	2000–2009	1,796 AD patients and 7.184 controls. Age >66.	First diagnosis (index date) of AD (ICD-9 (international classification of disease, ninth revision)	No dose-effect relation between BZDs and increased risk of AD was found in older people treated previously for more than 3 months.
Imfeld et al. (2015)	To assess the association of BZD use with risk of dementia.	1998–2013	16,823 subjects with AD and 9,636 subjects with vascular dementia and each being randomly matched (age, sex, general practice and duration of follow-up) with one control. Age ≥ 65. The time of study with these BZDs was 2 years from the diagnostic of AD and 3 years from vascular dementia	An algorithm based on recordings of specific dementia tests [e.g., Mini-Mental State Examination (MMSE), Clock Drawing Test (CDT), or Abbreviated Mental Test (7-Min Screen)], referrals to specialists, brain imaging [computed tomography (CT), magnetic resonance imaging (MRI), or single photon emission computed tomography (SPECT)] symptoms (memory impairment, aphasia, apraxia, or agnosia) supportive of a diagnosis of a specific dementia subtype.	Long-term BZDs use is not associated with an increased risk of AD or vascular dementia.
Gomm et al. (2016)	To explore the association between BDZ and Z-drug consumption and dementia in a large German population over 60 years old in German public health insurance data Allgemeine Ortskrankenkassen (AOK), which covers about 50% of the population at least 80 years old	2004–2011 follow-up.	21,145 cases (any dementia) and 84,580 controls, over 60 years of age.	Cognitive tests.	Regular use of BDZs and Z-drugs in the elderly induces a significantly increased risk of dementia.
Saarelainen et al. (2016)	The authors evaluated the effect of BZDs and Z-drugs administered 2 years before and three years after the diagnosis of AD. MEDALZ cohort in Finland.	2005–2011	51,981 patients with AD and 159.974 controls.	AD diagnoses based on the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association as well as the Diagnostic and Statistical Manual, Fourth Edition, criteria.	BZD use is higher in AD patients. BDZs could decrease the effectiveness of anti-AD drugs.

(Continued)

TABLE 1 | Continued

References	Objective	Intervention	Number of subjects	Main outcome measures	Findings
Billioti et al. (2017)	The association between former BDZ use and the risk of AD. Data from the Helsana Group, a large Swiss health insurance provider.	2013–2014	1,438 AD patients and 1,438 controls.	Diagnosis of AD in 2013 or 2014 *via* recorded first-time use of acetylcholinesterase inhibitors or the N-methyl-D-aspartate receptor antagonist memantine (agents commonly used to treat AD) using anatomic therapeutic chemical classification (ATC) codes N06DA02 for donepezil, N06DA03 for rivastigmine, N06DA04 for galantamine, or N06DX01 for memantine	BZD use in the 2 years preceding dementia diagnosis was not associated with an increased risk of developing AD.
Saarelainen et al. (2018)	To investigate the risk of death associated with new BZD and related drug (BZDR) use in a nationwide cohort of persons with AD. MEDALZ cohort in Finland. (Finland)	2005–2011	70,718 AD patients.	AD diagnoses based on the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association as well as the Diagnostic and Statistical Manual, Fourth Edition criteria.	BZD use is associated with an increased risk of death in persons with AD.
Tapiainen et al. (2018)	To assess the association between BDZ and related drug use and risk of AD, considering cumulative consumption and duration of treatment.	2005–2011	70,719 subjects with clinically verified AD diagnosis in 2005–2011 and 282,862 matched controls.	AD diagnosis based on DSM-IV and NINCDS-ADRDA criteria. Several confounding factors were considered, such as chronic diseases (COPD, asthma, cerebrovascular dementia, diabetes), abuse of other substances, socioeconomic status and the use of antidepressants or antipsychotics 5 years before the diagnosis of AD.	BZD and related drug use was associated with a modestly increased risk of AD. No major differences were observed among different subcategories of BZDs (BZDs, Z drugs, short-/medium-acting or long-acting BZDs)

of cases ($n = 73$) and controls ($n = 437$) prevented reaching statistical significance.

Wu et al. conducted two case–control studies in Taiwan using the National Health Insurance Research Database (NHIRD) with data of people aged ≥45 from 1997 to 2004 (Wu et al., 2009). The main conclusions of the study were that long-term use of BZDs or similar drugs might be associated with increased risk for dementia and cognitive alterations in prevalent and chronic users over a maximum follow-up of 8 years. The authors also suggested that risk of dementia was associated with higher cumulative dosage and longer duration of BZD exposure (Wu et al., 2009). In the second study, the same group stated that this association was reversible since BZD discontinuation could decrease the risk of dementia (Wu et al., 2011).

In 2012, Billioti de Gage et al. performed a study in a French population. The main conclusion was that the new use of BZDs was associated with an about 50% increase in the risk of AD (Billioti de Gage et al., 2012). In 2014, the same group assessed the effects of exposure to BZDs among 10 up to 5 years before the diagnosis of AD, considering both the doses and the reason for prescription (anxiety, depression, insomnia) in a population of aged individuals of Quebec (Canada) (Billioti de Gage et al., 2014). They concluded that the chronic use of BZDs was associated with a higher risk of AD when daily doses ranged between 91 and 180 mg/kg (cumulative dose expressed as prescribed daily, during 3–6 months), and increased further with doses higher than 180 mg/kg (during more than 6 months of exposure).

Gomm et al. reported that the risk of dementia increased by 21% in patients receiving regular hypnotic drug prescriptions when compared to non-users (odds ratio [OR] 1.21, 95% confidence interval [CI] 1.13–1.29; $p < 0.001$) (Gomm et al., 2016). The authors also reported the existence of a period of about 3 years from the first prescription of the BZDs to the diagnosis of dementia. This study did not create specific selection methods for those individuals with higher potential risk of AD, such as those with APOE4 allele or low educational level. Likewise, the study only focused on the analysis of regular hypnotic users.

Saarelainen et al. conducted a study in a Finnish cohort of 70,718 subjects diagnosed with AD between 2005 and 2011 in order to investigate the effects of BZDs and Z-drugs in a population of AD patients compared with matched controls (Saarelainen et al., 2016). The authors concluded that these psychotropic drugs could inhibit the beneficial effects of the drugs used in the treatment of AD, either anticholinesterases or memantine. Furthermore, AD patients treated with BZDs showed a risk of mortality up to 41% higher than those who did not use such drugs. Z-drugs did not increase the risk of death, but authors suggested that they could not be considered safer in persons with dementia (Saarelainen et al., 2018).

Another study was performed in a wide population of Finland for 6 years (Tapiainen et al., 2018). Currently, this is the largest study assessing the effect of BZDs and Z-drugs on AD risk. Moreover, the authors concluded that BZDs and Z-drugs modestly increased AD risk since the Off Ratios (OR) after adjusting for another concomitant psychotropic medication was

TABLE 2 | Cohort studies.

References	Objective	Intervention	Number of subjects	Main outcome measures	Findings
Lopez et al. (1999)	To examine the association of psychotropic medication use with cognitive, functional, and AD	1983–1988	179 patients with Alzheimer's disease age 82.2 mean 6.6	Cognitive impairment was evaluated using the Mini-Mental Status Evaluation (MMSE)	BZDs increase the risk of AD
Ellul et al. (2007)	To examine the effects of several drugs on the progression of disease in patients with Alzheimer's disease.	Not reported	257 patients with Alzheimer's disease age 82.2 mean 6.6 standard deviation	The diagnosis of Alzheimer's disease was made according to NINCDS-ADRDA criteria	Antipsychotics and BZDs were associated with a greater cognitive decline in patients treated with these drugs.
Rosenberg et al. (2012)	To examine the longitudinal association of psychotropic medication through the Persistency Index, which represents years of drug use divided by years of observation following AD diagnosis with cognitive, functional, and neuropsychiatric symptom among community-ascertained incident AD cases from the Cache County Dementia Progression Study	Not reported	335 participants were diagnosed with incident AD	Cognitive impairment was Mini-Mental State Evaluation (MMSE) and Clinical Dementia Rating	Psychotropic medication use was associated with more rapid cognitive and functional decline in AD
Hessmann et al. (2018)	To evaluate the continuity of BZDs prescriptions in patients with dementia insured in a German public sickness fund (Allgemeine Ortskrankenkasse AOK, 2018) in Lower Saxony, Germany	2014–2015	1,298 subjects with dementia.	Diagnosis of dementia in 2014, identified according to the International Classification of Diseases	The use of long acting BZD should be avoided in dementia patients.
Lee et al. (2018)	Association between sedative-hypnotic use and the risk of AD, in a Korean population through a retrospective cohort study from the National Health Insurance of Korea database	2002–2015 follow-up.	268,170 subjects. Age $\geq$ 50 The dosage of sedative-hypnotics was standardized by defined daily dose (DDD).	Comparison between the ever exposed, who were prescribed over 30 DDD of sedative-hypnotics and the non-exposed.	The risk of AD was higher in subjects exposed to sedative-hypnotics. (GABAA receptor agonists). Patients exposed to over 360 DDD of sedative-hypnotics showed a higher risk of dementia when compared to non-treated patients
Grande et al. (2019)	To investigate the effect of BDZs on first cognitive alterations in primary care patients suffering early cognitive alterations. Data comes from the REMIND—REteMilanese INtegrata per le Demenze—database.	Not reported	4,249 subjects (mean age 77.0 $\pm$ 8.2) enrolled by 353 General Practitioners (GPs) in the Milan metropolitan area.)	Evaluation of cognitive function by *ad hoc* trained GPs, using the Mini Mental State Examination (MMSE).	BZD use is not associated with an increased risk of poorer cognitive performance in primary care patients with first cognitive complaints.

1.06 (95% CI 1.04–1.08). They did not find significant differences between BZD subcategories (long or short action).

Controversially, there are some studies questioning the notion that BZDs/Z-drugs increase the risk of cognitive loss. For instance, in a study performed by Infeld et al., the results showed that long term use of BZDs did not increase the risk of AD (Imfeld et al., 2015). Adjusted odds ratios (aORs) were calculated with 95% confidence intervals (CIs) of developing AD or VaD in relation to previous BZD use, stratified by duration and benzodiazepine type. The OR of developing AD for those who started BZDs 1 year before AD diagnosis was 2.20 (1.91–2.53) and fell to the null for those who started between 2 and 3 years before [aOR 0.99 (0.84–1.17)] (Imfeld et al., 2015). In the same line, Biétry et al. evaluated the risk of AD after a period of 2 years of BZD and related drug treatment before AD diagnosis (Biétry et al., 2017). The results of the study indicated that the risk of developing AD was not associated with BZDs or Z-drugs. Likewise, the half-life of BZDs was not linked with AD risk (Biétry et al., 2017).

Cohort Studies

Data from studies reviewed in this section can be found in **Table 2**.

Several prospective and retrospective cohort studies which have assessed the association of BZDs/Z-drugs and related drugs on cognitive function reported controversial results.

For example, the study of Lopez et al. concluded that the use of BZDs in AD patients should be done with great caution and its use would not be adequate due to the risks of falls (Lopez et al., 1999). Also, in the treatment of insomnia, they suggested the use of other medications such as antihistaminic drugs. Also, Ellul et al. suggested that the prescription of antipsychotics and BZDs can accelerate cognitive decline in patients with AD (Ellul et al., 2007). In the prospective long-term "Caerphilly study," results also evidenced the association between the use of BZDs and the increased risk of developing both vascular and non-vascular dementia (Gallacher et al., 2012). The authors studied a representative sample of men with a follow-up rate over 22 years. They reported a significant higher risk for dementia with the use of BZDs. Moreover, Rosenberg et al. suggested that antipsychotics and BZDs showed an increase in cognitive loss associated with a high Persistency Index (Rosenberg et al., 2012). Lee et al. evaluated the risk of AD after the use of sedative-hypnotic antidepressants and antipsychotic drugs (Lee et al., 2018). Interestingly, the risk of AD was higher in patients receiving a defined daily dose over 30 defined daily dose (DDD). Likewise, in this study, different groups of BZDs were evaluated, and intermediate BZDs were associated with the highest risk of dementia. This study concluded that the risk of AD was associated with BZDs and sedative-hypnotic drugs, and that this association was dose-dependent (Lee et al., 2018). In another study performed in Taiwan, Chen et al. used data from the NHIRD, which covered 23 million registered patients from 1995 to 2010, accounting for 99% of the population (Chen et al., 2012). The authors clearly indicated the hypnotic drugs used which were classified into two groups: BZDs and Z-drugs. The results suggested that both hypnotics should be considered risk factors for dementia in patients with long-term insomnia. Likewise, an association between higher prescribed dosages of BZDs and Z-drugs and risks of dementia was found, which is in agreement with previous studies (Tapiainen et al., 2018).

It has been proposed that older people with anxiety may have a higher risk of AD development and involved an increase of Aβ levels in adults with MCI and AD (Pietrzak et al., 2015). Thus, Burke et al. investigated the role of anxiolytic drugs in the risk of AD. Likewise, they evaluated the association of APOE ε4, currently the most important risk factor in LOAD (Burke et al., 2018). One important finding of the study was that ε4 carriers had a statistically higher significant risk of AD development; however, this effect was moderated by the use of anxiolytics. Anxiolytics, alprazolam, lorazepam, paroxetine, or venlafaxine, specifically, may improve the association of anxiety on MCI and AD development. However, in the same study the authors reported that clonazepam conferred a statistically significant increased risk of MCI development among users of ε4 with anxiety, suggesting that there is a molecular mechanism on cognition that is altered by clonazepam.

Divergently, the prospective population-based cohort study published by Gray et al. investigated the risk of dementia associated with cumulative dosage of BZDs after an average follow-up of 7 years (Gray et al., 2016). They reported a small increased risk of dementia in people with low or moderate BZD treatment. However, the study concluded that BZDs do not increase the risk of AD (Gray et al., 2016). Furthermore, another team examined patients with recently diagnosed mild AD which were treated with anti-dementia medications such as acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine) and memantine. The authors reported an association between AD and an increased use of psychotropic drugs (Törmälehto et al., 2017). However, the administration of psychotropic medications was not related to alterations in cognitive performance (Törmälehto et al., 2017). Finally, Grande and collaborators showed that patients treated with short- and long-acting BZDs presented adjusted MMSE mean scores of 25.4; 95% CI 25.1–25.7, while non-treated patients had 25.9; 95% CI 25.3–26.4 (short acting BZDs); 25.3; 95% CI 25.2–25.5 (long-acting BZDs); ($p = 0.156$) (Grande et al., 2019). Therefore, the authors stated that the use of BZDs was not associated with an increase in cognitive loss in patients suffering initial cognitive alterations.

Longitudinal Studies

Data from studies reviewed in this section can be found in **Table 3**.

In a study performed in a young adult population, Boeuf-Cazou et al. concluded that, although long-term exposure to BZDs leads to specific impairment in long-term memory only in women, a longer period of observation is necessary to ascertain if these alterations are associated with a risk of developing dementia in old age (Boeuf-Cazou et al., 2011).

Yet, in a large population-based cohort study named "*The Three-City Study,*" Shash et al. compared short- vs. long-half-life BZDs as well as the effects of other psychotropic medications on dementia on non-institutionalized individuals aged $\geq$65 starting in 1999. The authors concluded that users of long half-life BZDs had a 60% increased risk of developing dementia (Shash et al., 2016). Also, it was examined whether the chronic use of BZDs over 4 years was associated with an increased risk of cognitive decline (Paterniti et al., 2002). The study demonstrated that prolonged use of BZDs was a significant risk factor for the cognitive decline in the elderly which was evaluated by the MMSE, the Trail Making Test, and the Digit Symbol Substitution. Finally, it had been described that BZDs decrease cognitive performance, although the effects were small. In addition, they suggested that higher BZD treatment duration and cumulative doses were responsible for the negative effects on cognition in elderly patients (Bierman et al., 2007).

Cross-Sectional Studies

Data from studies reviewed in this section can be found in **Table 4**.

Mura et al. conducted a cross-sectional and longitudinal study to evaluate the effects of chronic BZD use on cognitive decline in people over 65 years old in France (Mura et al., 2013). A total of 5,195 persons were included in the study, 969 of which were chronic users of BZDZs. The results showed that chronic BZD use was associated with poorer

TABLE 3 | Longitudinal studies.

References	Objective	Intervention	Number of subjects	Main outcome measures	Findings
Bierman et al. (2007)	To evaluate the effects of BZD use on cognitive function in the elderly. Data from the Longitudinal Aging Study Amsterdam (LASA), a population-based study	9 year follow-up	2,105 subjects aged 55 to 85 years.	General cognitive functioning measured by means of the Mini-mental State; Episodic memory measured with a Auditory Verbal Learning Test; Fluid intelligence measured by means of two sub-sets of 12 items (A and B) from Raven's Colored Progressive Matrices; Information processing speed measured by means of an adjusted version of the Coding task.	The duration of treatment and cumulative exposure to BZD use had a negative effect on the cognitive function of elderly people. However, this effect was small.
Boeuf-Cazou et al. (2011)	To investigate the impact of long-term BZD consumption on cognitive function Population from the VISAT study (Aging, Health and Work) (France). A prospective cohort study.	10 year follow-up	1,660 men and 1,577 women aged 32, 42, 52, and 62 years, classified according to the use of BDZs into non-users, occasional users and log-term users.	Cognitive function was assessed using five cognitive tests (immediate free recall test, delayed free recall test, recognition test, Digit Symbol Substitution Subtest and visual search speed test).	Long-term use of BDZs leads to specific impairment in long-term memory in women.

TABLE 4 | Cross-sectional studies.

References	Objective	Intervention	Number of subjects	Main outcome measures	Findings
Taipale et al. (2015)	To investigate the prevalence of BZD and related-drug consumption, especially those of long-term, and its associated factors among community dwelling individuals with and without AD.	2002–2006	The number of persons included in the study was 24,966 for individuals with AD and 24,985 for individuals without AD. The research was based on data from the MEDALZ-2005 (Medication use and Alzheimer's disease) cohort, which includes all community-dwelling individuals, diagnosed with AD in Finland at the end of 2005 and matched individuals without AD.	The diagnosis of AD based on the INCDS-ADRDA and DSM-IV criteria.	The long-term use of BDZs may impair cognition and may be associated with serious adverse events.
Hessmann et al., 2019	To evaluate the continuity of BZD prescriptions among dementia patients in Lower Saxony, Germany.	2014–2015	98 subjects with dementia.	Diagnosis of dementia in 2014, identified according to the International Classification of Diseases	The use of long acting BZD should be avoided by dementia patients.

cognitive performance, but not with accelerated cognitive decline with age. However, the authors stated that BZDs could deteriorate cognitive performance, increase the depletion of cognitive reserves and precipitate the onset of dementia (Mura et al., 2013). Later, another team aimed to evaluate the prevalence of BZD and Z-drug use in a population of Finland (Taipale et al., 2015). The authors concluded that approximately half of the people with AD used BZDs and Z-drugs during the 4 years of follow-up, and that AD patients used more BZDs in the long term that those without AD. In another study, the same group investigated the risk of any stroke (ischemic or hemorrhagic) associated with BZDs and Z-drugs in patients with AD taken from the MEDALZ-2005 population. They found an increase of 20% in the risk of ischemic stroke in AD patients using BZDs and Z-drugs. However, the risk of hemorrhagic stroke was not increased (Tolppanen et al., 2017).

Contrarily, Zhang et al. who collected data from the National Institute on Aging (NIA) ADCs, reported no association between BZDs and cognitive decline (Zhang et al., 2016).

Meta-Analyses

Zhong et al. investigated the association of long-term BZD use and dementia in a meta-analysis including five studies, which involved 45,391 participants and 1,891 dementia cases. In addition, the authors evaluated the potential risk of dementia associated with an increase of BZD dose by 22% (risk ratio 1:22, 95% CI 1.18–1.25). They concluded that long-term BZD users had an increased risk of dementia compared with non-users (Zhong et al., 2015).

In turn, He et al. pooled 10 studies to assess the association between BZDs and risk of dementia. PubMed and Embase databases were systematically searched for relevant publications up to September 2017. The literature search focused on observational studies that analyzed the relationship between the long-term use of BDZs and the risk of dementia. Their main findings pointed to a significantly increased risk of dementia in the elderly population using BZDs. This effect was associated with the use of BZDs with a longer half-life and with a longer treatment duration (He et al., 2019).

Finally, a meta-analysis of 12 prospective and retrospective cohort studies and case–control studies about the risk of BZDs and AD was reported. The team concluded that the use of BZDs—mainly those of long-action—and Z-drugs was associated with the development of dementia. However, the study showed some limitations since it did not differentiate between BZDs effects on AD and vascular dementias, neither between long- and short-acting BZDs (Lucchetta, 2018).

DISCUSSION

In the present review, we discussed the evidence pointing to BZDs as risks factors for cognitive decline in aging and AD (Paterniti et al., 2002; Zhang et al., 2016; Picton and Pharm, 2018).

Sleep disorders increase with age, altering clearance of toxic and waste products from the brain, including Aβ (Clinton et al., 2011). It has been reported that sleep disturbances could contribute to neurodegeneration and AD by altering physiological metabolic functions, increasing oxidative stress, and the accumulation of Aβ as well as due to the appearance of neuroinflammatory responses (Phan and Malkani, 2019) (**Figure 1**). In addition, insomnia is also associated with hypertension, diabetes, and a higher obesity risk; all of them contributing to AD (Clarke et al., 2018; Frozza et al., 2018). Bearing this in mind and considering the increase in insomnia diagnostics, especially in the elderly, this situation has led to higher consumption of hypnotic drugs in the adult population to improve sleep quality. Notwithstanding, and seeing the possible consequences derived from their intake, there needs to be a significant increase in awareness about their risks. In fact, there have been some studies reporting that the consumption of BZDs favors the appearance of cognitive affectations and increases the number of deaths in patients with AD (Imfeld et al., 2015; Saarelainen et al., 2017; Grande et al., 2019). Yet, as we have already assessed, there is controversy, and other researchers defend that BDZs have no such detrimental effects (Picton and Pharm, 2018). Thus, even though BZDs improve various measures of insomnia in the aged population, their clinical value is debatable, and melatonin agonists could be a much safer choice when trying to manage sleep disorders (Investigations, 2015). For the treatment of anxiety in the elderly, the administration of other medications such as serotonin uptake inhibitors (sertraline) or other compounds could be more adequate (Consensus and Statements, 2014; Investigations, 2015).

In the end, the mechanisms by which BZDs and Z-drugs could increase the risk of cognitive loss and AD remain to be clarified; here we have discussed some specific hypotheses. Thanks to molecular biology, the α1 subunit of the GABA has been shown to play an important role in BZD-mediated cognitive loss. Hence, it has been reported that higher activity at the α1GABA$_A$ receptors induced by positive allosteric modulation at the BZD site is responsible for spatial learning and memory incapacitation in preclinical models. Besides, activation of the α5 subunit, which is mainly expressed in the hippocampus, could in part explain the memory deficit states induced by BZDs. Therefore, α5GABA-targeting compounds could improve cognition, thus having therapeutic potential in AD and other dementias (Adrienn et al., 2017). It is conceivable that BZDs influence cognition and probably increase the risk of AD acting through hippocampal α5GABA$_A$, while Z-drugs (α5GABA$_A$-independent) confer a lower risk. In addition, the neuroinflammatory process is *per se* a risk factor for AD. The brain microglia play a prominent role in neuroinflammation, and it is associated with the secretion of pro-inflammatory cytokines. Likewise, α5GABA$_A$ receptor activity is enhanced by the inflammatory process, a fact that is probably critical in inflammation-induced memory deficits (Marczynski, 1998). Moreover, BZDs and other drugs may increase cognitive loss and AD risk through the phosphorylation of tau protein, which can also inhibit the signaling of the brain insulin receptor (Jovanovic, 2004; Whittington et al., 2019).

In spite of these described effects, it could be hypothesized that BZDs may indirectly exert a protective effect against the development of AD by improving sleep (through their clinical effects on sleep latency, number of awakenings, and duration and quality of sleep) (Guzmán et al., 2018). This paradigm states that the enhancement of GABA$_A$ receptor activity by BZDs could inhibit glutamatergic neurotransmission, thereby protecting against the excitotoxic effects of glutamate on the pathogenesis of AD (Fastbom et al., 1998). Moreover, it is noteworthy that some clinical trials reported no association between BZDs and risk of cognitive loss and AD.

In conclusion, we do not have enough data to ensure a causal relation between psychotropic drugs and cognitive loss. However, a therapeutic strategy based on BZDs and Z-drugs in elderly people should be extensively evaluated and monitored (Monzani et al., 2015; Yi et al., 2018). After reviewing the available data, a controversial question remains: is it safe to prescribe BZDs and Z-drugs to improve sleep in older patients, despite the potential cognitive loss risk? We strongly believe that there are enough data supporting an extremely cautious attitude with Z-drugs and the avoidance of BZD prescription in elderly people with AD.

AUTHOR CONTRIBUTIONS

All the co-authors of this research (ME, ACan, OB, PM, RC-T, CB-Z, MG, JO, CA, JF, and ACam) have directly participated in the planning, execution of the manuscript. All authors have read and approved the final version submitted.

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