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FRONTIERS IN AGING NEUROSCIENCE EDITOR'S PICK 2021

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SYSTEMATIC REVIEW
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Executive Functions in Alzheimer Disease: A Systematic Review

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Alzheimer's disease is a severe irreversible syndrome, characterized by a slow and progressive cognitive decline that interferes with the standard instrumental and essential functions of daily life. Promptly identifying the impairment of particular cognitive functions could be a fundamental condition to limit, through preventive or therapeutic interventions, the functional damages found in this degenerative dementia. This study aims to analyse, through a systematic review of the studies, the sensitivity of four experimental paradigms (Wisconsin Card Sorting Test, Stroop Task, Go/No-Go Task, and Flanker Task) considered as golden standard instruments for executive functions assessment in elderly subjects affected by Alzheimer dementia. This review was carried out according to the PRISMA method. Forty-five studies comparing the executive performance of patients with Alzheimer's dementia (diagnosed according to different classification criteria for dementia) and healthy elderly patients both over the age of sixty, were selected. For the research, PubMed, PsycINFO, PsycArticles databases were used. The study highlighted the importance of using standard protocols to evaluate executive dysfunction in Alzheimer's disease. The Stroop task allows discriminating better between healthy and pathological aging.

Keywords: alzheimer's disease, executive functions, wisconsin card sorting test, stroop task, go/no-go task, flanker task

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INTRODUCTION

The World Health Organization defines dementia as a "loss of intellectual capability of such severity as to interfere with the social or occupational functioning" (World Health Organization, 2010). It is a complex irreversible and chronic syndrome (Knapp et al., 2007) in which a slow and progressive cognitive decline affects the typical performance of the practical and essential functions of daily life (Boccardi, 2007).

The typical symptoms of dementia involve different cognitive domains, such as memory, spatial and temporal orienting, language and learning, comprehension, and communication skills (World Health Organization, 1992); moreover, alterations in emotional control, motivation and social behavior are often present (Kipps et al., 2009). This problem appears to be increasing in the general population (World Health Organization, 2012). It is estimated that about 46.8 million people worldwide, in 2015 had dementia; continually growing numbers that are expected to double every 20 years, reaching estimates of 74.7 million people in 2030 (Prince et al., 2016).

The calculation of the annual health costs related to dementias are similar to those of heart diseases, and they are much higher than those referred to cancer. These data would place dementia among the most expensive diseases in society (Prince et al., 2016). Most of these costs are attributable to home care and long-term institutionalization.

The most widespread form of dementia is Alzheimer's Dementia (AD), which is one of the main risk factors for death in the affected individuals (Alzheimer's Association, 2016).The average duration of the disease varies between 4 and 8 years, although some patients may survive up to 20 years after the onset of the AD (Xie et al., 2008). Alzheimer dementia is estimated to have increased by 35.4% in 2015, significantly raising the specific costs of this disease (Prince et al., 2016).

Alzheimer's disease includes a pre-dementia and a dementia phase (Taylor and Thomas, 2013). Pre-dementia represents the initial period of the disorder, in which the first symptoms associated with episodic memory loss begin (starting with the removal of the most recent memories and experiences), symptoms that however do not interfere with the management of the activities of the daily life (Förstl and Kurz, 1999). However, in this Mild Cognitive Impairment condition (MCI), not amnesic dysfunctions are also reported (Hodges et al., 2006). With the progression of the disease and the transition to the actual dementia phase, in addition to a worsening of the memory symptoms (which begin to affect even the most ancient memories and experiences), linguistic and spatial orienting deficits emerge that involves a severe functional difficulty (Hodges et al., 2006). High levels of anxiety and a general lack of motivation complete the clinical profile of AD (Steinberg et al., 2008; Dening and Sandilyan, 2015). On the other hand, in this first phase the procedural memory is still relatively preserved, but with the aggravation of the disease, there is a complete compromise of the entire memory domain (Pucci, 2004).

Balota and Faust (2002) have reported that individuals with AD present specific difficulties in selecting relevant information by separating them from irrelevant ones, highlighting their difficulty in dividing attention among multiple stimuli and in the attentional control. Likewise, lexical and semantic abilities would seem to be compromised, while phonological and syntax abilities would seem to be relatively conserved. As the disease progresses, the vocabulary tends to become impoverished and phonemic, and semantic paraphasias begin to appear, with a diminishment of expressive and understanding abilities (Pucci, 2004). In addition to language, the skills of spatial and temporal orienting, cognitive control of behavior, visuomotor integration, and executive functions are strongly compromised (Pucci, 2004).

Alzheimer's disease is characterized by a complex neuropsychological profile, associated with the gradual degeneration of the various cortical areas affected by this pathology. In the AD, the entorhinal cortex and the hippocampus seem to be compromised initially, that is, the structures involved in recording and consolidating information and in episodic memory (Du et al., 2001). Moreover, some studies have shown that patients with Alzheimer's disease showed severe lesions in the hippocampal and parahippocampal regions and the medial temporal lobe (Prvulovic et al., 2002; Machulda et al., 2003).

The AD, except the rare forms caused by genetic anomalies, derives from the presence and interaction of different conditions (Ngandu et al., 2015). Late age (Hebert et al., 2013), familiarity (Green et al., 2002) and the inheritance of the APOE-4 gene (Farrer et al., 1997) represent the risk factors most associated with the AD. Smoking, obesity (Beydoun et al., 2014), diabetes (Reitz et al., 2011), low levels of education and the inability to remain socially and mentally active, making it impossible to rely on their reserves cognitive (Wang et al., 2012) that, when are low, would be included among the risk factors indirectly associated with Alzheimer's disease. Regular physical activity (Sofi et al., 2011), a diet low in saturated fats (Loef and Walach, 2012), good cardiovascular health and the absence of brain lesions (McKee et al., 2013) represent protective factors for cognitive decline.

EXECUTIVE FUNCTIONS IN ALZHEIMER'S DISEASE

Executive functions represent a wide range of active cognitive processes, which allow responding in the appropriate way to environmental stimuli. This "umbrella term" includes verbal reasoning, problem-solving, planning, the ability to maintain sustained attention, resistance to interference, multitasking, cognitive flexibility, and the ability to cope with novelty (Stuss and Benson, 1986; Shallice, 1988; Damasio, 1995; Stuss et al., 1995; Grafman and Litvan, 1999; Burgess et al., 2000). To facilitate research in the field of Executive Functions, several authors (Miyake et al., 2000; Lehto et al., 2003; Diamond, 2013) have developed a tripartite classification that consists of:

- Inhibition, including inhibitory control, self-control (behavioral inhibition), and interference control (selective attention and cognitive inhibition). It includes the voluntary inhibition of dominant or automatic responses (Miyake et al., 2000) and would allow controlling behavior, thoughts and emotions, as well as attentional aspects, with the aim to respond appropriately to the needs of the task and specific objectives (Diamond, 2013);
- Updating, which allows keeping in mind and manipulating information. It involves the updating and the monitoring of the representations collected in the working memory (involvement of the Dorsolateral Prefrontal Cortex; Miyake et al., 2000), which allow responding appropriately to external tasks or stimuli, thanks to the processing of relevant information (Miyake et al., 2000);
- Cognitive flexibility (set-shifting), which allows modifying one's behavioral response to external stimuli (Baddeley and Hitch, 1994; Smith and Jonides, 1999; Diamond, 2013). It is characterized by the attentional shift between tasks or between different mental operations. This mechanism is commonly regarded as disengagement from an irrelevant task with subsequent anchorage on a relevant task to pursue a particular objective (Miyake et al., 2000). Diamond (2013) referring to this specific executive function uses the term Cognitive Flexibility, which allows underlining the ability to change the individual perspective not only from a spatial point of view but also by interpersonal and thoughtful perspectives.

Until the last 20 years, deficits in executive functions were rarely considered in the early stages of Alzheimer's disease (Allain et al., 2013). Some studies suggested that these were relatively preserved during the pre-clinical phase of the disorder (Broks et al., 1996; Razani et al., 2001). However, over the last years, this view has changed, and more recent studies have confirmed the presence in the AD of early impairment in a variety of tasks aimed at investigating executive functions (Binetti et al., 1996; Amieva et al., 2002; Bondi et al., 2002). These findings confirm that in the Alzheimer's disease executive functions are impaired from the early stages (Levy et al., 2002), primarily due to degeneration of the prefrontal cortex (Salat et al., 2001). In particular, the inhibitory abilities (Amieva et al., 2004), the attentional (Perry and Hodges, 1999) and the visuospatial functions (Cronin-Golomb and Amick, 2001) would be specifically compromised.

In patients with the AD, the attentional skills needed to resolve complex tasks would be impaired, such as divided attention, the ability to effectively disengage and shift attention (Perry and Hodges, 1999) and sustained attention (Berardi et al., 2005). Moreover, about the visuospatial functions the constructive praxia, visual-perceptive, and visual orienting abilities would seem to be damaged (Cronin-Golomb et al., 2007). When these cognitive deficits interfere with the performance of daily life activities, the patient can react to his/her cognitive impairment with mood swings, irritability and apathy. All these aspects outline the characteristic clinical profile associated with Alzheimer's disease.

Considering the relevance that the AD has on the life of patients affected by this disease, it is essential to understand the specific alterations involving the executive functioning thoroughly. With this purpose, recently, many researchers have focused on the use of experimental paradigms aimed at analyzing the deficits of executive functions in individuals affected by dementia (Sgaramella et al., 2001; Bullock and Lane, 2007; Cronin-Golomb et al., 2007; Ramanan et al., 2017). These studies highlighted how the various executive functions (Miyake et al., 2000) are differently affected by AD depending on the stage of the disease and by the personal characteristics of the patients.

COGNITIVE TASKS AND ASSESSMENT OF COGNITIVE FUNCTIONS IN ALZHEIMER'S DISEASE

Different experimental paradigms were used to evaluate executive functions in the AD. (Perry and Hodges, 1999) Given the heterogeneity of these paradigms and the vastness of studies aimed at investigating executive performance in patients with the AD, the objective of this review is to analyse the researches that address this issue through four specific behavioral tasks: Stroop Task, Wisconsin Card Sorting Test, Flanker Task, and Go/No-Go Task. These tasks were more commonly used to evaluate executive performance (Diamond, 2013).

Stroop Task (Stroop, 1935) is one of the most used paradigms for the study of executive functions. In particular, through the use of incongruent stimuli, it evaluates the management of the conflict and the inhibitory control of automatic responses. In the standard version of the Stroop Task, the stimuli are words written with colored inks. There are congruent (the word RED written in red ink) or incongruent (the word RED written in green ink) trials; the participant's goal is to respond by referring to the color of the ink ignoring the meaning of the word. In this way, two alternative and incompatible responses (color vs. word) are elicited, one of which is more spontaneous than the other (reading of the word vs. ink color denomination).

The Wisconsin Card Sorting Test (WCST) (Milner, 1963) is aimed to evaluate abstract reasoning, and cognitive flexibility understood as the ability to change one's strategies in response to environmental contingencies (Berg, 1948; Grant and Berg, 1948; Luria, 1973; Shallice, 1982). The WCST consists of four stimulus cards and two sets of 64 response cards. The cards vary in color, shape and number of elements represented. The test includes some ambiguous stimuli, and the pairing criteria vary according to a standardized order (Color, Form, Number). The task requires identifying the correct criterion with which to order the response cards to the stimulus cards; for each card placed by the participant, the experimenter provides feedback on the correctness of the performance. Based on the feedback from the experimenter, the participant can modify his/her behavior by identifying the appropriate strategy.

The Flanker Task (Eriksen and Eriksen, 1974) measures selective attention and the ability to control conflictual information. The task requires discriminating the central target stimulus between a series of lateral distractors (flanker). There are three types of conditions: the congruent trials, in which the target stimulus and the flankers have the same characteristics and required the same response; the incongruent trials, in which the target has different features with respect to the distractors, requiring an opposite response that generates conflict; finally, the neutral condition, in which the distractor is not confused with the targets presented in the task, and it does not cause conflict. The flanker effect (also called conflict or congruence effect) reveals the difficulty in ignoring the distractors due to the ambiguity of the stimuli used (Cohen and Shoup, 1997).

The Go/No-Go Task assesses sustained attention (vigilance) and impulsivity and allows obtaining information related to motor-type inhibitory control (Zahn et al., 1980, 1991; McGaughy and Sarter, 1995). The task consists in the presentation of a stimulus that requires a response from the participant (Go stimulus), and another stimulus for which the participant must, instead, inhibit any response (No-Go stimulus). A high percentage of errors indicates a difficulty in behavioral inhibition. Also, in this case, there are different versions of the task to investigate the inhibitory aspects and the influences of this ability from other variables, such as emotions (Schulz et al., 2007).

AIMS

The central aim of this review is to analyse the sensitivity of four experimental paradigms (Stroop Task, Flanker Task, Wisconsin Card Sorting Test and Go/No-Go Task) in the study of executive functions in elderly subjects suffering from Alzheimer's dementia, in order to be able to consider and define the applicability and

usefulness of these paradigms. Moreover, another objective of this work is to verify how the executive functioning in the AD is compromised concerning the normal operation of healthy elderly, with the aim of understanding how and where the cognitive impairment associated with dementia intervenes in a more evident way.

SYSTEMATIC REVIEW

The systematic review was conducted using the PRISMA method (Moher et al., 2009), but without recording the protocol. This review considered all the works that investigated the executive functioning through the use of the cognitive tasks defined in the introduction.

Most of the considered studies refer to the diagnostic criteria of Alzheimer Disease of the National Institute of Neurological and Communications Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984) for the classification of the AD. However, we considered also the studies that used the diagnosis criteria of DSM (American Psychiatric Association, 1980, 1994), the Cambridge Diagnostic Examination of Elderly (CAMDEX) (Roth et al., 1986) or National Institutes of Health and the Alzheimer's Association published revised guidelines (NIA-AA) (McKhann et al., 2011).

METHOD

The study was the result of systematic research in the PubMed, PsycArticles, and PsycINFO databases. The following keywords were used for the search: Stroop Task, Wisconsin Card Sorting Test, Go/No-Go Task, Flanker Task, and Alzheimer. Thespecific scripts are presented in **Table 1**.

All articles published on the topic up to the date of 1 July 2018 have been taken into account.

Two researchers performed the research independently, and the results were compared. The disagreements have been resolved with consensus methods. In case of lack of consensus among the researchers, a supervisor was used.

For the selection of the articles the following inclusion criteria were used: publications on "Peer Review-Journals"; use of the Stroop Task, the Flanker Task, the Wisconsin Card Sorting Test or, the Go/No-Go Task; the presence of a control group of healthy elderly.

The exclusion criteria were the following: (a) studies that did not present all the data useful for a critical analysis of the results; (b) the use of versions of the tasks that considered the emotional components of executive functions; (c) studies with methodological bias (for example with unspecified inclusion/exclusion criteria); (d) studies comparing the AD group with groups affected by other types of dementia or MCI without a healthy elderly group; single cases.

The initial results produced 858 articles. After the elimination of duplicates and irrelevant papers, by the title and abstract reading, 83 articles were read.

TABLE 1 | Scripts used in the systematic research.

	Script
Alzheimer and Stroop Task	("Alzheimer disease"[MeSH Terms] OR ("Alzheimer"[All Fields] AND "disease"[All Fields]) OR "Alzheimer disease"[All Fields] OR "Alzheimer"[All Fields]) AND ("Stroop test"[MeSH Terms] OR ("Stroop"[All Fields] AND "test"[All Fields]) OR "Stroop test"[All Fields] OR ("Stroop"[All Fields] AND "task"[All Fields]) OR "Stroop task"[All Fields]).
Alzheimer and Flanker Task	("Alzheimer disease"[MeSH Terms] OR ("Alzheimer"[All Fields] AND "disease"[All Fields]) OR "Alzheimer disease"[All Fields] OR "Alzheimer"[All Fields]) AND Flanker[All Fields] AND Task[All Fields].
Alzheimer and Go/No-Go	("Alzheimer disease"[MeSH Terms] OR ("Alzheimer"[All Fields] AND "disease"[All Fields]) OR "Alzheimer disease"[All Fields] OR "Alzheimer"[All Fields]) AND Go/No-Go[All Fields] AND Task[All Fields].
Alzheimer and Wisconsin Card Sorting Test	("Alzheimer disease"[MeSH Terms] OR ("Alzheimer"[All Fields] AND "disease"[All Fields]) OR "Alzheimer disease"[All Fields] OR "Alzheimer"[All Fields]) AND ("Wisconsin card sorting test"[MeSH Terms] OR ("Wisconsin"[All Fields] AND "card"[All Fields] AND "sorting"[All Fields] AND "test"[All Fields]) OR "Wisconsin card sorting test"[All Fields]).

At the end of the revision work, 45 articles were included in the review. The flowchart presented in **Figure 1** shows the selection of the studies.

The 45 selected articles have been categorized concerning the single paradigm used. Studies that used more experimental tasks were discussed in the different paragraphs concerning the results of each specific task. According to PICOS (Moher et al., 2009), information about participants, control groups, methods and results have been extracted. These data are presented in the different behavioral task tables.

RESULTS

Stroop Task

The systematic research has allowed the identification of 30 studies (see **Table 2**) that used different versions of the Stroop Task to evaluate inhibitory control and selective attention in patients with Alzheimer's disease.

Within the 30 studies, only five of these did not use the diagnostic criteria of the National Institute of Neurological and Communicative Disorders and the Alzheimer Disease Association (McKhann et al., 1984), in particular, Koss et al. (1984) used the criteria for the diagnosis of AD diagnosis of DSM-III, medical observation and scores obtained at the Dementia Rating Scale (DRS) (Mattis, 1988); Li and collaborators (Li et al., 2009, 2011), have used the diagnostic criteria of the DSM-IV; (Fisher et al., 1990)used the Blessed Dementia Scale (Blessed et al., 1968) and Hutchison and colleagues (Hutchison et al., 2010) used the Clinical Dementia Rating Scale (CDR) (Morris, 1993).

The patients with the AD and their control groups included all participants over 65 years old, who differed from each other

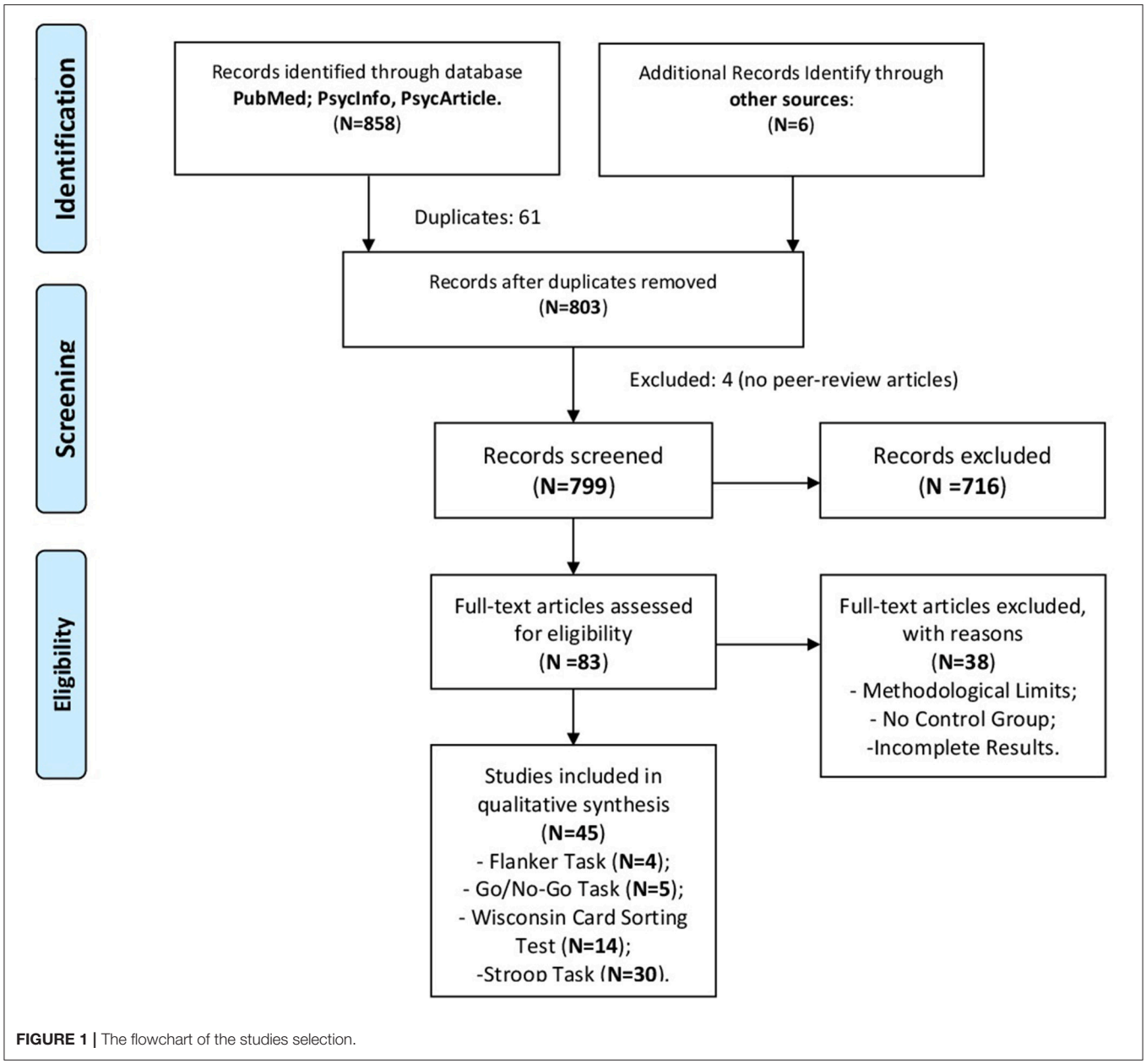

FIGURE 1 | The flowchart of the studies selection.

for the scores obtained at the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) or the DRS for the evaluation of the severity of dementia. Only in the study of Tse and colleagues (Tse et al., 2010), there was a significant difference between the age of patients with the AD and the healthy elderly group (patients with AD were older than people of the control group).

Compared to the Stroop Task analysis, all authors evaluated at least one of the following dependent variables: accuracy of responses, reaction times and interference effect (or Stroop effect).

In most of the studies, the analysis of accuracy showed worse performance in the patients with AD compared to healthy elderly (Koss et al., 1984; Spieler et al., 1996; Amieva et al., 2002, 2004; Bondi et al., 2002; Belleville et al., 2006, 2008; Duong et al., 2006; Stokholm et al., 2006; Collette et al., 2007; Doninger and Bylsma, 2007; Bélanger et al., 2010; Hutchison et al., 2010; McGuinness et al., 2010; Tse et al., 2010; Li et al., 2011; Stawarczyk et al., 2012; Chen et al., 2013; Peltsch et al., 2014; Sánchez-Benavides et al., 2014; Huang et al., 2017).

Furthermore, in all studies that also evaluated reaction times, it was found that patients with Alzheimer's disease are generally slower than healthy elderly people; (Koss et al., 1984; Spieler et al., 1996; Bondi et al., 2002; Amieva et al., 2004; Levinoff et al., 2004; Duong et al., 2006; Stokholm et al., 2006; Collette et al., 2007; Li et al., 2009; Bélanger et al., 2010; Hutchison et al., 2010; Tse et al., 2010) this result was not observed by Stawarczyk et al. (2012) that found similar reaction times in the two groups.

In the studies identified by systematic research, several indices were used to detect deficits in the inhibitory control

TABLE 2 | Some characteristics of the studies that have used the Stroop Task to assess executive dysfunction in patients with Alzheimer's disease.

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
Koss et al., 1984	CG = 11 (66.0) [5M;6F]	DRS4	DSM-III	Three conditions: Color (XXXs strings), Word (neutral word), Color-Word (naming the ink of the color word).	AD<CG	AD>CG	–
	Mild AD = 9 (62.3) [5M;4F]	Mild AD = 111.6					
	Moderate AD = 6 (67.6) [4M;2F]	Moderate AD = 73					
	MCI = 6 (60.7) [6M;0F]	MCI = 110					
Fisher et al., 1990	CG = 36 (72.9 ± 8.3) [13M;23F]	BDSe CG = 1.5 ± 6.1	NINCDS-ADRDA6	Three conditions: Color (cross), Word (Black Ink), Color-Word (Golden and Freshwater, 1978).	–	–	Interference Score: AD worse than AD
	AD = 36 (70.1 ± 8.9) [14M;22F]	AD = 15.5 ± 6.1					
Spieler et al., 1996	Older CG = 50 (85.6 ± 3.4)	CDR	NINCDS-ADRDA	Two conditions: Neutral word, Xs or symbols; Color-Word.	AD<CG	AD>CG	Facilitator Effect [(RT congruent-RT incongruent)/RT neutral]: AD>CG
	Young CG = 25 (70.5 ± 6.7)						Interference Effects [RT incongruent-RT neutral]: AD = CG
	Mild AD = 25 (73.2 ± 6.5)						
	Severe AD = 40 (73.7 ± 8.8)						
Amieva et al., 2002	CG = 28 (75.2 ± 6.6)	CG = 27.6 ± 1.8	NINCDS-ADRDA	Three Conditions: Color; Word; Color-Word.	AD<CG	AD>CG	Interference Ratio [RTon C-W task/RT on C task]: AD>CG
	Mild AD = 28 (75.8 ± 6.1)	Mild AD = 24.6 ± 1.9					
Bondi et al., 2002	CG = 51 (73.40 ± 7.43) [20M;31F]	DRS	NINCDS-ADRDA	Three conditions: Color (cross); Word (Black Ink); Color-Word (Golden and Freshwater, 1978).	AD<CG	AD>CG	–
	AD = 59 [31M;28F]	Very Mild = 119–139					
	Very mild AD =22 (71.7 ± 6.97)	Mild = 106–118					
	Mild AD = 25 (73.5 ± 5.76)	Moderate = 90–105					
	Moderate AD = 12 (72.1 ± 8.41)						

(Continued)

TABLE 2 | Continued

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
Amieva et al., 2004	CG-I = 22 (74.0 ± 4.7) [5M;17F]	CG-I = 27.9 ± 1.7	NINCDS-ADRDA	Four conditions: *Interference* (word-ink, naming the ink); *Reading* (word in black); *Color* (color patches) *Reverse Stroop Task* (reading the word).	AD<CG (in all condition)	AD>CG (in all condition)	–
	AD-I = 22 (74.0 ± 5.1) [5M;17F]	AD-I = 21.1 ±3.0					
	CG-R = 22 (74.9 ± 6.4) [6M;16F]	CG-R = 27.5 ± 1.7					
	AD-R = 22 (74.8 ± 6.3) [6M;16F]	AD-R = 21.4 ± 2.4					
Levinoff et al., 2004	CG = 23 (73.0 ± 6.1) [10M;13F]	CG = 28.8 ± 1.0	NINCDS-ADRDA	Two subtests: Color (dots), Color-Word (Stroop, 1935).	–	AD>RT	Interference Effect RT:
	AD = 30 (74.1 ± 8.3) [16M;14F]	AD = 22.4 ± 3.1					[RT C-W- RT C]: AD>CG
Belleville et al., 2006	CG = 12 (72.7 ± 4.6) [6M;6F]	CG = 28.2 ± 1.1	NINCDS-ADRDA	Three conditions; Word Condition (name of color in black ink), Color Condition (xxx in color ink), Interference Condition (color word in different color ink). Number of correct responses in 45 s (Golden, 1976).	AD<CG	–	Inhibition Score [Interference/ (Word+Color)/2]: AD>CG
	Young CG = 12 (22 ± 3.2) [4M;8F]	Young CG = N/A					
	AD = 12 (72.5 ± 5.9) [4M;8F]	AD = 22.9 ± 2.0					
Duong et al., 2006	CG = 60 (74.38 ± 5.74)	CG = 29.12 ± 0.97	NINCDS-ADRDA	*Classic Stroop Task*. Three conditions: dot (4 dots x 6 lines in four different colors), word (4 words x 6 lines, word in the same color as before), Color-Word (4 color words in incongruent ink x 6 line in the same color as before).	Stroop Task: AD<CG (in all conditions	Stroop Task: AD>CG (in all conditions)	Stroop Task: Interference dot-baseline (ACC e RT). AD worse than CG.
	AD = 36 (73.62 ± 8.94)	AD = 22.08 ± 3.76		*Stroop Pictures Naming*: Picture of animals and letter strings in the drawing. Four conditions: *Congruent* (letter string is the name of the animals drawing), *Incongruent/same item* (letter string is the name of a different animals in the task), *Incongruent/different item* (letter string is the name of an animal not presents in the task), *Neutral* (xxx on the animal drawing).	Stroop Pictures Naming:	Stroop Pictures Naming:	Interference word-baseline (ACC e RT). AD worse than CG.

(Continued)

TABLE 2 | Continued

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	**Reaction time**	**Other indices**
	MCI = 61 (74.68 ± 6.48)	MCI = 27.20 ± 2.15			AD<CG	AD>CG	Stroop Pictures Naming: Facilitation: AD =CG (in RT and n° of errors) Inhibition different AD = CG (RT), AD>CG (n° of errors) Inhibition similar AD = CG (RT), AD>CG (n° of errors)
Stokholm et al., 2006	CG = 32 (74.3 ± 4.2) AD = 36 (76 ± 5.6)	CG = 29.3 ± 0.9 AD = 25 ± 1.5	NINCDS-ADRDA	Color-Word condition (Stroop, 1935).	AD<CG	AD>CG	
Belleville et al., 2008	CG(AD) = 19 (72.42 ± 8.31) [4M;15F] AD = 19 (73.42 ± 9.18) [9M;10F] CG(MCI) = 25 (66.12 ± 10.09) [5M;20F] MCI = 25 (64.76 ± 10.83) [11M;14F]	CG(AD) = 28.74 ± 0.94 AD = 24.65 ± 3.60 CG(MCI) = 28.88 ± 0.99 MCI = 28.36 ± 1.98	NINCDS-ADRDA	Stroop Victoria (Spreen and Strauss, 1998). Short version (24 items for each condition), three conditions: Color, Neutral Word, Color-Word.	Incongruent: AD<CG		
Bracco et al., 2007	CG = 13 (70.46 ± 6.33) [5M;8F] AD = 50 (73.6 ± 7.1) [10M;40F] Very Mild AD = 22 (75.1 ± 6.86) [6M;16F] Mild AD =28 (72.4 ± 7.1) [4M;24F]	CG = 29.38 ± 0.87 AD = 21.3 ± 3.3 Very Mild AD = 23.7 ± 2.2 Mild AD = 19.5 ± 2.8	–	Three conditions: Word, Color, Color-Word (Stroop, 1935).	–	Interference (C-W) AD>CG	.
Collette et al., 2007	CG = 28 (70.6 ± 6.8) [13M;15F] AD = 25 (72.5 ± 5.8) [18M;17F] FTD = 13 (65.7 ± 7.5) [5M;8F]	DRS	NINCDS-ADRDA	Two sets: Color, Color-Word (interference, ink and word: incongruent color); naming the ink of the color word (Stroop, 1935).	Color naming: CG = AD Stroop: Task: AD<CG	Color naming: AD>CG Stroop: AD>CG	Interference ratio [RT Interference Condition/ RT Color Condition]: AD = CG Interference differential score [RT Interference Condition-RT Color Condition]:AD>CG
Doninger and Bylsma, 2007	CG = 30 (83.78 ± 7.81) [6M;24F] Mild AD = 24 (85.34 ± 6.09) [7M;17F]	GC = 29 ± 0.87 Mild AD = 25.25 ± 1.80	NINCDS-ADRDA	*Emotional Stroop Task*: emotive word in different color. Naming the color. *Color-Word Stroop* (Golden and Freshwater, 1978).			

(Continued)

TABLE 2 | Continued

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
	Moderate AD = 21 (79.75 ± 9.89) [10M;11F]	Moderate AD = 16.05 ± 3.94					
Belleville et al., 2008	CG = 25 (72.8 ± 7.6) [14M;11F]	CG-AD = 28.69 ± 0.8	NINCDS-ADRDA	Stroop Victoria (Regard, 1981).	–	In the C-W condition: AD>CG	–
	AD = 13 (73.2 ± 8.1) [6M;7F]	AD = 24.85 ± 4	DSM-IV	Short version (24 items for each condition), three conditions: Color, Neutral Word, Color-Word			
	MCI = 20 (66.3 ± 10.9) [8M;12F]	MCI = 28.15 ± 2.1					
Li et al., 2009	CG = 9 (65.2 ± 7.2) [4M;5F]	CG = 28.8 ± 0.9	DSM-IV	Three color names with three color inks (Leung et al., 2000).	AD<CG	AD>CG	–
	AD = 10 (65.8 ± 6.1) [5M;5F]	AD = 16.7 ± 2.6					
	MCI = 9 (63.4 ± 4.6) [5M;4F]	MCI = 26.4 ± 4.2					
Duchek et al., 2009	CG Older = 220 (71.75 ± 8.31)	CG Older = 29.09 ± 1.17	NINCDS-ADRDA	Two conditions: color word (*incongruent*) and neutral word (*congruent*) with ink color. Naming the ink of the word.	–	–	Congruent Effect RT [RT Congr- RT Incongr]: AD>CG
	Very Mild AD = 71 (75.25 ± 7.68)*	Very Mild AD = 26.95 ± 2.36					Congruent Effect ACC [Error Congr- Error Incongr]: AD>CG
Bélanger et al., 2010	CG Young = 20 (23.9 ± 4.7)	CG Young	NINCDS-ADRDA	Stroop version 1) Neutral Condition and Incongruent condition (two different blocks).	AD<CG	AD>CG	–
	CG Older = 20 (71.10 ± 7.5)	n.a		Stroop version 2 Congruent Condition and Incongruent Condition (mixed block: 75% congruent, 25% incongruent).			
	AD = 11 (75 ± 6.4)	CG Older 28.8 ± 1.4					
	MCI = 20 (72.7 ± 6.8)	AD 23.4 ± 3.7					
		MCI 27.4 ± 2.1					
Hutchison et al., 2010	Older CG = 64 (77.24 ± 9.80)	Older CG = 29.19	CDR	*Stroop Switch Task* [pc version; stimuli from (Spieler et al., 1996)]. Color word or neutral word. No congruent trial. Participant switching the responding (or color of the ink or reading of the word).	AD<CG	AD>CG	–

(Continued)

TABLE 2 | Continued

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
	Young CG = 30 (20.8 ± 1.5) AD = 32 (78.78 ± 5.89)	AD = 28.22					
McGuinness et al., 2010	CG = 28 (70.2 ± 7.9)	CG >28	NINCDS-ADRDA	Three conditions: Color (cross), Word (Black Ink), Color-Word (incongruent). Naming the ink of the word (Golden and Freshwater, 1978).	Color: AD<CG	–	–
	AD = 75 (77.7 ± 6.9) [24M;52F] VaD = 46 (75.9 ± 7.8) [22M;24F]	AD and VaD >12	NINDS- AIREN for Vascular Dementia		Word: AD<CG Color-Word: AD<CG		
Tse et al., 2010	CG = 246 (71.77±7.71)	CDR 0.5 = Very Mild AD	NINCDS-ADRDA	One condition: Color-Word, *congruent* (color and word are the same), *incongruent* (color and word are different), *neutral* (words are not color name). Naming the color ink.	.	.	Stroop-Effect [%errors incongruent trials- % errors congruent trials]= AD>CG
	Young CG = 32 (20.31±1.12) Very Mild AD = 74 (75.82 ± 781)*						Stroop-Effect [RT incongruent trials- RT congruent trials]= AD = CG
Coubard et al., 2011	CG Older = 17 (77.65 ± 7.72) [10M;7F]	–	–	Three conditions: Color (cross), Word (black ink), Color-Word (*incongruent* ink: suppressing the reading, naming the ink) (Godefroy, 2008).	–	–	Interference ratio n. correct responses in C-W condition/n. correct responses in C condition: AD = CG.
	CG Young = 18 (25.36±2.78) [8M;10F] AD = 17 (78.68±6.15) [3M;14F]						Interference error rate [% errors C-W condition- %errors C condition]: AD = CG
Li et al., 2011	CG = 8 (66) [3M;5F]	CG = 28.7	–	Three conditions: Word, Color, Color-Word (Stroop, 1935).	AD<CG		Interference Effect: AD>CG
	AD = 6 (68) [3M;3F] SVD =6 (66) [4M;2F]	Demented = 20.4					
Yun et al., 2011	CG = 54 (70.4±5.7)[66.7%F]	CG = 26.8±2.3	NINCDS-ADRDA	Three conditions: Word, Color, Color-Word. Number of responses in 45 s (Golden and Freshwater, 2002).	–	–	Interference Indices: AD worse than CG.
	AD = 136 (70.2±8.4) [75%F]	AD = 16.6±5.1	DSM-IV				

(Continued)

TABLE 2 | Continued

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
Stawarczyk et al., 2012	Study2	MMSE > 21	NINCDS-ADRDA	Three sets of stimuli: color string (neutral; %%%), congruent stimuli (ink and word same color), incongruent stimuli (ink and word different color); participant were asked to say aloud quickly and accurately as possible the name of the ink. Four conditions: Congruent, Incongruent, Positively Primed Congruent trials, Negatively Primed Incongruent Trials (Hogge et al., 2008).	Neutral: AD = CG	Neutral: AD = CG	Interferent effect RT [RT Neutral Condition- RT Interference Condition]: AD = CG
	CG = 16 (76.6 ± 10.6) [7M;9F]				Interferent: AD<CG	Interferent: AD = CG	Interferent effect ACC [ACC Neutral Condition- ACC Interference Condition]: AD>CG
	MildAD = 16 (75.3 ± 10.3) [7M;9F]						Negative Priming Effect [Negatively Primed Incongruent Trials RT- Positively Congruent Trials RT]
Chen et al., 2013	CG = 100 (75.4 ± 7.3) [68M;32F]	CG = 28.4 ± 1.7	NINCDS-ADRDA	Correct responses in 120 s of the task.	AD<CG	–	–
	aMCI = 120 (78.2 ± 7.7) [82M;38F]	aMCI = 26.6 ± 1.4	Petersen et al. (1999) Criteria For MCI				
	AD = 126 (78.9 ± 5.5) [88M;38F]	AD = 20.2 ± 3.6					
El Haj et al., 2013	CG Young = 18 (21.78 ± 3.56) [6M;12F]	CG Young n.a.	NINCDS-ADRDA	[pc-version]. Word, Color, Color-Word (*interference:* only incongruent trials).	–	–	Interference Effect: [Interference TR- (mean Color and Word TR)]: AD >CG
	CG Older = 18 (73.28 ± 6.35) [6M;12F]	CG Older = 28.28 ± 1.32	in the mild stage of severity dementia				
	AD = 18 (76.11 ± 5.92) [5M;13F]	AD = 23.23 ± 1.59					
Peltsch et al., 2014	CG = 72 (73 ± 6) [22M;50F]	CG = 29 ± 1	NINCDS- ADRDA	Two conditions: Color and Color-word.	No Cov: AD>CG Cov: AD<CG	–	–
	aMCI = 22 (76 ± 8) [10M;12F]	aMCI = 27 ± 2					
	Mild AD = 24 (76 ± 8) [9M;15F]	Mild AD = 27 ± 2					
Sánchez-Benavides et al., 2014	CG = 356 (64.9 ± 9.3) [144M;212F]	CG = 28.7 ± 1.5	NINCDS- ADRDA	Word, Color, Color-Word.	Color: AD<CG		

(Continued)

TABLE 2 | Continued

Studies	Participant N (mean age ± *SD*)	MMSEa	Diagnostic criteria for Adb	Stroop task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
	MCI = 79 (72.8 ± 6.5) [34M;45F] AD = 100 (74.4 ± 7.5) [35M;65F]	MCI = 25.7 ± 2.2 AD = 20.2 ± 4.0			Word: AD<CG Color-Word: AD<CG		
Huang et al., 2017	CG = 31 (76.5 ± 5.9) [45.2%F]	CG = 27.0 ± 1.2	NINCDS- ADRDA	One condition: *incongruent* (naming the ink of the color word) (Golden and Freshwater, 2002).	AD<CG	–	–
	Mild AD = 31 (78.9 ± 6.3) [64.5%F]	Mild AD = 21.2 ± 3.2	DSM-IV-TR CDR				

**There is a significant difference between groups. MMSE, Mini Mental State Examination; AD, Alzheimer Disease; CG, Control Group; DRS, Dementia Rating Scale; BDS, Blessed Dementia Scale; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; RT, Reaction Time; C-W, Naming incongruent ink color on the Stroop Task (Interference task); C, Naming Color Task on the Stroop Task; CG-I, Control Group Interference task; AD-I, Alzheimer Disease Interference task; CG-R, Control Group Reverse task; AD-R, Alzheimer Disease Reverse task; N/A, Data Not Available; MCI, Mild Cognitive Impairment; ACC, Accuracy; FTD, Frontotemporal Dementia; CDR, Clinical Dementia Rating Scale; VaD, Vascular Dementia; SVD, Subcortical Vascular Dementia.*

through the Stroop Task. The most commonly used is the Interference Effect Index, generally calculated through the ratio or subtraction of the performance values (on reaction times or Accuracy) between the Neutral condition (Color or Word) and the interference condition (Color-Word). The results showed a higher interference effect in the patients with AD groups than in the healthy elderly people (Fisher et al., 1990; Levinoff et al., 2004; Duong et al., 2006; Collette et al., 2007; Li et al., 2009; Tse et al., 2010; Yun et al., 2011; Stawarczyk et al., 2012). The interference effect can also be considered as a measure of inhibition, and patients with AD would exhibit lower inhibitory ability than healthy participants (Amieva et al., 2004; Belleville et al., 2006). In addition, some studies have evaluated additional indices and effects: among these, Spieler and colleagues (Spieler et al., 1996) have identified a higher facilitator effect in patients with AD in the congruent trials of the Stroop Task; while Amieva and collaborators (Amieva et al., 2004) have shown a higher difficulty of patients with Alzheimer's disease in inhibiting previously imposed rules.

Wisconsin Card Sorting Test

The systematic research allowed highlighting 14 studies (see **Table 3**) that used the WCST to investigate the executive function in patients with Alzheimer's disease. For this purpose, different versions of the test were used.

Almost all the studies used the diagnostic criteria NINCDS-ADRDA (McKhann et al., 1984) for the probable diagnosis of the AD. Hart et al. (1988) used non-specific criteria and the DRS score to define the AD; Bhutani et al. (1992) took into consideration the criteria of the Cambridge Diagnostic Examination of the Elderly (CAMDEX) (Roth et al., 1986); Chiu et al. (2014) used the criteria of the National Institutes of Health and the Alzheimer's Association, published revised guidelines (NIA-AA) (McKhann et al., 2011). Also, in this case, the participants were tested with the MMSE or DRS to verify the level of cognitive decline, except Chen et al. (2009) who took into account the IQ score to classify patients with the AD.

Concerning the characteristics of the groups, most of the studies matched healthy controls with patients with AD disease about age (considering an average age of over 65) and education. Redondo et al. (2016) and Peltsch et al. (2014) paired patients and healthy people only by considering years of education, groups of patients with AD were older than controls. In the Chiu study (2013), the AD group had lower education than healthy adults. Finally, the study by Kugo et al. (2007) considered patients with AD older and with lower levels of education compared to healthy controls.

Some of these studies compared the WCST performance of patients with the AD with that of patients with MCI, as well as with the healthy elderly group (Tei et al., 1997; Kugo et al., 2007; Chen et al., 2009; Chiu et al., 2014; Peltsch et al., 2014).

To evaluate performance in WCST, all studies used at least two of the following scores: number of completed categories, perseverative errors, total errors, and non-perseverative errors.

The results showed that patients with Alzheimer's disease complete fewer categories (Bondi et al., 1993; Paulsen et al., 1995; Paolo et al., 1996; Stokholm et al., 2006; Kugo et al., 2007; Chen

TABLE 3 | Some characteristics of the studies that have used the Wisconsin Card Sorting Test to assess executive dysfunction in patients with Alzheimer's disease.

Studies	Participant N (mean age ± *SD*)	MMSE	Diagnostic criteria for AD	WCST typologies	Differences between AD group and CG group
Hart et al., 1988	CG = 18 (70.6 ± 5.2) [78%F]	CDR	Non-specific criteria	mWCST (modified version: 72 cards): no ambiguous cards; after six correct responses there was the category shifting; no information about the shift in sorting (Nelson, 1976).	Achieved Categories: AD<CG
	DEP = 17 (69.3 ± 6.2) [65%F]				Number of Errors: AD>CG
	Mild AD = 18 (71.6 ± 6.4) [76%F]				Perseverative Errors: AD>CG
	Moderate AD = 16 (72.1 ± 6.8)				
Bhutani et al., 1992	CG = 12 (63.7 ± 8.27)	CG = 28. 8 ± 1.64	CAMBEX	WCST (original version: 128 cards) (Milner, 1963).	Achieved Categories: AD = CG
	MinimallyAD =11 (71.5 ± 7.97)	Minimally AD = 25.5 ± 3.69			Perseverative Errors: AD = CG
	Mildly AD = 6 (74 ± 12.88)	Mildly AD = 18.7 ± 2.73			Non-Perseverative Errors: AD = CG
	Moderately AD = 8 (79.7 ± 6.45)	Moderately AD = 11.81 ± 3.95			
Bondi et al., 1993	CG = 75 (71.1 ± 7.6) [48F]	CG = 28.9 ± 1.2	NINCDS-ADRDA	mWCST (modified version: 48 cards): no ambiguous cards; after six correct responses subject was informed of a shift in sorting principles (Nelson, 1976).	Achieved Categories: AD<CG
	Mild AD = 23 (72.7 ± 5.9) [12F]	Mild AD = 23.9 ± 2.3			Perseverative Errors: AD>CG
	Moderate AD = 33 (72.3 ± 5.9) [20F]	Moderate AD = 21.2 ± 2.9			Non-Perseverative Errors: AD>CG
	Severe AD = 31 (71.8 ± 7.9) [14F]	Severe AD = 17.8 ± 3.7			
Paulsen et al., 1995	CG MiddleAge = 20 (49.7 ± 13.9) [10M;10F]	DRS Middle Age CG = 141.3 ± 2.7	NINCDS-ADRDA	mWCST (modified version: 48 cards): no ambiguous cards; after six correct responses subject was informed of a shift in sorting principles (Nelson, 1976).	Achieved Categories: AD<CG
	Elderly CG = 20 (69.7 ± 8.2) [10M;10F] AD = 20 (70.0 ± 6.9) [10M;10]	Elderly CG = 140.1 ± 2.3			Correct Responses: AD<CG
	HD = 20 (69.7 ± 8.2) [12M;8F]	AD = 121.4 ± 8.6			Perseverative Errors: AD>CG
		HD = 120.3 ± 9.6			
Paolo et al., 1996	CG = 35 (71.34 ± 7.73) [22M;13F]	DRS	NINCDS-ADRDA	WCST-64: classical WCST rules, but in a short version (Heaton, 1981).	Achieved Categories, Trials to complete the 1 st category, Number of Errors, Perseverative Responses, Perseverative Errors, Non-Perseverative Errors, Percent Conceptual Level Response: AD worse than CG.
	PDN = 35 (70.51 ± 5.55) [22M;13F]				Failure to Maintain Set: AD = CG.
	PDD = 35 (71.77 ± 6.31) [22M;13F]				

(Continued)

TABLE 3 | Continued

Studies	Participant N (mean age ± SD)	MMSE	Diagnostic criteria for AD	WCST Typologies	Differences between AD group and CG group
	AD = 35 (71.06 ± 5.75) [22M;13F]				
Tei et al., 1997	CG= 30 (65.1±8.2)	CG = 29.1±0.6	NINCDS-ADRDA MSI = Hacinski ischemic score >7	WCST (original version: 128 cards) (Milner, 1963).	Achieved Categories: AD<CG
	AD = 22 (67.3±9.1)	AD = 23.6±1.4			Perseverative Errors: AD >CG
	MCI = 22 (68.6±7.3)	MCI = 24.6±2.0			
Nagahama et al., 2003	CG = 22 (70.8 ± 9.1)	CG = 29.1 ± 0.8	NINCDS- ADRDA	mWCST (computer version) 48 cards no ambiguous cards; participant was informed of the three possible categories before testing; after six correct responses there was the category shifting; no information about the shift in sorting (Nelson, 1976; Jenkins and Parsons, 1978).	Total Errors, Trials to complete the 1stcategory, Perseverative Errors: AD>CG;
	AD = 54 (74.2 ± 5.1)	AD = 20.8 ± 3.3	DSM-III-R		Achieved Categories, Non-perseverative Errors, Conceptual level responses: AD<CG.
	MCI= 17 (72.8 ± 5.4)	MCI = 26.4 ± 2.0			
Stokholm et al., 2006	CG = 32 (74.3 ± 4.2)	CG = 29.3 ± 0.9	NINCDS- ADRDA	mWCST (modified version: 48 cards): no ambiguous cards; after six correct responses subject was informed of a shift in sorting principles (Nelson, 1976).	Achieved Categories: AD>CG
	AD = 36 (76 ± 5.6)	AD = 25 ± 1.5			Number of Errors: AD = CG
					Perseverative Errors: AD = CG
Kugo et al., 2007	CG = 25 (63.7 ± 2.4) [12M;13F]	CG = 28.2 ± 1.9	NINCDS- ADRDA	KWCST (Keio version): participant was informed about the presences of three categories (Abe et al., 2004).	Achieved Categories: AD>CG
	AD = 58 (75.3 ± 7.8) [14M;44F]	AD = 19.3 ± 4.1	CDR		Perseverative Errors: AD = CG
	VaD = 24 (75.1 ± 9.3) [10M;14F]	VaD = 20.7 ± 4.7			
	FTD= 23 (64.7 ± 9.5) [9M;14F]	FTD = 19.6 ± 5.9			
Chen et al., 2009	CG = 16 (69 ± 8.4) [9M;7F]	CDR	NINCDS-ADRDA	mWCST (modified version: 48 cards): no ambiguous cards; after six correct responses subject was informed of a shift in sorting principles (Nelson, 1976).	Achieved Categories: AD<CG
	Early AD = 11 (76.7 ± 8.5) [7M;4F]		Mayo Clinic Criteria for aMCI		Perseverative Errors: AD>CG
	aMCI = 13 (73.2 ± 9.3) [8M;5F]				
Chiu et al., 2014	CG = 30 (64.4 ± 9.5) [13M;17F]	CG = 28.8 ± 1.6	NIA-AA	WCST (original version: 128 cards) (Milner, 1963).	Achieved Categories: AD<CG
	Early AD = 10 (69.3 ± 9.4) [4M;6F]	Early AD = 22.7 ± 3.6			Perseverative Errors: AD = CG
	MCI = 20 (71.2 ± 9.7) [9M;11F]	MCI = 26.3 ±2.7			
Peltsch et al., 2014	CG = 72 (73±6) [22M;50F]	CG = 29 ± 1	NINCDS-ADRDA	WCST (original version: 128 cards) (Milner, 1963).	% Errors: AD>CG
	aMCI = 22 (76±8) [10M;12F]	aMCI = 27 ± 2			
	Mild AD = 24 (76±8) [9M;15F]	Mild AD = 27 ± 2			

(Continued)

TABLE 3 | Continued

Studies	Participant N (mean age ± SD)	MMSE	Diagnostic criteria for AD	WCST Typologies	Differences between AD group and CG group
Huang et al., 2017	CG = 31 (76.5 ± 5.9) [45.2%F]	CG = 27.0 ± 1.2	NINCDS- ADRDA	WCST-64: classical WCST rules, but in a short version. (Heaton, 1981; Kongs et al., 2000).	Achieved Categories: AD = CG
	Mild AD = 31 (78.9 ± 6.3) [64.5%F]	Mild AD = 21.2 ± 3.2	DSM-IV-TR CDR		Perseverative Errors: AD = CG
Redondo et al., 2016	CG = 23 (70.92 ± 4.25) [11M;12F]	CG = 28±1.61	NINCDS-ADRDA	WCST (original version: 128 cards) (Milner, 1963).	% perseverative responses: AD>CG
	DiabetesG = 20 (70.82 ± 3.55) [12M;8F]	DiabetesG = 26.57 ±1.95			% perseverative errors: AD>CG
	AD = 22 (77.74 ± 3.90) [16M;6F]	AD = 23.71±4.25			

MMSE, Mini-Mental State Examination; AD, Alzheimer Disease Group; WCST, Wisconsin Card Sorting Test; CG, Control Group; DEP, Depression Group; CDR, Clinical Dementia Rating Scale; mWCST, Modified version of the Wisconsin Card Sorting Test; CAMBEX, Cambridge Diagnostic Examination of the Elderly; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; HD, Huntington's Disease; DRS, Dementia Rating Scale; PDN, Parkinson Disease without Dementia; PDD, Parkinson Disease with Dementia; MCI, Mild Cognitive Impairment; VaD, Vascular Dementia; FTD, Frontotemporal Dementia; KWCST, Keio Version of the Wisconsin Card Sorting Test; aMCI, Amnestic Mild Cognitive Impairment; NIA-AA, National Institute on Aging and Alzheimer's Association.

et al., 2009; Chiu et al., 2014) and commit more perseverative errors than healthy people (Hart et al., 1988; Bondi et al., 1993; Paulsen et al., 1995; Nagahama et al., 2003; Kugo et al., 2007; Chen et al., 2009; Redondo et al., 2016). Nevertheless, five studies (Bhutani et al., 1992; Tei et al., 1997; Stokholm et al., 2006; Chiu et al., 2014; Peltsch et al., 2014; Huang et al., 2017) do not identify differences between the numbers of perseverative errors committed by patients compared to those of the control group. Some of these studies (Bhutani et al., 1992; Tei et al., 1997; Peltsch et al., 2014; Huang et al., 2017) did not identify any difference between patients with the AD and the control group even when they considered the number of completed categories.

Redondo et al. (2016) identified a higher percentage of both perseverative errors and perseverative responses in AD patients compared to healthy older; while Nagahama et al. (2003) and Bhutani et al. (1992) showed fewer non-perseverative errors in patients with the AD than in healthy patients. This result could indicate a poor set-shifting capacity in patients affected by Alzheimer's disease that is expressed through a general perseveration of the responses.

Go/No-Go Task

To analyse the motor inhibition in patients with Alzheimer's disease, four studies (see **Table 4**) used different versions of the Go/No-Go Task. All the studies used the NINCDS-ADRDA diagnostic criteria for diagnosis (McKhann et al., 1984). All participants (mean age over 65 years in both groups) underwent an assessment of cognitive decline through MMSE (Amieva et al., 2002; Stawarczyk et al., 2012; Rochat et al., 2013) or DRS, (Collette et al., 2007) which confirmed the presence of a higher decline in patients with AD compared to healthy controls. Further, all studies (Amieva et al., 2002; Collette et al., 2007; Stawarczyk et al., 2012; Rochat et al., 2013) used a group of healthy elderly people matched by age and education to the patient group with the AD.

The accuracy analysis was evaluated in all studies by considering the number of errors in the No-Go trials (false alarms). Responding to the No-Go stimulus, in fact, is viewed as an error due to impulsivity. Concerning this result, Rochat et al. (2013) shows a higher number of false alarms in the AD group compared to healthy older adults. The other studies (Amieva et al., 2002; Collette et al., 2007; Stawarczyk et al., 2012) did not reveal any difference between patients and controls in the motor inhibition.

Reaction times analysis, in the Go trials, was used to evaluate the global processing speed (Amieva et al., 2002; Collette et al., 2007; Stawarczyk et al., 2012; Rochat et al., 2013). In particular, Collette et al. (2007) and Amieva et al. (2002) confirm a slower performance of patients with AD compared to controls, while Stawarczyk et al. (2012) and Rochat et al. (2013) do not report significant differences between the groups.

The study by Stawarczyk et al. (2012) also analyzed the preservation of inhibitory control through the analysis of reaction times. However, they do not show any difference between controls and patients with Alzheimer's disease. Moreover, Rochat et al. (2013) also considered the Counting coefficient (Standard Deviation/Mean reaction times of the Go Trial) and observed a higher score in patients with AD compared to healthy people, indicating a worse performance to the task.

Overall, the studies that used the Go/No-Go task to assess the motor inhibitory control of patients with Alzheimer's dementia would seem to show a specific heterogeneity in the results. Two of the four studies analyzed (Amieva et al., 2002; Collette et al., 2007) tend to show a general slowdown in response times, which would not indicate a specific deficit in motor inhibition in this task. In contrast, only the study by Rochat and colleagues (Rochat et al., 2013) indicates the presence of an evident executive-motor

TABLE 4 | Some characteristics of the studies that have used the Go/No-Go Task to assess executive dysfunction in patients with Alzheimer's disease.

Studies	Participant N (mean age ± *SD*)	MMSE	Diagnostic criteria for AD	Go/No-Go task typologies	Differences between AD group and CG group		
					Accuracy (No-Go)	Reaction time (Go)	Other indices
Amieva et al., 2002	CG = 28 (75.2 ± 6.6)	CG = 27.6 ± 1.8	NINCDS-ADRDA	*Go Trial (*red circle), *No-Go Trial* (blue triangle). Two errors: (1) wrongly remove the hand from starting point; (2) replace the hand or continue the action touching the No-Go stimulus. Four Data: RT latencies (Go Trial), Reaching time, I error score; II error score.	AD = CG	AD>CG	N/D[1]
	Mild AD = 28 (75.8 ± 6.1)	Mild AD = 24.6 ± 1.9					
Collette et al., 2007	CG = 28 (70.6 ± 6.8) [13M;15F]	DRS	NINCDS-ADRDA	*Go Trial* (red circle), *No-Go Trial* (blue triangle). Two errors: 1) wrongly remove the hand from starting point; (2) replace the hand or continue the action touching the No-Go stimulus. Four Data: RT latencies (Go Trial), Reaching time, I error score; II error score.	AD = CG	AD>CG	N/D
	AD = 25 (72.5 ± 5.8) [18M;17F]						
	FTD = 13 (65.7 ± 7.5) [5M;8F]						
Stawarczyk et al., 2012	Study2	MMSE > 21	NINCDS-ADRDA	Two conditions:	AD = CG	AD = CG	N/D
	CG = 16 (76.6 ± 10.6) [7M;9F]			*Simple RT task* (to a stimulus);			
	MildAD = 16 (75.3 ± 10.3) [7M;9F]			2) *Go/No-Go task* (responding as rapid as possible to the previous stimuli but not response to other). Two Data: RT of Go Trials; Accuracy of No-Go Trials (Zimmerman and Fimm, 1994).			
Rochat et al., 2013	CG = 30 (72.05±7.5)	CG = 27.87±1.61	NINCDS-ADRDA	SART: response to a rare target (a number). Three data: (1) number of false alarms (measuring of inhibition); (2) RT of correct responses (measuring processing speed); (3) Coefficient of Variation (measuring attention) (Gay et al., 2008).	AD<CG	AD = CG	CoV: AD>CG
	AD = 30 (72.03±5.9)	AD = 23.27±3.24					

NIA-AA, National Institute on Aging and Alzheimer's Association.

TABLE 5 | Some characteristics of the studies that have used the Flanker Task to assess executive dysfunction in patients with Alzheimer's disease.

Studies	Participant N (mean age ± *SD*)	MMSE	Diagnostic criteria for AD	Flanker task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
Collette et al., 2009	CG Young = 30 (22.4) [14M;16F]	DRS	NINCDS-ADRDA	Flankers and Target are words. Four different conditions: (1) SW: flankers and target are the same word; (2) NR: flankers are neutral word; (3) SC: flankers and target are of the same category; (4) DR: flankers are the opposite category of the task (incongruent condition)*.	AD = CG	AD = CG	Interference effect: ACC: AD>CG
	CG Older = 20 (72.3 ± 5.1) [5M;15F]	CG = 139.1±3.3					Facilitator effect: ACC: AD>CG
	AD = 20 (74±5.8) [6M;14F]	AD = 119.6 ± 8.9					
Stawarczyk et al., 2012	Study2	MMSE > 21	NINCDS-ADRDA	There are a central target (letters) and four flankers (asterisks or letters). Three conditions: (1) facilitator: flankers and target are associated to the same response key; (2) neutral: flanker are asterisks; (3) interferent: target and flankers are associated to different response key.	AD = CG	AD = CG	N/D
	CG = 16 (76.6 ± 10.6) [7M;9F]						
	MildAD = 16 (75.3 ± 10.3) [7M;9F]						
Wang et al., 2013	CG = 16 (69.3±1.8) [9M;7F]	CG = 29.3 ±0.5*	NINCDS-ADRDA	Target is an arrow, Flankers are of two types (dots or arrows). Four conditions: (1) target alone, (2) congruent (flankers and target are both arrows and go to the same direction); (3) neutral (flankers are dots); (4) incongruent (flankers and target are both arrows and go to the opposite direction).	AD<CG	AD>CG	Interference effects:
	MCI = 15 (72.9 ± 1.9) [9M;6F]	MCI = 27 ± 0.5*					RT = AD>CG ACC = AD< CG
	AD = 7 (68.6 ± 2.9) [3M;4F]	AD = 21.5 ± 0.8					
Chen et al., 2017	CG = 28 (73.7 ± 5.4) [12M;16F]*	CDRMC = 0.5	NINCDS-ADRDA	Two different Flanker Task.	N/D	AD>CG	N/D

(Continued)

TABLE 5 | Continued

Studies	Participant N (mean age ± *SD*)	MMSE	Diagnostic criteria for AD	Flanker task typologies	Differences between AD group and CG group		
					Accuracy	Reaction time	Other indices
	MCI = 33 (74.9 ± 5.6) [10M;23F]* AD = 26 (79.5 ± 6.1) [10M;16F]*	AD = 1		*Simple Flanker Task*: a target (arrow) and two flankers (dots). The request is to identify the direction of the arrow. *Flanker Reaction Time Task*: a target and two flankers (arrows), with two conditions: congruent (same direction) or incongruent (opposite direction).			

**There is a significant differences between groups. MMSE, Mini-Mental State Examination; AD, Alzheimer Disease Group; CG, Control Group; DRS, Dementia Rating Scale; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders; Association; SW, Same-word condition on the Flanker Task; NR, Neutral-response condition on the Flanker Task; SC, Same-category condition on the Flanker Task; DR, Different-response condition on the Flanker Task; ACC, Accuracy (number of correct response); N/D, Data not available for this variable; MCI, Mild Cognitive Impairment; RT, Reaction Time.*

deficit linked to inhibitory control in patients with Alzheimer's disease.

Flanker Task

Four studies (Collette et al., 2009; Stawarczyk et al., 2012; Wang et al., 2013; Chen et al., 2017) used different types of flanker tasks inspired by the classic paradigm of Eriksen and Eriksen (Eriksen and Eriksen, 1974), to analyses cognitive inhibition and conflict control in patients with Alzheimer's disease (**Table 2**).

All the studies used for the probable diagnosis of the AD the diagnostic criteria NINCDS-ADRDA (McKhann et al., 1984).

In all studies, both groups (healthy elderly and elderly with Alzheimer's disease) have an average age over 65 years. For the assessment of the level of cognitive impairment, the scores at MMSE and those of the DRS were taken into consideration. Although the authors do not report in detail the scores obtained by the different groups, they still attest to higher cognitive impairment in patients with the AD. Two of the studies (Wang et al., 2013; Chen et al., 2017), compared patients with the AD with both a healthy control group and a group of subjects classified as MCI.

All the authors recorded reaction times and accuracy of responses in the Flanker Task. Chen et al. (2017) compared the performance between groups only through reaction times; this methodological choice is justified by the fact that the authors consider reaction times as the significant markers of cognitive functioning because it is more closely associated with neural functioning.

The analysis of the reaction times highlighted inconsistent results. Wang et al. (2013) and Chen et al. (2017) reported slower reaction times in patients with Alzheimer compared to healthy elderly, while Collette et al. (2009) and Stawarczyk et al. (2012) did not find significant differences between the two groups.

Analyzing accuracy, only the study by Wang et al. (2013) showed a higher percentage of errors in patients with the AD than in the control group. However, both general reaction times and accuracy give a measure of selective attention, and they do not inform about executive function.

Some of the authors (Collette et al., 2009; Wang et al., 2013) also evaluated the flanker effect index, given by the difference between incongruent and congruent trials in reaction times or accuracy. Specifically, Collette et al. (2009), considering accuracy, reported a higher flanker effect in the patients with the AD than in the control group; Wang et al. (2013) has instead recorded a higher flanker effect in patients the AD compared to the healthy people by considering both reaction times and accuracy. Moreover, Collette et al. (2009) also evaluated the facilitator effect (comparing the accuracy of the congruent trials of the same word and same category conditions to the neutral response condition of Word Flanker Task; see **Table 5**), this effect was higher in patients with the AD than in the two control groups (young adults and elderly healthy subjects).

DISCUSSION

This systematic review was aimed to verify the sensitivity of four golden standard executive functions tasks in catching

dysfunctions in these domains in the Alzheimer's disease. Because executive deficits interfere with the performance of daily life activities, by worsening the quality of life of individuals with the AD (Wecker et al., 2000; Collette et al., 2009), it is essential to take this cognitive dimension into account. It is important to note that the recognition of an impairment in the executive functions in the AD is the result of a route change, in fact until a few years ago it was believed that the executive functions were not affected in the pre-clinical stages of dementia (Broks et al., 1996; Razani et al., 2001). Today it is known that these cognitive functions are already damaged prematurely (Binetti et al., 1996; Bondi et al., 2002; Amieva et al., 2004).

A correct choice of the cognitive tasks to use for the assessment of cognitive impairment is a crucial element to take into account. Those considered in this review are the most used in the study of executive functions (Alvarez and Emory, 2006; Duchek et al., 2009; Diamond, 2013) and are widely utilized for cognitive assessment in patients with AD compared to healthy elderly or other pathologies (Collette et al., 2007; Stawarczyk et al., 2012; Peltsch et al., 2014).

They specifically evaluate the capacities of the motor (Go/No-Go Task) and cognitive inhibition (Stroop Task), the conflict control (Flanker Task), and the cognitive flexibility (WCST), the ability to suppress automatic responses and the ability to "resist" to interference (Stroop Task and Flanker task respectively); all skills affected by cognitive decline that are specially compromise in AD.

The Stroop Task seems to be the paradigm that best discriminates between healthy and pathological aging, and it is the most widely used in the research on the AD (Spieler et al., 1996; Belleville et al., 2008; Duchek et al., 2009; Bélanger et al., 2010; McGuinness et al., 2010; Stawarczyk et al., 2012; Peltsch et al., 2014). A neuropsychological assessment including this test can assess the executive system. It could highlight the individual's ability to move the patient's cognitive set, providing a measure of cognitive inhibition and attentional control, and it gives information about the ability to inhibit an overlearned response (i.e., a dominant response, such as the reading) in favor of an unusual stimulus (Spreen and Strauss, 1998). One aspect to consider about this task is that some authors (Hutchison et al., 2010) believe that memory is entailed in the resolution process involved in this task, the decline of which may compromise the overall performance and hinder the ability to focusing attention on the target.

The results obtained with the other tests were inconsistent, but it should be noted that few studies have used them. Concerning the ability of the Wisconsin Card Sorting Test to catch deficits in the cognitive flexibility of Alzheimer's disease, the results are inconsistent. The most critical aspect related to WCST is the complexity of the task. For this reason, some authors (Hart et al., 1988; Bondi et al., 1993; Paulsen et al., 1995; Stokholm et al., 2006) have introduced modified and simplified forms of this test. Contrary to the standard version, on these modified versions of the WSCT, there are fewer cards, and in some case, the subject was informed about a shift in sorting principles. Moreover, there were not ambiguous cards, and the sorting criteria changed after six correct responses. These aspects make the task easier and allow identifying, with higher sensitivity, the cognitive flexibility deficits in the AD (Bondi et al., 1993; Paulsen et al., 1995; Stokholm et al., 2006; Chen et al., 2009). If we considered the modified versions of the test, WCST seems to discriminate between AD patients and healthy subjects (Hart et al., 1988; Bondi et al., 1993; Paulsen et al., 1995; Nagahama et al., 2003; Stokholm et al., 2006; Chen et al., 2009). However, even using a simplified version of the WCST, some studies did not find clear differences between healthy elderly and patients with the AD. Bhutani et al. (1992) believe that the WCST is characterized by a "floor effect" that would not allow discriminating the normal cognitive decline and deterioration typical of dementia, an aspect also reaffirmed by Huang et al. (2017). Moreover, if we consider the studies that compared the performance of patients with the AD with those of elderly people suffering from other dementia diseases (temporal dementia, vascular dementia, Parkinson's with dementia), there are no differences, suggesting that this task not allow discriminating among different forms of dementia (Paulsen et al., 1995; Paolo et al., 1996; Kugo et al., 2007; Li et al., 2011).

The results about the Flanker Task would seem to indicate that this paradigm is not able to highlight a difference between healthy and pathological elderly, especially when reaction times are considered (Collette et al., 2009; Stawarczyk et al., 2012). The analysis of accuracy, instead, has a higher discriminating ability to indicate the actual deterioration in selective attention in patients with the AD (Collette et al., 2009; Wang et al., 2013; Chen et al., 2017). However, if we consider the only two studies that analyzed the flanker effect, both found an impaired conflict control in patients with AD compared to healthy people. The characteristics of the sample represent a weak point of these studies. Two of the four studies did not perform an exact pairing for age and education between patients with the AD and healthy controls. Moreover, the number of participants was very reduced (AD group: $N = 26$ and Control group: $N = 28$; AD group: $N = 15$ and Control group: $N = 16$) (Wang et al., 2013; Chen et al., 2017). For these reasons, and considering the few studies present, it is not possible to exclude that the Flanker Task is sensitive to catch impairment in selective attention and conflict control in Alzheimer's dementia, especially in light of the results obtained at the Stroop Task, which involves attentional aspects similar to those assessed by the Flanker Task (Baddeley and Hitch, 1994).

Also, the results relative to the Go/No-Go task did not indicate whether a deficit in the control of motor inhibition is present or not in patients with AD compared to healthy elderly. Only one study (Rochat et al., 2013) showed a higher number of false alarms in the AD group compared to healthy older adults, indicating an impaired motor inhibition in the AD. The other studies (Amieva et al., 2002; Collette et al., 2007; Stawarczyk et al., 2012) did not reveal any difference between patients and controls in the motor inhibition, suggesting that there not be in AD an executive deficit of this type. This difference between the Rochat's study and the other studies could be due to a different version of the Go/No-Go Task, that required to respond to a rare target (a number); this fact involves a harder inhibitory control ability, that could explain the difference between AD patients and elderly healthy Control Group. However, the comparison between patients with Alzheimer's

dementia and patients with different types of dementia showed no difference (Collette et al., 2007; Kugo et al., 2007), suggesting a pattern of motor inhibition common to the different types of dementia.

LIMITS AND CONCLUSIONS

This review highlighted several limitations in the examination of executive functions in Alzheimer's disease. In particular, many studies have used a numerically insufficient sample (Koss et al., 1984; Bhutani et al., 1992; Belleville et al., 2006; Chen et al., 2009; Li et al., 2009, 2011; Coubard et al., 2011; Stawarczyk et al., 2012; El Haj et al., 2013; Wang et al., 2013) others did not consider the level of education of the participants (Spieler et al., 1996; Amieva et al., 2002; Belleville et al., 2006) although this is a variable to be taken into account when analyzing executive functions (Contador et al., 2017). A further problem is related to the assessment of cognitive decline. The different studies have used different scales to evaluate the cognitive decline, and in some studies, the average scores obtained by the various groups are not reported (Paolo et al., 1996; Spieler et al., 1996; Collette et al., 2007; Coubard et al., 2011). Furthermore, the severity of the decline within the AD groups varies a lot as regards the scores at the MMSE (see **Tables 2–5**). This condition does not allow controlling the influence of these variables on the performance of the tasks used. Furthermore, some studies not matching groups by age, gender and education, and they have not always controlled other aspects (such as the severity of the AD or comorbidity diseases) that could influence task performance (Spieler et al., 1996; Amieva et al., 2002; Collette et al., 2007; Duchek et al., 2009; McGuinness et al., 2010). These dimensions indeed reduce the sensitivity of the instruments in the identification of differences between groups, especially in the case of an analysis of functions that are subject to a physiological decline with age, as also shown by some of the studies considered here that compare elderly control groups with younger control groups (Spieler et al., 1996; Bélanger et al., 2010; Hutchison et al., 2010).

Another critical point of the reviewed studies concerns the use of multiple forms of the same experimental paradigm. These miscellanea of test does not allow for an explicit comparison between the various researches. Therefore, it is difficult to arrive at definite conclusions. This aspect is particularly evident in the studies that have used the WCST. In this case, simplified versions of the test have shown a higher ability to identify differences associated with the AD (Paulsen et al., 1995; Nagahama et al., 2003; Chen et al., 2009), probably because they allow participants to overcome the floor effect.

Concerning the Go/No-Go and the Flanker task, the results were weak in light of the few studies found. Therefore, the systematic use of these tests would be useful, to verify their sensitivity to capture deficits in conflict control and motor inhibition in the AD.

However, regardless of these limitations, at the end of this review emerges an effective executive deficit in the inhibitory control (Chen et al., 2013; Peltsch et al., 2014; Sánchez-Benavides et al., 2014; Huang et al., 2017), and partly also in the cognitive flexibility (Paulsen et al., 1995; Stokholm et al., 2006; Chiu et al., 2014) in the AD. These results allow us to suggest a plausible identikit of executive functioning in Alzheimer's disease, characterized by an impairment in inhibition and cognitive flexibility. There are differences in performance in the various tasks, which could reflect, as previously underlying, differences in the levels of deterioration of the various executive functions analyzed during the AD progression.

In the light of the results of this review that showed a more or less marked discriminatory capacity of the examined tasks for the identification of the executive deficits in Alzheimer's disease, it would be advisable to insert these tasks within neuropsychological batteries. This could allow investigating more entirely and articulately the cognitive functioning of patients affected by the AD.

Overall, this review highlighted the importance of a comprehensive neuropsychological evaluation to allow a clear delineation of the aging profile associated with Alzheimer's disease.

An evaluation of this type could be inserted into pathological aging prevention programs, and it could be useful as a form of monitoring of executive functioning in aging. Furthermore, it could allow identifying the presence of even slight deficits, such as a Mild Cognitive Impairment, that could predict a degenerative disease like the AD. MCI and AD have different diagnostic criteria and different levels of cognitive impairment, for these reasons MCI was not considered in this work. However, it could be useful to conduct a systematic review taking into account the executive performance in MCI specifically.

The results of this review would be to decline it in a meta-analysis that could allow to better understanding the profile of executive functions in the Alzheimer's disease. Furthermore, it would be advisable to carry out a comparative analysis of the different experimental paradigms used to investigate the individual executive functions. This comparison could allow to a better understanding of the results obtained in this work, consenting to conclude whether the results are univocal regardless of the task used or if there is an effect of the type of the task that reinforces or weakens the conclusions to which this review has arrived.

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REFERENCES

Abe, M., Suzuki, K., Okada, K., Miura, R., Fujii, T., Etsurou, M., et al. (2004). Normative data on tests for frontal lobe functions: trail making test, verbal fluency, Wisconsin card sorting test (Keio version). *No to shinkei* 56, 567–574.

Allain, P., Etcharry-Bouyx, F., and Verny, C. (2013). Executive functions in clinical and preclinical Alzheimer's disease. *Rev. Neurol.* 169, 695–708. doi: 10.1016/j.neurol.2013.07.020

Alvarez, J. A., and Emory, E. (2006). Executive function and the frontal lobes: a meta-analytic review. *Neuropsychol. Rev.* 16, 17–42. doi: 10.1007/s11065-006-9002-x

Alzheimer's Association (2016). 2016 Alzheimer's disease facts and figures. *Alzheimer Dement.* 12, 459–509. doi: 10.1016/j.jalz.2016.03.001

American Psychiatric Association (1980). *Diagnostic and Statistical Manual of Mental Disorders DSM-III*. Washington, DC: APA Diagnostic.

American Psychiatric Association (1994). *Statistical Manual of Mental Disorders*. Washington, DC: APA Diagnostic.

Amieva, H., Lafont, S., Auriacombe, S., Le Carret, N., Dartigues, J. F., Orgogozo, J. M., et al. (2002). Inhibitory breakdown and dementia of the Alzheimer type: a general phenomenon. *J Clin Exp Neuropsychol.* 24, 503–516. doi: 10.1076/jcen.24.4.503.1034

Amieva, H., Lafont, S., Rouch-Leroyer, I., Rainville, C., Dartigues, J. F., Orgogozo, J. M., et al. (2004). Evidencing inhibitory deficits in Alzheimer's disease through interference effects and shifting disabilities in the Stroop test. *Arch. Clin. Neuropsychol.* 19, 791–803. doi: 10.1016/j.acn.2003.09.006

Baddeley, A. D., and Hitch, G. J. (1994). Developments in the concept of working memory. *Neuropsychology*, 8, 485.

Balota, D. A., and Faust, M. (2002). "Attention in Alzheimer's disease," in *Handbook of Neuropsychology*, Vol. 6, 2nd Edn, eds F. Boller and S. Cappa (New Brunswick, NJ: Elsevier Science), 51–80.

Bélanger, S., Belleville, S., and Gauthier, S. (2010). Inhibition impairments in Alzheimer's disease, mild cognitive impairment and healthy aging: effect of congruency proportion in a Stroop task. *Neuropsychologia* 48, 581–590. doi: 10.1016/j.neuropsychologia.2009.10.021

Belleville, S., Bherer, L., Lepage, É., Chertkow, H., and Gauthier, S. (2008). Task switching capacities in persons with Alzheimer's disease and mild cognitive impairment. *Neuropsychologia* 46, 2225–2233. doi: 10.1016/j.neuropsychologia.2008.02.012

Belleville, S., Rouleau, N., and Van der Linden, M. (2006). Use of the Hayling task to measure inhibition of prepotent responses in normal aging and Alzheimer's disease. *Brain Cogn.* 62, 113–119. doi: 10.1016/j.bandc.2006.04.006

Berardi, A. M., Parasuraman, R., and Haxby, J. V. (2005). Sustained attention in mild Alzheimer's disease. *Dev. Neuropsychol.* 28, 507–537. doi: 10.1207/s15326942dn2801_4

Berg, E. A. (1948). A simple objective technique for measuring flexibility in thinking. *J. Gen. Psychol.* 39, 15–22. doi: 10.1080/00221309.1948.9918159

Beydoun, M. A., Beydoun, H. A., Gamaldo, A. A., Teel, A., Zonderman, A. B., and Wang, Y. (2014). Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. *BMC Public Health* 14:643. doi: 10.1186/1471-2458-14-643

Bhutani, G. E., Montaldi, D., Brooks, D. N., and McCulloch, J. (1992). A neuropsychological investigation into frontal lobe involvement in dementia of the Alzheimer type. *Neuropsychology* 6:211. doi: 10.1037/0894-4105.6.3.211

Binetti, G., Magni, E., Padovani, A., Cappa, S. F., Bianchetti, A., and Trabucchi, M. (1996). Executive dysfunction in early Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatr.* 60, 91–93. doi: 10.1136/jnnp.60.1.91

Blessed, G., Tomlinson, B. E., and Roth, M. (1968). The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *Br. J. Psychiatry* 114, 797–811. doi: 10.1192/bjp.114.512.797

Boccardi, M. (2007). *La riabilitazione Nella Demenza Grave. Manuale pratico per operatori e Caregiver*. Trento: Edizioni Erickson.

Bondi, M. W., Monsch, A. U., Butters, N., Salmon, D. P., and Paulsen, J. S. (1993). Utility of a modified version of the Wisconsin Card Sorting Test in the detection of dementia of the Alzheimer type. *Clin. Neuropsychol.* 7, 161–170. doi: 10.1080/13854049308401518

Bondi, M. W., Serody, A. B., Chan, A. S., Eberson-Shumate, S. C., Delis, D. C., Hansen, L. A., et al. (2002). Cognitive and neuropathologic correlates of Stroop Color-Word Test performance in Alzheimer's disease. *Neuropsychology* 16:335. doi: 10.1037//0894-4105.16.3.335

Bracco, L., Bessi, V., Piccini, C., Mosconi, L., Pupi, A., and Sorbi, S. (2007). Metabolic correlates of executive dysfunction. *J. Neurol.* 254:1052. doi: 10.1007/s00415-006-0488-1

Broks, P., Lines, C., Atchison, L., Challenor, J., Traub, M., Foster, C., et al. (1996). Neuropsychological investigation of anterior and posterior cortical function in early-stage probable Alzheimer's disease. *Behav. Neurol.* 9, 135–148. doi: 10.3233/BEN-1996-93-405

Bullock, R., and Lane, R. (2007). Executive dyscontrol in dementia, with emphasis on subcortical pathology and the role of butyrylcholinesterase. *Curr. Alzheimer Res.* 4, 277–293. doi: 10.2174/156720507781077313

Burgess, P. W., Veitch, E., de Lacy Costello, A., and Shallice, T. (2000). The cognitive and neuroanatomical correlates of multitasking. *Neuropsychologia* 38, 848–863. doi: 10.1016/S0028-3932(99)00134-7

Chen, K. C., Weng, C. Y., Hsiao, S., Tsao, W. L., and Koo, M. (2017). Cognitive decline and slower reaction time in elderly individuals with mild cognitive impairment. *Psychogeriatrics* 17, 364–370. doi: 10.1111/psyg.12247

Chen, N. C., Chang, C. C., Lin, K. N., Huang, C. W., Chang, W. N., Chang, Y. T., et al. (2013). Patterns of executive dysfunction in amnestic mild cognitive impairment. *Int. Psychogeriatr.* 25, 1181–1189. doi: 10.1017/S1041610213000392

Chen, T. F., Chen, Y. F., Cheng, T. W., Hua, M. S., Liu, H. M., and Chiu, M. J. (2009). Executive dysfunction and periventricular diffusion tensor changes in amnesic mild cognitive impairment and early Alzheimer's disease. *Hum. Brain Mapp.* 30, 3826–3836. doi: 10.1002/hbm.20810

Chiu, M. J., Chen, Y. F., Chen, T. F., Yang, S. Y., Yang, F. P. G., Tseng, T. W., et al. (2014). Plasma tau as a window to the brain—negative associations with brain volume and memory function in mild cognitive impairment and early alzheimer's disease. *Hum. Brain Mapp.* 35, 3132–3142. doi: 10.1002/hbm.22390

Cohen, A., and Shoup, R. (1997). Perceptual dimensional constraints in response selection processes. *Cogn. Psychol.* 32, 128–181. doi: 10.1006/cogp.1997.0648

Collette, F., Amieva, H., Adam, S., Hogge, M., Van der Linden, M., Fabrigoule, C., et al. (2007). Comparison of inhibitory functioning in mild Alzheimer's disease and frontotemporal dementia. *Cortex* 43, 866–874. doi: 10.1016/S0010-9452(08)70686-5

Collette, F., Schmidt, C., Scherrer, C., Adam, S., and Salmon, E. (2009). Specificity of inhibitory deficits in normal aging and Alzheimer's disease. *Neurobiol. Aging* 30, 875–889. doi: 10.1016/j.neurobiolaging.2007.09.007

Contador, I., del Ser, T., Llamas, S., Villarejo, A., Benito-León, J., and Bermejo-Pareja, F. (2017). Impact of literacy and years of education on the diagnosis of dementia: A population-based study. *J. Clin. Exp. Neuropsychol.* 39, 112–119. doi: 10.1080/13803395.2016.1204992

Coubard, O. A., Ferrufino, L., Boura, M., Gripon, A., Renaud, M., and Bherer, L. (2011). Attentional control in normal aging and Alzheimer's disease. *Neuropsychology* 25:353. doi: 10.1037/a0022058

Cronin-Golomb, A., and Amick, M. (2001). Spatial abilities in aging, Alzheimer's disease and Parkinson's disease," in *Handbook of Neuropsychology*, Vol. 6, 2nd Edn, eds F. Boller and S. Cappa (Amsterdam: Elsevier), 119–143.

Cronin-Golomb, A., Gilmore, G. C., Neargarder, S., Morrison, S. R., and Laudate, T. M. (2007). Enhanced stimulus strength improves visual cognition in aging and Alzheimer's disease. *Cortex* 43, 952–966. doi: 10.1016/S0010-9452(08)70693-2

Damasio, A. R. (1995). REVIEW: toward a neurobiology of emotion and feeling: operational concepts and hypotheses. *Neuroscientist* 1, 19–25. doi: 10.1177/107385849500100104

Dening, T., and Sandilyan, M. B. (2015). Dementia: definitions and types. *Nurs. Stand.* 29:37. doi: 10.7748/ns.29.37.37.e9405

Diamond, A. (2013). Executive functions. *Annu. Rev. Psychol.* 64, 135–168. doi: 10.1146/annurev-psych-113011-143750

Doninger, N. A., and Bylsma, F. W. (2007). Inhibitory control and affective valence processing in dementia of the Alzheimer type. *J. Neuropsychol.* 1, 65–83. doi: 10.1348/174866407X.180828

Du, A. T., Schuff, N., Amend, D., Laakso, M. P., Hsu, Y. Y., Jagust, W. J., et al. (2001). Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatr.* 71, 441–447. doi: 10.1136/jnnp.71.4.441

Duchek, J. M., Balota, D. A., Tse, C. S., Holtzman, D. M., Fagan, A. M., and Goate, A. M. (2009). The utility of intraindividual variability in selective attention tasks as an early marker for Alzheimer's disease. *Neuropsychology* 23:746. doi: 10.1037/a0016583

Duong, A., Whitehead, V., Hanratty, K., and Chertkow, H. (2006). The nature of lexico-semantic processing deficits in mild cognitive impairment. *Neuropsychologia* 44, 1928–1935. doi: 10.1016/j.neuropsychologia.2006.01.034

El Haj, M., Postal, V., and Allain, P. (2013). Destination memory in Alzheimer's disease: when i imagine telling ronald reagan about Paris. *Cortex* 49, 82–89. doi: 10.1016/j.cortex.2011.11.014

Eriksen, B. A., and Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Percept. Psychophys.* 16, 143–149. doi: 10.3758/BF03203267

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

Fisher, L. M., Freed, D. M., and Corkin, S. (1990). Stroop Color-Word Test performance in patients with Alzheimer's disease. *J. Clin. Exp. Neuropsychol.* 12, 745–758. doi: 10.1080/01688639008401016

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.

Förstl, H., and Kurz, A. (1999). Clinical features of Alzheimer's disease. *Eur. Arch. Psychiatry Clin. Neurosci. Eur.* 249, 288–290.

Gay, P., Rochat, L., Billieux, J., d'Acremont, M., and Van der Linden, M. (2008). Heterogeneous inhibition processes involved in different facets of self-reported impulsivity: Evidence from a community sample. *Acta Psychol.* 129, 332–339. doi: 10.1016/j.actpsy.2008.08.010

Godefroy, O. (2008). *Fonctions Exécutives et Pathologies Neurologiques et Psychiatriques: Évaluation en Pratique Clinique*. Groupe de Boeck.

Golden, C. J. (1976). Identification of brain disorders by the Stroop Color and Word Test. *J. Clin. Psychol.* 32, 654–658. doi: 10.1002/1097-4679(197607)32:3<654::AID-JCLP2270320336>3.0.CO;2-Z

Golden, C. J., and Freshwater, S. M. (1978). *Stroop Color and Word Test: A Manual for Clinical and Experimental Uses*. Wood Dale: Stoelting Company.

Golden, C. J., and Freshwater, S. M. (2002). The Stroop Color and Word Test: A Manual for Clinical and Experimental Uses. Chicago: Stoelting.

Grafman, J., and Litvan, I. (1999). Importance of deficits in executive functions. *Lancet* 354, 1921–1923. doi: 10.1016/S0140-6736(99)90438-5

Grant, D. A., and Berg, E. (1948). A behavioral analysis of degree of reinforcement and ease of shifting to new responses in a Weigl-type card-sorting problem. *J. Exp. Psychol.* 38:404. doi: 10.1037/h0059831

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

Hart, R. P., Kwentus, J. A., Wade, J. B., and Taylor, J. R. (1988). Modified Wisconsin Sorting Test in elderly normal, depressed and demented patients. *Clin. Neuropsychol.* 2, 49–56. doi: 10.1080/13854048808520085

Heaton, R. K. (1981). *Wisconsin Card Sorting Test manual*. Odessa, FL: Psychological Assessment Resources Inc.

Hebert, L. E., Weuve, J., Scherr, P. A., and Evans, D. A. (2013). Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. *Neurology* 80, 1778–1783. doi: 10.1212/WNL.0b013e31828726f5

Hodges, J. R., Erzinçliolu, S., and Patterson, K. (2006). Evolution of cognitive deficits and conversion to dementia in patients with mild cognitive impairment: a very-long-term follow-up study. *Dement. Geriatr. Cogn. Disord.* 21, 380–391. doi: 10.1159/000092534

Hogge, M., Salmon, E., and Collette, F. (2008). Interference and negative priming in normal aging and in mild Alzheimer's disease. *Psychol. Belg.* 48, 1–23. doi: 10.5334/pb-48-1-1

Huang, S. F., Liu, C. K., Chang, C. C., and Su, C. Y. (2017). Sensitivity and specificity of executive function tests for Alzheimer's disease. *Appl. Neuropsychol. Adult* 24, 493–504. doi: 10.1080/23279095.2016.1204301

Hutchison, K. A., Balota, D. A., and Ducheck, J. M. (2010). The utility of Stroop task switching as a marker for early-stage Alzheimer's disease. *Psychol. Aging* 25:545. doi: 10.1037/a0018498

Jenkins, R. L., and Parsons, O. A. (1978). Cognitive deficits in male alcoholics as measured by a modified Wisconsin Card Sorting Test. *Alcohol Tech. Reports* 7, 76–83.

Kipps, C. M., Mioshi, E., and Hodges, J. R. (2009). Emotion, social functioning and activities of daily living in frontotemporal dementia. *Neurocase*, 15, 182–189. doi: 10.1080/13554790802632892

Knapp, M., Prince, M., Albanese, E., Banerjee, S., Dhanasiri, S., Fernandez, J. L., et al. (2007). *Dementia UK: a report into the prevalence and cost of dementia prepared by the Personal social Services Research Unit (PCSSRU) at the London School of Economics and Institute of Psychiatry at King's College London for the Alzheimer's Society*. London: Alzheimer's Society.

Kongs, S. K., Thompson, L. L., Iverson, G. L., and Heaton, R. K. (2000). *Wisconsin Card Sorting Test-64 Card Version (WCST-64)*. Odessa, FL: Psychological Assessment Resources.

Koss, E., Ober, B. A., Delis, D. C., and Friedland, R. P. (1984). The Stroop color-word test: indicator of dementia severity. *Int. J. Neurosci.* 24, 53–61. doi: 10.3109/00207458409079534

Kugo, A., Terada, S., Ata, T., Ido, Y., Kado, Y., Ishihara, T., et al. (2007). Japanese version of the frontal assessment battery for dementia. *Psychiatry Res.* 153, 69–75. doi: 10.1016/j.psychres.2006.04.004

Lehto, J. E., Juujärvi, P., Kooistra, L., and Pulkkinen, L. (2003). Dimensions of executive functioning: evidence from children. *Br. J. Dev. Psychol.* 21, 59–80. doi: 10.1348/026151003321164627

Leung, H. C., Skudlarski, P., Gatenby, J. C., Peterson, B. S., and Gore, J. C. (2000). An event-related functional MRI study of the Stroop color word interference task. *Cereb. Cortex* 10, 552–560. doi: 10.1093/cercor/10.6.552

Levinoff, E. J., Li, K. Z., Murtha, S., and Chertkow, H. (2004). Selective attention impairments in Alzheimer's disease: evidence for dissociable components. *Neuropsychology* 18:580. doi: 10.1037/0894-4105.18.3.580

Levy, G., Jacobs, D. M., Tang, M. X., Côté, L. J., Louis, E. D., Alfaro, B., ., et al. (2002). Memory and executive function impairment predict dementia in Parkinson's disease. *Mov. Disord.* 17, 1221–1226. doi: 10.1002/mds.10280

Li, C., Zheng, J., Wang, J., and Gui, L. (2011). Comparison between Alzheimer's disease and subcortical vascular dementia: attentional cortex study in functional magnetic resonance imaging. *J. Int. Med. Res.* 39, 1413–1419. doi: 10.1177/147323001103900428

Li, C., Zheng, J., Wang, J., Gui, L., and Li, C. (2009). An fMRI stroop task study of prefrontal cortical function in normal aging, mild cognitive impairment, and Alzheimer's disease. *Curr. Alzheimer Res.* 6, 525–530. doi: 10.2174/156720509790147142

Loef, M., and Walach, H. (2012). The combined effects of healthy lifestyle behaviors on all cause mortality: a systematic review and meta-analysis. *Prev. Med.* 55, 163–170. doi: 10.1016/j.ypmed.2012.06.017

Luria, A. R. (1973). *The Working Brain*. London: Penguin.

Machulda, M. M., Ward, H. A., Borowski, B., Gunter, J. L., Cha, R. H., O'brien, P. C., et al. (2003). Comparison of memory fMRI response among normal, MCI, and Alzheimer's patients. *Neurology* 61, 500–506. doi: 10.1212/01.WNL.0000079052.01016.78

Mattis, S. (1988). *Dementia Rating Scale DRS: Professional Manual*. Odessa, FL: Psychological Assessment Resources.

McGaughy, J., and Sarter, M. (1995). Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology* 117, 340–357. doi: 10.1007/BF02246109

McGuinness, B., Barrett, S. L., Craig, D., Lawson, J., and Passmore, A. P. (2010). Attention deficits in Alzheimer's disease and vascular dementia. *J. Neurol. Neurosur. Psychiatry* 81, 157–159. doi: 10.1136/jnnp.2008.164483

McKee, A. C., Stein, T. D., Nowinski, C. J., Stern, R. A., Daneshvar, D. H., Alvarez, V. E., et al. (2013). The spectrum of disease in chronic traumatic encephalopathy. *Brain* 136, 43–64. doi: 10.1093/brain/aws307

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease report of the NINCDS-ADRDA work group* under the auspices of department of health

and human services task force on Alzheimer's disease. *Neurology* 34, 939–939. doi: 10.1212/WNL.34.7.939

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack Jr, C. R., Kawas, C. H., et al. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers. Dement.* 7, 263–269. doi: 10.1016/j.jalz.2011.03.005

Milner, B. (1963). Effects of different brain lesions on card sorting: the role of the frontal lobes. *Arch. Neurol.* 9, 90–100. doi: 10.1001/archneur.1963.00460070100010

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., and Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: a latent variable analysis. *Cogn. Psychol.* 41, 49–100. doi: 10.1006/cogp.1999.0734

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann. Intern. Med.* 151, 264–269. doi: 10.7326/0003-4819-151-4-200908180-00135

Morris, J. C. (1993). The clinical dementia rating (CDR): current version and scoring rules. *Neurology*. 43, 2412–2414.

Nagahama, Y., Okina, T., Suzuki, N., Matsuzaki, S., Yamauchi, H., Nabatame, H., et al. (2003). Factor structure of a modified version of the wisconsin card sorting test: an analysis of executive deficit in Alzheimer's disease and mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* 16, 103–112. doi: 10.1159/000070683

Nelson, H. E. (1976). A modified card sorting test sensitive to frontal lobe defects. *Cortex* 12, 313–324. doi: 10.1016/S0010-9452(76)80035-4

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

Paolo, A. M., Tröster, A. I., Blackwell, K. T., Koller, W. C., and Axelrod, B. N. (1996). Utility of a wisconsin card sorting test short form in persons with Alzheimer's and Parkinson's disease. *J. Clin. Exp. Neuropsychol.* 18, 892–897. doi: 10.1080/01688639608408310

Paulsen, J. S., Salmon, D. P., Monsch, A. U., Butters, N., Swenson, M. R., and Bondi, M. W. (1995). Discrimination of cortical from subcortical dementias on the basis of memory and problem-solving tests. *JCLP* 51, 48–58.

Peltsch, A., Hemraj, A., Garcia, A., and Munoz, D. P. (2014). Saccade deficits in amnestic mild cognitive impairment resemble mild Alzheimer's disease. *Eur. J. Neurosci.* 39, 2000–2013. doi: 10.1111/ejn.12617

Perry, R. J., and Hodges, J. R. (1999). Attention and executive deficits in Alzheimer's disease: a critical review. *Brain* 122, 383–404. doi: 10.1093/brain/122.3.383

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., and Kokmen, E. (1999). Mild cognitive impairment: clinical characterization and outcome. *Arch. Neurol.* 56, 303–308. doi: 10.1001/archneur.56.3.303

Prince, M., Ali, G. C., Guerchet, M., Prina, A. M., Albanese, E., and Wu, Y. T. (2016). Recent global trends in the prevalence and incidence of dementia, and survival with dementia. *Alzheimers. Res. Ther.* 8:23. doi: 10.1186/s13195-016-0188-8

Prvulovic, D., Hubl, D., Sack, A. T., Melillo, L., Maurer, K., Frölich, L., et al. (2002). Functional imaging of visuospatial processing in Alzheimer's disease. *Neuroimage* 17, 1403–1414. doi: 10.1006/nimg.2002.1271

Pucci, E. (2004). *Malattia Di Alzheimer: Manuale Per Gli Operatori, Vol. 54.* Milano: FrancoAngeli.

Ramanan, S., Bertoux, M., Flanagan, E., Irish, M., Piguet, O., Hodges, J. R., et al. (2017). Longitudinal executive function and episodic memory profiles in behavioral-variant frontotemporal dementia and Alzheimer's disease. *J. Int. Neuropsychol. Soc.* 23, 34–43. doi: 10.1017/S1355617716000837

Razani, J., BOONE, K. B., Miller, B. L., Lee, A., and Sherman, D. (2001). Neuropsychological performance of right-and left-frontotemporal dementia compared to Alzheimer's disease. *J. Int. Neuropsychol. Soc.* 7, 468–480. doi: 10.1017/S1355617701744037

Redondo, M. T., Beltrán-Brotóns, J. L., Reales, J. M., and Ballesteros, S. (2016). Executive functions in patients with Alzheimer's disease, type 2 diabetes mellitus patients and cognitively healthy older adults. *Exp. Gerontol.* 83, 47–55. doi: 10.1016/j.exger.2016.07.013

Regard, M. (1981). *Stroop Test–Victoria Version*. Victoria, BC: Neuropsychological Laboratory, University of Victoria.

Reitz, C., Brayne, C., and Mayeux, R. (2011). Epidemiology of Alzheimer disease. *Nat. Rev. Neurol.* 7:137. doi: 10.1038/nrneurol.2011.2

Rochat, L., Billieux, J., Van der Linden, A. C. J., Annoni, J. M., Zekry, D., Gold, G., et al. (2013). A multidimensional approach to impulsivity changes in mild Alzheimer's disease and control participants: cognitive correlates. *Cortex* 49, 90–100. doi: 10.1016/j.cortex.2011.08.004

Roth, M., Tym, E., Mountjoy, C. Q., Huppert, F. A., Hendrie, H., Verma, S., and Goddard, R. (1986). CAMDEX: a standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. *Br J Psychiatry* 149, 698–709. doi: 10.1192/bjp.149.6.698

Salat, D. H., Kaye, J. A., and Janowsky, J. S. (2001). Selective preservation and degeneration within the prefrontal cortex in aging and Alzheimer disease. *Arch. Neurol.* 58, 1403–1408. doi: 10.1001/archneur.58.9.1403

Sánchez-Benavides, G., Peña-Casanova, J., Casals-Coll, M., Gramunt, N., Molinuevo, J. L., Gómez-Ansón, B., et al. (2014). Cognitive and neuroimaging profiles in mild cognitive impairment and Alzheimer's disease: data from the spanish multicenter normative studies (NEURONORMA project). *J. Alzheimers. Dis.* 41, 887–901. doi: 10.3233/JAD-132186

Schulz, K. P., Fan, J., Magidina, O., Marks, D. J., Hahn, B., and Halperin, J. M. (2007). Does the emotional go/no-go task really measure behavioral inhibition? Convergence with measures on a non-emotional analog. *Arch. Clin. Neuropsychol.* 22, 151–160. doi: 10.1016/j.acn.2006.12.001

Sgaramella, T. M., Borgo, F., Mondini, S., Pasini, M., Toso, V., and Semenza, C. (2001). Executive deficits appearing in the initial stage of Alzheimer's disease. *Brain Cogn.* 46, 264–268. doi: 10.1016/S0278-2626(01)80080-4

Shallice, T. (1982). Specific impairments of planning. *Phil. Trans. R. Soc. Lond. B* 298, 199–209. doi: 10.1098/rstb.1982.0082

Shallice, T. (1988). *From Neuropsychology to Mental Structure*. Cambridge: Cambridge University Press.

Smith, E. E., and Jonides, J. (1999). Storage and executive processes in the frontal lobes. *Science* 283, 1657–1661. doi: 10.1126/science.283.5408.1657

Sofi, F., Valecchi, D., Bacci, D., Abbate, R., Gensini, G. F., Casini, A., et al. (2011). Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. *J. Intern. Med.* 269, 107–117. doi: 10.1111/j.1365-2796.2010.02281.x

Spieler, D. H., Balota, D. A., and Faust, M. E. (1996). Stroop performance in healthy younger and older adults and in individuals with dementia of the Alzheimer's type. *J. Exp. Psychol. Hum. Percept. Perform.* 22:461. doi: 10.1037/0096-1523.22.2.461

Spreen, O., and Strauss, E. A. (1998).*Compendium of Neuropsychologial Tests. Administration, Norms, and Commentary.* New York, NY: Oxford University Press.

Stawarczyk, D., Grandjean, J., Salmon, E., and Collette, F. (2012). Perceptual and motor inhibitory abilities in normal aging and Alzheimer disease (AD): A preliminary study. *Arch. Gerontol. Geriatr.* 54, e152–e161. doi: 10.1016/j.archger.2011.12.004

Steinberg, M., Shao, H., Zandi, P., Lyketsos, C. G., Welsh-Bohmer, K. A., Norton, M. C., et al. (2008). Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: the Cache County Study. *Int. J. Geriatr. Psychiatry* 23, 170–177. doi: 10.1002/gps.1858

Stokholm, J., Vogel, A., Gade, A., and Waldemar, G. (2006). Heterogeneity in executive impairment in patients with very mild Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 22, 54–59.doi: 10.1159/000093262

Stroop, J.R. (1935). Studies of interference in serial verbal reactions. *J. Exp. Psychol.* 18:643. doi: 10.1037/0096-3445.121.1.15

Stuss, D. T., and Benson, D. F. (1986). *The Frontal Lobes*. New York, NY: Raven Press.

Stuss, D. T., Shallice, T., Alexander, M. P., and Picton, T. W. (1995). A multidisciplinary approach to anterior attentional functions. *Ann. N.Y. Acad. Sci.* 769, 191–212. doi: 10.1111/j.1749-6632.1995.tb38140.x

Taylor, J. P., and Thomas, A. (2013). "Alzheimer's disease," in *Textbook of Old Age Psychiatry*, eds. T. Dening and A. Thomas (Oxford: Oxford University Press), 431–455.

Tei, H., Miyazaki, A., Iwata, M., Osawa, M., Nagata, Y., and Maruyama, S. (1997). Early-stage Alzheimer's disease and multiple subcortical infarction with mild cognitive impairment: neuropsychological comparison using an easily applicable test battery. *Dement. Geriatr. Cogn. Disord.* 8, 355–358. doi: 10.1159/000106655

Tse, C. S., Balota, D. A., Yap, M. J., Duchek, J. M., and McCabe, D. P. (2010). Effects of healthy aging and early stage dementia of the Alzheimer's type on components of response time distributions in three attention tasks. *Neuropsychology* 24:300. doi: 10.1037/a0018274

Wang, H. X., Xu, W., and Pei, J. J. (2012). Leisure activities, cognition and dementia. *Biochim. Biophys. Acta* 1822, 482–491. doi: 10.1016/j.bbadis.2011.09.002

Wang, P., Zhang, X., Liu, Y., Liu, S., Zhou, B., Zhang, Z., et al. (2013). Perceptual and response interference in Alzheimer's disease and mild cognitive impairment. *Clin. Neurophysiol.* 124, 2389–2396. doi: 10.1016/j.clinph.2013.05.014

Wecker, N. S., Kramer, J. H., Wisniewski, A., Delis, D. C., and Kaplan, E. (2000). Age effects on executive ability. *Neuropsychology* 14:409. doi: 10.1037/0894-4105.14.3.409

World Health Organization (1992). *The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines (Vol. 1)*. Geneva: World Health Organization.

World Health Organization (2010). *International Classification of Disease*. Geneva: World Health Organization.

World Health Organization (2012). *Dementia: A Public Health Priority*. Geneva: World Health Organization.

Xie, J., Brayne, C., and Matthews, F. E. (2008). Survival times in people with dementia: analysis from population based cohort study with 14 year follow-up. *BMJ* 336, 258–262. doi: 10.1136/bmj.39433.616678.25

Yun, J. Y., Lee, D. Y., Seo, E. H., Choo, I. H., Park, S. Y., Kim, S. G., et al. (2011). Neural correlates of stroop performance in Alzheimer's disease: a FDG-PET study. *Dement. Geriatr. Cogn. Disord. Extra* 1, 190–201. doi: 10.1159/000329517

Zahn, T. P., Kruesi, M. J., and Rapoport, J. L. (1991). Reaction time indices of attention deficits in boys with disruptive behavior disorders. *J. Abnorm. Cild Psychol.* 19, 233–252. doi: 10.1007/BF00909980

Zahn, T. P., Rapoport, J. L., and Thompson, C. L. (1980). Autonomic and behavioral effects of dextroamphetamine and placebo in normal and hyperactive prepubertal boys. *J. Abnorm. Child Psychol.* 8, 145–160. doi: 10.1007/BF00919060

Zimmerman, P., and Fimm, B. (1994). *Tests D'évalutazione de L'attention (TEA)*. Würselen: Psytest.

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Incidence of Mild Cognitive Impairment and Dementia in Parkinson's Disease: The Parkinson's Disease Cognitive Impairment Study

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Background: Cognitive impairment in Parkinson's disease (PD) includes a spectrum varying from Mild Cognitive Impairment (PD-MCI) to PD Dementia (PDD). The main aim of the present study is to evaluate the incidence of PD-MCI, its rate of progression to dementia, and to identify demographic and clinical characteristics which predict cognitive impairment in PD patients.

Methods: PD patients from a large hospital-based cohort who underwent at least two comprehensive neuropsychological evaluations were retrospectively enrolled in the study. PD-MCI and PDD were diagnosed according to the Movement Disorder Society criteria. Incidence rates of PD-MCI and PDD were estimated. Clinical and demographic factors predicting PD-MCI and dementia were evaluated using Cox proportional hazard model.

Results: Out of 139 enrolled PD patients, 84 were classified with normal cognition (PD-NC), while 55 (39.6%) fulfilled the diagnosis of PD-MCI at baseline. At follow-up (mean follow-up 23.5 ± 10.3 months) 28 (33.3%) of the 84 PD-NC at baseline developed MCI and 4 (4.8%) converted to PDD. The incidence rate of PD-MCI was 184.0/1000 pyar (95% CI 124.7–262.3). At multivariate analysis a negative association between education and MCI development at follow-up was observed (HR 0.37, 95% CI 0.15–0.89; $p = 0.03$). The incidence rate of dementia was 24.3/1000 pyar (95% CI 7.7–58.5). Out of 55 PD-MCI patients at baseline, 14 (25.4%) converted to PDD, giving an incidence rate of 123.5/1000 pyar (95% CI 70.3–202.2). A five time increased risk of PDD was found in PD patients with MCI at baseline (RR 5.09, 95% CI 1.60–21.4).

Conclusion: Our study supports the relevant role of PD-MCI in predicting PDD and underlines the importance of education in reducing the risk of cognitive impairment.

Keywords: mild cognitive impairment, dementia, Parkinson's disease, incidence, neuropsychological assessment

INTRODUCTION

Although Parkinson's Disease (PD) has been classically considered a movement disorder, non-motor symptoms, such as cognitive impairment, represent very common features of the disease (Munhoz et al., 2015). Cognitive impairment encompasses a spectrum varying from Mild Cognitive Impairment (MCI) to dementia, and MCI is considered as an intermediate condition between "normal aging" and dementia (Petersen et al., 2001). This concept was originally used to early capture subjects at risk to develop Alzheimer's disease (Petersen et al., 2001), and recently was extended and adapted to PD patients (Litvan et al., 2012). While a subtle cognitive impairment configuring MCI in PD (PD-MCI) could be diagnosed even in incident PD, a condition of overt dementia in PD (PDD) usually occurs in advanced stages with a prevalence close to 30% (Aarsland et al., 2017). Several risk factors have been associated with PDD occurrence, including old age at onset, long disease duration, severe motor impairment, and MCI (Hanagasi et al., 2017). Considering that PDD has a substantial negative effect on patient's well-being and caregiver's burden, the early detection of patients at risk to develop PDD deserves relevant prognostic and therapeutic implications (Aarsland et al., 2017).

To accurately identifying MCI in subjects with PD, in 2012 a task force of the Movement Disorder Society (MDS) proposed a standardized set of diagnostic criteria to be used both in daily clinical practice and research settings (Litvan et al., 2012). Depending on the comprehensiveness of neuropsychological testing, the MDS criteria provided two different diagnostic levels (i.e., Level I and Level II). Level I criteria allow for the diagnosis of PD-MCI through the administration of an "abbreviated" neuropsychological assessment, while Level II criteria recommend the administration of a "comprehensive" neuropsychological battery which permits the classification of PD-MCI into different subtypes, according to the cognitive domains impaired. Moreover, the identification of PD-MCI subtypes not only increases the diagnostic sensitivity but also allows hypothesizing MCI evolution and prognosis (Litvan et al., 2012). Studies carried out using these criteria have reported frequencies of PD-MCI ranging from 14.8 to 42.5% in patients with *newly diagnosed* PD (Yarnall et al., 2004; Poletti et al., 2012; Weintraub et al., 2018).

This study is part of The PArkinson's disease COgnitive impairment Study (PACOS), an observational study involving two centers located in southern Italy (Sicily), aimed to evaluate frequency, clinical features and biomarkers associated with MCI in a large hospital-based cohort of PD patients (Baschi et al., 2018; Monastero et al., 2018). The PACOS cohort included 659 non-demented PD patients. In agreement with other studies, according to the MDS criteria, the prevalence of PD-MCI was 39.6% in the whole sample and 31.7% among *newly diagnosed* patients (disease duration $\leq$ 1 year). Amnestic MCI multidomain phenotype was the most frequent subtype recorded in 39.1% of the overall sample and 43.9% in *newly diagnosed* PD (Monastero et al., 2018).

Although several cross-sectional studies have evaluated the prevalence of cognitive impairment in PD, few longitudinal studies have assessed the incidence of PD-MCI according to the MDS criteria (Broeders et al., 2013; Pedersen et al., 2013, 2017; Domellof et al., 2015; Hobson and Meara, 2015; Pigott et al., 2015; Santangelo et al., 2015; Cholerton et al., 2018). Furthermore, only few studies adopted Level II MDS criteria for PD-MCI (Broeders et al., 2013; Domellof et al., 2015; Santangelo et al., 2015; Cholerton et al., 2018).

The aim of the present study was to evaluate the incidence of PD-MCI and PDD, the rate of progression from PD-MCI to PDD, and to identify demographic and clinical characteristics which predict cognitive impairment in a well-defined cohort of PD patients.

MATERIALS AND METHODS

Study Population

Patients affected by PD diagnosed according to the Brain Bank criteria (Gibb and Lees, 1988) who attended the Neurologic Unit of the "Policlinico Vittorio Emanuele" in Catania and the Memory and Parkinson's disease Center of the "Policlinico Paolo Giaccone" in Palermo, over a six-year period (2011–2016), were retrospectively enrolled in the PACOS cohort. The population included 659 non-demented PD subjects at baseline. All participants underwent a standard neurological workup, including a comprehensive neuropsychological assessment. Background and methods have been extensively reported elsewhere (Monastero et al., 2018).

Between 2014 and 2017 we retrospectively enrolled all PD patients who underwent at least two comprehensive neuropsychological evaluations (baseline and follow-up) during a period of maximum 48 months (between 12 and 48). All participants provided written informed consent prior to entering the study, which was approved by the Ethical Committee of the University Hospital of Palermo, P. Giaccone (approval number: 14:03/2018) and was in accordance with the Declaration of Helsinki.

Clinical Assessment

All patients, at baseline and follow-up, underwent a standard neurological examination performed by neurologists experienced in movement disorders. Demographic, clinical and pharmacological data were collected from patient's medical records. PD severity was evaluated with the Unified Parkinson Disease Rating Scale – Motor Evaluation (UPDRS-ME) and the Hoehn and Yahr (HY) scale. All patients were evaluated in "off" state. The clinical phenotype was attributed according to the classification in Tremor Dominant (TD), Postural Instability Gait Difficulty (PIGD) and Undetermined using scores from part II and III of UPDRS (Jankovic et al., 1990).

Neuropsychological and Behavioral Assessment

All the enrolled patients, at baseline and follow-up, underwent a comprehensive neuropsychological assessment when in "on" state. Neuropsychological evaluations were performed by

neurologists with a specific expertise in neuropsychology and dementia, and the same rater performed both baseline and follow-up assessments.

Patients underwent a Level I MDS criteria evaluation of global cognition using the following tests: the Mini Mental State Examination (MMSE) (Folstein et al., 1975), the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), and the Frontal Assessment Battery (FAB) (Dubois et al., 2000).

According to MDS Level II criteria (Litvan et al., 2012), two tests for cognitive domains have been performed. The memory domain has been assessed with the Rey's Auditory Verbal Learning Test (Carlesimo et al., 1996) and the Prose recall test with a delayed recall condition (Novelli et al., 1986a); the attention domain with the Stroop color-word test (Uttl and Graf, 1997) and the Trail Making Test part A (TMT-A) (Giovagnoli et al., 1996); the executive function domain with the Verbal fluency letter test (COWAT) (Novelli et al., 1986b) and the Colored Raven's Progressive Matrices (Carlesimo et al., 1996); the visuo-spatial function domain with the Clock drawing test (CDT) (Shulman, 2000) and the Copy of figures (Carlesimo et al., 1996); lastly, the language domain has been assessed with the Aachener Aphasie Test-Naming item (Luzzatti et al., 1996) and the short version of the Token test (De Renzi and Vignolo, 1962).

For each test, details regarding administration procedures and Italian normative data for score adjustment (based on age, gender and education) were used. Neuropsychological performances were considered as impaired when the subject scored 2 standard deviation (SD) below normality cut-off values.

Mild cognitive impairment was diagnosed when patients scored below the cut-off values in at least two neuropsychological tests. MCI subtypes were defined as follows: amnestic MCI single domain (aMCIsd), when two of the memory tests were altered without impairment of other domains; non-amnestic MCI single domain (naMCIsd), when there were at least two tests altered within one single domain other than memory; amnestic MCI multi domain (aMCImd), when at least one memory test plus at least one test in any other domain were altered; non-amnestic MCI multiple domain (naMCImd), when two tests were altered in two different domains, without the involvement of the memory domain. The diagnosis of probable PDD was made according to the MDS criteria (Emre et al., 2007).

Functional independence was assessed using the Basic Activities of Daily Living (BADL) (Katz et al., 1963) and the Instrumental Activities of Daily Living (IADL) (Hughes et al., 1982). Lastly, Depression was evaluated using the Hamilton Depression Rating Scale, considering a cut-off scores > 9, as suggested by the MDS (Hamilton, 1960; Schrag et al., 2007).

Statistical Analysis

Data were analyzed using STATA 12.1 software packages (StataCorp, College Station, TX, United States). Data cleaning was performed before the data analysis considering both range and consistence checks. Quantitative variables were described using mean and standard deviation. The difference between means and proportions was evaluated by the *t*-test and the Chi square test, respectively. In case of a not normal distribution, appropriate non-parametric tests were performed.

To calculate incidence rates of PD-MCI and PDD, we divided the number of cases with PD-MCI or PDD by the total number of person-years at risk during follow-up. We estimated person-years at risk (pyar) as the total follow-up time until PD-MCI or PDD. For incident PDD cases, we assigned time of dementia onset to the midpoint of the interval between assessments at which dementia was diagnosed. Because PD-MCI, in contrast to PDD, may be reversible or fluctuate over time, we set time of onset of incident PD-MCI to the exact date at which PD-MCI was first diagnosed. Incidence rates were also estimated considering only *newly diagnosed* patients (disease duration ≤ 1 year).

Kaplan–Meier survival analysis was carried out to estimate the cumulative proportion from normal cognition to any cognitive impairment (MCI or dementia) as well as the progression rate from MCI to dementia. The log-rank test was used to compare survival curves.

In order to identify possible predictors associated with the probability of progression from normal cognition to any cognitive impairment (MCI or Dementia) among the clinical and demographic characteristics, Cox proportional-hazards regression model was used for both the univariate and multivariate analyses. Variables with p-value < 0.1 at univariate analysis were included in the final multivariate Cox models. Schoenfeld residuals test was used for testing the proportional hazard. 95% confidence interval (CI), and p-value (two-tailed test, $a = 0.05$) were calculated. Analysis was also restricted to *newly diagnosed* PD patients.

Whenever variables were dichotomized or polychotomized, the cut-offs were derived from the pooled distribution of cases and control subjects (e.g., using the median value). To evaluate the role of dopaminergic therapy the levodopa equivalent daily dose (LED) was calculated for those patients taking dopamine agonists or levodopa in combination with dopamine agonists (Tomlinson et al., 2010).

RESULTS

The PACOS cohort consists of 659 non-demented PD patients (Monastero et al., 2018). Of 659 subjects, 139 PD patients (men 87, 62.6%) with a mean disease duration of 3.0 ± 2.8 years who underwent at least two neuropsychological evaluations between 12 and 48 months from 2014 to 2017 were enrolled in the present study. No significant differences in demographic and clinical characteristics were found between groups, apart from a borderline significant difference in disease duration between the two groups (see **Supplementary Table S1**). Of the 139 patients at baseline (first neuropsychological evaluation), 84 (60.4%) were classified as PD-NC, while 55 (39.6%) fulfilled the diagnosis of PD-MCI. Concerning the MCI subtypes, 4 (7.3%) patients had aMCIsd, 28 (50.9%) aMCImd, 12 (21.8%) naMCIsd and 11 (20.0%) naMCImd. Fifty-three (38.1%) of the 139 PD patients were *newly diagnosed* patients with a disease duration ≤ 1 year and of these 20 (37.7%) were classified as PD-MCI at the baseline evaluation. Baseline characteristics are shown in **Table 1**.

TABLE 1 | Clinical and demographic characteristics at baseline.

	PD-NC *N* = 84	PD-MCI *N* = 55	Total *N* = 139	*p*-value
Men, *n* (%)	52 (61.9)	35 (63.6)	87 (62.6)	0.8
Age, years	64.4 ± 10.4	67.5 ± 7.4	65.7 ± 9.4	0.07
Age at onset, years	61.6 ± 11.0	64.5 ± 7.8	62.8 ± 10.0	0.09
Education, years	9.3 ± 4.4	8.3 ± 4.6	8.9 ± 4.6	0.2
UPDRS-ME score	25.4 ± 14.5	27.4 ± 11.9	26.2 ± 13.5	0.4
HY stage	1.9 ± 0.6	2.2 ± 0.7	2.0 ± 0.7	0.02
Disease duration, years	3.0 ± 2.9	3.0 ± 2.7	3.0 ± 2.8	0.9
Depression, *n* (%)	29 (34.5)	22 (40.0)	51 (36.7)	0.4
LED mg/day	437.2 ± 463.8	397.9 ± 408.8	421.8 ± 442.0	0.6
Phenotype (%)				
TD	32 (38.1)	11 (20.0)	43 (30.9)	/
PIGD	47 (55.9)	39 (70.9)	86 (61.9)	/
Mixed	5 (5.9)	5 (9.1)	10 (7.2)	0.07

PD: Parkinson's Disease; PD-NC: PD with Normal Cognition; PD-MCI: PD with Mild Cognitive Impairment; UPDRS-ME: Unified Parkinson's Disease Rating Scale Motor Examination; HY: Hoehn and Yahr; LED: Levodopa Equivalent Daily Dose; TD: Tremor Dominant; PIGD: Postural Instability Gait Difficulty.

Incidence of MCI

Considering the 84 PD-NC at baseline, 28 (33.3%) fulfilled the diagnosis of PD-MCI, while 4 (4.8%) fulfilled the diagnosis of PDD at follow-up (mean follow-up time 23.5 ± 10.3 months). A slightly longer and borderline significant follow-up time was recorded among PD patients who developed MCI (25.7 ± 9.8 *versus* 21.3 ± 9.7 months; *p*-value 0.05), while a significantly longer follow-up was observed in the four patients who developed PDD (38.0 ± 9.6 months; *p*-value 0.004).

Regarding the MCI subtypes, 3 (10.7%) out of the 28 patients developed an aMCIsd, 8 (28.6%) naMCIsd, 10 (35.7%) aMCImd and 7 (25.0%) naMCImd. The incidence rate of MCI among PD-NC at baseline was 184.0/1000 pyar (95% CI 124.7–262.3) (total person time at risk 152.2 years), without significant difference between sex [185.6/1000 pyar for men and 181.2/1000 pyar for women; relative risk (RR) 1.02, 95% CI 0.45–2.48; $p = 0.5$] (see **Figure 1**).

Out of the 84 PD-NC, 33 (39.3%) were *newly diagnosed* patients and of these 10 (30.3%) developed PD-MCI at follow-up. The incidence of MCI in *newly diagnosed* patients was 155.8/1000 pyar (95% CI 79.1–277.6) (total person time at risk 64.2 years). There was no significant difference between the incidence in the whole cohort and the incidence rate of *newly diagnosed* PD patients (RR 1.18, 95% CI 0.56–2.73; $p = 0.3$).

At univariate Cox proportional-hazards regression models, PD patients who developed MCI at follow-up were significantly older and with a lower level of education compared to those who preserved normal cognition (see **Table 2**). Multivariate analysis confirmed the strong protective effect of education in the development of MCI at follow-up with a Hazard Ratio (HR) of 0.37 for PD patients with more than 8 years of schooling (95% CI 0.15–0.89; $p = 0.03$) (see **Table 2**).

According to Kaplan–Meier survival analysis, 94.8% (95% CI 91.3–99.8) of PD patients were free of MCI at 1 year of follow-up, 73.8% (95% CI 59.9–83.5) at 2 years of follow-up and 45.3% (95% CI 27.8–61.2) at 3 years as shown in **Figure 2A**. Close rates have been recorded when analysis was restricted to *newly diagnosed* patients [90.7% (95% CI 73.1–96.8) were free of MCI at 1 year, 76.7% (95% CI 54.5–89.0) at 2 years and 39.4% (95% CI 13.6–64.8) at 3 years]. A significant difference in survival curves, according to the log-rank test, has been found by age (>66 years and <66 years; $p = 0.007$) and education (years of schooling >8 years and <8 years; $p = 0.008$) as shown in **Figures 3A,B**.

Four of the 84 PD-NC developed dementia, giving an incidence rate of 24.3/1000 pyar (95% CI 7.7–58.5) (total person time at risk 164.8). These 4 patients presented a significantly higher UPDRS-ME (42.0 ± 21.5 *versus* 24.5 ± 13.7; $p = 0.01$) and a lower, although not significant, educational level (mean years of schooling 6.7 ± 4.5 *versus* 9.5 ± 3.5; $p = 0.2$).

Incidence of PDD

Considering the entire sample of 139 PD patients, 18 fulfilled the diagnosis of PDD at follow-up (mean follow-up time 24.0 ± 10.2 months). A significantly longer follow-up time was recorded among PD patients who developed PDD (29.0 ± 11.1 months *versus* 23.3 ± 9.9 months; *p*-value 0.02). The incidence rate of PDD was 64.7/1000 pyar (95% CI 39.5–100.3) (total person-time at risk 278.2 years) with three times higher risk for men (70.1/1000 pyar for men and 56.1.0/1000 pyar for women; RR 3.2, 95% CI 1.11–10.4; $p = 0.009$).

Fifty-three (38.1%) of the 139 PD patients were *newly diagnosed* patients, of whom (11.3%) developed PDD with an incidence rate of 53.3/1000 pyar (95% CI 21.6–110.8) (total person time at risk 112.6 years). No significant difference has been recorded between the incidence in the whole cohort and incidence rate among the *newly diagnosed* PD patients (RR 1.21, 95% CI 0.46–3.73; $p = 0.3$).

At univariate analysis, Cox proportional-hazards regression model, PD patients who developed PDD at follow-up were significantly older, with a borderline but significantly higher UPDRS-ME score and a significantly lower education level compared to subjects who do not developed dementia (see

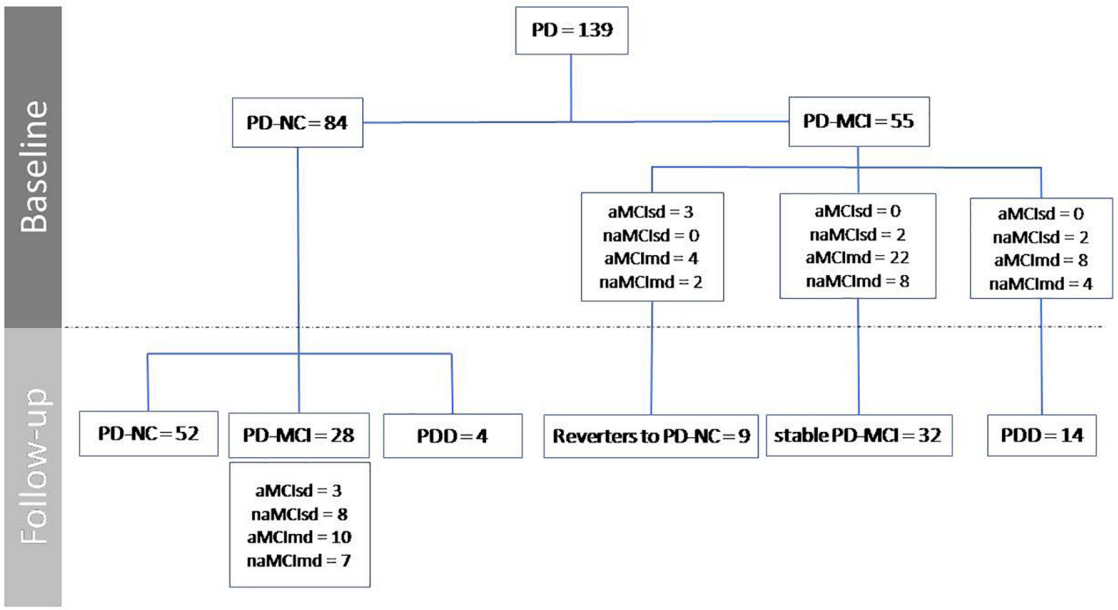

FIGURE 1 | Progression of PD-NC and PD-MCI from baseline to follow-up.

TABLE 2 | Development of PD-MCI considering the 84 PD-NC at baseline.

			Univariate analysis			Multivariate analysis		
	PD-MCI ***N* = 28**	**PD No-MCI** ***N* = 52**	**HR**	**95%CI**	***p*-value**	**HR**	**95%CI**	***p*-value**
Sex, Men (%)	18 (64.3)	33 (63.4)	1.30	0.58–2.90	0.6	0.83	0.36–1.96	0.7
Age, years	68.5 ± 9.8	62.2 ± 10.3	1.04	1.00–1.09	0.04	1.04	0.99–1.08	0.07
Age ≤ 66 years	8 (28.6)	33 (63.5)	1	/	/			
Age > 66 years	20 (71.4)	19 (36.5)	2.81	1.24–6.41	0.01			
Age at onset, years	65.2 ± 10.8	59.6 ± 11.0	1.04	1.00–1.08	0.06			
Age at onset ≤ 50 years	2 (7.1)	10 (19.2)	1	/	/			
Age at onset > 50 years	26 (92.9)	42 (80.8)	2.10	0.49–8.99	0.3			
UPDRS-ME	24.4 ± 13.0	24.6 ± 14.2	0.98	0.95–1.00	0.2			
HY stage	2.1 ± 0.9	1.9 ± 0.5	1.01	0.73–1.40	0.9			
Disease duration, years	3.3 ± 3.1	2.6 ± 2.7	1.01	0.90–1.14	0.8			
Education, years	7.5 ± 4.8	10.5 ± 4.1	0.90	0.82–0.98	0.02			
Education ≤ 8 years	21 (75.0)	22 (42.3)	1	/	/	1	/	/
Education > 8 years	7 (25.0)	30 (57.7)	0.35	0.15–0.82	0.02	0.37	0.15–0.89	0.03
LED mg/day	480.8 ± 569.5	425.0 ± 415.6	1.00	0.99–1.00	0.5			
Depression	13 (46.4)	45 (31.8)	1.80	0.83–3.88	0.1			
Phenotype								
TD	11 (39.3)	19 (42.2)	1					
PIGD	15 (53.6)	24 (53.3)	0.75	0.34–1.65	0.5			
Mixed	2 (7.1)	2 (4.4)	3.50	0.72–16.9	0.1			

Cox proportional-hazards regression models. PD: Parkinson's Disease; PD-NC: PD with Normal Cognition; PD-MCI: PD with Mild Cognitive Impairment; HR: Hazard Ratio; UPDRS-ME: Unified Parkinson's Disease Rating Scale Motor Examination; HY: Hoehn and Yahr; LED: Levodopa Equivalent Daily Dose; TD: Tremor Dominant; PIGD: Postural Instability Gait Difficulty.

Table 3). At univariate analysis the presence of MCI at baseline was the most important factor associated with the development of PDD (univariate HR 4.37, 95% CI 1.42–13.5; $p = 0.01$). This association was even stronger at multivariate analysis adjusting by age, sex *(a priori confounder)*, UPDRS-ME and education (HR 6.24, 95% CI 1.81–21.5; $p = 0.004$).

According to Kaplan–Meier survival analysis, 99.2% (95% CI 94.7–99.9) PD-MCI were free of dementia at 1 year of

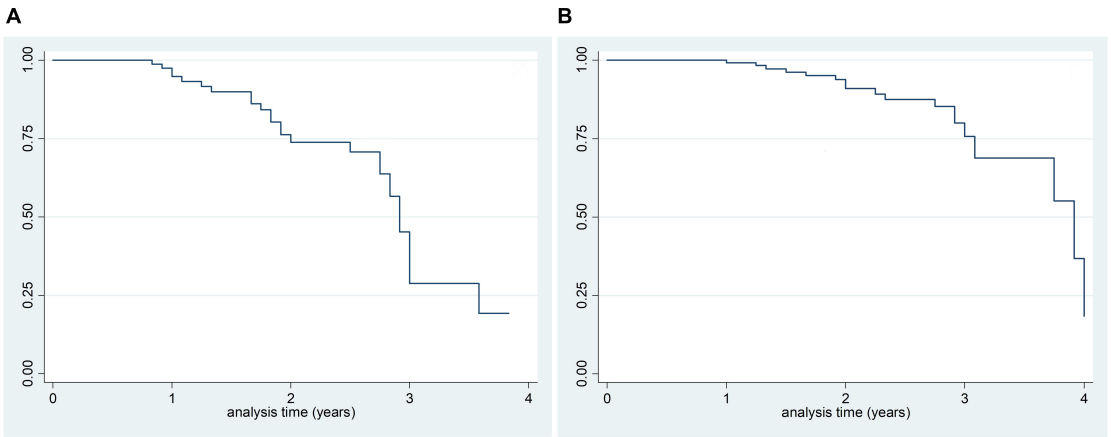

FIGURE 2 | Kaplan–Meier survival analysis of PD-NC at baseline who developed PD-MCI at follow-up **(A)** and survival estimates of PD-MCI who developed PDD at follow-up **(B)**.

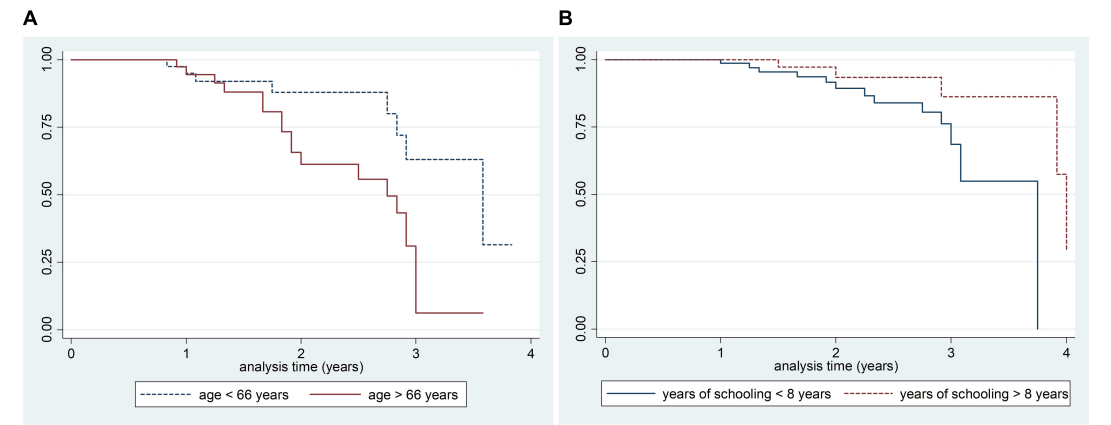

FIGURE 3 | Kaplan–Meier survival estimates of PD-NC at baseline who developed PD-MCI at follow-up by age (**A**: Log rank test *p*-value 0.007) and education (**B**: Log rank test *p*-value 0.008).

follow-up, 91.0% (95% CI 82.5–95.5) at 2 years, and 75.7% (95% CI 59.8–86.0) at 3 years (**Figure 2B**). A significant difference in survival curves was observed after stratifying for the presence of MCI at baseline (p = 0.005) and education (years of schooling > 8 years and ≤ 8 years; p = 0.04) as shown in **Figures 4A,B**. In particular, according to Kaplan–Meier survival analysis among PD-NC at baseline, the 100% were free of dementia at 1 and 2 years of follow-up, and 92.5% (95% CI 72.8–98.1) at 3 years. Considering PD-MCI patients, 98.1% (95% CI 87.1–99.7) were free of dementia at 1 year, 79.7% (95% CI 63.2–89.4) at 2 years, and only 55.0% (95% CI 28.2–75.4) at 3 years (**Figure 4A**).

Concerning the MCI subtypes, 14 out of the 18 patients who developed PDD had MCI at baseline; of these 12 (85.7%) were classified as MCI multi-domain (8 aMCImd and 4 naMCImd). A higher risk of developing PDD was recorded for those patients who had naMCImd at baseline with an adjusted HR of 21.1 (95% CI 3.89–114.7; p < 0.0001), followed by aMCImd (adjusted HR 8.70, 95% CI 2.01–38.0; p = 0.004). Overall, the risk of PDD was higher among patients with mdMCI, compared to those with sdMCI as shown in **Table 4**.

Concerning the five different domains evaluated, in our sample a higher risk of dementia was recorded among PD patients presenting at least one impaired test in executive function at the baseline evaluation (HR 7.76, 95% CI 2.31–5.64; p = 0.001), followed by attention (HR 4.75, 95% CI 1.44–15.6; p = 0.01). Due to the presence of just two PD patients who presented an impaired language at baseline (one developed dementia at follow-up and one did not) with the consequent wide 95% CIs, the role of this domain is difficult to evaluate as shown in **Table 4**.

Progression From MCI to PDD

Considering only the 55 patients with PD-MCI at baseline, 14 (25.4%) developed PDD at follow-up (mean follow-up time 24.7 ± 10.0 months), while 9 (16.3%) reverted to PD-NC. The incidence rate of PDD in patients with MCI at baseline was 123.5/1000 pyar (95%

TABLE 3 | Development of PDD.

			Univariate analysis			Multivariate analysis		
	PDD ***N* = 18**	**No-PDD** ***N* = 121**	**HR**	**95%CI**	***p*-value**	**HR**	**95%CI**	***p*-value**
Sex, Men (%)	12 (66.7)	75 (62.0)	1.21	0.44–3.37	0.7	2.42	0.76–7.74	0.1
Age, years	68.3 ± 8.4	65.3 ± 9.5	1.07	1.00–1.15	0.04	1.06	0.99–1.14	0.1
Age ≤ 67 years	7 (38.9)	62 (52.1)	1	/	/			
Age > 67 years	11 (61.1)	59 (48.8)	3.49	1.09–11.2	0.03			
Age at onset, years	64.9 ± 8.4	62.4 ± 10.1	1.05	0.99–1.12	0.1			
Age at onset ≤ 50 years	1 (5.6)	11 (10.0)	1	/	/			
Age at onset > 50 years	17 (94.4)	99 (90.0)	1.12	0.15–8.62	0.9			
UPDRS-ME	33.1 ± 16.5	25.1 ± 12.7	1.03	1.00–1.05	0.06	1.04	1.00–1.08	0.03
UPDRS ≤ 25	7 (38.9)	71 (58.7)	1	/	/			
UPDRS > 26	11 (61.1)	50 (41.3)	2.61	0.90–7.55	0.08			
HY stage	1.9 ± 0.6	2.1 ± 0.7	0.77	0.40–1.47	0.4			
Disease duration, years	3.4 ± 2.8	2.9 ± 2.8	1.07	0.92–1.25	0.3			
Education, years	7.4 ± 4.8	9.2 ± 4.5	0.88	0.78–0.99	0.04			
Education ≤ 8 years	13 (72.2)	69 (57.0)	1	/	/	1	/	/
Education > 8 years	5 (27.8)	52 (43.0)	0.28	0.08–1.03	0.06	0.36	0.09–1.52	0.2
LED mg/day	353.8 ± 298.4	432.1 ± 459.8	1.00	1.00–1.001	0.9			
Cognition baseline								
NC	4 (22.2)	80 (66.1)	1	/	/	1	/	/
MCI	14 (77.8)	41 (33.9)	4.37	1.42–13.5	0.01	6.24	1.81–21.5	0.004
Depression	9 (50.0)	42 (34.7)	1.28	0.50–3.24	0.6			
Phenotype (%)								
TD	6 (33.3)	37 (30.6)	1	/	/			
PIGD	12 (66.7)	74 (61.2)	1.01	0.38–2.76	0.9			
Mixed	0	10 (8.3)	/	/	/			

Cox proportional-hazards regression models. PDD: Parkinson's Disease Dementia; HR: Hazard Ratio; UPDRS-ME: Unified Parkinson's Disease Rating Scale Motor Examination; HY: Hoehn and Yahr; LED: Levodopa Equivalent Daily Dose; NC: Normal Cognition; MCI: Mild Cognitive Impairment; TD: Tremor Dominant; PIGD: Postural Instability Gait Difficulty.

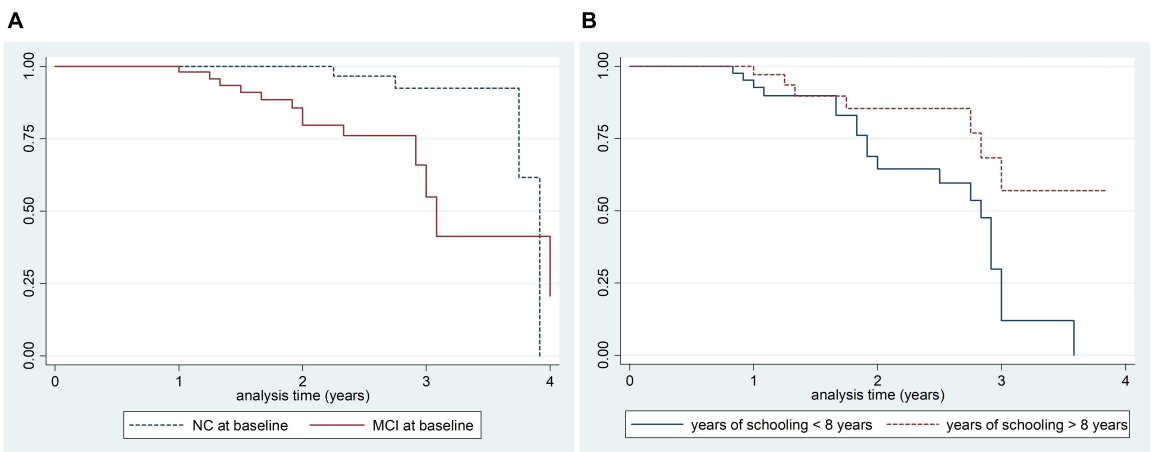

FIGURE 4 | Kaplan–Meier survival estimates of patients who developed PDD at follow-up by the presence of MCI at follow-up (**A**: Log rank test *p*-value 0.004) and years of schooling (**B**: Log rank test *p*-value 0.005).

CI 70.3–202.2) (total person-time at risk 113.4), while the incidence rate of PDD among PD-NC at baseline was 24.3/1000 (95% CI 7.7–58.5), giving a RR of 5.09 (95% CI 1.60–21.4; $p = 0.0009$).

Of the 9 reverters, 3 (33.3%) were aMCIsd, 4 (44.4%) were aMCImd and 2 (22.2%) were naMCImd (see **Figure 1**). No significant differences regarding clinical and demographic characteristics at baseline between reverters and PD-MCI,

TABLE 4 | PD-MCI subtypes, domains and risk of PDD.

			Multivariate analysis		
	PDD ***N*** **= 18**	**No-PDD** ***N*** **= 121**	**HR**	**95%CI**	***p*-value**
Model 1: MCI subtypes					
Normal Cognition	4 (22.2)	80 (66.1)	1		
Amnestic MCI single domain	/	5 (4.13)	/	/	/
Non-amnestic MCI single domain	2 (11.1)	10 (8.3)	4.77	0.71–31.9	0.1
Amnestic MCI multi-domain	8 (44.4)	19 (15.7)	8.70	2.01–38.0	0.004
Non-amnestic MCI multi-domain	4 (22.2)	7 (5.8)	21.1	3.89–114.7	<0.0001
Model 2: MCI single domain *versus* MCI multi-domain					
Normal Cognition	4 (22.2)	80 (66.1)	1		
MCI single domain	2 (11.1)	15 (12.4)	2.51	0.39–15.8	0.3
MCI multiple domain	12 (66.7)	26 (21.4)	11.7	2.91–47.3	0.001
Model 3: Amnestic MCI *versus* non-amnestic MCI					
Normal Cognition	4 (22.2)	80 (66.1)	1		
Amnestic MCI	8 (44.4)	24 (19.8)	6.18	1.51–25.2	0.01
Non-amnestic MCI	6 (33.3)	17 (14.0)	8.58	2.11–15.8	0.003
Impaired domain (at baseline)*					
Memory	8 (44.4)	33 (27.4)	1.88	0.62–5.64	0.3
Executive function	12 (66.7)	28 (23.1)	7.76	2.31–5.64	0.001
Attention	9 (50.0)	31 (25.6)	4.75	1.44–15.6	0.01
Visuo-spatial function	4 (22.2)	19 (15.7)	1.33	0.39–4.56	0.6
Language	1 (5.6)	1 (0.8)	38.2	3.31–441.3	0.004

*Cox proportional-hazards multivariate regression models. PD: Parkinson's Disease; PD-MCI: PD with Mild Cognitive Impairment; PDD: PD with Dementia; HR: Hazard Ratio; HR adjusted by age sex, UPDRS-ME and education. *Impaired domain at baseline = at least one impaired test; for each domain the reference group is "not impaired."*

irrespective of whether the patients developed PDD or not, were found.

DISCUSSION

In the present study we evaluated the incidence rate of PD-MCI and PDD and the risk of progression from PD-MCI to dementia in the PACOS cohort. At follow-up more than 33% of PD-NC at baseline developed MCI with an incidence rate of 184.0/1000 pyar, while more than 12% converted to PDD with an incidence rate of 24.3/1000 pyar. Conversely, the incidence rate of PDD among patients with MCI at baseline was 123.5/1000 pyar, giving a five time increased risk of developing dementia. Lastly, a significant negative association between education and PD-MCI was observed. PD-NC who converted to PD-MCI at follow-up were significantly less educated than non-converters. Moreover, the presence of MCI at baseline, in particular the naMCImd subtype, was strongly associated with PDD conversion, increasing the risk of dementia more than five times.

Mild cognitive impairment is considered an intermediate state between normal cognitive aging and early dementia. Several cross-sectional studies, the majority of which are multicenter studies, have been carried out to evaluate the prevalence of MCI during the last decade reporting ratios ranging from 18.9 to 35.2% (Foltynie et al., 2004; Aarsland et al., 2009). Differences in study designs, the definition of PD-MCI and the neuropsychological assessment adopted have greatly contributed to the wide variations in the reported estimates of PD-MCI. However, a high variability has also been reported across studies using the MDS criteria (Litvan et al., 2012) with MCI prevalence ranging from 20 to 41% (Broeders et al., 2013; Domellof et al., 2015; Pedersen et al., 2017; Weintraub et al., 2018). In agreement with these studies, in the PACOS cohort the prevalence of PD-MCI was 39.1% and MCI was associated with age and motor scores while a strong negative association was observed with educational level (Monastero et al., 2018).

Nonetheless, few prospective studies based on the MDS criteria for PD-MCI (Litvan et al., 2012) and PDD (Emre et al., 2007) have been performed until now, in order to evaluate the progression from normal cognition to MCI and the incidence of dementia (Broeders et al., 2013; Domellof et al., 2015; Pigott et al., 2015; Santangelo et al., 2015; Pedersen et al., 2017; Cholerton et al., 2018). Of these, four studies (Broeders et al., 2013; Domellof et al., 2015; Santangelo et al., 2015; Cholerton et al., 2018) have adopted Level II MDS criteria for the diagnosis of PD-MCI.

Progression From Normal Cognition to MCI

Throughout the entire sample of 139 non-demented PD patients, the prevalence of PD-MCI at baseline was 44.6% and 39.2% considering only *newly diagnosed* patients; these rates were close to those reported for the whole PACOS cohort

(Monastero et al., 2018), as well as those regarding other studies (Broeders et al., 2013; Domellof et al., 2015; Santangelo et al., 2015).

A lower frequency of MCI at baseline (20.2%) was reported in the Norwegian study (Pedersen et al., 2017), while the study by Cholerton et al. (2018) reported a higher prevalence of MCI. The latter result is probably due to the lower cut-off point used for the impairment on specific neuropsychological test (1 SD below normative data).

According to literature data, the most frequent type of MCI at baseline was the multiple domain (Santangelo et al., 2015), both amnestic and non-amnestic, representing the 49.1% and 20.0%, respectively.

At follow-up, 33.3% of PD-NC at the baseline developed MCI and considering only the *newly diagnosed* patients the frequency was 30.3%. These similar rates probably account for the short disease duration and mild motor impairment of the patients enrolled in the study. To the best of our knowledge, incidence rate of MCI among PD-NC was estimated only for the Norwegian study (Pedersen et al., 2017) where an incidence rate of 68.9/1000 pyar was recorded. This rate was lower with respect to our study, but it should be underlined that also a lower frequency of MCI at baseline was reported.

The results of survival analysis have demonstrated that approximately 5% of PD-NC developed MCI at 1 year, 26% at 2 years; these estimates are close to those reported by previous longitudinal studies conducted on PD-MCI, which had adopted MDS Level II criteria (Broeders et al., 2013; Santangelo et al., 2015; Pedersen et al., 2017). Nonetheless, approximately 55% of PD-NC developed MCI at 3 years, an estimate which is slightly higher than those reported in the literature (Broeders et al., 2013; Pigott et al., 2015; Pedersen et al., 2017). This difference can be in part explained by the enrolment of PD patients with a disease duration (mean disease duration: about 3 years) which is slightly longer than other cohorts (Broeders et al., 2013; Santangelo et al., 2015; Pedersen et al., 2017). At any rate when survival analysis was restricted to *newly diagnosed* patients close rates were found. Furthermore, it should be noted that PD patients enrolled in the present study had a lower educational level compared with other cohorts (Pigott et al., 2015; Santangelo et al., 2015; Pedersen et al., 2017) and this lower educational level probably contributed to a higher risk of developing MCI. Indeed, in agreement with these prospective studies (Santangelo et al., 2015; Pedersen et al., 2017), a strong protective effect of education was recorded with an almost 70% reduced risk of MCI in patients with more than 8 years of schooling.

Progression From PD-NC and PD-MCI to Dementia

Incidence rate of PDD in the whole cohort (PD-NC and PD-MCI) was 64.7/1000 pyar and 53.3/1000 pyar among *newly diagnosed* patients. Again, the lack of differences between the whole sample and the *newly diagnosed* patients is probably due to the short disease duration of the entire sample. Only two prospective studies have evaluated the incidence rate of PDD reporting similar estimates. In particular, a close rate of 62.6/1000 pyar has been reported by Domellof et al. (2015), while a slightly lower rate of 38.1/1000 pyar was found in the Norwegian cohort (Pedersen et al., 2017). Nonetheless, comparison with this latter study is limited by the lower frequency of MCI reported at baseline (20.2%).

A significantly higher incidence rate of PDD was recorded among PD-MCI at baseline with respect to PD-NC, resulting in a five time increased risk of PDD among PD-MCI. Only two prospective studies based on MDS criteria, have evaluated the incidence rate of PDD among PD-NC patients and patients with MCI at baseline, both reporting very close results. In particular, similar rates were reported by Domellof et al. (2015) where the incidence rate of PDD was 18.8/1000 pyar among PD-NC and 142/1000 pyar among PD-MCI at baseline, leading to a 6.5 times increased risk of developing dementia among patients with MCI at baseline. Close results have also been reported in the Norwegian cohort, where incidence rate of PDD among PD patients presenting MCI at baseline was 120.8/1000 pyar, while the incidence in the whole cohort was 38.1/1000 pyar (Pedersen et al., 2017). A clear contribution of PD-MCI to the hazard of PDD was finally reported by an international study including longitudinal data from four different cohorts assessing cognition according to MDS Level II criteria. In this very recent study, only 6.4% of PD-NC developed dementia, while 50% of the PD-MCI group developed PDD (Hoogland et al., 2017). In agreement with previous studies (Domellof et al., 2015; Hoogland et al., 2017; Pedersen et al., 2017), the presence of MCI at baseline in the present study was the main predictor of PDD regardless of age, sex, educational level, and motor impairment as demonstrated by multivariate analysis.

In agreement with previous reports (Domellof et al., 2015; Pigott et al., 2015), in our cohort the risk of PDD was significantly associated with older age and motor impairment (borderline significant) at univariate analysis, and inversely associated with educational level. Of interest, at multivariate analysis and except for the presence of MCI at baseline, only motor impairment (UPDRS–ME) was still significantly associated with the risk of developing dementia.

In our study a three times increased risk of dementia among men (70.1/1000 pyar *versus* 56.1.0/1000 pyar) was found. The role of gender in the risk of cognitive impairment in PD is still debated, although several studies have suggested that male gender is a risk factor (Picillo et al., 2017). In a large multicenter case-control study, conducted in central-southern Italy, the association between PD and cognitive impairment was stronger among men compared to women (adjusted OR 5.44 for men and 2.82 for women) (Nicoletti et al., 2017). Nonetheless, and considering longitudinal studies, only a few studies have demonstrated a high risk of PDD among men (Pigott et al., 2015; Cholerton et al., 2018) and in particular in a recent study the principal predictive factor in the transition from PD-NC to PD-MCI or PDD was male sex with an OR of 4.47 (Cholerton et al., 2018).

Regarding the impact of specific MCI subtypes and cognitive domain in the progression from PD-NC to PD-MCI and dementia, naMCImd was the most important predictor of PDD after multivariate regression analysis, followed by aMCImd. To

the best of our knowledge, none of the prospective studies based on MDS Level II criteria for the diagnosis of MCI have evaluated the role of the different MCI subtypes as a predictor for PDD development. Concerning specific cognitive domains (at least one impaired test at the baseline evaluation), executive functions and attention were strongly associated with the development of PDD. To date there has been no agreement regarding which type of impaired cognitive domain is a predictor of PDD. Indeed, according to the "dual syndrome hypothesis," the impaired "cholinergic" visuo-spatial domain was more likely to evolve into later dementia, while the "dopaminergic" executive dysfunction was not (Kehagia et al., 2013). On the other hand, a recent study has confirmed the role of the cholinergic system in the maintenance of attention and executive functions as well (Lee et al., 2014). These neuroanatomical bases could support the role of the executive dysfunction as a significant predictor of PDD, as previously reported (Levy et al., 2002; Chung et al., 2017) and confirmed by our study.

The presence of at least one impaired test in the language domain in the present study was also strongly related to the development of PDD. However, it should be noted that only two subjects, both classified as PD-MCI at baseline, were impaired in the language domain, and only one developed PDD at follow-up. Accordingly, we believe that accuracy of this finding is questionable as confirmed by the wide CIs obtained. Furthermore, the frequency of the impairment of the language domain is generally rather low in subjects with PD-MCI (Santangelo et al., 2015).

According to previous reports, about 10% of patients classified as PD-MCI reverted to normal cognition over a period of years (Koepsell and Monsell, 2012). A possible explanation for this "inconstant" cognitive impairment could be: the effects of practice-related learning, normal fluctuation in cognition, depression, poor motivation, mild psychiatric symptoms, other medical condition or daytime sleepiness (Koepsell and Monsell, 2012). This complex and sometimes fluctuating course of cognitive impairment in PD increases the diagnostic uncertainly of the PD-MCI construct. In this study nine (16%) PD-MCI at baseline reverted to NC at follow-up. In non-PD patients, almost 40% MCI patients reverted to normal cognition during follow-up. Non-amnestic MCI and, more generically, single domain MCI have been reported to revert with high frequency (Roberts et al., 2014). Reversion in our cohort was not associated with a specific MCI subtype, although the small number of reverters does not provide accurate estimates.

Although the data presented in this paper relating to the incidence of PD-MCI and the progression from PD-MCI to PDD are close to those reported by other studies, comparisons should be interpreted with caution. Indeed, a wide variation in estimates has been reported by studies which used the MDS criteria. This variability in estimates should not only be due to the different study design: prevalent (Pigott et al., 2015) *versus* incident cases (Broeders et al., 2013; Domellof et al., 2015; Santangelo et al., 2015; Pedersen et al., 2017); population-based (Domellof et al., 2015; Pedersen et al., 2017) *versus* hospital-based (Broeders et al., 2013; Pigott et al., 2015; Santangelo et al., 2015), but also to the different level of the MDS criteria adopted [Level I criteria (Hobson and Meara, 2015; Pigott et al., 2015; Pedersen et al., 2017) *versus* Level II criteria (Broeders et al., 2013; Domellof et al., 2015; Santangelo et al., 2015; Cholerton et al., 2018)]. Furthermore, the different neuropsychological assessment adopted to evaluate cognitive impairment in PD may also account for the variability in estimates. Lastly, another relevant source of variability related to the MDS criteria, is the possibility of using different cut-off levels to consider a test as *impaired*. Indeed, the MDS has proposed a range of cut-off scores (1 and 2 Standard Deviations below normative data), but the choice of cut-offs levels impacts on prevalence estimates (Roberts et al., 2014).

The major strength of our study lies in the large cohort size of the PACOS study at baseline (Monastero et al., 2018), that allowed us to identify several non-demented PD patients, suitable to be re-evaluated at follow-up. Furthermore, a comprehensive neuropsychological assessment was used, fulfilling the requirements of Level II MDS criteria. Lastly, we used a cut-off score of 2 SD which produces reliable sensitivity and specificity levels, and its use in this field is, therefore, recommended (Goldman et al., 2013).

Nonetheless, several limits should be taken into account in interpreting our data. First, a possible selection bias cannot be excluded due to the hospital-based study design. Regarding other hospital-based cohorts (Foltynie et al., 2004; Aarsland et al., 2009; Hoogland et al., 2017), the presence of more severe cases attending the two hospital centers involved in the study cannot be excluded, and this may possibly have contributed to the high estimate of MCI at baseline. Nonetheless and, as previously reported (Monastero et al., 2018), the average HY score and the short disease duration recorded in the PACOS cohort have revealed a mild to moderate stage of disease. Second, although analyses were adjusted for major potential confounders, residual confounding (e.g., medical and neuropsychiatric comorbidity, the use of psychotropic drugs) cannot be excluded. Lastly, due to the small samples of some of the MCI subtypes in our longitudinal analysis, our results need to be confirmed and strengthened in larger cohorts.

In conclusion, despite the difference sources of variability across the few prospective studies conducted in PD-MCI based on MDS criteria, our data are in line with those previously reported. This supports the relevant role of MCI in the risk of developing dementia in PD patients and it underlines the importance of education in reducing the risk of cognitive impairment. Furthermore, the results of the present study may have relevant clinical and therapeutic implications. Indeed, considering the high risk of developing dementia, PD-MCI patients should be carefully monitored in order to benefit from both early pharmacological (Mamikonyan et al., 2015) and non-pharmacological interventions (Dibilio et al., 2017). Prospective data relating to large populations are required to confirm the risk of cognitive impairment in male subjects with PD, in addition to the specific cognitive phenotype, which is associated with the PD progression to MCI and dementia.

AUTHOR CONTRIBUTIONS

AN designed and conceptualized the study, analyzed and interpreted the data, and drafted the manuscript for intellectual content. AL collected and interpreted the data, and drafted the manuscript for intellectual content. RB and CEC collected the data and revised the manuscript for intellectual content. GM, MD, LP, VR and MZ interpreted the data and revised the manuscript for intellectual content. RM designed and conceptualized the study, interpreted the data, and drafted the manuscript for intellectual content. All authors approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2019.00021/full#supplementary-material

REFERENCES

Aarsland, D., Bronnick, K., Larsen, J., Tysnes, O. B., and Alves, G. (2009). Norwegian parkwest study group, cognitive impairment in incident, untreated Parkinson disease: the norwegian parkwest study. *Neurology* 72, 1121–1126. doi: 10.1212/01.wnl.0000338632.00552

Aarsland, D., Creese, B., Politis, M., Chaudhuri, K. R., Ffytche, D. H., Weintraub, D., et al. (2017). Cognitive decline in Parkinson disease. *Nat. Rev. Neurol.* 4, 217–231. doi: 10.1038/nrneurol.2017.27

Baschi, R., Nicoletti, A., Restivo, V., Recca, D., Zappia, M., and Monastero, R. (2018). Frequency and correlates of subjective memory complaints in Parkinson's disease with and without mild cognitive impairment: data from the Parkinson's disease cognitive impairment study. *J. Alzheimers Dis.* 63, 1015–1024. doi: 10.3233/JAD-171172

Broeders, M., de Bie, R. M., Velseboer, D. C., Speelman, J. D., Muslimovic, D., and Schmand, B. (2013). Evolution of mild cognitive impairment in Parkinson disease. *Neurology* 81, 346–352. doi: 10.1212/WNL.0b013e31829c5c86

Carlesimo, G. A., Caltagirone, C., and Gainotti, G. (1996). The mental deterioration battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment. The group for the standardization of the mental deterioration battery. *Eur. Neurol.* 36, 378–384. doi: 10.1159/000117297

Cholerton, B., Johnson, C. O., Fish, B., Quinn, J. F., Chung, K. A., Peterson-Hiller, A. L., et al. (2018). Sex differences in progression to mild cognitive impairment and dementia in Parkinson's disease. *Parkinsonism Relat. Disord.* 50, 29–36. doi: 10.1016/j.parkreldis.2018.02.007

Chung, S. J., Shin, J. H., Cho, K. H., Lee, Y., Sohn, Y. H., Seong, J. K., et al. (2017). Subcortical shape analysis of progressive mild cognitive impairment in Parkinson's disease. *Mov. Disord.* 32, 1447–1456. doi: 10.1002/mds.27106

De Renzi, E., and Vignolo, L. A. (1962). The token test: a sensitive test to detect receptive disturbances in aphasia. *Brain* 85, 665–678. doi: 10.1093/brain/85.4.665

Dibilio, V., Nicoletti, A., Mostile, G., Portaro, G., Luca, A., Patti, F., et al. (2017). Computer-assisted cognitive rehabilitation on freezing of gait in Parkinson's disease: a pilot study. *Neurosci. Lett.* 654, 38–41. doi: 10.1016/j.neulet.2017.06.006

Domellof, M. E., Ekman, U., Forsgren, L., and Elgh, E. (2015). Cognitive function in the early phase of parkinson's disease, a five-year follow-up. *Acta Neurol. Scand.* 132, 79–88. doi: 10.1111/ane.12375

Dubois, B., Slachevsky, A., Litvan, I., and Pillon, B. (2000). The FAB: frontal assessment battery at bedside. *Neurology* 55, 1621–1626. doi: 10.1212/WNL.55.11.1621

Emre, M., Aarsland, D., Brown, R., Burn, D. J., Duyckaerts, C., Mizuno, Y., et al. (2007). Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov. Disord.* 22, 1689–1707. doi: 10.1002/mds.21507

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). 'Mini-mental state': a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Foltynie, T., Brayne, C. E., Robbins, T. W., and Barker, R. A. (2004). The cognitive ability of an incident cohort of Parkinson's patients in the UK. The CamPaIGN study. *Brain* 127, 550–560.

Gibb, W. R., and Lees, A. J. (1988). The relevance of the lewy body to the pathogenesis of idiopathic Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 51, 745–752. doi: 10.1136/jnnp.51.6.745

Giovagnoli, A. R., Del Pesce, M., Mascheroni, S., Simoncelli, M., Laiacona, M., and Capitani, E. (1996). Trail making test: normative values from 287 normal adult controls. *Ital. J. Neurol. Sci.* 1996, 305–309. doi: 10.1007/BF01997792

Goldman, J. G., Holden, S., Bernard, B., Ouyang, B., Goetz, C. G., and Stebbins, G. T. (2013). Defining optimal cutoff scores for cognitive impairment using movement disorder society task force criteria for mild cognitive impairment in Parkinson's disease. *Mov. Disord.* 28, 1972–1979. doi: 10.1002/mds.25655

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

Hanagasi, H. A., Tufekcioglu, Z., and Emre, M. (2017). Dementia in Parkinson's disease. *J. Neurol. Sci.* 374, 26–31. doi: 10.1016/j.jns.2017.01.012

Hobson, P., and Meara, J. (2015). Mild cognitive impairment in Parkinson's disease and its progression onto dementia: a 16-year outcome evaluation of the denbighshire cohort. *Int. J. Geriatr. Psychiatry* 30, 1048–1055. doi: 10.1002/gps.4261

Hoogland, J., Boel, J., de Bie, R., Geskus, R. B., Schmand, B. A., Dalrymple-Alford, J. C., et al. (2017). Mild cognitive impairment as a risk factor for Parkinson's disease dementia. *Mov. Disord.* 32, 1056–1065. doi: 10.1002/mds.27002

Hughes, C. P., Berg, L., Danziger, W. L., Coben, L. A., and Martin, R. L. (1982). A new clinical scale for the staging of dementia. *Br. J. Psychiatry* 140, 566–572. doi: 10.1192/bjp.140.6.566

Jankovic, J., McDermott, M., Carter, J., Gauthier, S., Goetz, C., Golbe, L., et al. (1990). Variable expression of Parkinson's disease: a base-line analysis of the datatop cohort. The parkinson study group. *Neurology* 40, 1529–1534. doi: 10.1212/WNL.40.10.1529

Katz, S., Ford, A. B., Moskowitz, R. W., Jackson, B. A., and Jaffe, M. W. (1963). Studies of illness in the aged. the index of ADL: a standardized measure of biological and psychosocial function. *JAMA* 185, 914–919. doi: 10.1001/jama.1963.03060120024016

Kehagia, A. A., Barker, R. A., and Robbins, T. W. (2013). Cognitive impairment in Parkinson's disease: the dual syndrome hypothesis. *Neurodegener. Dis.* 11, 79–92. doi: 10.1159/000341998

Koepsell, T. D., and Monsell, S. E. (2012). Reversion from mild cognitive impairment to normal or near-normal cognition: risk factors and prognosis. *Neurology* 79, 1591–1598. doi: 10.1212/WNL.0b013e31826e26b7

Lee, J. E., Cho, K. H., Song, S. K., Kim, H. J., Lee, H. S., Sohn, Y. H., et al. (2014). Exploratory analysis of neuropsychological and neuroanatomical correlates of progressive mild cognitive impairment in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 85, 7–16. doi: 10.1136/jnnp-2013-305062

Levy, G., Jacobs, D. M., Tang, M. X., Côté, L. J., Louis, E. D., Alfaro, B., et al. (2002). Memory and executive function impairment predict dementia in Parkinson's disease. *Mov. Disord.* 17, 1221–1226. doi: 10.1002/mds.10280

Litvan, I., Goldman, J. C., Tröster, A. I., Schmand, B. A., Weintraub, D., Petersen, R. C., et al. (2012). Diagnostic criteria for mild cognitive impairment in Parkinson's disease: movement disorder society task force guidelines. *Mov. Disord.* 27, 349–356. doi: 10.1002/mds.24893

Luzzatti, C., Willmes, K., and De Bleser, R. (1996). *Aachener Aphasie Test (AAT, versione italiana) Manuale e Dati Normativi*. Firenze: Organizzazioni Speciali.

Mamikonyan, E., Xie, S. X., Melvin, E., and Weintraub, D. (2015). Rivastigmine for mild cognitive impairment in Parkinson disease: a placebo-controlled study. *Mov. Disord.* 30, 912–918. doi: 10.1002/mds.26236

Monastero, R., Cicero, C. E., Baschi, R., Davì, M., Luca, A., Restivo, V., et al. (2018). Mild cognitive impairment in Parkinson's disease: the Parkinson's disease

cognitive study (PaCoS). *J. of Neurol.* 265, 1050–1058. doi: 10.1007/s00415-018-8800-4

Munhoz, R. P., Moro, A., Silveira-Moriyama, L., and Teive, H. A. (2015). Non-motor signs in Parkinson's disease: a review. *Arq. Neuropsiquiatr.* 73, 454–462. doi: 10.1590/0004-282X20150029

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53, 695–699. doi: 10.1111/j.1532-5415.2005.53221.x

Nicoletti, A., Vasta, R., Mostile, G., Nicoletti, G., Arabia, G., Iliceto, G., et al. (2017). Gender effect on non-motor symptoms in Parkinson's disease: are men more at risk? Parkinsonism relat. *Disorder* 35, 69–74. doi: 10.1016/j.parkreldis.2016.12.008

Novelli, G., Papagno, C., Capitani, E., Cappa, S. F., and Vallar, G. (1986a). Tre test clinici di memoria verbale a lungo termine. *Arch. Psicol. Neurol. Psichiatr.* 47, 278–296.

Novelli, G., Papagno, C., Capitani, E., Laiacona, M., Vallar, G., and Cappa, S. F. (1986b). Tre test clinici di ricerca e produzione lessicale. Taratura su soggetti normali. *Arch. Psicol. Neurol. Psichiatr.* 47, 477–506.

Pedersen, K. F., Larsen, J. P., Tysnes, O. B., and Alves, G. (2013). Prognosis of mild cognitive impairment in early Parkinson disease: the norwegian parkwest study. *JAMA Neurol.* 70, 580–586. doi: 10.1001/jamaneurol.2013.2110

Pedersen, K. F., Larsen, J. P., Tysnes, O. B., and Alves, G. (2017). Natural course of mild cognitive impairment in Parkinson disease: a 5-year population-based study. *Neurology* 88, 767–774. doi: 10.1212/WNL.0000000000003634

Petersen, R. C., Doody, R., Kurz, A., Mohs, R. C., Morris, J. C., Rabins, P. V., et al. (2001). Current concepts in mild cognitive impairment. *Arch. Neurol.* 58, 1985–1992. doi: 10.1001/archneur.58.12.1985

Picillo, M., Nicoletti, A., Fetoni, V., Garavaglia, B., Barone, P., and Pellecchia, M. T. (2017). The relevance of gender in Parkinson's disease: a review. *J. Neurol.* 264, 1583–1607. doi: 10.1007/s00415-016-8384-9

Pigott, K., Rick, J., Xie, S. X., Hurtig, H., Chen-Plotkin, A., Duda, J. E., et al. (2015). Longitudinal study of normal cognition in Parkinson disease. *Neurology* 85, 1276–1282. doi: 10.1212/WNL.0000000000002001

Poletti, M., Frosini, D., Pagni, C., Baldacci, F., Nicoletti, V., Tognoni, G., et al. (2012). Mild cognitive impairment and cognitive-motor relationships in newly diagnosed drug-naive patients with Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 83, 601–606. doi: 10.1136/jnnp-2011-301874

Roberts, R. O., Knopman, D. S., Mielke, M. M., Cha, R. H., Pankratz, V. S., Christianson, T. J., et al. (2014). Higher risk of progression to dementia in mild cognitive impairment cases who revert to normal. *Neurology* 82, 317–325. doi: 10.1212/WNL.0000000000000055

Santangelo, G., Vitale, C., Picillo, M., Moccia, M., Cuoco, S., Longo, K., et al. (2015). Mild Cognitive Impairment in newly diagnosed Parkinson's disease: a longitudinal prospective study. *Parkinsonism Relat. Disord.* 21, 1219–1226. doi: 10.1016/j.parkreldis.2015.08.024

Schrag, A., Barone, P., Brown, R. G., Leentjens, A. F., McDonald, W. M., Starkstein, S., et al. (2007). Depression rating scales in Parkinson's disease: critique and recommendations. *Mov. Disord.* 22, 1077–1092. doi: 10.1002/mds.21333

Shulman, K. (2000). Clock-drawing: is it the ideal cognitive screening test? *Int. J. Geriatr. Psychiatry* 15, 548–561. doi: 10.1002/1099-1166(200006)15:6<548::AID-GPS242>3.0.CO;2-U

Tomlinson, C. L., Stowe, R., Patel, S., Rick, C., Gray, R., and Clarke, C. E. (2010). Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov. Disord.* 25, 2649–2653. doi: 10.1002/mds.23429

Uttl, B., and Graf, P. (1997). Color-Word Stroop test performance across the adult life span. *J. Clin. Exp. Neuropsychol.* 19, 405–420. doi: 10.1080/01688639708403869

Weintraub, D., Tröster, A. I., Marras, C., and Stebbins, G. (2018). Initial cognitive changes in Parkinson's disease. *Mov. Disord.* 33, 511–519. doi: 10.1002/mds.27330

Yarnall, A. J., Breen, D. P., Duncan, G. W., Khoo, T. K., Coleman, S. Y., Firbank, M. J., et al. (2004). Characterizing mild cognitive impairment in incident Parkinson disease. The ICICLE-PD Study. *Neurology* 82, 308–316. doi: 10.1212/WNL.0000000000000066

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Pathological Tau From Alzheimer's Brain Induces Site-Specific Hyperphosphorylation and SDS- and Reducing Agent-Resistant Aggregation of Tau *in vivo*

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Neurofibrillary tangles (NFTs) made up of hyperphosphorylated tau are a histopathological hallmark of Alzheimer's disease (AD) and related tauopathies. Hyperphosphorylation of tau is responsible for its loss of normal physiological function, gain of toxicity and its aggregation to form NFTs. Injection of misfolded tau seeds into mouse brain induces tau aggregation, but the nature of tau phosphorylation in pathologic tau seeded pathology is unclear. In the present study, we injected hyperphosphorylated and oligomeric tau isolated from AD brain (AD P-tau) into hippocampus of human tau transgenic mice and found that in addition to tau aggregation/pathology, tau was hyperphosphorylated at Ser202/Thr205, Thr212, Ser214, Thr217, Ser262, and Ser422 in AD P-tau injected hippocampus and at Ser422 in the contralateral hippocampus and in the ipsilateral cortex. AD P-tau-induced AD-like high molecular weight aggregation of tau that was SDS- and reducing agent-resistant and site-specifically hyperphosphorylated in the ipsilateral hippocampus. There were no detectable alterations in levels of tau phosphatases or tau kinases in AD P-tau-injected brains. Furthermore, we found that hyperphosphorylated tau was easier to be captured by AD P-tau and that aggregated tau was more difficult to be dephosphorylated than the non-aggregated tau by protein phosphatase 2A (PP2A). Based on these findings, we speculate that AD P-tau seeds hyperphosphorylated tau to form aggregates, which resist to the dephosphorylation by PP2A, resulting in hyperphosphorylation and pathology of tau.

Keywords: Alzheimer's disease, AD P-tau, hyperphosphorylation of tau, tau pathology, propagation of tau pathology

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INTRODUCTION

Alzheimer's disease (AD) is multifactorial and involves different etiopathogenic mechanisms (Iqbal et al., 2005, 2016; Iqbal and Grundke-Iqbal, 2010). Histopathologically, AD is characterized by intraneuronal neurofibrillary tangles (NFTs) and extracellular deposits of β-amyloid plaques. Clinicopathological correlation studies have shown that the number of NFTs, but not of amyloid

plaques, correlates with the degree of dementia in AD patients (Tomlinson et al., 1970; Alafuzoff et al., 1987; Arriagada et al., 1992; Quiroz et al., 2018).

NFTs initiate in subcortical regions, transentorhinal area, and entorhinal cortex, then appear in the hippocampal formation and some parts of the neocortex, followed by most of the neocortex—the Braak stages—whereas the distribution of NFTs correlates with the progression of the disease (Braak and Braak, 1991; Braak and Del Tredici, 2011). Tau pathology in AD develops progressively in regions of the brain with known synaptic connectivity. Recently, tau tracer retention measured by positron emission tomography also showed similar stages (Johnson et al., 2016; Schöll et al., 2016; Schwarz et al., 2016). Thus, the regional distribution of tau pathology is apparently associated with the disease progression.

NFTs are composed of hyperphosphorylated and aggregated microtubule-associated protein tau (Grundke-Iqbal et al., 1986a,b), major function of which is to promote microtubule assembly and maintain microtubule structure. This biological activity of tau is regulated by its degree of phosphorylation. In AD brain, tau is abnormally hyperphosphorylated. The hyperphosphorylation inhibits the activity of tau to promote microtubule assembly (Lindwall and Cole, 1984; Iqbal et al., 1986; Alonso et al., 1994). Unlike normal tau, the hyperphosphorylated and oligomeric tau isolated from AD brain (AD P-tau) sequesters/captures normal tau and templates it into filaments *in vitro* (Alonso et al., 1994). This phenomenon was recently termed prion-like property of pathological tau.

Injection of brain extract from tau_{P301S}-expressing mice into the brain of transgenic wild-type tau-expressing mice induces tau aggregation not only at the injection sites, but also in the anatomically connected brain regions in a time-dependent manner, introducing the concept of "propagation of tau pathology" (Clavaguera et al., 2009). Subsequently, several studies reported the induction of tau pathology by intrahippocampal injection of misfolded tau seeds (Liu et al., 2012; de Calignon et al., 2012; Iba et al., 2013; Ahmed et al., 2014; Dujardin et al., 2014; Peeraer et al., 2015). We showed that injection of AD P-tau into the hippocampi of Tg/hTau and 3xTg-AD mice induces AD-like NFTs, which can be labeled by various phosphorylation-dependent and site-specific anti-tau antibodies (Hu et al., 2016; Dai et al., 2018). However, whether AD P-tau induces tau hyperphosphorylation is not documented and the possible mechanism(s) involved is unknown. In the present study, we analyzed tau phosphorylation in AD P-tau-injected hippocampus in Tg/hTau mice and found site-specific hyperphosphorylation and SDS- and reducing agent-resistant high molecular weight smears of tau, but no alteration in the levels of tau phosphatases or kinases in AD P-tau injected hippocampus. Thus, the AD P-tau-seeded tau aggregation/pathology apparently maintains its characteristics.

MATERIALS AND METHODS

Animals

The hemizygous human tau transgenic [Tg/hTau, B6.Cg-Mapttm1 (EGFP)Klt Tg(MAPT) 8cPdav/J] mice with murine tau knockout (tau-/-) background (Duff et al., 2000) and Tau-/- mice (Tucker et al., 2001) were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and generated by crossing Tg/hTau and Tau-/-. The mice were housed under a 12-h light/dark cycle, with access to food and water *ad libitum*. All animal handling and use were as per the protocol approved by Institutional Animal Care and Use Committee at New York State Institute for Basic Research in Developmental Disabilities in accordance with the PHS Policy on Human Care and Use of Laboratory Animals.

Preparation of Hyperphosphorylated and Oligomeric Tau (AD P-tau), Heat-Stable Tau (HS-tau) and Sarkosyl Insoluble Tau (SI-tau) From AD Brain

Frozen brain tissue samples from autopsied and histopathologically confirmed AD cases were obtained from the Brain Tissue Resource Center, McLean Hospital, Belmont, MA, USA. The use of autopsied frozen human brain tissue was in accordance with the National Institutes of Health guidelines and was exempted by the Institutional Review Board (IRB) of New York State Institute for Basic Research in Developmental Disabilities because "the research does not involve intervention or interaction with the individuals" nor "is the information individually identifiable."

Hyperphosphorylated and oligomeric tau (AD P-tau) was isolated from autopsied and frozen AD cerebral cortex as described by us previously (Köpke et al., 1993; Hu et al., 2016). Briefly, 10% brain homogenate prepared in the buffer (20 mM Tris-HCl, pH 8.0, 0.32 M sucrose, 10 mM β-mercaptoethanol, 5 mM $MgSO_4$, 1 mM EDTA, 10 mM glycerophosphate, 1 mM Na_3VO_4, 50 mM NaF, 2.0 mM benzamidine, 1.0 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), and 10 μg/ml each of aprotinin, leupeptin, and pepstatin) was centrifuged at 27,000× *g* for 30 min. The pellet was saved for sarkosyl insoluble tau (SI-tau) preparation. The supernatant was further centrifuged at 235,000× *g* for 45 min, and the resulting pellet, i.e., AD P-tau, was collected and washed three times and then resuspended in saline. The supernatant was used for heat stable tau (HS-tau) preparation.

HS-tau preparation: the supernatant from above 235,000× *g* was adjusted to 0.75 M NaCl and 10 mM β-mercaptoethanol, heated for 5 min at 100°C, and centrifuged at 25,000× *g* for 30 min. The supernatant was dialyzed against 10 mM Tris-HCl, pH 7.6, and concentrated by five times.

Sarkosyl insoluble aggregated tau (SI-tau) preparation: the pellet from above 27,000× *g* was homogenized in the homogenization buffer containing 0.1% sarkosyl and centrifuged at 10,000× *g* for 10 min. The supernatant was adjusted to 1% sarkosyl, incubated for 1 h at room temperature, and centrifuged at 235,000× *g* for 45 min. The pellet was collected as SI-tau after washing with 50 mM Tris-HCl for two times.

Stereotaxic Injection

AD P-tau was injected into the right hippocampus in Tg/hTau mice as described previously (Hu et al., 2016; Dai et al., 2018). Briefly, mice were deeply anesthetized with 1.25% Avertin

(Sigma, St. Louis, MO, USA) and placed on a stereotaxic frame. After craniotomy, 1 mm in diameter, was made with a motorized mini-drill, the tau seeds were injected using a 10 μl Hamilton syringe custom made with a 30 gauge/0.5 inch/hypodermic needle (Hamilton Syringe Co., Reno, NV, USA). AD P-tau was unilaterally injected into the right hippocampus (0.55 μg in 2.0 μl saline per hippocampus) in 9–11-month-old Tg/hTau or Tau-/-mice. The coordinates were as follows: −2.5 mm anterior/posterior, +2.0 mm medial/lateral to Bregma, and −1.67 mm dorsal/ventral to dura surface. AD P-tau was injected at a rate of 1.25 μl/min, and the needle was kept in position for three additional minutes before slow withdrawal to prevent leakage of the liquid infused. Saline was injected into Tg/hTau or Tau-/- mice of the same age as vehicle controls. The skin was sutured after injection, and the mice were allowed to completely recover on a soft warming pad before they were returned to their home cages.

Immunohistochemistry

At 10 weeks after injection, mice were deeply anesthetized and transcardially perfused with saline followed by buffered 4% paraformaldehyde. The whole brain was collected, post fixed in the same fixative overnight at 4°C, and dehydrated in buffered 30% sucrose solution. The brains were then cut into 40-μm serial coronal sections using a freezing microtome, and the free-floating sections were preserved in antifreeze solution at −20°C until used for immunohistochemical staining. Sections were washed with PBS, permeabilized with 0.3% Triton X-100, blocked with normal goat serum, and then incubated with primary antibody overnight at 4°C. Then, the sections were incubated with Alexa Fluor 555- or 488-goat anti-mouse or rabbit IgG (1:1,000, Life Technologies, Rockford, IL, USA) or a combination where appropriate. Images were captured with an EZ-C1 laser scanning confocal microscope (Nikon Instruments, Melville, NY, USA) and the Z-stack function was used to reveal the morphology of tangles. Control staining with samples which are known to be positive/negative for target antigen and the control with the absence of primary antibody were included in each experiment. AT8 staining was thresholded using Yen's arithmetic and quantified using the ImageJ software package. Area of AT8-positive somatodendritic profiles in the hippocampus was measured based on 3–4 sections each of Tg/hTau mice injected with AD P-tau.

Western Blots

Ipsilateral and contralateral hippocampi and cortices were dissected and homogenized in cold buffer consisting of 50 mM Tris-HCl, pH 7.4, 2.0 mM EDTA, 2.0 mM EGTA, 10 mM β-mercaptoethanol, 150 mM NaCl, 1.0 mM Na_3VO_4, 50 mM NaF, 10 μg/ml aprotinin, leupeptin and pepstatin, and 0.5 mM AEBSF. The homogenates were boiled in 1× Laemmli buffer (125 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 2.5% β-mercaptoethanol, 0.004% bromophenol blue) for 5 min.

TABLE 1 | Primary antibodies used in the present study.

Antibody	Type	Species	Specificity	Source/reference (catalog/lot #)
R134d	Poly-	R	Total tau	In-house (Tatebayashi et al., 1999)
Anti-pS199	Poly-	R	pSer199	Invitrogen (44734G)/0300A
Anti-pT205	Poly-	R	pThr205	Invitrogen (44–738G)/RJ239402
AT8	Mono-	M	pSer202/pThr205	Thermo Scientific (MN1020/PI205175)
Anti-pT212	Poly-	R	pThr212	Invitrogen (44740G)/1709582A
Anti-pS214	Poly-	R	pSer214	Invitrogen (44–742G)0500B
Anti-pT217	Poly-	R	pSer217	Invitrogen (44–744)/785771A
Anti-pS262	Poly-	R	pSer262	Invitrogen (44–750G)/QK220618
Anti-pS396	Poly-	R	pSer396	Invitrogen (44752G)/567847B
Anti-pS404	Poly-	R	pSer404	Invitrogen (44–758G)/5G255476
R145d	Poly-	R	pSer422	In-house (Pei et al., 1999)
PHF-1	Mono-	M	pSer396/pSer404	Dr. Peter Davies
Tau-1	Mono-	M	Up-tau (195–202)	Dr. Binder, L. I.
Anti-pERK	Poly-	R	pThr202/pTyr204	Cell Signaling (4377S/10)
Anti-pJNK	Poly-	R	pThr183/pTyr185	Cell Signaling (9251S/10)
Anti-pAKT	Poly-	R	pSer473	Cell Signaling (4058L/30)
Anti-pGSK-3β	Mono-	R	pSer9	Cell Signaling (9323S/13)
Anti-pAMPK	Mono-	R	pThr172	Cell Signaling (2535L/16)
Anti-pP70S6K	Mono-	R	pThr389	Cell Signaling (9234S/11)
Anti-PKAcα	Poly-	R	Total PKAc	Santa Cruz (SC-9031/B1111)
Anti-Cdk5	Mono-	M	Total Cdk5	Santa Cruz (SC-249/G1817)
8D9	Mono-	M	Total Dyrk1A	In-house (Wegiel et al., 2004)
CK1ε	Mono-	M	Total CK1ε	Santa Cruz (SC-81446)
Anti-PP2Ac	Mono-	M	Total PP2Ac	BD Transduction (610556/26637)
Anti-DM-PP2Ac	Mono-	M	Demethylated Lys309	Santa Cruz (SC-80990/F2512)
R126	Poly-	R	Total PP2B	In-house (Pei et al., 1998)
Anti-PP5	Poly-	R	Total PP5	Bahl et al. (2001)
PP1	Poly-	R	Total PP1	Santa-Cruz (sc-7482/1032)
Anti-GAPDH	Poly-	R	GAPDH	Sigma (G9545/015M4824V)
Anti-β-actin	Mono-	M	β-actin	Sigma (A1978/046M4789V)

Abbreviations: Mono-, monoclonal; p-, phosphorylated; up-, unphosphorylated; Poly-, polyclonal; M, Mouse; R, Rabbit.

Protein concentration was quantified by using A660 Protein Assay kit (Pierce, Rockford, IL, USA). The same amount of brain homogenate proteins was separated by SDS-PAGE and electrically blotted onto polyvinylidene fluoride membrane (PVDF, Millipore). After blocking with 5% milk in Tris-HCl buffered saline (TBS), the membrane was incubated with primary antibodies (**Table 1**) and followed by the species-matched peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA). The blots were then developed by using ECL kit (Thermo Fisher Scientific) and exposed to HyBlot CLr autoradiography film (Denville Scientific, Inc., Holliston, MA, USA). Immunoblotting image was quantified by using the Multi Gauge software V3.0 from Fuji Film (Minato, Tokyo, Japan).

Dephosphorylation by Protein Phosphatase 2A (PP2A)

Heat stable tau (HS-tau) and sarkosyl insoluble tau (SI-tau) were dephosphorylated with protein phosphatase 2A (PP2A) for various time points in the buffer (100 mM Tris-HCl, pH7.4, 1 mM $MnCl_2$, 10 mM β-mercaptoethanol). The dephosphorylation products were then analyzed for phosphorylation by dot-blots developed with anti-pS199-tau (**Table 1**).

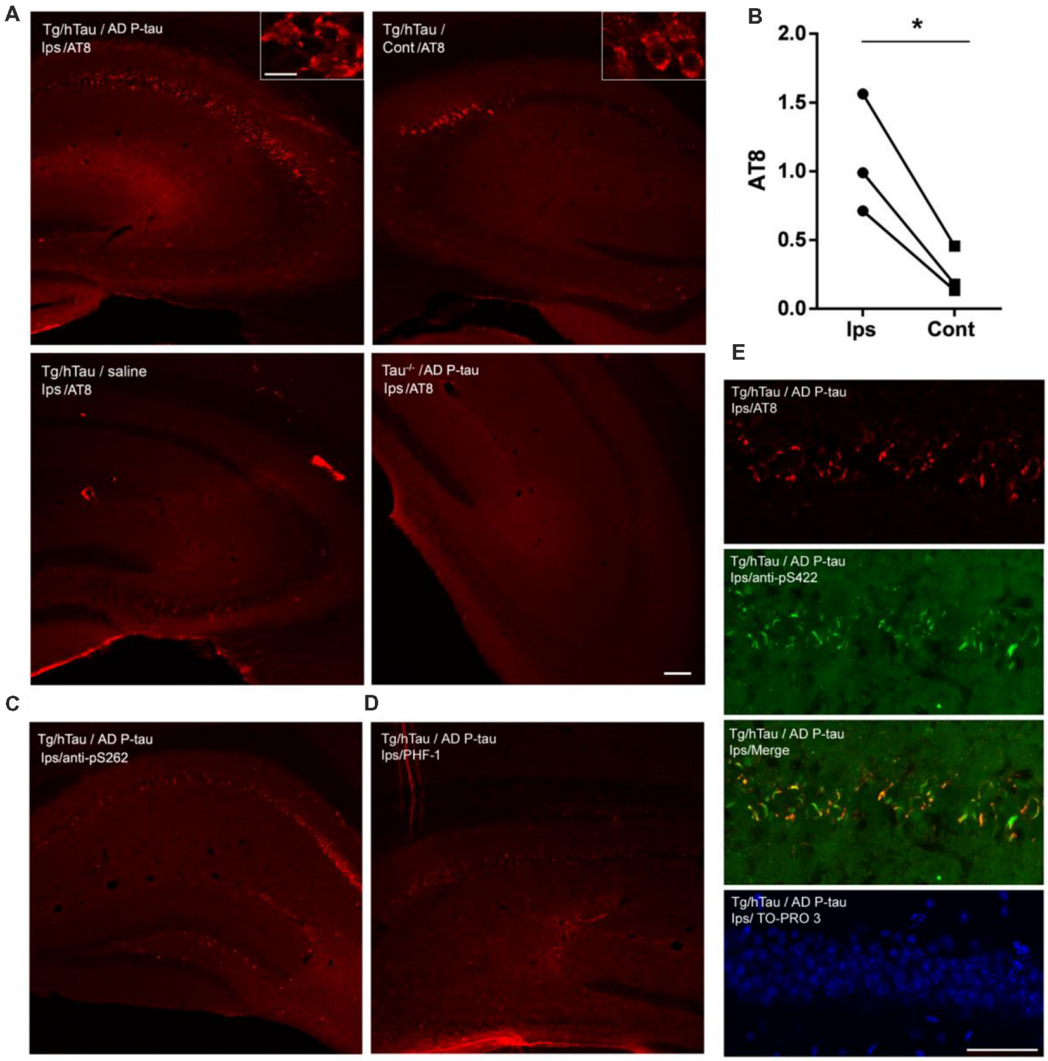

FIGURE 1 | Alzheimer's hyperphosphorylated and oligomeric tau (AD P-tau)-seeded tau aggregates/pathology in the hippocampus in Tg/hTau mice. **(A–D)** AD P-tau (0.55 μg) or as a control saline was injected unilaterally into the hippocampus of 9–11-month-old Tg/hTau or Tau-/- mice. Coronal brain sections were immunostained with tau antibodies, AT8 (pSer202/Thr205-tau) **(A)**, anti-pSer262-tau **(C)**, and PHF-1 (pSer396/404) **(D)** 10 weeks after injection and immunostaining of ipsilateral (Ips) and contralateral (Cont) hippocampi of Tg/hTau or Tau-/- mice were captured. AT8 staining in 3–4 sections of both ipsilateral and contralateral hippocampi was quantified with ImageJ software from each of three Tg/hTau mice injected with AD P-tau and statistically analyzed with paired student *t*-test **(B)**, $^*p < 0.05$. **(E)** CA1 region of Tg/hTau mouse ipsilateral hippocampus double immunostained with AT8 (Red) and anti-pSer422-tau (Green) and counterstained with TO-PRO 3 iodide for nucleus. Scale bar 100 μm and insert scale bar 20 μm.

Tau Capture Assay

HEK-293FT cells were transfected with pCI/HA-Tau$_{441}$ for 48 h. The cells were lysed in PBS containing protease and phosphatase inhibitors by probe sonication. The debris was removed by centrifugation at 15,000× *g* for 5 min. The cell extract was aliquoted and stored at −80°C.

Various amounts of AD P-tau were dotted on nitrocellulose membrane. After drying for 1 h at 37°C, the membrane was blocked with 5% milk in TBS and incubated with HEK-293FT/HA-tau$_{441}$ cell extract overnight at room temperature. After washing, the captured tau was analyzed by incubating with anti-HA followed by peroxidase-conjugated secondary antibody and developed by using ECL kit as described above.

Statistical Analysis

The GraphPad Prism 6 software was used for statistical analysis. Results were analyzed by one- or two-way analyses of variance (ANOVA) for multiple-group analysis followed with Tukey's or Sidak's multiple comparisons test and by the unpaired or paired two-tailed Student's *t*-test for two-group comparison.

RESULTS

AD P-Tau Induces Tau Aggregation in Tg/hTau Mouse Brains

We previously found that injection of 0.12 μg AD P-tau into the hippocampus in 3-month-old Tg/hTau mouse induces robust tau pathology 9 months after injection (Hu et al., 2016). In the present study, we injected 0.55 μg AD P-tau unilaterally into the hippocampus of 9-11-month-old Tg/hTau mice, in which no detectable tau pathology occurs at this age (Hu et al., 2016). Similar age tau knockout (Tau-/-) mice were used as a control. Coronal brain sections were immunostained with site-specific and phosphorylation dependent anti-tau antibodies 10 weeks post AD P-tau injection. Robust tau aggregates/pathology was observed in both ipsilateral and contralateral (**Figure 1A**) hippocampi immunostained with tau antibodies, AT8 (pSer202/Thr205; **Figure 1A**), anti-pS262-tau (**Figure 1C**) and PHF-1 (pSer396/404; **Figure 1D**). No tau pathology was detected in the hippocampus in Tg/hTau mice injected with vehicle or in Tau-/- mice injected with AD P-tau (**Figure 1A**). AD P-tau seeded tau pathology in the contralateral hippocampus was milder than that in the ipsilateral hippocampus of Tg/hTau mice (**Figure 1B**). Tau aggregates were co-labeled by AT8 and anti-pS422-tau (**Figure 1E**). Thus, AD P-tau was able to induce tau aggregation and pathology in Tg/hTau mouse brain 10 weeks post AD P-tau injection, and the tau aggregates were phosphorylated at multiple sites including Ser202/205, Ser262, Ser396/404, and Ser422.

AD P-Tau Induces Site-Specific Hyperphosphorylation of Tau in Tg/hTau Mouse Brains

NFTs are made up of abnormally hyperphosphorylated tau (Grundke-Iqbal et al., 1986a,b). AD P-tau-seeded tau pathology was phosphorylated at multiple sites (**Figure 1**). To determine whether AD P-tau induces tau hyperphosphorylation, we analyzed the hippocampi of Tg/hTau and Tau-/- mice 10 weeks post AD P-tau injection by Western blots developed with site

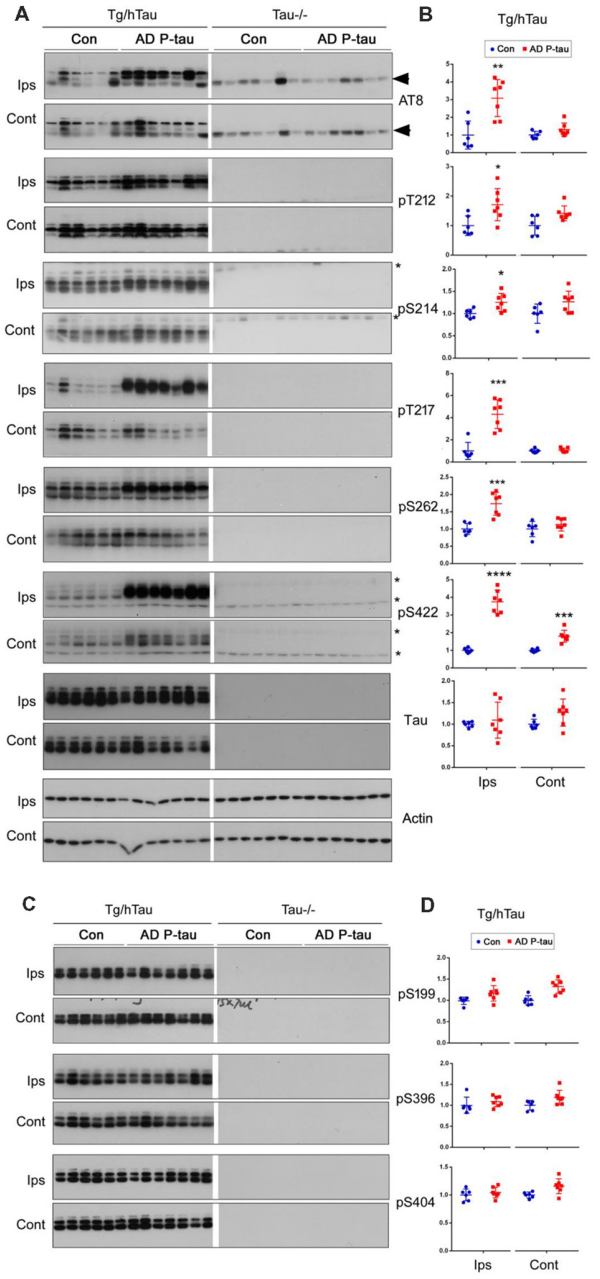

FIGURE 2 | AD P-tau-induced site-specific hyperphosphorylation of tau in the hippocampus in Tg/hTau mice. AD P-tau (0.55 μg) was injected unilaterally into the hippocampus in 9–11-month-old Tg/hTau and Tau-/- mice. Ten weeks post injection, phosphorylation of tau in the hippocampus was analyzed by Western blots developed with the indicated phosphorylation-dependent and site-specific tau antibodies **(A,C)**, normalized with total tau and analyzed with unpaired student *t*-test, and are presented as scattered dots with mean ± SD **(B,D)**, and analyzed with unpaired student *t*-test; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. Arrow head of AT8 blots indicates the heavy chain of IgG (50 kDa) and *indicates the non-specific band.

specific- and phosphorylation-dependent tau antibodies. Tau protein level was found to be similar in the hippocampus in Tg/hTau mice injected with AD P-tau and that in vehicle injected mice (**Figures 2A,B**). However, phosphorylation of tau was markedly increased at Ser202/Thr205 (AT8), Thr217, Ser262 and Ser422 (**Figures 2A,B**), was slightly increased at Thr212 and Ser214 (**Figures 2A,B**), and was not altered at Ser199, Ser396 and Ser404 (**Figures 2C,D**) in the AD P-tau injected hippocampus in Tg/hTau mice. These results suggest that AD P-tau induces site-specific hyperphosphorylation of tau *in vivo*.

In the contralateral hippocampus, tau phosphorylation was increased only at Ser422 and showed a trend to increase at Thr212 and Ser214 (**Figures 2A,B**). Ser422 phosphorylation may be an early event in AD P-tau-induced tau hyperphosphorylation. No tau was detected in tau-/- mouse hippocampus (**Figure 2A**), except a ∼50 kDa IgG heavy chain and non-specific bands in the blots developed with AT8 and anti-Ser422, respectively (**Figure 2A**), confirming immuno-specificity of tau and phosphorylated tau antibodies.

Tau pathology templated by misfolded tau seeds can be propagated to other brain regions (Liu et al., 2012; de Calignon et al., 2012). To study the spread of tau pathological alterations in the cortex, we analyzed tau phosphorylation by Western blots. Similar as in the contralateral hippocampus, phosphorylation of tau at Ser422, but not at Ser199, Thr205, Ser214, Thr217, Ser262, Ser396, or Ser404, was significantly increased in the ipsilateral cortex of Tg/hTau mice injected with AD P-tau (**Figure 3**). No significant alteration of tau phosphorylation was observed in the contralateral cortex (data not shown). These results also support that phosphorylation of tau at Ser422 may be an early event in AD P-tau templated tau pathogenesis.

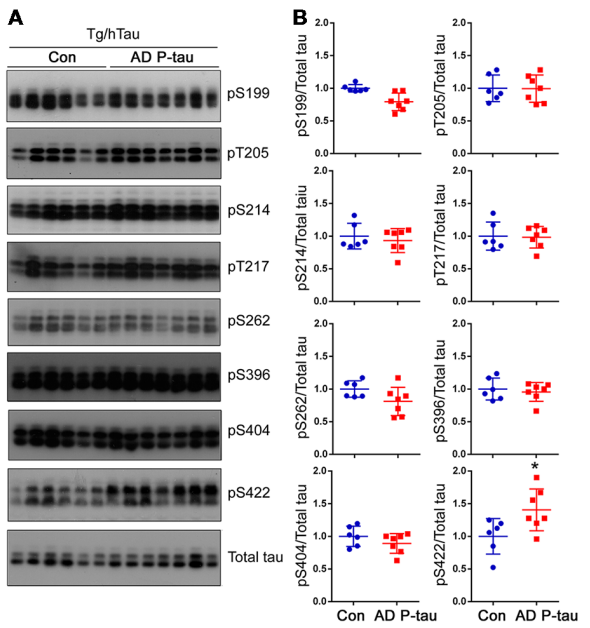

FIGURE 3 | Tau phosphorylation in the ipsilateral cortex in Tg/hTau mice injected with AD P-tau. Phosphorylation of tau at Ser199, Thr205, Ser214, Thr217, Ser262, Ser396, Ser404 and Ser422 in the ipsilateral cortex was analyzed by Western blots **(A)** and analyzed with unpaired student *t*-test. Data are presented as scattered dots with mean ± SD **(B)**; $^*p < 0.05$.

AD P-Tau Seeds Tau to Form SDS- and Reducing Agent-Resistant High-Molecular Weight Aggregation

High molecular tau smear (HMW-tau) in Western blots is only seen in AD brains but not in control human brains, indicating that SDS- and reducing agent-resistant HMW-tau aggregation may be the features of pathological alteration of tau (Zhou et al., 2018). To determine whether AD P-tau is able to induce the formation of HMW-tau in Western blots, we exposed above blots to X-ray film for extended time. We found obvious HMW-tau smears in the blots developed with anti-pT217-tau, anti-pS262-tau and anti-pS422-tau in the ipsilateral hippocampus and no or much less HMW-tau in contralateral hippocampus (**Figures 4A,C**). Interestingly, we also observed a trace amount of HMW-tau in the ipsilateral hippocampus in the PHF-1 blot (**Figures 4A,C**). Consistently, phosphorylation of tau at Thr217 and Ser262 was increased only in ipsilateral hippocampus, but at Ser422 was increased in both ipsilateral and contralateral hippocampi (**Figures 4A,B**). However, compared to saline-injected and contralateral hippocampi, tau phosphorylation at Thr217, Ser262, and Ser422, but not at Ser396/404 (PHF-1), was increased in the ipsilateral hippocampus (**Figures 4A,B**). Thus, in addition to site-specific hyperphosphorylation, *in vivo* treatment with AD P-tau induces site-specific formation of AD-like SDS- and β-mercaptoethanol-resistant and site-specifically hyperphosphorylated HMW-tau.

To learn that SDS- and β-mercaptoethanol-resistant AD-like HMW-tau in the AD P-tau-injected hippocampus is not the injected exogenous AD P-tau, we analyzed ipsilateral hippocampus of Tg/hTau and Tau-/- mice injected with AD P-tau by Western blots developed with various anti-phospho-tau antibodies. There are obvious HMW-tau seen in anti-pT212, anti-pT217, anti-pS262, and anti-pS422 blots in AD P-tau injected hippocampus of Tg/hTau mice (**Figure 5**). However, no SDS- and β-mercaptoethanol-resistant HMW-tau in any blots developed with above antibodies was detected in ipsilateral hippocampus of Tau-/- mice injected with AD P-tau (**Figure 5**). Thus, these data indicate that HMW-tau in Western blots in AD P-tau injected hippocampus is not exogenous protein and it is endogenous tau aggregates induced by AD P-tau.

Expression of Tau Phosphatases and Kinases in AD P-Tau Injected Hippocampus

PP2A is the major tau phosphatase (Liu et al., 2005). Methylation of PP2A catalytic subunit is required for it to dephosphorylate tau (Sontag et al., 1999, 2004). To learn whether PP2A is involved in AD P-tau-induced site-specific hyperphosphorylation of tau,

we analyzed PP2A catalytic subunit and its methylation by Western blots in the hippocampus. We found that levels of PP2A and demethylated PP2A were similar in AD P-tau injected hippocampus as compared with vehicle treatment in Tg/hTau and Tau-/- mice (**Figures 6A,B**), suggesting that PP2A may not be involved in AD P-tau-induced hyperphosphorylation of tau.

To determine the roles of other tau phosphatases in AD P-tau-induced tau hyperphosphorylation, we analyzed the expression of PP1, PP2B and PP5 by Western blots. Compared with saline injected mice, we did not find any changes of PP1, PP2B or PP5 in the AD P-tau injected hippocampus in Tg/hTau and Tau-/- mice (**Figures 6A,C**), suggesting that none of these phosphatases are responsible for the hyperphosphorylation of tau. Interestingly,

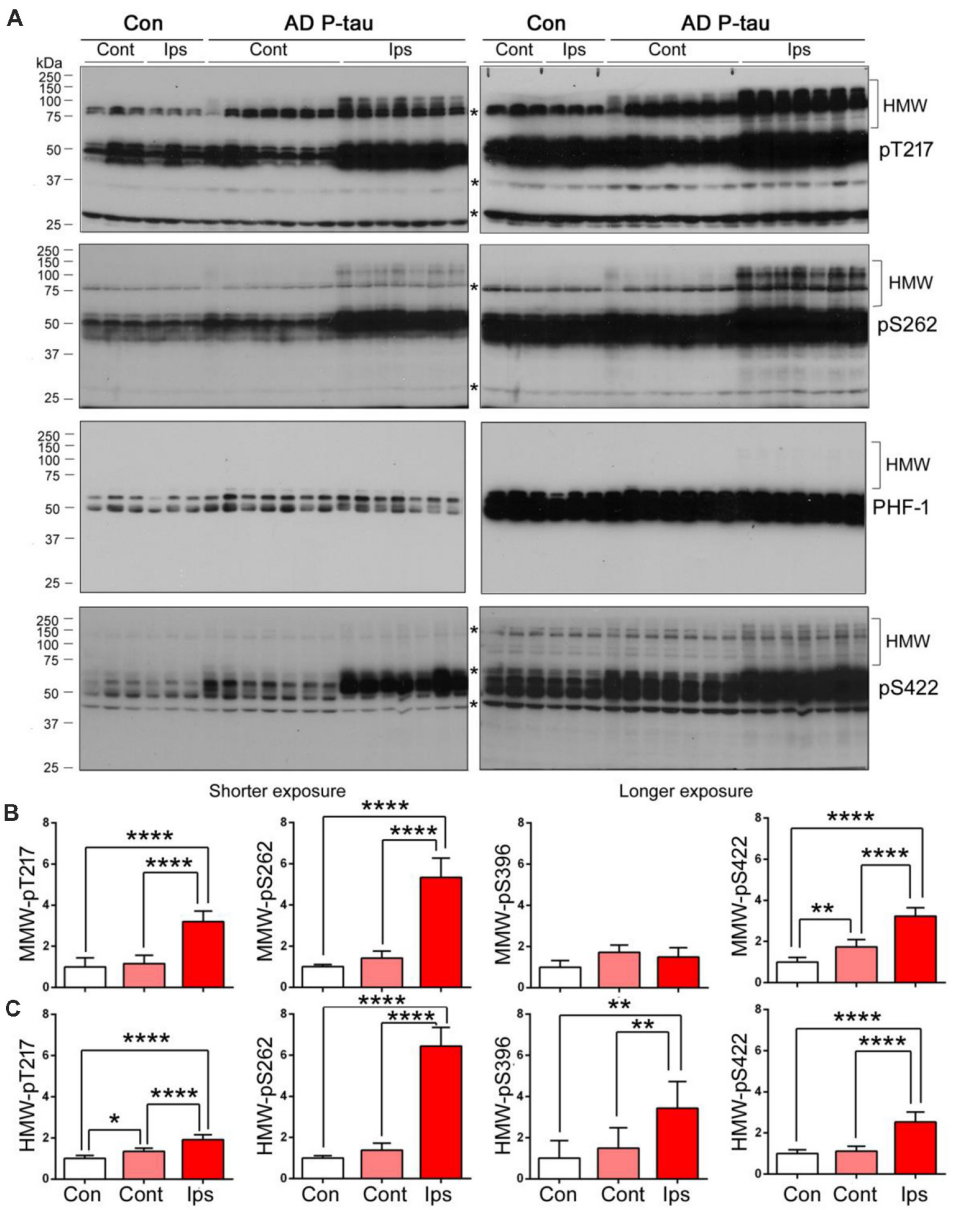

FIGURE 4 | SDS- and β-mercaptoethanol-resistant high molecular weight tau (HMW-tau) in AD P-tau injected hippocampus. Phosphorylation of tau and SDS- and β-mercaptoethanol-resistant HMW-tau in saline or AD P-tau injected hippocampus was analyzed by Western blots developed with anti-pThr217, anti-pSer262, PHF-1, and anti-pSer422 antibodies with shorter (left) and longer (right) exposure to X-ray film **(A)**. Shorter exposure blots were used for quantification of phosphorylation of middle molecular weight tau (MMW-tau), whereas longer exposure blots were used for quantification of SDS- and β-mercaptoethanol-resistant HMW-tau. *Indicates a non-specific band. The data from ipsilateral and contralateral hippocampi in Tg/hTau mice injected with saline were pooled as control since there was no significant difference in tau phosphorylation between them. Levels of phosphorylated MMW-tau **(B)** and HMW-tau **(C)** were statistically analyzed with one-way ANOVA post Tukey's multiple comparisons test and are presented as mean ± SD. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

we found that the levels of PP1, PP2B, and PP5 were higher in Tau-/- mouse hippocampus than that in Tg/hTau mouse hippocampus (**Figures 6A,C**).

AD P-tau-induced hyperphosphorylation of tau at Ser202/Thr205 (AT8), Thr212, Ser214, Thr217, Ser262, and Ser422 (**Figures 2A,B**), suggesting that both proline-directed protein kinases (PDPKs) and non-PDPKs may participate in the hyperphosphorylation of tau in the AD P-tau injected mouse brains. We analyzed the levels of tau kinases by Western blots. We found that in the AD P-tau-injected hippocampi of Tg/htau and Tau-/- mice, levels of PDPKs, Cdk5, inactive form of GSK-3β (phosphorylated GSK-3β at Ser9), Dyrk1A, active form of Erk (phosphorylated Erk at Thr202/Tyr204), and active form of Jnk/SAPK (phosphorylated

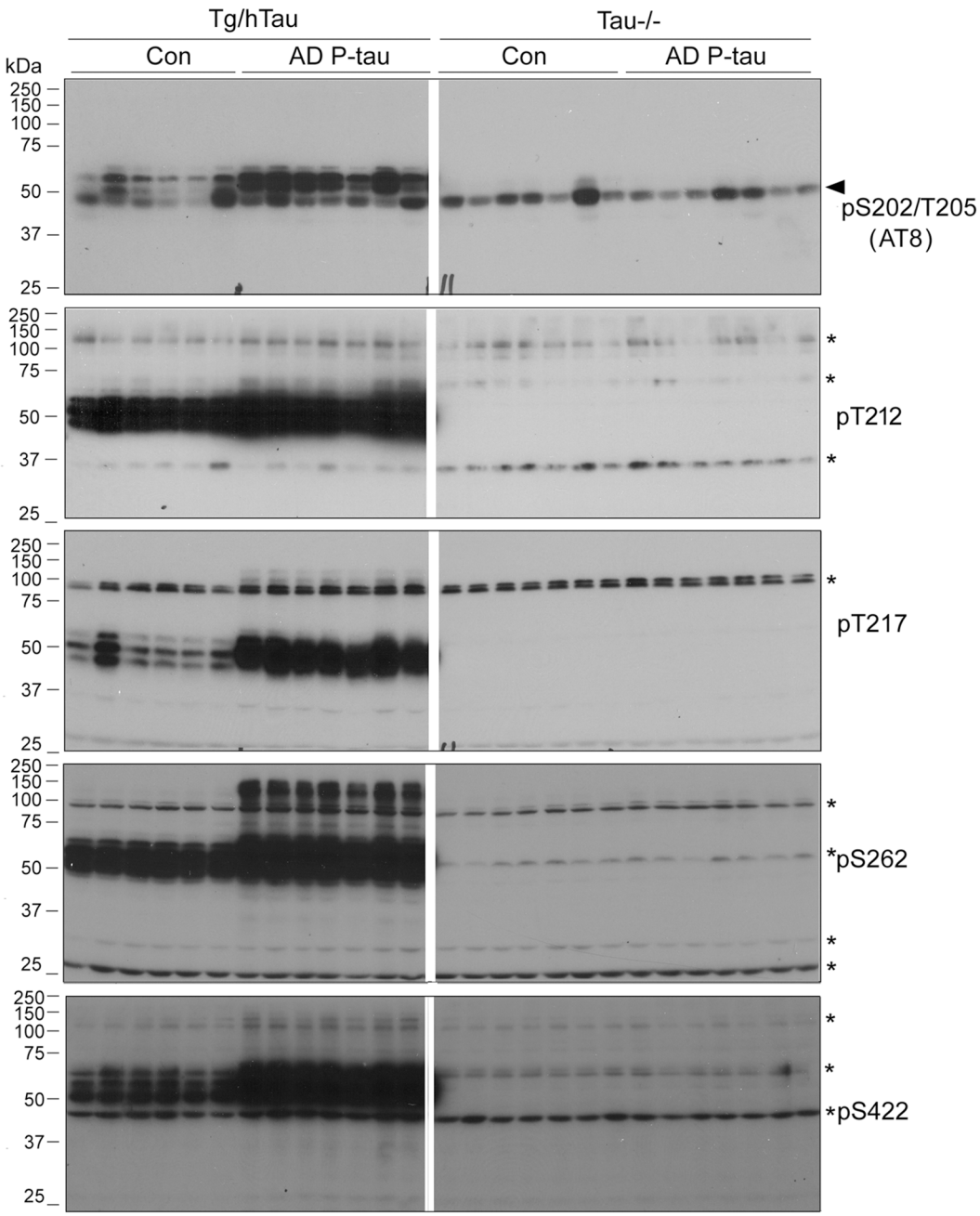

FIGURE 5 | SDS- and β-mercaptoethanol-resistant HMW-tau in AD P-tau injected hippocampus of Tg/hTau mice. Ipsilateral hippocampi from Tg/hTau and Tau-/- mice injected with AD P-tau were analyzed by Western blots developed with AT8 (anti-pS202/T205-tau), anti-pT212-tau, anti-pT217-tau, anti-pS262-tau, and anti-pS422-tau to study site-specific SDS- and β-mercaptoethanol-resistant HMW-tau. *Points the non-specific bands and arrow head indicates the heavy chain of IgG.

Jnk/SAPK at Thr183/Tyr185), were not altered as compared with that in the saline-injected hippocampus (**Figures 7A,B**), suggesting that they may not contribute to AD P-tau-induced tau hyperphosphorylation. Furthermore, the active form of AMPK (phosphorylated AMPK at Thr172), PKA catalytic subunit, active form of AKT (phosphorylated at Ser473), active form of P70S6K (phosphorylated P70S6K at Thr389), and CK1ε were also not changed in AD P-tau-injected hippocampus as compared with those in corresponding controls (**Figure 7C**), indicating that these non-PDPKs may not be responsible for the hyperphosphorylation of tau too. Compared with that in the Tg/hTau mouse hippocampus, we found that levels of Dyrk1A, phosphorylated AKT, and phosphorylated P70S6K were increased in the Tau-/- mouse hippocampus (**Figures 7A–C**).

Effective Capture of Hyperphosphorylated Tau by AD P-Tau

Above studies suggest no significant alteration of tau phosphatases and tau kinases in the AD P-tau injected hippocampus. To study the possible mechanisms by which AD P-tau induces site-specific hyperphosphorylation of tau *in vivo*, we first assumed that AD P-tau may template phosphorylated tau more effectively. We used overlay capture assay to determine the effect of tau phosphorylation on its capture by AD P-tau, as described previously (Alonso et al., 1996). We overexpressed tau_{441} tagged with HA in HEK-293FT cells and treated the cells with 100 nM okadaic acid (OA) for 2 h to induce tau hyperphosphorylation (Qian et al., 2010). Then, we dotted various amounts of AD P-tau on nitrocellulose membrane and incubated the membrane with the cell-extract of OA-treated (OA-tau) and control-treated (Con-tau) HEK-293FT/tau_{441}. AD P-tau captured tau was analyzed by anti-HA and ECL. We found that much more tau was captured by AD P-tau from the extract of OA-tau cells than that from control treated cells (**Figures 8A,B**), suggesting that hyperphosphorylated tau is captured more effectively by AD P-tau.

Difficult Dephosphorylation of Aggregated Tau by PP2A

It is well known that aggregated tau is sarkosyl insoluble and heat treatment removes aggregated tau (Greenberg and Davies, 1990; Planel et al., 2007). To learn whether aggregated tau resists to dephosphorylation, we isolated sarkosyl insoluble tau (SI-tau), AD P-tau and heat stable tau (HS-tau) from AD brain and analyzed them by Western blots. These three forms of tau from AD brain showed different patterns in Western blots developed with R134d (pan-tau), anti-pS199-tau and PHF-1 (**Figure 8C**). SDS- and β-mercaptoethanol-resistant HMW-tau aggregates were present in SI-tau and AD P-tau, but not in

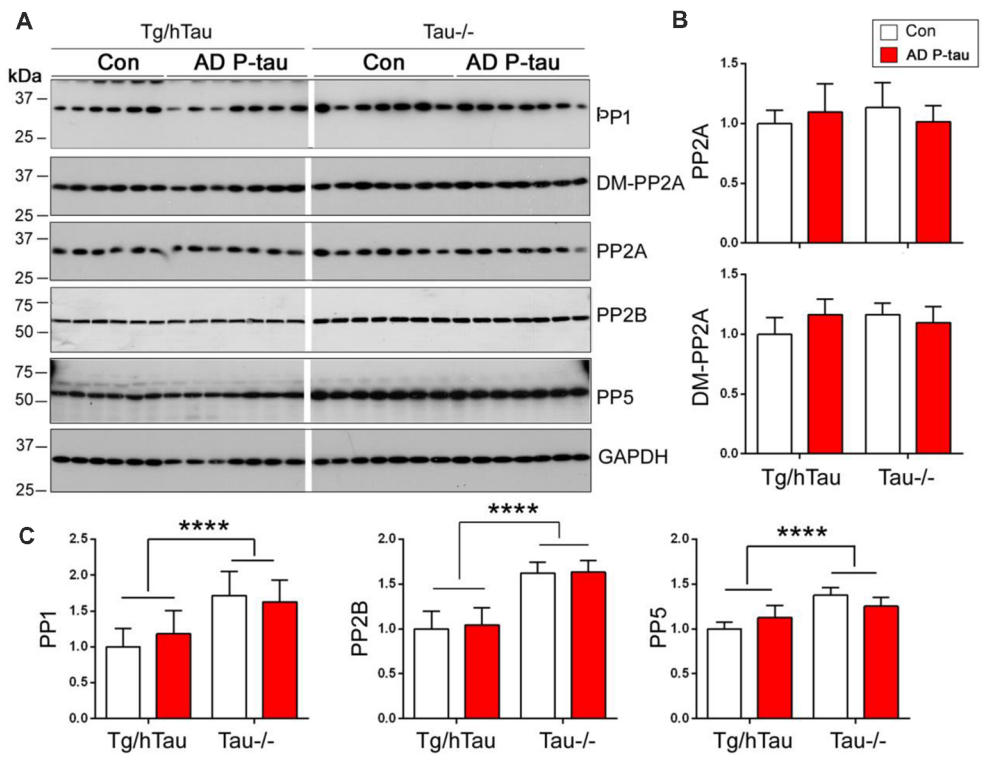

FIGURE 6 | Expression of tau phosphatases in AD P-tau-injected hippocampus. PP1, protein phosphatase 2A (PP2A), demethylated PP2A, PP2B and PP5 in the ipsilateral hippocampus in Tg/hTau and Tau-/- mice injected with AD P-tau or saline were analyzed by Western blots developed with antibodies indicated **(A)**. The levels of demethylated PP2A and PP2A **(B)** and PP1, PP2B and PP5 **(C)** were statistically analyzed with two-way ANOVA post Sidak's multiple comparisons test after normalized with PP2A (for DM-PP2A) or GAPDH (for PPs) and are presented as mean ± SD. $^{****}p < 0.0001$.

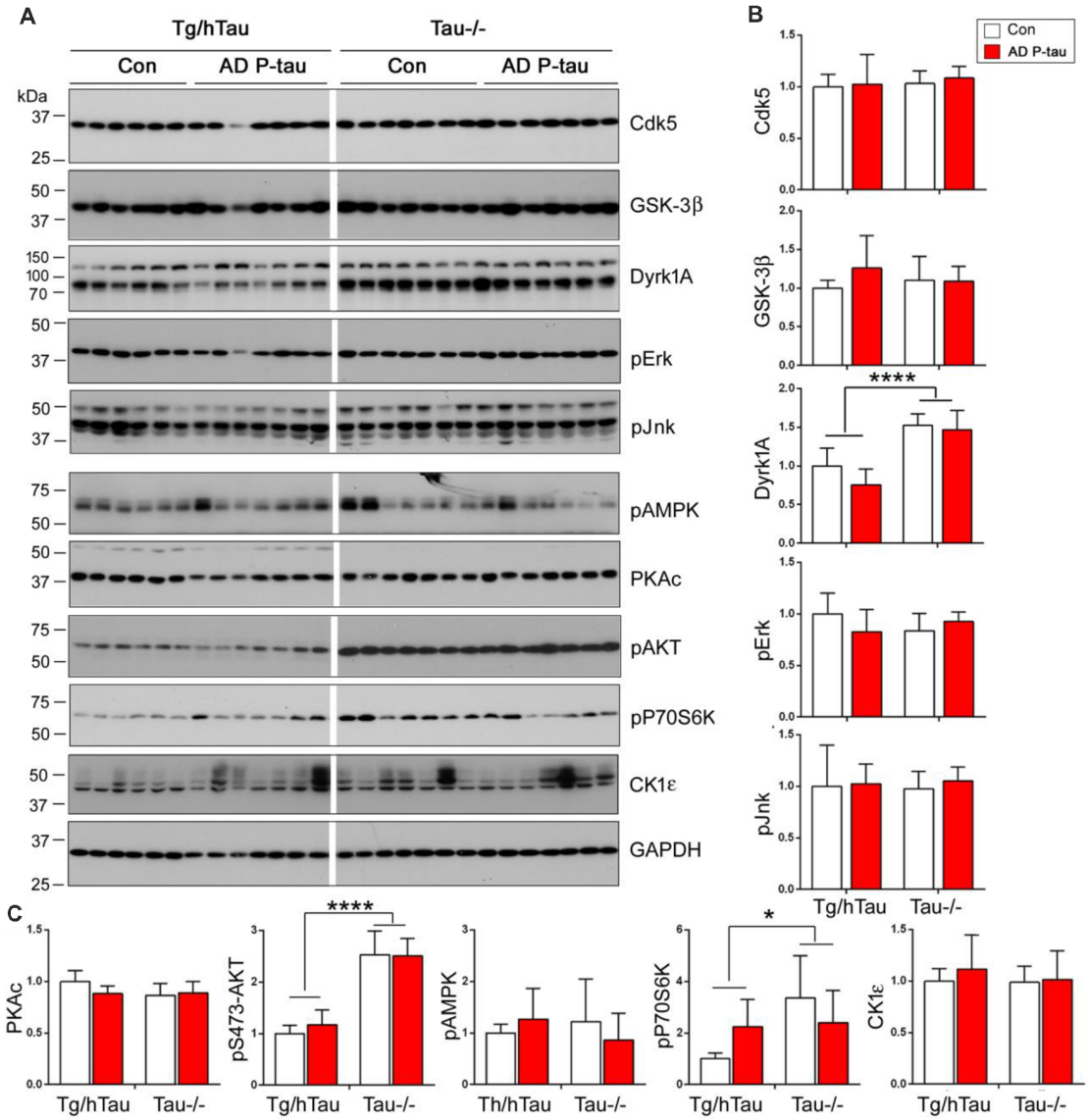

FIGURE 7 | Expression of tau kinases in AD P-tau-injected hippocampi in Tg/hTau and Tau-/- mice. Cdk5, inactive form of GSK-3β (phospho-GSK-3β), Dyrk1A, active form of Erk (phospho-Erk), active form of Jnk/SAPK (phospho-Jnk/SAPK), active form of AMPK (phospho-AMPK), PKA catalytic subunit, active form of AKT (phosphorylated at Ser473), (phospho-AKT), active form of P70S6k (phospho-P70S6K), and CK1ε in ipsilateral hippocampus of Tg/htau and Tau-/- mice injected with AD P-tau or saline were analyzed by Western blots **(A)**. The levels of proline-directed protein kinases (PDPKs; **B)** and non-PDPKs **(C)** were statistically analyzed with two-way ANOVA post Sidak's multiple comparisons test and are presented as mean ± SD. *$p < 0.05$; ****$p < 0.0001$.

HS-tau (**Figure 8A**), confirming aggregated tau in SI-tau and AD P-tau and the non-aggregated nature of HS-tau (Grundke-Iqbal et al., 1986a; Greenberg and Davies, 1990). AD P-tau was used for above capture assay. SI-tau and HS-tau were used as aggregated tau and non-aggregated tau to study efficiency of dephosphorylation by PP2A. We dephosphorylated SI-tau and HS-tau with PP2A for various time points and analyzed dephosphorylation efficiency by dot blots developed with anti-pS199-tau since HS-tau was phosphorylated at Ser199. Even PP2A could not dephosphorylate tau at Ser199 effectively (Liu et al., 2005), we found that Ser199 phosphorylation of SI-tau and of HS-tau was decreased in time dependent manner by PP2A (**Figures 8D,E**), and HS-tau was dephosphorylated more rapidly than SI-tau (**Figures 8D,E**), suggesting PP2A dephosphorylates the non-aggregated tau more effectively than the aggregated tau.

DISCUSSION

Tau oligomers recently have emerged as the pathogenic species in tauopathies. AD P-tau isolated from AD brain is hyperphosphorylated and oligomeric (Köpke et al., 1993). It serves as potent tau seeds, sequesters/captures normal tau *in vitro* (Alonso et al., 1996) and induces tau pathology *in vivo* (Hu et al., 2016; Dai et al., 2018). In

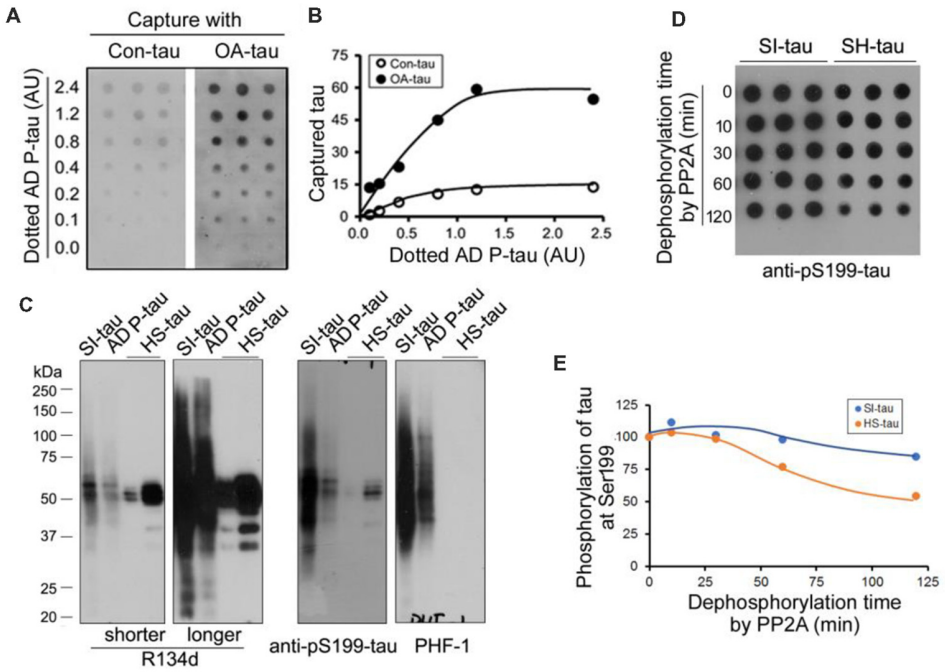

FIGURE 8 | Effects of hyperphosphorylation of tau on its capture by AD P-tau and aggregation of tau on its dephosphorylation by PP2A. **(A,B)** Effect of hyperphosphorylation of tau on its capture by AD P-tau. Tau_{441} tagged with HA was overexpressed in HEK-293FT cells for 48 h. The cells were treated with 100 nM okadaic acid (OA) for 2 h to induce hyperphosphorylation of tau and lysed in PBS containing a cocktail of proteinase and phosphatase inhibitors by probe sonication. AD P-tau was dotted on nitrocellulose membrane and incubated with the 15,000× *g* extract from OA treated cells (OA-tau) or control treated cells (Con-tau) overnight. AD P-tau captured HA-tau_{441} was analyzed by anti-HA, followed by HRP-anti-mouse IgG and ECL **(A)** and plotted against various amount of AD P-tau **(B)**. **(C,D)** Effect of aggregation of tau on its dephosphorylation by PP2A. Sarkosyl insoluble tau (SI-tau), AD P-tau, and heat stable monomeric tau (HS-tau) were isolated from AD cerebral cortex and analyzed by Western blots developed with R134d (pan-tau), anti-pS199-tau and PHF-1 **(C)**. SI-tau and HS-tau were incubated with PP2A (20 mU/ml) for various time points. The phosphorylation of tau at Ser199 was analyzed by dot-blots **(D)** and plotted against time points of dephosphorylation reaction **(E)**.

the present study, we unilaterally injected AD P-tau into the hippocampus of 9–11-month-old Tg/hTau mice and found for the first time that in addition to the induction of tau aggregation/pathology, AD P-tau led to site-specific hyperphosphorylation and SDS- and reducing agent-resistant and AD-like high-molecular weight tau *in vivo* 10 weeks post injection. Tau aggregation/pathology was observed in both hippocampi, but four-times less in contralateral sites. In the AD P-tau injected hippocampus, tau was abnormally hyperphosphorylated at Ser202/Thr205, Thr212, Ser214, Thr217, Ser262, and Ser422. The SDS- and reducing agent-resistant and AD-like HMW-tau was hyperphosphorylated at Ser212, Ser217, Ser262, and Ser422. Different from ipsilateral hippocampus, tau was hyperphosphorylated at Ser422 in the contralateral hippocampus and the ipsilateral cortex. No detectable alteration in levels of tau phosphatases and kinases was observed in AD P-tau injected hippocampus. However, we found that hyperphosphorylated tau was more effectively captured by AD P-tau and aggregated tau was relatively resistant to dephosphorylation by PP2A. Thus, we speculate that AD P-tau seeds site-specifically hyperphosphorylated tau to form aggregates, and the aggregated tau resists to dephosphorylation by PP2A, leading to hyperphosphorylation and pathology of tau.

Abnormally hyperphosphorylated tau is the major component of NFTs (Grundke-Iqbal et al., 1986b). Tau aggregates induced by misfolded tau seeds are labeled by various site-specific and phosphorylation-dependent antibodies (Clavaguera et al., 2009; Hu et al., 2016), but the state of tau phosphorylation in misfolded tau seeds-injected brains had not been documented biochemically. In the present study, we found that tau phosphorylation was increased at Ser202/Thr205, Thr212, Ser214, Thr217, Ser262, and Ser422, but not at Ser199, Ser396 and Ser404, in the AD P-tau-injected hippocampus, suggesting that AD P-tau induces site-specific hyperphosphorylation of tau *in vivo*. Since these hyperphosphorylated sites are followed by both proline and non-proline residues, both PDPK or non-PDPK may be involved in AD P-tau-induced hyperphosphorylation of tau. The major PDPK of tau is GSK-3β, which phosphorylates tau at Ser199, Ser202, Thr205, Thr212, Thr217, Ser396, and Ser404 with Ser199, Thr205 and Ser396 being the most favorable sites in cells (Liu et al., 2006; Qian et al., 2010). However, Ser199 and Ser396 phosphorylation was not increased, suggesting that GSK-3β may not be involved in the hyperphosphorylation induced by AD P-tau *in vivo*. Moreover, we did not find a significant alteration in phospho-Ser9 of GSK-3β in AD P-tau injected hippocampus, supporting that GSK-3β probably does

not contribute to AD P-tau-induced tau hyperphosphorylation. In case of PKA, it phosphorylates Ser214 more effectively than Ser262, but more increase of tau phosphorylation at Ser262 than Ser214 was seen in the AD P-tau-injected hippocampus. Similarly, other kinases, including Cdk5, Erk1, Jnk/SAPK, Dyrk1A, P70S6K, AMPK, AKT, and CK1ε, may not be involved in AD P-tau-induced hyperphosphorylation was evidenced by the site-specific phosphorylation and their expression levels. Interestingly, we found that levels of PP1, PP2B, PP5, phospho-AKT, Dyrk1A and phospho-P70S6k were increased in tau-/- hippocampus, suggesting that tau may influence the expression or/and degradation of these proteins directly or indirectly, which remain to be studied.

PP2A is the major tau phosphatase (Liu et al., 2005) and it dephosphorylates multiple sites of tau with different efficiencies (Liu et al., 2005). PP2A also dephosphorylates GSK-3β, resulting in its activation (Qian et al., 2010; Wang et al., 2015a). Inhibition of PP2A increases tau phosphorylation directly and indirectly through activating GSK-3β (Qian et al., 2010). Methylation of PP2A catalytic subunit at Lys 309 enhances its activity to dephosphorylate tau (Sontag et al., 1999, 2004). However, no alteration of methylated PP2Ac or Ser9 phosphorylation of GSK-3β was observed in AD P-tau injected hippocampus, suggesting PP2A may not be course of hyperphosphorylation of tau in AD P-tau-injected mouse brain. Similarly, similar levels of PP1, PP2B and PP5 in AD P-tau-injected and saline-injected hippocampi suggest that they may not play roles in AD P-tau-induced tau phosphorylation.

Among these hyperphosphorylation sites induced by AD P-tau *in vivo*, phosphorylation of tau at Ser422 was very interesting. Increase of Ser422 phosphorylation was found in the contralateral hippocampus and the ipsilateral cortex, where very limited tau pathology were observed. Thus, Ser422 phosphorylation may be an early event in AD P-tau-induced tau pathology. Several kinases are able to phosphorylate Ser422, including Jnk/SAPK (Wang and Liu, 2008). PP2A and PP5 effectively dephosphorylate Ser422 (Liu et al., 2002). Treatment of brain slices with OA induces tau hyperphosphorylation at multiple sites, with the most increase at Ser422 (Gong et al., 2000; Qian et al., 2010). Ser422 is abnormally hyperphosphorylated in AD brain (Liu et al., 2009). Normal tau protein has been proposed to have a "paper clip" structure, in which the N- and C-terminal ends fold over the microtubule-binding domain to prevent the protein from self-aggregation (Mandelkow et al., 2007). Ser422 phosphorylation may make tau C-terminus to stretch out and expose the microtubule-binding domain, thereby leading to tau captured easily by AD P-tau. Specially, Ser422 was the only site to be hyperphosphorylated in the ipsilateral and contralateral hippocampi and the ipsilateral cortex of AD P-tau injected Tg/hTau mice. Thus, Ser422 phosphorylation may be critical in AD P-tau-induced aggregation/tau pathology, but this remains to be determined in future studies.

AD P-tau sequesters tau *in vitro* and induces tau aggregation *in vivo* (Alonso et al., 1996; Hu et al., 2016). We found that hyperphosphorylated tau induced by OA in cells was captured by AD P-tau more effectively than normal tau from control treated cells. OA is a PP2A inhibitor (Bialojan et al., 1988). Treatment of brain slices with OA induces tau hyperphosphorylation at multiple sites, including Ser422 and Ser262 phosphorylation (Gong et al., 2000; Qian et al., 2010). Tau in NFTs is hyperphosphorylated (Grundke-Iqbal et al., 1986b). In the present study, we found that PP2A dephosphorylated heat-stable monomeric tau more effectively than aggregated sarkosyl-insoluble tau. We previously reported that tau is rapidly dephosphorylated during postmortem delay (Wang et al., 2015b). In AD brain, SI-tau was hyperphosphorylated at all the sites studied, but HS-tau was phosphorylated only at Ser199, which also supports that aggregated tau may resist to dephosphorylation during postmortem period. Dephosphorylation with alkaline phosphatase abolishes the ability of AD P-tau to aggregate with normal tau and prevents tangle formation (Alonso et al., 1996). From these findings, we speculate that AD P-tau captures phosphorylated tau and that aggregated tau is resistant to dephosphorylation, leading to tau hyperphosphorylation. Hyperphosphorylated tau aggregates capture and template tau aggregation and eventually leading to tau pathology.

AUTHOR CONTRIBUTIONS

JM, RS, LL, FC, YZ, YT and WH performed experiments and analyzed the results. C-XG and KI provided the reagents, discussed results and edited the manuscript. FL designed and performed experiments, analyzed and interpreted results, and wrote the manuscript.

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REFERENCES

Ahmed, Z., Cooper, J., Murray, T. K., Garn, K., McNaughton, E., Clarke, H., et al. (2014). A novel *in vivo* model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. *Acta Neuropathol.* 127, 667–683. doi: 10.1007/s00401-014-1254-6

Alafuzoff, I., Iqbal, K., Friden, H., Adolfsson, R., and Winblad, B. (1987). Histopathological criteria for progressive dementia disorders: clinical-pathological correlation and classification by multivariate data analysis. *Acta Neuropathol.* 74, 209–225. doi: 10.1007/BF00688184

Alonso, A. D., Grundke-Iqbal, I., and Iqbal, K. (1996). Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat. Med.* 2, 783–787.

Alonso, A. C., Zaidi, T., Grundke-Iqbal, I., and Iqbal, K. (1994). Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *Proc. Natl. Acad. Sci. U S A* 91, 5562–5566.

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., and Hyman, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* 42, 631–639.

Bahl, R., Bradley, K. C., Thompson, K. J., Swain, R. A., Rossie, S., and Meisel, R. L. (2001). Localization of protein Ser/Thr phosphatase 5 in rat brain. *Brain Res. Mol.* 90, 101–109. doi: 10.1016/s0169-328x(01)00089-4

Bialojan, C., Rüegg, J. C., and Takai, A. (1988). Effects of okadaic acid on isometric tension and myosin phosphorylation of chemically skinned guinea-pig taenia coli. *J. Physiol.* 398, 81–95. doi: 10.1113/jphysiol.1988.sp017030

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 82, 239–259.

Braak, H., and Del Tredici, K. (2011). Alzheimer's pathogenesis: is there neuron-to-neuron propagation? *Acta Neuropathol.* 121, 589–595. doi: 10.1007/s00401-011-0825-z

Clavaguera, F., Bolmont, T., Crowther, R. A., Abramowski, D., Frank, S., Probst, A., et al. (2009). Transmission and spreading of tauopathy in transgenic mouse brain. *Nat. Cell Biol.* 11, 909–913. doi: 10.1038/ncb1901

Dai, C.-L., Hu, W., Tung, Y. C., Liu, F., Gong, C.-X., and Iqbal, K. (2018). Tau passive immunization blocks seeding and spread of Alzheimer hyperphosphorylated Tau-induced pathology in 3 x Tg-AD mice. *Alzheimers Res. Ther.* 10:13. doi: 10.1186/s13195-018-0341-7

de Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D. H., Kopeikina, K. J., et al. (2012). Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* 73, 685–697. doi: 10.1016/j.neuron.2011.11.033

Duff, K., Knight, H., Refolo, L. M., Sanders, S., Yu, X., Picciano, M., et al. (2000). Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. *Neurobiol. Dis.* 7, 87–98. doi: 10.1006/nbdi.1999.0279

Dujardin, S., Bégard, S., Caillierez, R., Lachaud, C., Delattre, L., Carrier, S., et al. (2014). Ectosomes: a new mechanism for non-exosomal secretion of tau protein. *PLoS One* 9:e100760. doi: 10.1371/journal.pone.0100760

Gong, C. X., Lidsky, T., Wegiel, J., Zuck, L., Grundke-Iqbal, I., and Iqbal, K. (2000). Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease. *J. Biol. Chem.* 275, 5535–5544. doi: 10.1074/jbc.275.8.5535

Greenberg, S. G., and Davies, P. (1990). A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. *Proc. Natl. Acad. Sci. U S A* 87, 5827–5831. doi: 10.1073/pnas.87.15.5827

Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y. C., Zaidi, M. S., and Wisniewski, H. M. (1986a). Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J. Biol. Chem.* 261, 6084–6089.

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986b). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U S A* 83, 4913–4917. doi: 10.1073/pnas.83.13.4913

Hu, W., Zhang, X., Tung, Y. C., Xie, S., Liu, F., and Iqbal, K. (2016). Hyperphosphorylation determines both the spread and the morphology of tau pathology. *Alzheimers Dement.* 12, 1066–1077. doi: 10.1016/j.jalz.2016.06.627

Iba, M., Guo, J. L., McBride, J. D., Zhang, B., Trojanowski, J. Q., and Lee, V. M. (2013). Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. *J. Neurosci.* 33, 1024–1037. doi: 10.1523/JNEUROSCI.2642-12.2013

Iqbal, K., Alonso Adel, C., Chen, S., Chohan, M. O., El-Akkad, E., Gong, C.-X., et al. (2005). Tau pathology in Alzheimer disease and other tauopathies. *Biochim. Biophys. Acta* 1739, 198–210. doi: 10.1016/j.bbadis.2004.09.008

Iqbal, K., and Grundke-Iqbal, I. (2010). Alzheimer's disease, a multifactorial disorder seeking multitherapies. *Alzheimers Dement.* 6, 420–424. doi: 10.1016/j.jalz.2010.04.006

Iqbal, K., Grundke-Iqbal, I., Zaidi, T., Merz, P. A., Wen, G. Y., Shaikh, S. S., et al. (1986). Defective brain microtubule assembly in Alzheimer's disease. *Lancet* 2, 421–426. doi: 10.1016/s0140-6736(86)92134-3

Iqbal, K., Liu, F., and Gong, C. X. (2016). Tau and neurodegenerative disease: the story so far. *Nat. Rev. Neurol.* 12, 15–27. doi: 10.1038/nrneurol.2015.225

Johnson, K. A., Schultz, A., Betensky, R. A., Becker, J. A., Sepulcre, J., Rentz, D., et al. (2016). Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Ann. Neurol.* 79, 110–119. doi: 10.1002/ana.24546

Köpke, E., Tung, Y. C., Shaikh, S., Alonso, A. C., Iqbal, K., and Grundke-Iqbal, I. (1993). Microtubule-associated protein tau Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J. Biol. Chem.* 268, 24374–24384.

Lindwall, G., and Cole, R. D. (1984). Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J. Biol. Chem.* 259, 5301–5305.

Liu, L., Drouet, V., Wu, J. W., Witter, M. P., Small, S. A., Clelland, C., et al. (2012). Trans-synaptic spread of tau pathology *in vivo*. *PLoS One* 7:e31302. doi: 10.1371/journal.pone.0031302

Liu, F., Grundke-Iqbal, I., Iqbal, K., and Gong, C. X. (2005). Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur. J. Neurosci.* 22, 1942–1950. doi: 10.1111/j.1460-9568.2005.04391.x

Liu, F., Liang, Z., Shi, J., Yin, D., El-Akkad, E., Grundke-Iqbal, I., et al. (2006). PKA modulates GSK-3β- and cdk5-catalyzed phosphorylation of tau in site- and kinase-specific manners. *FEBS Lett.* 580, 6269–6274. doi: 10.1016/j.febslet.2006.10.033

Liu, F., Shi, J., Tanimukai, H., Gu, J., Grundke-Iqbal, I., Iqbal, K., et al. (2009). Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. *Brain.* 132, 1820–1832. doi: 10.1093/brain/awp099

Liu, F., Zaidi, T., Iqbal, K., Grundke-Iqbal, I., and Gong, C. X. (2002). Aberrant glycosylation modulates phosphorylation of tau by protein kinase A and dephosphorylation of tau by protein phosphatase 2A and 5. *Neuroscience* 115, 829–837. doi: 10.1016/s0306-4522(02)00510-9

Mandelkow, E., von Bergen, M., Biernat, J., and Mandelkow, E. M. (2007). Structural principles of tau and the paired helical filaments of Alzheimer's disease. *Brain Pathol.* 17, 83–90. doi: 10.1111/j.1750-3639.2007.00053.x

Peeraer, E., Bottelbergs, A., Van Kolen, K., Stancu, I. C., Vasconcelos, B., Mahieu, M., et al. (2015). Intracerebral injection of preformed synthetic tau fibrils initiates widespread tauopathy and neuronal loss in the brains of tau transgenic mice. *Neurobiol. Dis.* 73, 83–95. doi: 10.1016/j.nbd.2014.08.032

Pei, J. J., Braak, E., Braak, H., Grundke-Iqbal, I., Iqbal, K., Winblad, B., et al. (1999). Distribution of active glycogen synthase kinase 3β (GSK-3β) in brains staged for Alzheimer disease neurofibrillary changes. *J. Neuropathol. Exp. Neurol.* 58, 1010–1019. doi: 10.1097/00005072-199909000-00011

Pei, J.-J., Gong, C.-X., Iqbal, K., Grundke-Iqbal, I., Wu, Q. L., Winblad, B., et al. (1998). Subcellular distribution of protein phosphatases and abnormally phosphorylated τ in the temporal cortex from Alzheimer's disease and control brains. *J. Neural. Transm.* 105, 69–83. doi: 10.1007/s007020050039

Planel, E., Tatebayashi, Y., Miyasaka, T., Liu, L., Wang, L., Herman, M., et al. (2007). Insulin dysfunction induces *in vivo* tau hyperphosphorylation through distinct mechanisms. *J. Neurosci.* 27, 13635–13648. doi: 10.1523/jneurosci.3949-07.2007

Qian, W., Shi, J., Yin, X., Iqbal, K., Grundke-Iqbal, I., Gong, C.-X., et al. (2010). PP2A regulates tau phosphorylation directly and also indirectly via activating GSK-3β. *J. Alzheimers Dis.* 19, 1221–1229. doi: 10.3233/jad-2010-1317

Quiroz, Y. T., Sperling, R. A., Norton, D. J., Baena, A., Arboleda-Velasquez, J. F., Cosio, D., et al. (2018). Association between amyloid and tau accumulation in young adults with autosomal dominant Alzheimer disease. *JAMA Neurol.* 75, 548–556. doi: 10.1001/jamaneurol.2017.4907

Schöll, M., Lockhart, S. N., Schonhaut, D. R., O'Neil, J. P., Janabi, M., Ossenkoppele, R., et al. (2016). PET imaging of tau deposition in the aging human brain. *Neuron* 89, 971–982. doi: 10.1016/j.neuron.2016.01.028

Schwarz, A. J., Yu, P., Miller, B. B., Shcherbinin, S., Dickson, J., Navitsky, M., et al. (2016). Regional profiles of the candidate tau PET ligand 18F-AV-1451 recapitulate key features of Braak histopathological stages. *Brain* 139, 1539–1550. doi: 10.1093/brain/aww023

Sontag, E., Hladik, C., Montgomery, L., Luangpirom, A., Mudrak, I., Ogris, E., et al. (2004). Downregulation of protein phosphatase 2A carboxyl methylation

and methyltransferase may contribute to Alzheimer disease pathogenesis. *J. Neuropathol. Exp. Neurol.* 63, 1080–1091. doi: 10.1093/jnen/63.10.1080

Sontag, E., Nunbhakdi-Craig, V., Lee, G., Brandt, R., Kamibayashi, C., Kuret, J., et al. (1999). Molecular interactions among protein phosphatase 2A, tau and microtubules. *J. Biol. Chem.* 274, 25490–25498. doi: 10.1074/jbc.274.36.25490

Tatebayashi, Y., Iqbal, K., and Grundke-Iqbal, I. (1999). Dynamic regulation of expression and phosphorylation of tau by fibroblast growth factor-2 in neural progenitor cells from adult rat hippocampus. *J. Neurosci.* 19, 5245–5254. doi: 10.1523/jneurosci.19-13-05245.1999

Tomlinson, B. E., Blessed, G., and Roth, M. (1970). Observations on the brains of demented old people. *J. Neurol. Sci.* 11, 205–242. doi: 10.1016/0022-510X(70)90063-8

Tucker, K. L., Meyer, M., and Barde, Y. A. (2001). Neurotrophins are required for nerve growth during development. *Nat Neurosci.* 4, 29–37. doi: 10.1038/82868

Wang, J. Z., and Liu, F. (2008). Microtubule-associated protein tau in development, degeneration and protection of neurons. *Prog. Neurobiol.* 85, 148–175. doi: 10.1016/j.pneurobio.2008.03.002

Wang, Y., Yang, R., Gu, J., Yin, X., Jin, N., Xie, S., et al. (2015a). Cross talk between PI3K-AKT-GSK-3β and PP2A pathways determines tau hyperphosphorylation. *Neurobiol. Aging* 36, 188–200. doi: 10.1016/j.neurobiolaging.2014.07.035

Wang, Y., Zhang, Y., Hu, W., Xie, S., Gong, C.-X., Iqbal, K., et al. (2015b). Rapid alteration of protein phosphorylation during postmortem: implication in the study of protein phosphorylation. *Sci Rep.* 5:15709. doi: 10.1038/srep15709

Wegiel, J., Kuchna, I., Nowicki, K., Frackowiak, J., Dowjat, K., Silverman, W. P., et al. (2004). Cell type- and brain structure-specific patterns of distribution of minibrain kinase in human brain. *Brain Res.* 1010, 69–80. doi: 10.1016/j.brainres.2004.03.008

Zhou, Y., Shi, J., Chu, D., Hu, W., Guan, Z., Gong, C. X., et al. (2018). Relevance of phosphorylation and truncation of tau to the etiopathogenesis of Alzheimer's disease. *Front. Aging Neurosci.* 10:27. doi: 10.3389/fnagi.2018.00027

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REVIEW
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Influence of Normal Aging on Brain Autophagy: A Complex Scenario

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Misfolded proteins are pathological findings in some chronic neurodegenerative disorders including Alzheimer's, Parkinson's, and Huntington's diseases. Aging is a major risk factor for these disorders, suggesting that the mechanisms responsible for clearing misfolded proteins from the brain, the ubiquitin-proteasome system and the autophagy-lysosomal pathway, may decline with age. Although autophagic mechanisms have been found to decrease with age in many experimental models, whether they do so in the brain is unclear. This review examines the literature with regard to age-associated changes in macroautophagy and chaperone-mediated autophagy (CMA) in the central nervous system (CNS). Beclin 1, LC3-II, and the LC3-II/LC3-I ratio have frequently been used to examine changes in macroautophagic activity, while lamp2a and HSPA8 (also known as hsc70) have been used to measure CMA activity. Three gene expression analyses found evidence for an age-related downregulation of macroautophagy in human brain, but no published studies were found of age-related changes in CMA in human brain, although cerebrospinal fluid concentrations of HSPA8 were reported to decrease with age. Most studies of age-related changes in brain autophagy in experimental animals have found age-related declines in macroautophagy, and macroautophagy is necessary for normal lifespan in *Caenorhabditis elegans*, *Drosophila*, and mice. However, the few studies of age-related changes in brain CMA in experimental animals have produced conflicting results. Investigations of the influence of aging on macroautophagy in experimental animals in systems other than the CNS have generally found an age-related decrease in Beclin 1, but conflicting results for LC3-II and the LC3-II/LC3-I ratio, while CMA decreases with age in most models. CONCLUSION: while indirect evidence suggests that brain autophagy may decrease with normal aging, this issue has not been investigated sufficiently, particularly in human brain. Measuring autophagic activity in the brain can be challenging because of differences in basal autophagic activity between experimental models, and the inability to include lysosomal inhibitors when measuring the LC3-II/LC3-I ratio in postmortem specimens. If autophagy does decrease in the brain with aging, then pharmacological interventions and/or lifestyle alterations to slow this decline could reduce the risk of developing age-related neurodegenerative disorders.

Keywords: aging, autophagy, brain, chaperone-mediated autophagy, macroautophagy

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INTRODUCTION

More than 30% of newly synthesized proteins are misfolded because of errors in translation or post-translational processes (Schubert et al., 2000). Molecular chaperones, primarily heat shock proteins, recognize these proteins and attempt to facilitate their refolding (Ciechanover and Kwon, 2017). Two proteolytic mechanisms, the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP), are responsible for cellular clearance of misfolded proteins which are unable to be refolded. The ALP includes three autophagic processes in mammals, namely macroautophagy, chaperone-mediated autophagy (CMA), and microautophagy (Orenstein and Cuervo, 2010), which use different mechanisms to deliver their cargo to lysosomes for enzymatic degradation. Misfolded proteins are initially subjected to removal by the UPS and selected proteins may also be removed by CMA. Misfolded proteins which avoid clearance by these mechanisms may be removed by macroautophagy (Ciechanover and Kwon, 2015). Protein oligomers, soluble aggregates, and intact organelles are too large to enter the proteasome's narrow opening, so they can be degraded by the ALP but not by the UPS (Bence et al., 2001; Wu et al., 2015). Cross-talk is present between CMA and macroautophagy so that if one mechanism is impaired, the activity of the other may increase in an effort to maintain protein homeostasis (Massey et al., 2006a; Wu et al., 2015). Clearance of misfolded proteins is critical for cell survival because misfolding alters a protein's three-dimensional structure, impairing its biological activities and increasing its propensity to form toxic aggregates (Hartl, 2017). Misfolded and aggregated proteins are key pathological findings in several chronic neurodegenerative diseases including Alzheimer's disease (AD) (Selkoe, 2004), Parkinson's disease (PD) (Tan et al., 2009), Huntington's disease (Hatters, 2008), amyotrophic lateral sclerosis (Mulligan and Chakrabartty, 2013), and prion disorders (Kupfer et al., 2009); not surprisingly, dysregulation of the UPS and the ALP has been reported in some of these disorders (Bandyopadhyay and Cuervo, 2007; Wang et al., 2009; Alvarez-Erviti et al., 2010, 2013; Salminen et al., 2013; Murphy et al., 2015; Zheng et al., 2016; Budini et al., 2017; Guo et al., 2018). Because aging is the most consistent known risk factor for progressive neurodegenerative disorders (Jeppesen et al., 2011), the impairment of proteolytic mechanisms in these disorders may be due in part to an age effect (Lipinski et al., 2010; Xilouri and Stefanis, 2016).

The biological changes that occur in aging cells include alterations in proteostatic mechanisms, chromosome regulation, transcriptional regulation, protein translation, mitochondrial functioning, and cytoskeletal integrity (DiLoreto and Murphy, 2015). This review will focus on studies that have examined age-related changes in macroautophagy and CMA in the central nervous system (CNS) including the brain, cerebrospinal fluid (CSF), and retina. For comparative purposes, findings from studies which have investigated age-associated changes in these processes in non-CNS experimental systems will also be summarized.

AUTOPHAGY OVERVIEW

Autophagy involves degradation of cytoplasmic contents within the lysosome, followed by recycling of the resulting macromolecular constituents including amino acids, sugars, lipids, and nucleic acids (Feng et al., 2014; Wu et al., 2015; Hewitt and Korolchuk, 2017). Macroautophagy (**Figure 1**), the best studied of these mechanisms, involves sequestering of cytoplasmic cargo into an autophagosome followed by lysosomal degradation of the cargo (Klionsky et al., 2016). A double-membraned structure, the phagophore, initially forms around bulk cytoplasm and its constituents ("non-selective macroautophagy") or specific cytoplasmic targets including organelles ("selective macroautophagy"). The origin of the components which comprise the phagophore membrane is unclear; it has been suggested to derive from multiple sources including plasma membrane, endoplasmic reticulum, mitochondria, and the Golgi apparatus (Mari et al., 2011). Once autophagy is initiated, double-membrane nucleation allows formation of a phagophore which elongates, sequesters the cargo, and closes to form an autophagosome. The autophagosome then fuses with a lysosome to form an autolysosome (Seglen et al., 1990; Mizushima, 2007; Rubinsztein et al., 2011). Sequestered constituents are rapidly degraded by lysosomal hydrolytic enzymes. The term "autophagic flux," which is a measure of autophagic degradation activity (Loos et al., 2014), is used to refer to the complete process which starts with autophagosome formation and ends with the release of macromolecules into the cytosol (Zhang et al., 2013). Quantification of autophagic flux requires multiple measurements of macroautophagy markers over time to determine the rate of the process (Loos et al., 2014).

Much of what is known about the induction of macroautophagy comes from studies with yeast, where 41 autophagy-related proteins, known as Atgs, have been identified to date (Noda and Mizushima, 2016; Pöggeler et al., 2018). Approximately half of these proteins are known to have homologs in higher eukaryotes (Klionsky and Codogno, 2013). The proteins necessary for autophagosome formation are referred to as "core molecular machinery" (Xie and Klionsky, 2007). The proteins involved in macroautophagy in mammals are similar but not identical to those in yeast; several reviews of the process in mammalian systems have appeared (Eskelinen, 2008; Mehrpour et al., 2010; Yang and Klionsky, 2010; Abounit et al., 2012). The core molecular machinery has been divided into functional groups (Yang and Klionsky, 2010; Noda and Mizushima, 2016): (a) the Atg1 kinase complex [in mammals, the Atg1/unc-51-like kinase (ULK) complex], (b) the Atg12-conjugation system, (c) the Atg8/microtubule-associated protein light chain 3 (LC3)-conjugation system, (d) the Vps34 phosphoinositide 3-kinase (PI3 kinase) complex, (e) the Atg9 and Atg2-Atg18 complex, and (f) vacuole-membrane-protein-1 (VMP1). ULK-1 and ULK-2 are mammalian proteins with close homology to Atg1. The Atg1/ULK complex, composed of ULK1 or ULK2, mAtg13 (the mammalian homolog of Atg13), Atg101, and FIP200 (the homolog of yeast Atg17), regulates macroautophagic activity by detecting changes in concentrations of constituents such as glucose, growth factors, nitrogen, amino

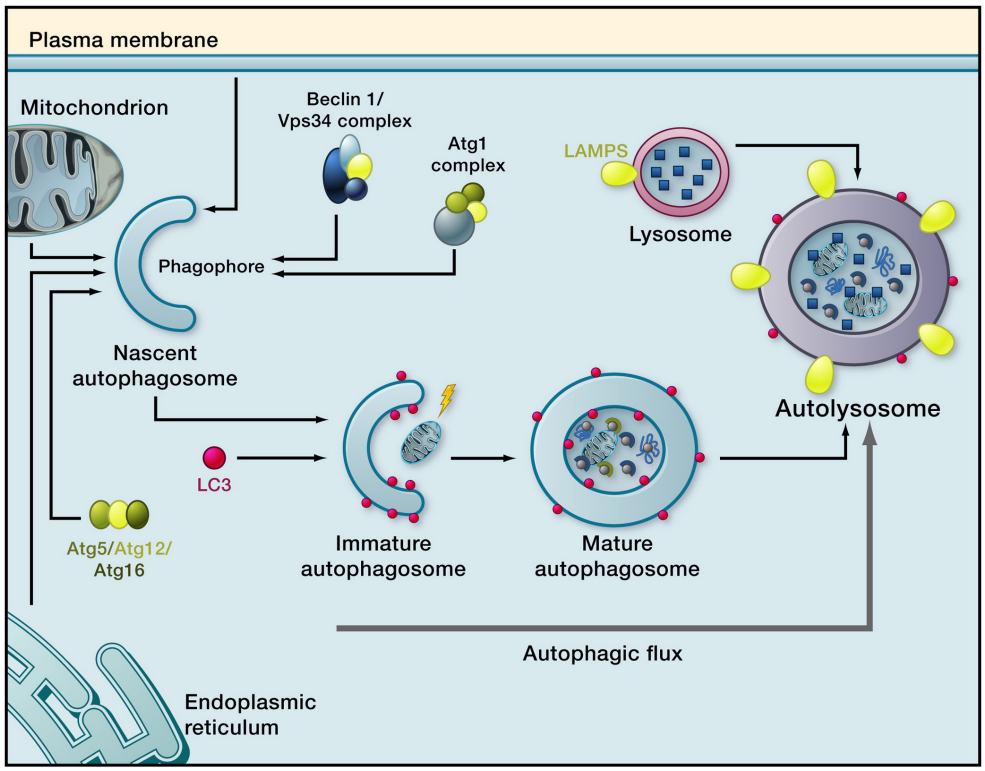

FIGURE 1 | Macroautophagy. Induction of macroautophagy results in phagophore formation, which requires the kinase Vps34 (functioning in a complex that contains Atg6, whose mammalian homolog is Beclin 1). This is followed by phagophore membrane elongation, which is regulated by LC3-II. The phagophore closes around sequestered cargo, resulting in formation of a mature autophagosome. The autophagosome moves along microtubules to a lysosome and fuses with it. Cargo is then degraded by lysosomal enzymes and breakdown products are recycled to the cytosol. The complete process is termed autophagy flux (Reprinted from Rubinsztein et al., 2011).

acids, and reactive oxygen species (Kim et al., 2013). When nutrients are sufficient, mammalian TOR complex 1 (mTORC1) is included in this complex, but mTORC1 dissociates from it when nutrients are limiting. Macroautophagy can be induced by multiple mechanisms (Rubinsztein et al., 2011) including caloric restriction or treatment with rapamycin, both of which inhibit mTORC1. A decrease in mTOR's kinase activity results in dephosphorylation of ULK1, ULK2, and mAtg13, which activates ULK1 and ULK2. ULK1 and ULK2 phosphorylate mAtg13 and FIP200, which initiates autophagosome formation (Hosokawa et al., 2009). Beclin 1, the mammalian homolog of Atg6, participates with other subunits in forming the PI3 kinase complex (Wu et al., 2015). The Atg12 and Atg8/LC3 conjugation systems are involved with elongation and expansion of the phagophore membrane (Yang and Klionsky, 2010). The Atg8-conjugation system conjugates Atg8 (and, in mammals, LC3) to phosphatidylethanolamine (PE). PE-conjugated LC3 is referred to as LC3-II, whereas its unconjugated counterpart is known as LC3-I. LC3-II regulates phagophore membrane elongation (Abeliovich et al., 2000) and facilitates membrane tethering and hemifusion (Nakatogawa et al., 2007). The Vps34 PI3 kinase complex is a positive regulator of macroautophagy; its activity generates phosphatidylinositol 3-phosphate, which recruits the Atg2–Atg18 complex to bind to Atg9 on autophagic membranes (Obara and Ohsumi, 2008). Atg9 also carries membranes from donor organelles between the developing autophagosome and the periphery. VMP1, a mammalian protein which does not appear to have an equivalent in yeast, induces autophagy even when nutrients are adequate by binding to Beclin 1, leading to the formation of a complex with Vps34 (Molejon et al., 2013). UV radiation-associated resistance gene (UVRAG) and Beclin 1-associated autophagy related key regulator (Barkor) stabilize the Beclin 1/Vps34 complex (Liang et al., 2008; Sun et al., 2008). Other important regulators of macroautophagy include AMP-activated protein kinase (AMPK) (Meley et al., 2006) and the PI3K/Akt/mTOR pathway (Woo et al., 2017). AMPK is activated by low ATP or glucose concentrations. Its activation can induce macroautophagy by inactivating TORC1 and by phosphorylating ULK1 (Hardie, 2011). Conversely, signaling from the PI3K/Akt/mTOR complex leads to phosphorylation, and thus activation, of mTOR (Triplett et al., 2015), which inhibits macroautophagy (Cao et al., 2014).

Markers which have been used to measure macroautophagy (reviewed by Klionsky et al., 2016) include the proteins encoded by Atg5 (Codogno and Meijer, 2006) and Atg12 (Hanada and Ohsumi, 2005), Beclin 1 (Kang et al., 2011), mTOR (Brown et al., 1994), and LC3 (Sugawara et al., 2004), including LC3-I,

LC3-II, and the LC3-II/LC3-I ratio. LC3-II is initially found on inner and outer layers of the phagophore membrane. During autophagosome maturation, it is removed from the outer (cytosolic) surface due to cleavage of PE by Atg4 (Yu et al., 2012), but remains on the inner (lumenal) surface of the autophagosome. LC3-II levels therefore correlate with autophagosome numbers (Mizushima and Yoshimori, 2007; Wu et al., 2015). The LC3-II/LC3-I ratio is often examined by Western blot at a single time point to assess changes in macroautophagic activity, but this procedure does not actually measure autophagic flux because no rate of change is measured (Loos et al., 2014). Alterations in LC3-II and in the LC3-II/LC3-I ratio can be difficult to interpret, because an increase in the LC3-II level may be due to either activation of macroautophagy or a decrease in autophagosomal-lysosomal fusion or lysosomal degradation of cargo; similarly, a reduction in LC3-II may be due to inhibition of macroautophagy or to an increase in autophagic flux (Linton et al., 2015). Preventing lysosomal degradation of autophagosomal LC3-II, by including inhibitors of autophagosome-lysosome fusion or of lysosomal proteases in the experimental system, may be necessary to interpret LC3-II and LC3-II/LC3-I measurements (Klionsky et al., 2016; Orhon and Reggiori, 2017). Other markers that have been used to investigate changes in macroautophagy include the lysosomal protease cathepsin D (Tatti et al., 2012) and the autophagic adaptor proteins p62/Sequestosome-1 (known as p62/SQSTM1 or p62) (Lippai and Löw, 2014) and its functional homolog, neighbor of BRCA1 (NBR1) (Kirkin et al., 2009). The latter two proteins are macroautophagy substrates; however, similar to the situation with LC3-II, single measurements of these proteins provides no information about their rate of turnover (Loos et al., 2014).

Chaperone-mediated autophagy (**Figure 2**), a more selective autophagic mechanism than macroautophagy which has been identified only in mammals, does not involve vesicle formation (Cuervo, 2010). Its selectivity is due to the requirement for its substrate proteins to contain amino acid sequences biochemically similar to Lys-Phe-Glu-Arg-Gln (KFERQ), which is present in approximately 30% of cytosolic proteins (Dice et al., 1986; Chiang and Dice, 1988; Chiang et al., 1989). Proteins bearing this sequence are bound by the molecular chaperone heat shock 70 kDa protein 8 (HSPA8, also known as hsc70 and hsc73; hereafter, "HSPA8/hsc70") in association with co-chaperones (Agarraberes and Dice, 2001). The protein-HSPA8/hsc70 complex then binds to a lysosomal surface receptor, lysosome-associated membrane glycoprotein 2a (lamp2a), which facilitates its translocation into the lysosomal lumen. Binding of the protein-HSPA8/hsc70 complex to lamp2a

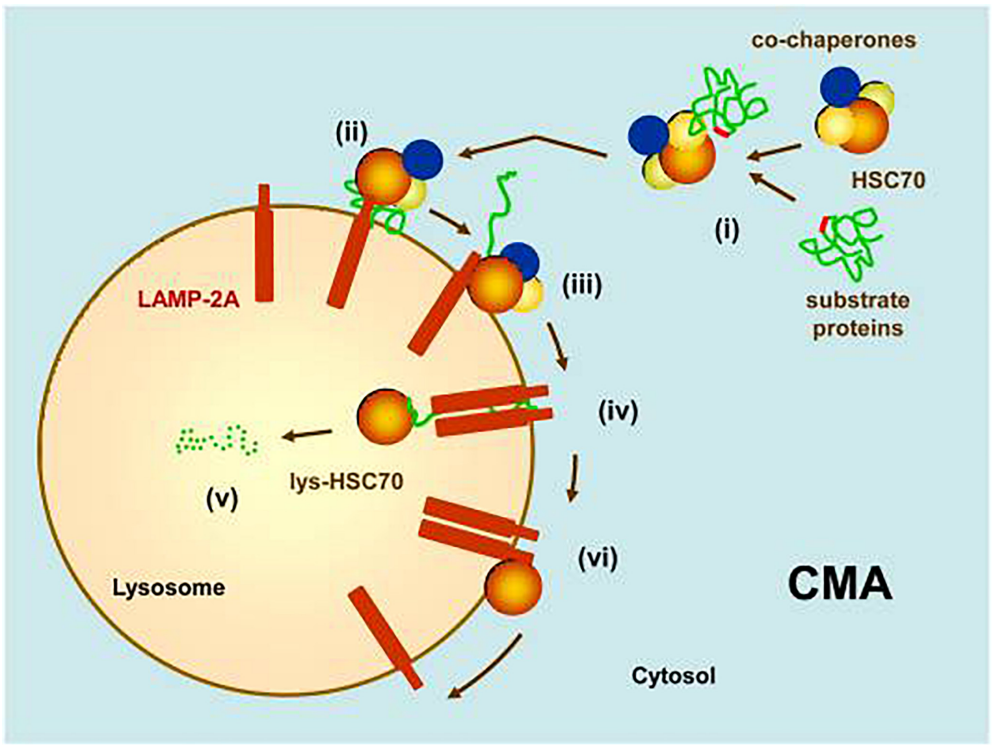

FIGURE 2 | Chaperone-mediated autophagy (CMA). (i) Substrate proteins with an amino acid sequence biochemically similar to KFERQ are recognized by HSPA8/hsc70 in association with co-chaperones. (ii) This complex binds to the monomeric form of a lysosomal surface receptor, lamp2a, which will facilitate its translocation into the lumen of the lysosome. (iii) Binding of the substrate to lamp2a causes lamp2a to multimerize into a translocation complex. (iv) After unfolding, the substrate translocates into the lysosomal lumen. The presence of a lysosomal luminal form of HSPA8/hsc70 is required for this translocation to occur. (v) The substrate is quickly degraded by lysosomal hydrolytic enzymes. (vi) Lamp2a disassembles from its translocation complex, facilitated by HSPA8/hsc70 (Reprinted from Cuervo, 2010).

is rate-limiting for CMA (Cuervo and Dice, 1996) so the concentration of lamp2a on lysosomal membranes is considered to be a marker for CMA (Cuervo and Dice, 2000b; Patel and Cuervo, 2015). In CMA, substrate proteins must unfold before their translocation into the lysosomal lumen. This unfolding is facilitated by HSPA8/hsc70 and its co-chaperones (Salvador et al., 2000). Only lysosomes containing HSPA8/hsc70 on the lumenal side of the lysosomal membrane are capable of performing CMA (Patel and Cuervo, 2015). The percentage of CMA-competent lysosomes in the rat liver is 20–30% under basal conditions but can increase to 80% during starvation (Cuervo et al., 1997).

Microautophagy has been less studied than the other autophagic mechanisms. In this process the lysosomal membrane directly engulfs portions of cytoplasm and the constituents within it. This process can be non-selective (degradation of randomly sequestered cytosol) or selective (degradation of specific organelles such as mitochondria, portions of the nucleus, or peroxisomes) (Farré et al., 2009; Mijaljica et al., 2011). Little is known about the factors that regulate microautophagy in mammalian cells (Mijaljica et al., 2011).

CROSS-TALK BETWEEN AUTOPHAGIC MECHANISMS

Both CMA and macroautophagy are maximally activated by stress conditions such as starvation and oxidative stress (Kaushik et al., 2008). Cross-talk occurs between these two autophagic mechanisms (**Figure 3**) (reviewed by Wu et al., 2015); if the activity of one is reduced, the activity of the other may increase in an effort to maintain protein homeostasis (Cuervo et al., 2004; Massey et al., 2006a,b, 2008; Kaushik et al., 2008; Wang et al., 2010). For example, while macroautophagy is initially upregulated due to nutrient deprivation, if this condition continues it becomes downregulated, with compensatory activation of CMA (Cuervo et al., 1995; Massey et al., 2006a).

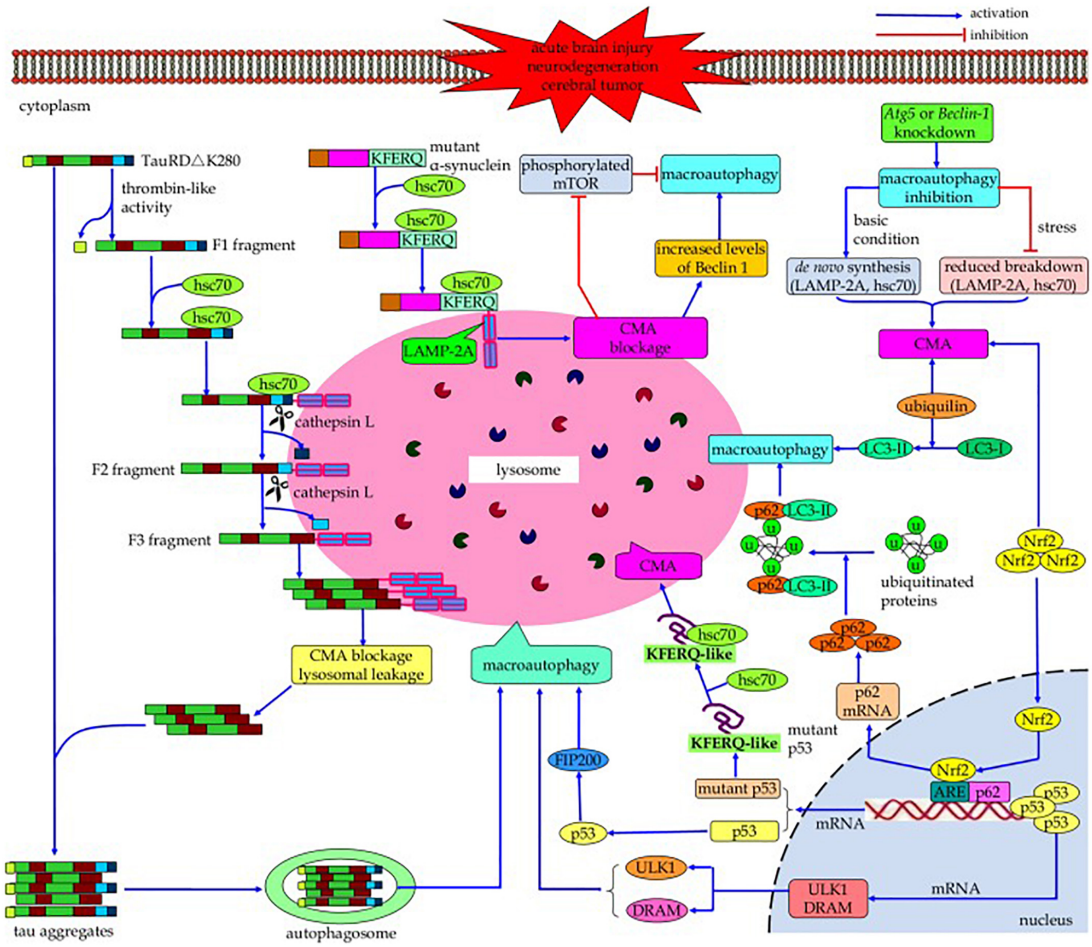

FIGURE 3 | Cross-talk between macroautophagy and CMA. If the activity of one of these autophagic processes is reduced, the activity of the other may increase in a compensatory manner. Continued blockage of CMA by inhibitory RNA targeting of lamp2a results in activation of macroautophagy as indicated by increased levels of Beclin 1; conversely, while nutrient deprivation initially upregulates macroautophagy, continued nutrient deprivation downregulates it with compensatory activation of CMA. However, this compensation may not be bi-directional, and the upregulation of one mechanism may not fully compensate for impairment of the other one. The signaling mechanisms involved in this cross-talk, while poorly understood, may include HSPA8/hsc70, p53, Nrf2, and/or ubiquilin. (Reprinted from Wu et al., 2015).

Conversely, blockage of CMA by inhibitory RNA targeting of lamp2a initially results in a decline in macroautophagy due to increased mTOR activity, but if CMA blockage continues then macroautophagy becomes activated as indicated by increased levels of Beclin 1. In a cell model of tauopathy, failure of CMA to clear mutated tau led to formation of tau aggregates, which could be cleared by macroautophagy (Wang et al., 2010). The signaling mechanisms in this cross-talk are incompletely understood; proteins suggested by Wu et al. (2015) to be involved in this process include HSPA8/hsc70 (Vakifahmetoglu-Norberg et al., 2013), p53 (Tasdemir et al., 2008), nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Gan et al., 2012), and ubiquilin (Rothenberg et al., 2010). Reduced phosphorylation of mTOR may play a role in the compensatory increase in macroautophagy when CMA is impaired (Massey et al., 2008), and when macroautophagy is inhibited, compensatory upregulation of CMA can occur through increasing lysosomal concentrations of lamp2a and HSPA8/hsc70 (Kaushik et al., 2008; Koga et al., 2011). Upregulation of CMA may not fully compensate for impairment in macroautophagy, and vice versa (Massey et al., 2006b; Wu et al., 2015). The cross-talk between the autophagic processes is not always bidirectional. Rodríguez-Muela et al. (2013) found an age-associated decrease in macroautophagy (decreased LC3-II/LC3-I ratio and reduced mRNA expression of Beclin 1 and Atg7) in the mouse retina, which was accompanied by an increase in CMA (increased lysosomal levels of lamp2a and HSPA8/hsc70). A similar result was obtained *in vitro* by shRNA-mediated knockdown of Atg7 expression in the 661W mouse retinal cell line. However, inhibition of CMA in these cells (by downregulating lamp2a) did not result in an increase in macroautophagy. The interactions between CMA and macroautophagy have been suggested to delay the accumulation of abnormal proteins, perhaps contributing to the association between aging and neurodegenerative disorders (Cuervo and Wong, 2014).

INFLUENCE OF NORMAL AGING ON AUTOPHAGY IN HUMAN BRAIN AND CSF

Few studies have been performed on the effects of normal aging on autophagy in human brain or CSF. The findings in these studies are summarized in **Table 1**. Three gene expression studies with human brain have found age-associated reductions in macroautophagy markers. Shibata et al. (2006) found downregulation of Beclin 1, and Lipinski et al. (2010) found decreases in autophagy-regulating genes including Atg5 and Atg7. Guebel and Torres (2016), investigating the effects of gender and aging on gene transcription in the hippocampus, reported decreased expression of LC3, HDAC6 (a deacetylase required for autophagosomal maturation and fusion with lysosomes) (Lee J.H. et al., 2010), and PINK1 (a mitochondrial kinase whose activity is crucial to mitochondrial function) (Qu et al., 2015) in older women. In older men expression of Bcl-2, which inhibits Beclin 1 (Liang et al., 1999), was increased, suggesting a decrease in macroautophagic activity.

TABLE 1 | Influence of normal aging on autophagy in human brain and CSF.

Study	Results
Shibata et al., 2006	Beclin 1 expression decreased in age-dependent fashion in human brains.
Lipinski et al., 2010	Genome-wide analysis found transcriptional downregulation of key macroautophagy genes including Atg5 and Atg7 in older (>70 years old) vs. younger (<40 years old) human brains.
Baird et al., 2012	Proteomics study on CSF from cognitively normal adults; three proteins in autophagy category of gene ontological analysis were positively associated with age.
Guebel and Torres, 2016	Influence of sexual dimorphism and aging on gene transcription in hippocampus: expression of LC3 and HDAC6 decreased in older women; in older men, expression of bcl-2, which inhibits Beclin 1, was increased. However, BAG-2, which can trigger PARKIN-mediated mitophagy, also increased in older men.
Loeffler et al., 2016	HSPA8/hsc70 in CSF from healthy subjects was negatively correlated with age.
Loeffler et al., 2018	Lamp2 concentration in CSF changed little during normal aging.

Three gene expression studies on human postmortem brain specimens have found age-associated decreases in macroautophagy markers. No studies of the effects of normal aging on CMA activity in human brain were found. Three studies of age-related changes in CSF autophagy proteins have produced conflicting results.

Conversely, expression of BAG-2, which inhibits PINK1 degradation by blocking its ubiquitination, and therefore triggers PARKIN-mediated mitophagy (Qu et al., 2015), was also increased in older men, suggesting activation of mitophagy. Notably, no studies were found of the effects of normal aging on CMA activity in human brain.

Three studies have examined the effects of normal aging on autophagy-related proteins in CSF. A proteomics analysis found CSF concentrations of three proteins with the gene ontology classification of "autophagy," namely myoglobin, MMP8, and HMW kininogen, to be positively correlated with age (Baird et al., 2012). Conversely, the CSF concentration of HSPA8/hsc70 was found to decrease with age (Loeffler et al., 2016) while the CSF level of lamp2 changed little (Loeffler et al., 2018). Lamp2 is not a marker for a specific autophagic process, because in addition to the previously discussed role of lamp2a in CMA (Cuervo and Dice, 2000a), lamp2 has two other isoforms which also participate in autophagic processes (Nishino et al., 2000; Fujiwara et al., 2015).

INFLUENCE OF NORMAL AGING ON AUTOPHAGY IN THE CNS IN EXPERIMENTAL MODELS

Studies of the relationship between aging and brain macroautophagy, including age-related changes in macroautophagy markers and the effects of impairing macroautophagy (for example, by introducing mutations in Atg genes) on lifespan, are shown in **Table 2** for experimental

animal models. This table is not all-inclusive; additional references can be found in recent reviews by Hansen et al. (2018) and Nakamura and Yoshimori (2018). Macroautophagy has been shown to be required for normal lifespan in *Caenorhabditis elegans* and *Drosophila* (Meléndez et al., 2003; Hars et al., 2007; Simonsen et al., 2008; Minnerly et al., 2017) and enhancement of macroautophagic activity, for example by upregulation of AMPK or rapamycin-mediated inhibition of TOR, increases lifespan in these models (Vellai et al., 2003; Simonsen et al., 2008; Bjedov et al., 2010; Ulgherait et al., 2014). However, conflicting results were found by Hashimoto et al. (2009), who reported that knockout of some autophagy genes in daf-2 mutant *C. elegans* mutants increased rather than decreased their lifespan. Also in C. elegans, Saha et al. (2015) found an age-associated loss of macroautophagic function in dopamine neurons. A recent review by Nakamura and Yoshimori (2018) concluded that "basal level of autophagic activity is elevated in many longevity paradigms and the activity is required for lifespan extension." The effects of aging on brain macroautophagy have also been investigated in mice (Hara et al., 2006; Komatsu et al., 2006; Gamerdinger et al., 2009; Kaushik et al., 2012; Triplett et al., 2015; Ott et al., 2016), rats (Yu et al., 2017), and cows (De Biase et al., 2017). Mice lacking Atg7 in the CNS developed neuronal loss and died within 28 weeks (Komatsu et al., 2006), and mice deficient for Atg5 in neural cells developed progressive motor deficits in conjunction with neuronal inclusion bodies (Hara et al., 2006). An association was reported between activation of macroautophagy and retention of spatial memory during aging; mice that were subjected to caloric restriction for 10 months, beginning at 6 weeks of age, performed better on Morris water maze testing than mice fed a high calorie diet, and the mice subjected to caloric restriction had higher hippocampal levels of Beclin 1, LC3, and cathepsin B and lower levels of mTOR and p62 than the high-calorie group (Dong et al., 2015). Kaushik et al. (2012) and Ott et al. (2016) reported age-related decreases in macroautophagy markers in mouse brain, and similar findings were reported by Yu et al. (2017) in rat brain. Brain levels of Beclin 1 were found to decline in old naked mole-rats, the longest-lived rodent, suggesting an age-associated decrease in macroautophagy in this species as well (Triplett et al., 2015). Rodríguez-Muela et al. (2013), examining age-related changes in autophagic mechanisms in mouse retina, found lower Beclin 1, and lower LC3-II after

TABLE 2 | Influence of normal aging on CNS macroautophagy in experimental systems.

Study	Results
Meléndez et al., 2003	Knockdown of bec-1 (*C. elegans* homolog of mammalian APG6/VPS30/beclin1) prevented lifespan extension in *daf-2 C. elegans* mutants.
Vellai et al., 2003	TOR deficiency in *C. elegans* increases its lifespan.
Hara et al., 2006	Mice deficient for Atg5 in neural cells developed progressive motor deficits together with neuronal inclusion bodies.
Komatsu et al., 2006	Mice lacking Atg7 in the CNS developed behavioral defects and died within 28 weeks. Neuronal loss was present in cerebral and cerebellar cortices, with age-related accumulation of polyubiquitinated proteins as neuronal inclusion bodies.
Hars et al., 2007	Knockdown of atg7 and atg12 shortened the lifespan of both wild type and insulin/IGF-1 receptor *daf-2* mutant *C. elegans*.
Simonsen et al., 2008	Expression of several autophagy genes in *Drosophila* neural tissues decreases with aging. Atg8a mutations reduce lifespan and increase sensitivity to oxidative stress.
Hashimoto et al., 2009	Knockout of macroautophagy genes tended to reduce lifespan in wild type *C. elegans*, but knockout of any of seven autophagy genes in *daf-2 C. elegans* mutants increased lifespan.
Gamerdinger et al., 2009	Increased BAG-3 (regulator of macroautophagy) and decreased BAG-1 (regulator of proteasomal pathways) were found in brain regions of old mice.
Bjedov et al., 2010	Inhibition of TOR by rapamycin increased lifespan in *Drosophila*.
Kaushik et al., 2012	22-month-old mice had lower levels of Atg7, LC3-II, p62, and NBR1 in hypothalamus than 3-month-old mice.
Rodríguez-Muela et al., 2013	No differences in basal levels of LC3-II in retinas between 3- and 22-month-old mice, but in contrast to findings in young mice, no increase in LC3-II occurred in the old mice after lysosomal blockage. mRNA for beclin-1 and Atg7 was decreased in the older animals, and protein levels of the macroautophagy substrate p62 were increased.
Dong et al., 2015	An association was found between induction of macroautophagy activity (by caloric restriction) and retention of spatial memory during aging in mice. Caloric restriction resulted in higher hippocampal levels of Beclin 1, total LC3, and cathepsin B and lower levels of mTOR and p62.
Saha et al., 2015	Age-related inhibition of macroautophagy in dopaminergic neurons after *C. elegans* finished reproductive period.
Ott et al., 2016	Beclin 1, p62, and ATG5-ATG12 decreased with age, and mTOR increased with age, in C57BL6 mouse brain.
Triplett et al., 2015	Beclin 1 decreased in whole-brain homogenates from old naked mole-rats, while the LC3-II/LC3-I ratio did not change with age.
De Biase et al., 2017	Old cow brain specimens had higher LC3 immunoreactivity, lower Beclin 1 immunoreactivity, and increased LC3-II/LC3-I ratio compared to young cow brain specimens.
Minnerly et al., 2017	A mutation in the atg-18 gene reduces *C. elegans* lifespan. Expression of atg-18 in neurons or intestinal cells restores normal lifespan, with similar results in *daf-2* mutants.
Yu et al., 2017	24-month old Wistar rats had lower hippocampal Beclin 1 and LC3-II than 5-month-old rats.

Most studies in C. elegans and Drosophila have reported decreased brain macroautophagy with aging. Macroautophagy has been shown to be required for normal lifespan in these models. In mouse brain, macroautophagy markers decrease with aging. Knockout of critical Atgs results in neurodegeneration and shortened lifespan in mice.

lysosomal blockage, in older mice, and De Biase et al. (2017) found lower Beclin 1 but an increase in the LC3-II/LC3-I ratio in old cow brain specimens. In conflict with these results is a study by Gamerdinger et al. (2009) which reported increased total cathepsin and cathepsin B activity in old mouse brain. In that study, aging was associated with a switch of regulatory control over degradation of polyubiquitinated proteins in mouse brain from Bcl-2-associated athanogene-1 (BAG1), a regulator of proteasomal degradation pathways (Lüders et al., 2000), to BAG3, a stimulator of macroautophagy (Carra et al., 2008), suggesting that aged cells may rely more on macroautophagy for degrading polyubiquitinated proteins.

Few studies have investigated the effects of normal aging on CMA in the CNS in experimental models. The results from these studies are summarized in **Table 3**. Three studies examined the influence of normal aging on the concentration of HSPA8/hsc70 in rat brain. Two of these reported an increase (Unno et al., 2000; Calabrese et al., 2004) while the third one found a decrease (Gleixner et al., 2014). In the study discussed above in which an age-related decrease in macroautophagy was found in mouse retina (Rodríguez-Muela et al., 2013), CMA was reported to increase with normal aging, possibly to compensate for the decrease in macroautophagy.

INFLUENCE OF NORMAL AGING ON AUTOPHAGY IN EXPERIMENTAL SYSTEMS OTHER THAN THE CNS

The experimental models that have been used most frequently to examine the effects of aging on macroautophagy in non-CNS systems are human fibroblast cultures (Tashiro et al., 2014; Ott et al., 2016; Pernodet et al., 2016; Romero et al., 2016) and rat liver, in which Bergamini and colleagues have extensively investigated the effects of caloric restriction on macroautophagy and its endocrine regulation (Vittorini et al., 1999; Cavallini et al., 2001, 2007; Donati et al., 2001a,b, 2008, 2009; Del Roso et al., 2003). Studies have also been performed in mouse liver (Terman, 1995), rat heart (Wohlgemuth et al., 2007), mouse muscle (Masiero et al., 2009; Carnio et al., 2014), mouse heart (Inuzuka et al., 2009; Taneike et al., 2010; Boyle et al., 2011; Zhou et al., 2017), rat nucleus pulposis (Ye et al., 2011), and human muscle biopsies (Carnio et al., 2014). The results in these studies are summarized in **Table 4**. Most studies using Beclin 1 as a marker for macroautophagy have found an age-related decrease, while conflicting results have been obtained with measurements of LC3-II and LC3-II/LC3-I.

Studies on the effects of aging on CMA in experimental systems other than the CNS are summarized in **Table 5**. The initial studies on CMA were done by Dice and his colleagues (Dice, 1982; Okada and Dice, 1984; McElligott et al., 1985; Dice et al., 1986; Chiang and Dice, 1988; Chiang et al., 1989; Cuervo and Dice, 1996). Cuervo and Dice (2000a) reported that CMA decreased in lysosomes from aged rat livers and late-passage human fibroblasts, in association with an age-associated decrease in lamp2a on lysosomal membranes. Their finding of an age-related decrease in CMA in rat liver has been confirmed and extended in subsequent studies (Kaushik et al., 2007; Kiffin et al., 2007; Zhang and Cuervo, 2008; Bandyopadhyay et al., 2010; Rodríguez-Navarro and Cuervo, 2012; Schneider et al., 2015). Age-related changes in CMA have also been studied in mouse skeletal and cardiac muscle (Zhou et al., 2017) and rat nucleus pulposis (Ye et al., 2011), with conflicting results. An age-associated decrease in lamp2 gene expression was reported for human leukocytes (Huang et al., 2012) but as discussed above, lamp2 measurements are not specific for CMA.

TABLE 3 | Effects of normal aging on CNS CMA in experimental systems.

Study	Results
Unno et al., 2000	HSPA8/hsc70 in pons, medulla, striatum, and thalamus was higher in 24- than in 6-month-old Wistar rats.
Calabrese et al., 2004	Age-related increase in HSPA8/hsc70 in rat brain between 6 and 28 months of age; highest levels in hippocampus and substantia nigra, followed by cerebellum, cortex, septum and striatum.
Rodríguez-Muela et al., 2013	An age-related increase was found in lamp2a and HSPA8/hsc70 concentrations in mouse retina, possibly to compensate for the age-related decrease in macroautophagy. However, in cultured cone retinal cells, CMA blockade did not result in an increase in macroautophagy.
Gleixner et al., 2014	HSPA8/hsc70 was lower in striatum, but not in substantia nigra, of old vs. young female rats.

Few studies have been performed to investigate the effects of normal aging on CMA in the CNS in experimental animal models. Two studies have reported an age-related increase in HSPA8/hsc70 in rat brain while a third one found a decrease. In mouse retina, an age-related increase in CMA was detected, possibly in compensation for an age-related decrease in macroautophagy.

INFLUENCE OF NORMAL AGING ON LYSOSOMAL ACTIVITY

Normal aging has been associated with decreased lysosomal activity (Reeg and Grune, 2015; Carmona-Gutierrez et al., 2016). Successful autophagic removal of proteins requires an efficiently functioning lysosomal pool, so a decline in lysosomal activity could play a prominent role in age-related deficits in macroautophagy and CMA. Lysosomal hydrolytic enzymes include proteases, nucleases, lipases, sulfatases, and phosphatases, whose optimal pH range is 4.5–5 (Carmona-Gutierrez et al., 2016). In yeast, lysosomal pH is a regulator of lifespan and mitochondrial function (Hughes and Gottschling, 2012; Ruckenstuhl et al., 2014). Lysosome numbers and their size increase in senescent cells (Robbins et al., 1970; Brunk et al., 1973; Kurz et al., 2000), possibly due to accumulation of non-degradable constituents such as lipofuscin in autophagic vacuoles (Brunk and Terman, 1999). Lipofuscin accumulates during normal aging in lysosomes of neurons and other post-mitotic cells, where it may exert deleterious effects on autophagy if lysosomal enzymatic activity is expended in an effort to degrade it (Brunk and Terman, 2002). The literature contains conflicting results with regard to age-related changes

TABLE 4 | Influence of normal aging on macroautophagy in experimental systems other than the CNS.

Study	Results
Terman, 1995	Decreased rate of formation of autophagic vacuoles, and decreased rate of their elimination, in hepatocytes from old (20–21 months) CBA mice.
Vittorini et al., 1999	Reduced hepatic macroautophagy in old Sprague-Dawley rats, partially prevented by dietary restriction.
Donati et al., 2001a	Maximum rate of autophagic proteolysis reached at 6 months in Sprague-Dawley rats and declined thereafter.
Donati et al., 2001b	Autophagy in isolated liver cells of Sprague-Dawley rats exhibited age-related decline after 6 months.
Del Roso et al., 2003	Autophagic-proteolytic response of liver to an anti-lipolytic agent was maximal in 1-month-old Sprague-Dawley rats, decreased in 6-month-old rats, and almost negligible in older rats.
Cavallini et al., 2007	Oxidized mitochondria accumulated in older Sprague-Dawley rat liver rather than degradation by autophagy.
Wohlgemuth et al., 2007	Beclin 1 increased in heart from 26-month old vs. 6-month old Fisher 344 rats.
Donati et al., 2008	Autophagic response to glucagon and insulin decreased in isolated hepatocytes from older rats.
Ye et al., 2011	Increased LC3-II and LC3-II/LC3-I ratio in nucleus pulposis of 24 month-old vs. 3 month-old Sprague-Dawley rats.
Carnio et al., 2014	Age-associated decrease in LC3-II and Atg7 in muscle from older humans and mice.
Tashiro et al., 2014	Increased autophagosome number and LC3-II, but no change in Beclin 1 or Atg5, in skin fibroblasts from older women.
Ott et al., 2016	Decreased LC3-II/LC3-I ratio, p62, ATG5–ATG12, and beclin-1 in older (60 doublings) vs. younger (20 doublings) human dermal fibroblasts; higher mTOR (macroautophagy inhibitor) in old fibroblasts.
Pernodet et al., 2016	Reduced mRNA expression of LC3-II in synchronized aged normal human skin fibroblasts compared to young skin fibroblasts.
Romero et al., 2016	Reduced induction of autophagy in aged human primary lung fibroblasts.
Zhou et al., 2017	Decreased autophagic degradation of p62 in skeletal and cardiac muscle in 27-month old vs. 5-month old mice, and decreased LC3-II/LC3-I ratio in skeletal muscle from aged mice.

Many studies have been performed to examine the effects of aging on macroautophagy in rat liver and human fibroblasts. Most studies have found an age-related decrease.

TABLE 5 | Effects of normal aging on CMA in experimental systems other than the CNS.

Study	Results
Okada and Dice, 1984	Reduced ability of late-passage confluent human fibroblasts to degrade long-lived, but not short-lived, proteins in absence of growth factors.
Cuervo and Dice, 2000a	CMA decreased in lysosomal membranes from aged (22 month-old) vs. young (3 month-old) rat liver, and in higher-passage (52 population doublings) vs. lower passage (22 population doublings) human fibroblasts. Age-related reduction in lamp2a at lysosomal membrane in both systems. Increased hsc73 (HSPA8/hsc70) at hepatic lysosomal membrane in old rats; may be compensatory response to age-related reduction in CMA.
Kiffin et al., 2007	Age-related decline in CMA in rat liver due to altered dynamics and stability of lamp2a at lysosomal membrane. Rate of transcription of lamp2a unchanged with age.
Kaushik et al., 2007	Age-related changes in lipid composition of discrete microdomains at the lysosomal membrane found to be responsible for reduced lysosomal levels of lamp2a with aging in rat hepatocytes.
Zhang and Cuervo, 2008	Age-related decline in CMA prevented in transgenic mouse in which lysosomal membrane lamp2a concentration was modulated. Preservation of autophagic activity resulted in reduced intracellular accumulation of damaged proteins.
Ye et al., 2011	Increased lamp2a, but decreased HSPA8/hsc70, in nucleus pulposis of 24 month-old vs. 3 month-old Sprague-Dawley rats.
Huang et al., 2012	Age-related decrease in lamp2 gene expression in human leukocytes.
Rodríguez-Navarro et al., 2012	Chronic exposure to high-fat diet or acute exposure to cholesterol-enriched diet decreased hepatic CMA in mice due to lower lamp2a at lysosomal membrane. Changes in lipid composition of the lysosomal membrane of lipid-challenged animals were similar to changes caused by aging.
Schneider et al., 2015	In a mouse model with liver-specific CMA deficits, other proteolytic systems were able to compensate for these deficits in young mice but not in older mice.
Zhou et al., 2017	Lamp2a and HSPA8/hsc70 decreased in skeletal muscle of aged (27 months old) vs. young (5 months old) C57BL/6 mice; in cardiac muscle of aged mice, lamp2a increased while HSPA8/hsc70 was unchanged.

Most studies have examined this issue in rat liver, and have reported an age-related decrease in CMA. Similar findings were reported for late-passage human fibroblasts, while conflicting results have been found for mouse skeletal and cardiac muscle and rat nucleus pulposis.

in the activities of different lysosomal proteases in the CNS. Kenessey et al. (1989) and Nakamura et al. (1989) reported that cathepsin D activity increased in the aged rat brain, and Nakanishi et al. (1994) found increases in cathepsin D, E, and B activities, but decreased cathepsin L activity (although not in cathepsin L protein), in brain regions from older rats. Cathepsin D localized primarily to lysosomes in young rat cerebral cortical neurons, but diffuse cytosolic immunoreactivity was also present in older rats, possibly contributing to age-related cell death (Jung et al., 2010). (See Stoka et al., 2016 for a review of the regulation of lysosomal cathepsins in aging and neurodegeneration and their contribution to apoptotic cell death.) Conversely, Sarkis et al. (1988) reported age-related decreases in the activities of cathepsins D, Ce1, and Ce2 in *C. elegans*. Finally, Banay-Schwartz et al. (1992), measuring cathepsin D in 50 brain regions of adult and older individuals, found the levels of this enzyme to be significantly increased with age in 14 regions and decreased in two regions. The findings in these studies suggest that an age-related decline in lysosomal function is not necessarily accompanied by decreases in the activities of lysosomal proteases.

DISCUSSION AND CONCLUSION

Autophagic processes have often been stated to decrease with age (Martinez-Lopez et al., 2005; Martinez-Vicente et al., 2005; Cuervo, 2008; He et al., 2013; Cuervo and Wong, 2014; Schroeder et al., 2014). This conclusion is supported by most studies that have examined this issue in experimental systems other than the CNS (**Tables 4**, **5**). The literature also suggests an age-related decline in macroautophagy in the CNS in experimental models, particularly *C. elegans*, *Drosophila*, and mice (**Table 2**). But because of the few studies on this issue in the human CNS (**Table 1**), no conclusion is currently possible as to whether autophagic processes in human brain decrease with normal aging, despite indirect evidence for this possibility from the studies in experimental systems. A similar conclusion was reached in a recent review by Hansen et al. (2018) of the connection between macroautophagy and longevity: ". . .further work is required to more rigorously test the hypothesis that autophagic activity may decline in an age-dependent fashion in the nervous system."

The three gene expression studies which have investigated this issue in human brain found age- associated decreases in macroautophagy proteins, although the study by Guebel and Torres (2016) suggested differences between men and women for these parameters. However, confirmation that macroautophagy decreases in human brain during normal aging requires similar results with other methodologies. The lack of studies on age-associated changes in CMA in human brain specimens is a critical gap in the literature; unfortunately, the few CSF studies on this issue do not help to resolve the situation. Although the study by Baird et al. (2012) found the concentrations of three autophagy-related proteins to correlate with age, none of the proteins plays a major role in autophagy. The reports of an age-associated decrease in CSF HSPA8/hsc70 (Loeffler et al., 2016) and no change with normal aging in CSF lamp2 levels (Loeffler et al., 2018) provide no information about the effects of aging on specific autophagic mechanisms in the CNS. HSPA8/hsc70 participates in many processes in addition to CMA (Stricher et al., 2013; Liao and Tang, 2014) and measurement of total lamp2 is not specific for a particular autophagic mechanism. CSF studies are unlikely to be of value for determining the effects of aging on brain autophagy because (a) changes in the CSF concentration of a protein may not correlate with changes in its brain concentration, (b) the levels of autophagy-related proteins in CSF provide no information about their concentrations on lysosomal membranes; (c) it is unclear if the markers commonly used to assess changes in autophagic processes can be detected in CSF; and (d) measurement of the LC3-II/LC3-I ratio in the presence of inhibitors of lysosomal proteases or autophagosomal-lysosomal fusion is not possible in humans.

Some of these same issues raise concerns about the findings in the studies that have examined the effects of aging on brain autophagic processes in vertebrate models. Many of the studies on age-related changes in macroautophagy in experimental systems (**Table 2**) included measurements of total LC3, LC3-II, or the LC3-II/LC3-I ratio. The results in most of these studies are difficult to interpret with regard to autophagic flux because autophagic inhibitors were not included (exceptions: Kaushik et al., 2012; Rodríguez-Muela et al., 2013; Tashiro et al., 2014; Ott et al., 2016), and because they represent findings at only one time point. None of the studies on rat brain listed in **Table 3** (effects of normal aging on CNS CMA in experimental systems) were performed on lysosome-enriched fractions, so the extent to which the reported levels of HSPA8/hsc70 reflected lysosomal concentrations of this protein is unknown. An inherent difficulty with comparing autophagy studies between different models is that basal autophagic activity in experimental animals can vary according to age, sex, and genetic background (Klionsky et al., 2016).

Comprehensive reviews of methods for assessing macroautophagy and CMA have recently been published (Patel and Cuervo, 2015; Klionsky et al., 2016). Appropriate procedures for investigating the effects of aging on these processes in the brain include transmission electron microscopy (TEM), western blot, immunohistochemical and immunofluorescent staining, and gene expression analyses. Combinations of these methods should be used (Klionsky et al., 2016). In addition to potential difficulties with data interpretation resulting from agonal changes and postmortem autolysis, studies on postmortem brain specimens provide only a "snapshot" of autophagic activity at a single time point, with no information about autophagic flux. TEM can detect and quantify morphological changes in autophagosomes, autolysosomes, amphisomes, and sequestered organelles (Eskelinen et al., 2011). Western blot is often used for examining changes in LC3-I and LC3-II levels (Tanida et al., 2005), but there are some caveats to the use of this procedure: (a) the concentration of LC3-I is higher than LC3-II in the brain, so LC3-II may be difficult to detect by western blot in crude homogenates (Chu et al., 2009; Yang et al., 2011); (b) LC3-I is more susceptible than LC3-II to degradation in SDS sample buffer or by exposure to freeze/thaw cycles; and (c) LC3-II levels measured by western blot should be compared to the levels of one or more loading control proteins rather than to LC3-I (Klionsky et al., 2016). Specific markers for CMA are presently limited to lamp2a and HSPA8/hsc70, but only the lysosomal membrane levels of these proteins correlate with CMA activity so their detection by Western blot or RT-PCR (Zhang et al., 2006; Jesus et al., 2013) should optimally be performed on lysosome-enriched fractions (Klionsky et al., 2016). Immunodetection of lamp2a should be done with antibodies which are specific for lamp2a (Patel and Cuervo, 2015).

Determining whether autophagic mechanisms decrease in the brain during normal aging has important clinical ramifications. Lifestyle modifications such as caloric restriction (Morselli et al., 2010; Rodríguez-Navarro and Cuervo, 2012; Rodríguez-Navarro et al., 2012) and exercise (He et al., 2012) have been shown to increase autophagic activity in experimental animals. Exercise reduces brain pathology in some mouse models of PD (Lau et al., 2011; Patki and Lau, 2011) and AD (Adlard et al., 2005; Nichol et al., 2008; Um et al., 2008), perhaps by induction of autophagy. Note, however, that a study in the P301S mouse model of tauopathy which examined the influence of long-term exercise on autophagy as well as tau pathology

found reduced levels of full-length and hyperphosphorylated tau in the hippocampus and spinal cord despite no significant changes in the concentrations of LC3-II or p62/SQSTM1 (Ohia-Nwoko et al., 2014), suggesting that the neuroprotective effects of exercise in that study may have been due to factors other than induction of macroautophagy. Exercise can also exert neuroprotective effects by increasing synaptic protein levels, cell proliferation, and neurotrophic factors in the brain (Molteni et al., 2002; Naylor et al., 2005; Huang et al., 2006; Marlatt et al., 2012). In transgenic mouse models of AD, reversal of autophagic deficits reduces brain Aβ deposition and prevents learning and memory deficits (Caccamo et al., 2010; Spilman et al., 2010; Yang et al., 2011; Steele et al., 2013), and a reduction in α-synuclein aggregation and mitochondrial dysfunction has been found when autophagy is increased in experimental animal models of PD (reviewed by Moors et al., 2017). Taken together, these findings suggest that pharmacologic interventions and/or lifestyle alterations which increase brain autophagy might be useful for treatment and possibly even prevention of age-associated neurodegenerative disorders. The development of autophagy-inducing agents is an active field of research (reviewed by Levine et al., 2015; Vakifahmetoglu-Norberg et al., 2015). This approach is not without risk, because autophagy has been associated with increased tumor cell survival in some studies (reviewed by Gelino and Hansen, 2012). Further, in patients with impairments in autophagosomal-lysosomal fusion, such as AD patients with presenilin gene mutations (Lee J.Y. et al., 2010), treatment with autophagy- inducing agents might produce further accumulation of neurotoxic polyubiquitinated protein aggregates (Levine et al., 2015). Nevertheless, if autophagic mechanisms can be conclusively shown to decrease in the brain during normal aging, then interventions to prevent this decrease might reduce the risk for some age-related neurodegenerative disorders, as suggested by Cuervo and Wong (2014) and Loos et al. (2017).

AUTHOR CONTRIBUTIONS

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REFERENCES

Abeliovich, H., Dunn, W. A. Jr., Kim, J., and Klionsky, D. J. (2000). Dissection of autophagosome biogenesis into distinct nucleation and expansion steps. *J. Cell Biol.* 151, 1025–1034. doi: 10.1083/jcb.151.5.1025

Abounit, K., Scarabelli, T. M., and McCauley, R. B. (2012). Autophagy in mammalian cells. *World J. Biol. Chem.* 3, 1–6. doi: 10.4331/wjbc.v3.i1.1

Adlard, P. A., Perreau, V. M., Pop, V., and Cotman, C. W. (2005). Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J. Neurosci.* 25, 4217–4221. doi: 10.1523/JNEUROSCI.0496-05.2005

Agarraberes, F. A., and Dice, J. F. (2001). A molecular chaperone complex at the lysosomal membrane is required for protein translocation. *J. Cell Sci.* 114, 2491–2499.

Alvarez-Erviti, L., Rodriguez-Oroz, M. C., Cooper, J. M., Caballero, C., Ferrer, I., Obeso, J. A., et al. (2010). Chaperone-mediated autophagy markers in Parkinson disease brains. *Arch. Neurol.* 67, 1464–1472. doi: 10.1001/archneurol.2010.198

Alvarez-Erviti, L., Seow, Y., Schapira, A. H., Rodriguez-Oroz, M. C., Obeso, J. A., and Cooper, J. M. (2013). Influence of microRNA deregulation on chaperone-mediated autophagy and α-synuclein pathology in Parkinson's disease. *Cell Death Dis.* 4:e545. doi: 10.1038/cddis.2013.73

Baird, G. S., Nelson, S. K., Keeney, T. R., Stewart, A., Williams, S., Kraemer, S., et al. (2012). Age-dependent changes in the cerebrospinal fluid proteome by slow off-rate modified aptamer array. *Am. J. Pathol.* 180, 446–456. doi: 10.1016/j.ajpath.2011.10.024

Banay-Schwartz, M., DeGuzman, T., Kenessey, A., Palkovits, M., and Lajtha, A. (1992). The distribution of cathepsin D activity in adult and aging human brain regions. *J. Neurochem.* 58, 2207–2211. doi: 10.1111/j.1471-4159.1992.tb10965.x

Bandyopadhyay, U., and Cuervo, A. M. (2007). Chaperone-mediated autophagy in aging and neurodegeneration: lessons from alpha-synuclein. *Exp. Gerontol.* 42, 120–128. doi: 10.1016/j.exger.2006.05.019

Bandyopadhyay, U., Sridhar, S., Kaushik, S., Kiffin, R., and Cuervo, A. M. (2010). Identification of regulators of chaperone-mediated autophagy. *Mol. Cell* 39, 535–547. doi: 10.1016/j.molcel.2010.08.004

Bence, N. F., Sampat, R. M., and Kopito, R. R. (2001). Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292, 1552–1555. doi: 10.1126/science.292.5521.1552

Bjedov, I., Toivonen, J. M., Kerr, F., Slack, C., Jacobson, J., Foley, A., et al. (2010). Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* 11, 35–46. doi: 10.1016/j.cmet.2009.11.010

Boyle, A. J., Shih, H., Hwang, J., Ye, J., Lee, B., Zhang, Y., et al. (2011). Cardiomyopathy of aging in the mammalian heart is characterized by myocardial hypertrophy, fibrosis and a predisposition towards cardiomyocyte apoptosis and autophagy. *Exp. Gerontol.* 46, 549–559. doi: 10.1016/j.exger.2011.02.010

Brown, E. J., Albers, M. W., Shin, T. B., Ichikawa, K., Keith, C. T., Lane, W. S., et al. (1994). A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* 369, 756–758. doi: 10.1038/369756a0

Brunk, U. T., Ericsson, J. L. E., Ponten, J., and Westermark, B. (1973). Residual bodies and 'aging' in cultured human glia cells. *Exp. Cell Res.* 79, 1–14. doi: 10.1016/0014-4827(73)90484-9

Brunk, U. T., and Terman, A. (1999). "The mitochondrial-lysosomal axis theory of cellular aging," in *Understanding the Process of Aging: The Roles of Mitochondria, Free Radicals and Antioxidants*, eds E. Cadenas and L. Packer (New York, NY: Marcel Dekker Inc), 229–249.

Brunk, U. T., and Terman, A. (2002). Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic. Biol Med.* 33, 611–619. doi: 10.1016/S0891-5849(02)00959-0

Budini, M., Buratti, E., Morselli, E., and Criollo, A. (2017). Autophagy and its impact on neurodegenerative diseases: new roles for TDP-43 and C9orf72. *Front. Mol. Neurosci.* 10:170. doi: 10.3389/fnmol.2017.00170

Caccamo, A., Majumder, S., Richardson, A., Strong, R., and Oddo, S. (2010). Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments. *J. Biol. Chem.* 285, 13107–13120. doi: 10.1074/jbc.M110.100420

Calabrese, V., Scapagnini, G., Ravagna, A., Colombrita, C., Spadaro, F., Butterfield, D. A., et al. (2004). Increased expression of heat shock proteins in rat brain during aging: relationship with mitochondrial function and glutathione redox state. *Mech. Ageing Dev.* 125, 325–335. doi: 10.1016/j.mad.2004.01.003

Cao, B., Li, J., Zhou, X., Juan, J., Han, K., Zhang, Z., et al. (2014). Clioquinol induces pro-death autophagy in leukemia and myeloma cells by disrupting the mTOR signaling pathway. *Sci. Rep.* 4:5749. doi: 10.1038/srep05749

Carmona-Gutierrez, D., Hughes, A. L., Madeo, F., and Ruckenstuhl, C. (2016). The crucial impact of lysosomes in aging and longevity. *Ageing Res. Rev.* 32, 2–12. doi: 10.1016/j.arr.2016.04.009

Carnio, S., LoVerso, F., Baraibar, M. A., Longa, E., Khan, M. M., Maffei, M., et al. (2014). Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging. *Cell Rep.* 8, 1509–1521. doi: 10.1016/j.celrep.2014.07.061

Carra, S., Seguin, S. J., Lambert, H., and Landry, J. (2008). HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J. Biol. Chem.* 283, 1437–1444. doi: 10.1074/jbc.M706304200

Cavallini, G., Donati, A., Gori, Z., Pollera, M., and Bergamini, E. (2001). The protection of rat liver autophagic proteolysis from the age-related decline co-varies with the duration of anti-ageing food restriction. *Exp. Gerontol.* 36, 497–506. doi: 10.1016/S0531-5565(00)00224-2

Cavallini, G., Donati, A., Taddei, M., and Bergamini, E. (2007). Evidence for selective mitochondrial autophagy and failure in aging. *Autophagy* 3, 26–27. doi: 10.4161/auto.3268

Chiang, H. I., and Dice, J. F. (1988). Peptide sequences that target proteins for enhanced degradation during serum withdrawal. *J. Biol. Chem.* 263, 6797–6805.

Chiang, H. L., Terlecky, S. R., Plant, C. P., and Dice, J. F. (1989). A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science* 246, 382–385. doi: 10.1126/science.2799391

Chu, C. T., Plowey, E. D., Dagda, R. K., Hickey, R. W., Cherra, S. J. III, and Clark, R. S. (2009). Autophagy in neurite injury and neurodegeneration: in vitro and in vivo models. *Methods Enzymol.* 453, 217–249. doi: 10.1016/S0076-6879(08)04011-1

Ciechanover, A., and Kwon, Y. T. (2015). Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Exp. Mol. Med.* 47:e147. doi: 10.1038/emm.2014.117

Ciechanover, A., and Kwon, Y. T. (2017). Protein quality control by molecular chaperones in neurodegeneration. *Front. Neurosci.* 11:185. doi: 10.3389/fnins.2017.00185

Codogno, P., and Meijer, A. J. (2006). Atg5: more than an autophagy factor. *Nat. Cell Biol.* 8, 1045–1047. doi: 10.1038/ncb1006-1045

Cuervo, A., Dice, J., and Knecht, E. (1997). A lysosomal population responsible for the hsc73-mediated degradation of cytosolic proteins in lysosomes. *J. Biol. Chem.* 272, 5606–5615. doi: 10.1074/jbc.272.9.5606

Cuervo, A. M. (2008). Autophagy and aging: keeping that old broom working. *Trends Genet.* 24, 604–612. doi: 10.1016/j.tig.2008.10.002

Cuervo, A. M. (2010). Chaperone-mediated autophagy: selectivity pays off. *Trends Endocrinol. Metab.* 21, 142–150. doi: 10.1016/j.tem.2009.10.003

Cuervo, A. M., and Dice, J. F. (1996). A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* 273, 501–503. doi: 10.1126/science.273.5274.501

Cuervo, A. M., and Dice, J. F. (2000a). Age-related decline in chaperone-mediated autophagy. *J. Biol. Chem.* 275, 31505–31513.

Cuervo, A. M., and Dice, J. F. (2000b). Regulation of lamp2a levels in the lysosomal membrane. *Traffic* 1, 570–583. doi: 10.1034/j.1600-0854.2000.010707.x

Cuervo, A. M., Knecht, E., Terlecky, S. R., and Dice, J. F. (1995). Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. *Am. J. Physiol.* 269, C1200–C1208. doi: 10.1152/ajpcell.1995.269.5.C1200

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T., and Sulzer, D. (2004). Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science* 305, 1292–1295. doi: 10.1126/science.1101738

Cuervo, A. M., and Wong, E. (2014). Chaperone-mediated autophagy: roles in disease and aging. *Cell Res.* 24, 92–104. doi: 10.1038/cr.2013.153

De Biase, D., Costagliola, A., Pagano, T. B., Piegari, G., Wojcik, S., Dziewiątkowski, J., et al. (2017). Amyloid precursor protein, lipofuscin accumulation and expression of autophagy markers in aged bovine brain. *BMC Vet. Res.* 13:102. doi: 10.1186/s12917-017-1028-1

Del Roso, A., Vittorini, S., Cavallini, G., Donati, A., Gori, Z., Masini, M., et al. (2003). Ageing-related changes in the in vivo function of rat liver macroautophagy and proteolysis. *Exp. Gerontol.* 38, 519–527. doi: 10.1016/S0531-5565(03)00002-0

Dice, J. F. (1982). Altered degradation of proteins microinjected into senescent human fibroblasts. *J. Biol. Chem.* 257, 14624–14627.

Dice, J. F., Chiang, H. L., Spencer, E. P., and Backer, J. M. (1986). Regulation of catabolism of microinjected ribonuclease A. Identification of residues 7-11 as the essential pentapeptide. *J. Biol. Chem.* 261, 6853–6859.

DiLoreto, R., and Murphy, C. T. (2015). The cell biology of aging. *Mol. Biol. Cell* 26, 4524–4531. doi: 10.1091/mbc.E14-06-1084

Donati, A., Cavallini, G., and Bergamini, E. (2009). Methods for inducing and monitoring liver autophagy relative to aging and antiaging caloric restriction in rats. *Methods Enzymol.* 452, 441–455. doi: 10.1016/S0076-6879(08)03626-4

Donati, A., Cavallini, G., Paradiso, C., Vittorini, S., Pollera, M., Gori, Z., et al. (2001a). Age-related changes in the regulation of autophagic proteolysis in rat isolated hepatocytes. *J. Gerontol. A Biol. Sci. Med. Sci.* 56, B288–B293. doi: 10.1093/gerona/56.7.B288

Donati, A., Cavallini, G., Paradiso, C., Vittorini, S., Pollera, M., Gori, Z., et al. (2001b). Age-related changes in the autophagic proteolysis of rat isolated liver cells: effects of antiaging dietary restrictions. *J. Gerontol. A Biol. Sci. Med. Sci.* 56, B375–B383.

Donati, A., Recchia, G., Cavallini, G., and Bergamini, E. (2008). Effect of aging and anti-aging caloric restriction on the endocrine regulation of rat liver autophagy. *J. Gerontol. A Biol. Sci. Med. Sci.* 63, 550–555. doi: 10.1093/gerona/63.6.550

Dong, W., Wang, R., Ma, L. N., Xu, B. L., Zhang, J. S., Zhao, Z. W., et al. (2015). Autophagy involving age-related cognitive behavior and hippocampus injury is modulated by different caloric intake in mice. *Int. J. Clin. Exp. Med.* 8, 11843–11853.

Eskelinen, E. L. (2008). New insights into the mechanisms of macroautophagy in mammalian cells. *Int. Rev. Cell Mol. Biol.* 266, 207–247. doi: 10.1016/S1937-6448(07)66005-5

Eskelinen, E. L., Reggiori, F., Baba, M., Kovács, A. L., and Seglen, P. O. (2011). Seeing is believing: the impact of electron microscopy on autophagy research. *Autophagy* 7, 935–956. doi: 10.4161/auto.7.9.15760

Farré, J. C., Krick, R., Subramani, S., and Thumm, M. (2009). Turnover of organelles by autophagy in yeast. *Curr. Opin. Cell Biol.* 21, 522–530. doi: 10.1016/j.ceb.2009.04.015

Feng, Y., He, D., Yao, Z., and Klionsky, D. J. (2014). The machinery of macroautophagy. *Cell Res.* 24, 24–41. doi: 10.1038/cr.2013.168

Fujiwara, Y., Hase, K., Wada, K., and Kabuta, T. (2015). An RNautophagy/DNautophagy receptor, LAMP2C, possesses an arginine-rich motif that mediates RNA/DNA-binding. *Biochem. Biophys. Res. Commun.* 460, 281–286. doi: 10.1016/j.bbrc.2015.03.025

Gamerdinger, M., Hajieva, P., Kaya, A. M., Wolfrum, U., Hartl, F. U., and Behl, C. (2009). Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J.* 28, 889–901. doi: 10.1038/emboj.2009.29

Gan, L., Vargas, M. R., Johnson, D. A., and Johnson, J. A. (2012). Astrocyte-specific overexpression of Nrf2 delays motor pathology and synuclein aggregation throughout the CNS in the alpha-synuclein mutant (A53T) mouse model. *J. Neurosci.* 32, 17775–17787. doi: 10.1523/JNEUROSCI.3049-12.2012

Gelino, S., and Hansen, M. (2012). Autophagy - an emerging anti-aging mechanism. *J. Clin. Exp. Pathol.* Suppl 4:006.

Gleixner, A. M., Pulugulla, S. H., Pant, D. B., Posimo, J. M., Crum, T. S., and Leak, R. K. (2014). Impact of aging on heat shock protein expression in the substantia nigra and striatum of the female rat. *Cell Tissue Res.* 357, 43–54. doi: 10.1007/s00441-014-1852-6

Guebel, D. V., and Torres, N. V. (2016). Sexual dimorphism and aging in the human hyppocampus: identification, validation, and impact of differentially expressed genes by factorial microarray and network analysis. *Front. Aging Neurosci.* 8:229. doi: 10.3389/fnagi.2016.00229

Guo, F., Liu, X., Cai, H., and Le, W. (2018). Autophagy in neurodegenerative diseases: pathogenesis and therapy. *Brain Pathol.* 28, 3–13. doi: 10.1111/bpa.12545

Hanada, T., and Ohsumi, Y. (2005). Structure-function relationship of Atg12, a ubiquitin-like modifier essential for autophagy. *Autophagy* 1, 110–118. doi: 10.4161/auto.1.2.1858

Hansen, M., Rubinsztein, D. C., and Walker, D. W. (2018). Autophagy as a promoter of longevity: insights from model organisms. *Nat. Rev. Mol. Cell Biol.* 19, 579–593. doi: 10.1038/s41580-018-0033-y

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* 441, 885–889. doi: 10.1038/nature04724

Hardie, D. G. (2011). AMPK and autophagy get connected. *EMBO J.* 30, 634–635. doi: 10.1038/emboj.2011.12

Hars, E. S., Qi, H., Ryazanov, A. G., Jin, S., Cai, L., Hu, C., et al. (2007). Autophagy regulates ageing in *C. elegans*. *Autophagy* 3, 93–95. doi: 10.4161/auto.3636

Hartl, F. U. (2017). Protein misfolding diseases. *Annu. Rev. Biochem.* 86, 21–26. doi: 10.1146/annurev-biochem-061516-044518

Hashimoto, Y., Ookuma, S., and Nishida, E. (2009). Lifespan extension by suppression of autophagy genes in *Caenorhabditis elegans*. *Genes Cells* 14, 717–726. doi: 10.1111/j.1365-2443.2009.01306.x

Hatters, D. M. (2008). Protein misfolding inside cells: the case of huntingtin and Huntington's disease. *IUBMB Life* 60, 724–728. doi: 10.1002/iub.111

He, C., Sumpter, R. Jr., and Levine, B. (2012). Exercise induces autophagy in peripheral tissues and in the brain. *Autophagy* 8, 1548–1551. doi: 10.4161/auto.21327

He, L. Q., Lu, J. H., and Yue, Z. Y. (2013). Autophagy in ageing and ageing-associated diseases. *Acta Pharmacol. Sin.* 34, 605–611. doi: 10.1038/aps.2012.188

Hewitt, G., and Korolchuk, V. I. (2017). Repair, reuse, recycle: the expanding role of autophagy in genome maintenance. *Trends Cell Biol.* 27, 340–351. doi: 10.1016/j.tcb.2016.11.011

Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., et al. (2009). Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell* 20, 1981–1991. doi: 10.1091/mbc.E08-12-1248

Huang, A. M., Jen, C. J., Chen, H. F., Yu, L., Kuo, Y. M., and Chen, H. I. (2006). Compulsive exercise acutely upregulates rat hippocampal brain-derived neurotrophic factor. *J. Neural Transm.* 113, 803–811. doi: 10.1007/s00702-005-0359-4

Huang, J., Xu, J., Pang, S., Bai, B., and Yan, B. (2012). Age-related decrease of the LAMP-2 gene expression in human leukocytes. *Clin. Biochem.* 45, 1229–1232. doi: 10.1016/j.clinbiochem.2012.06.01

Hughes, A. L., and Gottschling, D. E. (2012). An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature* 492, 261–265. doi: 10.1038/nature11654

Inuzuka, Y., Okuda, J., Kawashima, T., Kato, T., Niizuma, S., Tamaki, Y., et al. (2009). Suppression of phosphoinositide 3-kinase prevents cardiac aging in mice. *Circulation* 120, 1695–1703. doi: 10.1161/CIRCULATIONAHA.109.871137

Jeppesen, D. K., Bohr, V. A., and Stevnsner, T. (2011). DNA repair deficiency in neurodegeneration. *Prog. Neurobiol.* 94, 166–200. doi: 10.1016/j.pneurobio.2011.04.013

Jesus, T. F., Inácio, A., and Coelho, M. C. (2013). Different levels of hsp70 and hsc70 mRNA expression in Iberian fish exposed to distinct river conditions. *Genet. Mol. Biol.* 36, 61–69. doi: 10.1590/S1415-47572013000100009

Jung, C. H., Ro, S. H., Cao, J., Otto, N. M., and Kim, D. H. (2010). mTOR regulation of autophagy. *FEBS Lett.* 584, 1287–1295. doi: 10.1016/j.febslet.2010.01.017

Kang, R., Zeh, H. J., Lotze, M. T., and Tang, D. (2011). The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ.* 18, 571–580. doi: 10.1038/cdd.2010.191

Kaushik, S., Arias, E., Kwon, H., Lopez, N. M., Athonvarangkul, D., Sahu, S., et al. (2012). Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. *EMBO Rep.* 13, 258–265. doi: 10.1038/embor.2011.260

Kaushik, S., Kiffin, R., and Cuervo, A. M. (2007). Chaperone-mediated autophagy and aging: a novel regulatory role of lipids revealed. *Autophagy* 3, 387–389. doi: 10.4161/auto.4246

Kaushik, S., Massey, A. C., Mizushima, N., and Cuervo, A. M. (2008). Constitutive activation of chaperone-mediated autophagy in cells with impaired macroautophagy. *Mol. Biol. Cell* 19, 2179–2192. doi: 10.1091/mbc.e07-11-1155

Kenessey, A., Banay-Schwartz, M., DeGuzman, T., and Lajtha, A. (1989). Increase in cathepsin D activity in rat brain in aging. *J. Neurosci. Res.* 23, 454–456. doi: 10.1002/jnr.490230412

Kiffin, R., Kaushik, S., Zeng, M., Bandyopadhyay, U., Zhang, C., Massey, A. C., et al. (2007). Altered dynamics of the lysosomal receptor for chaperone-mediated autophagy with age. *J. Cell Sci.* 120(Pt. 5), 782–791. doi: 10.1242/jcs.001073

Kim, S. G., Buel, G. R., and Blenis, J. (2013). Nutrient regulation of the mTOR complex 1 signaling pathway. *Mol. Cells* 35, 463–473. doi: 10.1007/s10059-013-0138-2

Kirkin, V., Lamark, T., Sou, Y. S., Bjørkøy, G., Nunn, J. L., Bruun, J. A., et al. (2009). A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol. Cell* 33, 505–516. doi: 10.1016/j.molcel.2009.01.020

Klionsky, D. J., Abdelmohsen, K., Abe, A., Abedin, M. J., Abeliovich, H., Acevedo Arozena, A., et al. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* 12, 1–222. doi: 10.1080/15548627.2015.1100356

Klionsky, D. J., and Codogno, P. (2013). The mechanism and physiological function of macroautophagy. *J. Innate Immun.* 5, 427–433. doi: 10.1159/000351979

Koga, H., Martinez-Vicente, M., Arias, E., Kaushik, S., Sulzer, D., and Cuervo, A. M. (2011). Constitutive upregulation of chaperone-mediated autophagy in Huntington's disease. *J. Neurosci.* 31, 18492–18505. doi: 10.1523/JNEUROSCI.3219-11.2011

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441, 880–884. doi: 10.1038/nature04723

Kupfer, L., Hinrichs, W., and Groschup, M. (2009). Prion protein misfolding. *Curr. Mol. Med.* 9, 826–835. doi: 10.2174/156652409789105543

Kurz, D. J., Decary, S., Hong, Y., and Erusalimsky, J. D. (2000). Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J. Cell Sci.* 113, 3613–3622.

Lau, Y. S., Patki, G., Das-Panja, K., Le, W. D., and Ahmad, S. O. (2011). Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. *Eur. J. Neurosci.* 33, 1264–1274. doi: 10.1111/j.1460-9568.2011.07626.x

Lee, J. H., Yu, W. H., Kumar, A., Lee, S., Mohan, P. S., Peterhoff, C. M., et al. (2010). Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell* 141, 1146–1158. doi: 10.1016/j.cell.2010.05.008

Lee, J. Y., Koga, H., Kawaguchi, Y., Tang, W., Wong, E., Gao, Y. S., et al. (2010). HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy. *EMBO J.* 29, 969–980. doi: 10.1038/emboj.2009.405

Levine, B., Packer, M., and Codogno, P. (2015). Development of autophagy inducers in clinical medicine. *J. Clin. Invest.* 125, 14–24. doi: 10.1172/JCI73938

Liang, C., Lee, J. S., Inn, K. S., Gack, M. U., Li, Q., Roberts, E. A., et al. (2008). Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat. Cell Biol.* 10, 776–787. doi: 10.1038/ncb1740

Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., et al. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402, 672–676. doi: 10.1038/45257

Liao, Y., and Tang, L. (2014). The critical roles of HSC70 in physiological and pathological processes. *Curr. Pharm. Des.* 20, 101–107. doi: 10.2174/13816128113199990585

Linton, P. J., Gurney, M., Sengstock, D., Mentzer, R. M. Jr., and Gottlieb, R. A. (2015). This old heart: Cardiac aging and autophagy. *J. Mol. Cell. Cardiol.* 83, 44–54. doi: 10.1016/j.yjmcc.2014.12.017

Lipinski, M. M., Zheng, B., Lu, T., Yan, Z., Py, B. F., Ng, A., et al. (2010). Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14164–14169. doi: 10.1073/pnas.1009485107

Lippai, M., and Löw, P. (2014). The role of the selective adaptor p62 and ubiquitin-like proteins in autophagy. *Biomed. Res. Int.* 2014:832704. doi: 10.1155/2014/832704

Loeffler, D. A., Klaver, A. C., Coffey, M. P., and Aasly, J. O. (2018). Cerebrospinal fluid concentration of key autophagy protein Lamp2 changes little during normal aging. *Front. Aging Neurosci.* 10:130. doi: 10.3389/fnagi.2018.00130

Loeffler, D. A., Klaver, A. C., Coffey, M. P., Aasly, J. O., and LeWitt, P. A. (2016). Age-related decrease in heat shock 70-kDa protein 8 in cerebrospinal fluid is associated with increased oxidative stress. *Front. Aging Neurosci.* 8:178. doi: 10.3389/fnagi.2016.00178

Loos, B., du Toit, A., and Hofmeyr, J. H. (2014). Defining and measuring autophagosome flux -concept and reality. *Autophagy* 10, 2087–2096. doi: 10.4161/15548627.2014.973338

Loos, B., Klionsky, D. J., and Wong, E. (2017). Augmenting brain metabolism to increase macro- and chaperone-mediated autophagy for decreasing neuronal proteotoxicity and aging. *Prog. Neurobiol.* 156, 90–106. doi: 10.1016/j.pneurobio.2017.05.001

Lüders, J., Demand, J., and Höhfeld, J. (2000). The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and

the proteasome. *J. Biol. Chem.* 275, 4613–4617. doi: 10.1074/jbc.275.7.4613

Mari, M., Tooze, S. A., and Reggiori, F. (2011). The puzzling origin of the autophagosomal membrane. *F1000 Biol. Rep.* 3:25. doi: 10.3410/B3-25

Marlatt, M. W., Potter, M. C., Lucassen, P. J., and van Praag, H. (2012). Running throughout middle-age improves memory function, hippocampal neurogenesis, and BDNF levels in female C57BL/6J mice. *Dev. Neurobiol.* 72, 943–952. doi: 10.1002/dneu.22009

Martinez-Lopez, N., Martinez-Vicente, M., Sovak, G., and Cuervo, A. M. (2005). Protein degradation and aging. *Exp. Gerontol.* 40, 622–633. doi: 10.1016/j.exger.2005.07.005

Martinez-Vicente, M., Sovak, G., and Cuervo, A. M. (2005). Protein degradation and aging. *Exp. Gerontol.* 40, 622–633. doi: 10.1016/j.exger.2005.07.005

Masiero, E., Agatea, L., Mammucari, C., Blaauw, B., Loro, E., Komatsu, M., et al. (2009). Autophagy is required to maintain muscle mass. *Cell Metab.* 10, 507–515. doi: 10.1016/j.cmet.2009.10.008

Massey, A. C., Follenzi, A., Kiffin, R., Zhang, C., and Cuervo, A. M. (2008). Early cellular changes after blockage of chaperone-mediated autophagy. *Autophagy* 4, 442–456. doi: 10.4161/auto.5654

Massey, A. C., Kaushik, S., and Cuervo, A. M. (2006a). Lysosomal chat maintains the balance. *Autophagy* 2, 325–327.

Massey, A. C., Kaushik, S., Sovak, G., Kiffin, R., and Cuervo, A. M. (2006b). Consequences of the selective blockage of chaperone-mediated autophagy. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5805–5810. doi: 10.1073/pnas.0507436103

McElligott, M. A., Miao, P., and Dice, J. F. (1985). Lysosomal degradation of ribonuclease A and ribonuclease S-protein microinjected into the cytosol of human fibroblasts. *J. Biol. Chem.* 260, 11986–11993.

Mehrpour, M., Esclatine, A., Beau, I., and Codogno, P. (2010). Overview of macroautophagy regulation in mammalian cells. *Cell Res.* 20, 748–762. doi: 10.1038/cr.2010.82

Meléndez, A., Tallóczy, Z., Seaman, M., Eskelinen, E. L., Hall, D. H., and Levine, B. (2003). Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* 301, 1387–1391. doi: 10.1126/science.1087782

Meley, D., Bauvy, C., Houben-Weerts, J. H., Dubbelhuis, P. F., Helmond, M. T., Codogno, P., et al. (2006). AMP-activated protein kinase and the regulation of autophagic proteolysis. *J. Biol. Chem.* 281, 34870–34879. doi: 10.1074/jbc.M605488200

Mijaljica, D., Prescott, M., and Devenish, R. J. (2011). Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. *Autophagy* 7, 673–682. doi: 10.4161/auto.7.7.14733

Minnerly, J., Zhang, J., Parker, T., Kaul, T., and Jia, K. (2017). The cell non-autonomous function of ATG-18 is essential for neuroendocrine regulation of Caenorhabditis elegans lifespan. *PLoS Genet.* 13:e1006764. doi: 10.1371/journal.pgen.1006764

Mizushima, N. (2007). Autophagy: process and function. *Genes Dev.* 21, 2861–2873. doi: 10.1101/gad.1599207

Mizushima, N., and Yoshimori, T. (2007). How to interpret LC3 immunoblotting. *Autophagy* 3, 542–545. doi: 10.4161/auto.4600

Molejon, M. I., Ropolo, A., Re, A. L., Boggio, V., and Vaccaro, M. I. (2013). The VMP1-Beclin 1 interaction regulates autophagy induction. *Sci. Rep.* 3:1055. doi: 10.1038/srep01055

Molteni, R., Ying, Z., and Gómez-Pinilla, F. (2002). Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur. J. Neurosci.* 16, 1107–1116. doi: 10.1046/j.1460-9568.2002.02158.x

Moors, T. E., Hoozemans, J. J., Ingrassia, A., Beccari, T., Parnetti, L., Chartier-Harlin, M. C., et al. (2017). Therapeutic potential of autophagy-enhancing agents in Parkinson's disease. *Mol. Neurodegener.* 12:11. doi: 10.1186/s13024-017-0154-3

Morselli, E., Maiuri, M. C., Markaki, M., Megalou, E., Pasparaki, A., Palikaras, K., et al. (2010). Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction f autophagy. *Cell Death Dis.* 1:e10. doi: 10.1038/cddis.2009.8

Mulligan, V. K., and Chakrabartty, A. (2013). Protein misfolding in the late-onset neurodegenerative diseases: common themes and the unique case of amyotrophic lateral sclerosis. *Proteins* 81, 1285–1303. doi: 10.1002/prot.24285

Murphy, K. E., Gysbers, A. M., Abbott, S. K., Spiro, A. S., Furuta, A., Cooper, A., et al. (2015). Lysosomal-associated membrane protein 2 isoforms are differentially affected in early Parkinson's disease. *Mov. Disord.* 30, 1639–1647. doi: 10.1002/mds.26141

Nakamura, S., and Yoshimori, T. (2018). Autophagy and longevity. *Mol. Cells* 41, 65–72. doi: 10.14348/molcells.2018.2333

Nakamura, Y., Takeda, M., Suzuki, H., Morita, H., Tada, K., Hariguchi, S., et al. (1989). Age-dependent change in activities of lysosomal enzymes in rat brain. *Mech. Ageing Dev.* 50, 215–225. doi: 10.1016/0047-6374(89)90101-2

Nakanishi, H., Tominaga, K., Amano, T., Hirotsu, I., Inoue, T., and Yamamoto, K. (1994). Age-related changes in activities and localizations of cathepsins D, E, B, and L in the rat brain tissues. *Exp. Neurol.* 126, 119–128. doi: 10.1006/exnr.1994.1048

Nakatogawa, H., Ichimura, Y., and Ohsumi, Y. (2007). Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 130, 165–178. doi: 10.1016/j.cell.2007.05.021

Naylor, M., Bowen, K. K., Sailor, K. A., Dempsey, R. J., and Vemuganti, R. (2005). Preconditioning-induced ischemic tolerance stimulates growth factor expression and neurogenesis in adult rat hippocampus. *Neurochem. Int.* 47, 565–572. doi: 10.1016/j.neuint.2005.07.003

Nichol, K. E., Poon, W. W., Parachikova, A. I., Cribbs, D. H., Glabe, C. G., and Cotman, C. W. (2008). Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid. *J. Neuroinflammation* 5:13. doi: 10.1186/1742-2094-5-13

Nishino, I., Fu, J., Tanji, K., Yamada, T., Shimojo, S., Koori, T., et al. (2000). Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature* 406, 906–910. doi: 10.1038/35022604

Noda, N. N., and Mizushima, N. (2016). Atg101: not just an accessory subunit in the autophagy-initiation complex. *Cell Struct. Funct.* 41, 13–20. doi: 10.1247/csf.15013

Obara, K., and Ohsumi, Y. (2008). Dynamics and function of PtdIns(3)P in autophagy. *Autophagy* 4, 952–954. doi: 10.4161/auto.6790

Ohia-Nwoko, O., Montazari, S., Lau, Y. S., and Eriksen, J. L. (2014). Long-term treadmill exercise attenuates tau pathology in P301S tau transgenic mice. *Mol. Neurodegener.* 9:54. doi: 10.1186/1750-1326-9-54

Okada, A. A., and Dice, J. F. (1984). Altered degradation of intracellular proteins in aging human fibroblasts. *Mech. Ageing Dev.* 26, 341–356. doi: 10.1016/0047-6374(84)90105-2

Orenstein, S. J., and Cuervo, A. M. (2010). Chaperone-mediated autophagy: molecular mechanisms and physiological relevance. *Semin. Cell Dev. Biol.* 21, 719–726. doi: 10.1016/j.semcdb.2010.02.005

Orhon, I., and Reggiori, F. (2017). Assays to monitor autophagy progression in cell cultures. *Cells* 6:E20. doi: 10.3390/cells6030020

Ott, C., König, J., Höhn, A., Jung, T., and Grune, T. (2016). Macroautophagy is impaired in old murine brain tissue as well as in senescent human fibroblasts. *Redox Biol.* 10, 266–273. doi: 10.1016/j.redox.2016.10.015

Patel, B., and Cuervo, A. M. (2015). Methods to study chaperone-mediated autophagy. *Methods* 75, 133–140. doi: 10.1016/j.ymeth.2015.01.003

Patki, G., and Lau, Y. S. (2011). Impact of exercise on mitochondrial transcription factor expression and damage in the striatum of a chronic mouse model of Parkinson's disease. *Neurosci. Lett.* 505, 268–272. doi: 10.1016/j.neulet.2011.10.036

Pernodet, N., Dong, K., and Pelle, E. (2016). Autophagy in human skin fibroblasts: Comparison between young and aged cells and evaluation of its cellular rhythm and response to Ultraviolet A radiation. *J. Cosmet. Sci.* 67, 13–20.

Pöggeler, S., Nowrousian, M., Teichert, I., Beier, A., and Kück, U. (2018). "Fruiting-body development in ascomycetes," in *The Mycota. A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. XV. Physiology and Genetics: Selected Basic and Applied Aspects*, 2nd Edn, eds T. Anke and A. Schüffler (Basel: Springer International Publishing AG), 26. doi: 10.1007/978-3-319-71740-1_1

Qu, D., Hage, A., Don-Carolis, K., Huang, E., Joselin, A., Safarpour, F., et al. (2015). BAG2 gene-mediated regulation of PINK1 protein is critical for mitochondrial translocation of PARKIN and neuronal survival. *J. Biol. Chem.* 290, 30441–30452. doi: 10.1074/jbc.M115.677815

Reeg, S., and Grune, T. (2015). Protein oxidation in aging: does it play a role in aging progression? *Antioxid. Redox Signal.* 23, 239–255. doi: 10.1089/ars.2014.6062

Robbins, E., Levine, E. M., and Eagle, H. (1970). Morphologic changes accompanying senescence of cultured human diploid cells. *J. Exp. Med.* 131, 1211–1222. doi: 10.1084/jem.131.6.1211

Rodríguez-Muela, N., Koga, H., García-Ledo, L., de la Villa, P., de la Rosa, E. J., Cuervo, A. M., et al. (2013). Balance between autophagic pathways preserves retinal homeostasis. *Aging Cell* 12, 478–488. doi: 10.1111/acel.12072

Rodríguez-Navarro, J. A., and Cuervo, A. M. (2012). Dietary lipids and aging compromise chaperone-mediated autophagy by similar mechanisms. *Autophagy* 8, 1152–1154. doi: 10.4161/auto.20649

Rodríguez-Navarro, J. A., Kaushik, S., Koga, H., Dall'Armi, C., Shui, G., Wenk, M. R., et al. (2012). Inhibitory effect of dietary lipids on chaperone-mediated autophagy. *Proc. Natl. Acad Sci. U.S.A.* 109, E705–E714. doi: 10.1073/pnas.1113036109

Romero, Y., Bueno, M., Ramirez, R., Álvarez, D., Sembrat, J. C., Goncharova, E. A., et al. (2016). mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. *Aging Cell* 15, 1103–1112. doi: 10.1111/acel.12514

Rothenberg, C., Srinivasan, D., Mah, L., Kaushik, S., Peterhoff, C. M., Ugolino, J., et al. (2010). Ubiquilin functions in autophagy and is degraded by chaperone-mediated autophagy. *Hum. Mol. Genet.* 19, 3219–3232. doi: 10.1093/hmg/ddq231

Rubinsztein, D. C., Mariño, G., and Kroemer, G. (2011). Autophagy and aging. *Cell* 146, 682–695. doi: 10.1016/j.cell.2011.07.030

Ruckenstuhl, C., Netzberger, C., Entfellner, I., Carmona-Gutierrez, D., Kickenweiz, T., and Stekovic, S. (2014). Autophagy extends lifespan via vacuolar acidification. *Microb. Cell* 1, 160–162. doi: 10.15698/mic2014.05.147

Saha, S., Ash, P. E., Gowda, V., Liu, L., Shirihai, O., and Wolozin, B. (2015). Mutations in LRRK2 potentiate age-related impairment of autophagic flux. *Mol. Neurodegener.* 10:26. doi: 10.1186/s13024-015-0022-y

Salminen, A., Kaarniranta, K., Kauppinen, A., Ojala, J., Haapasalo, A., Soininen, H., et al. (2013). Impaired autophagy and APP processing in Alzheimer's disease: the potential role of Beclin 1 interactome. *Prog. Neurobiol.* 106-107, 33–54. doi: 10.1016/j.pneurobio.2013.06.002

Salvador, N., Aguado, C., Horst, M., and Knecht, E. (2000). Import of a cytosolic protein into lysosomes by chaperone-mediated autophagy depends on its folding state. *J. Biol. Chem.* 275, 27447–27456. doi: 10.1074/jbc.M001394200

Sarkis, G. J., Ashcom, J. D., Hawdon, J. M., and Jacobson, L. A. (1988). Decline in protease activities with age in the nematode *Caenorhabditis elegans*. *Mech. Ageing Dev.* 45, 191–201. doi: 10.1016/0047-6374(88)90001-2

Schneider, J. L., Villarroya, J., Diaz-Carretero, A., Patel, B., Urbanska, A. M., Thi, M. M., et al. (2015). Loss of hepatic chaperone-mediated autophagy accelerates proteostasis failure in aging. *Aging Cell* 14, 249–264. doi: 10.1111/acel.12310

Schroeder, S., Zimmermann, A., Carmona-Gutierrez, D., Eisenberg, T., Ruckenstuhl, C., Andryushkova, A., et al. (2014). Metabolites in aging and autophagy. *Microb. Cell* 1, 110–114. doi: 10.15698/mic2014.04.142

Schubert, U., Antón, L. C., Gibbs, J., Norbury, C. C., Yewdell, J. W., and Bennink, J. R. (2000). Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 404, 770–774. doi: 10.1038/35008096

Seglen, P. O., Gordon, P. B., and Holen, I. (1990). Non-selective autophagy. *Semin. Cell Biol.* 1, 441–448.

Selkoe, D. J. (2004). Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. *Nat. Cell Biol.* 6, 1054–1061. doi: 10.1038/ncb1104-1054

Shibata, M., Lu, T., Furuya, T., Degterev, A., Mizushima, N., Yoshimori, T., et al. (2006). Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. *J. Biol. Chem.* 281, 14474–14485. doi: 10.1074/jbc.M600364200

Simonsen, A., Cumming, R. C., Brech, A., Isakson, P., Schubert, D. R., and Finley, K. D. (2008). Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. *Autophagy* 4, 176–184. doi: 10.4161/auto.5269

Spilman, P., Podlutskaya, N., Hart, M. J., Debnath, J., Gorostiza, O., Bredesen, D., et al. (2010). Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. *PLoS One* 5:e9979. doi: 10.1371/journal.pone.0009979

Steele, J. W., Lachenmayer, M. L., Ju, S., Stock, A., Liken, J., Kim, S. H., et al. (2013). Latrepirdine improves cognition and arrests progression of neuropathology in an Alzheimer's mouse model. *Mol. Psychiatry* 18, 889–897. doi: 10.1038/mp.2012.106

Stoka, V., Turk, V., and Turk, B. (2016). Lysosomal cathepsins and their regulation in aging and neurodegeneration. *Ageing Res. Rev.* 32, 22–37. doi: 10.1016/j.arr.2016.04.010

Stricher, F., Macri, C., Ruff, M., and Muller, S. (2013). HSPA8/HSC70 chaperone protein: structure, function, and chemical targeting. *Autophagy* 9, 1937–1954. doi: 10.4161/auto.26448

Sugawara, K., Suzuki, N. N., Fujioka, Y., Mizushima, N., Ohsumi, Y., and Inagaki, F. (2004). The crystal structure of microtubule-associated protein light chain 3, a mammalian homologue of *Saccharomyces cerevisiae* Atg8. *Genes Cells* 9, 611–618. doi: 10.1111/j.1356-9597.2004.00750.x

Sun, Q., Fan, W., Chen, K., Ding, X., Chen, S., and Zhong, Q. (2008). Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19211–19216. doi: 10.1073/pnas.0810452105

Tan, J. M., Wong, E. S., and Lim, K. L. (2009). Protein misfolding and aggregation in Parkinson's disease. *Antioxid. Redox Signal.* 11, 2119–2134. doi: 10.1089/ARS.2009.2490

Taneike, M., Yamaguchi, O., Nakai, A., Hikoso, S., Takeda, T., Mizote, I., et al. (2010). Inhibition of autophagy in the heart induces age-related cardiomyopathy. *Autophagy* 6, 600–606. doi: 10.4161/auto.6.5.11947

Tanida, I., Minematsu-Ikeguchi, N., Ueno, T., and Kominami, E. (2005). Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy* 1, 84–91. doi: 10.4161/auto.1.2.1697

Tasdemir, E., Chiara Maiuri, M., Morselli, E., Criollo, A., D'Amelio, M., Djavaheri-Mergny, M., et al. (2008). A dual role of p53 in the control of autophagy. *Autophagy* 4, 810–814. doi: 10.4161/auto.6486

Tashiro, K., Shishido, M., Fujimoto, K., Hirota, Y., Yo, K., Gomi, T., et al. (2014). Age-related disruption of autophagy in dermal fibroblasts modulates extracellular matrix components. *Biochem. Biophys. Res. Commun.* 443, 167–172. doi: 10.1016/j.bbrc.2013.11.066

Tatti, M., Motta, M., Di Bartolomeo, S., Scarpa, S., Cianfanelli, V., Cecconi, F., et al. (2012). Reduced cathepsins B and D cause impaired autophagic degradation that can be almost completely restored by overexpression of these two proteases in Sap C-deficient fibroblasts. *Hum. Mol. Genet.* 21, 5159–5173. doi: 10.1093/hmg/dds367

Terman, A. (1995). The effect of age on formation and elimination of autophagic vacuoles in mouse hepatocytes. *Gerontology* 41(Suppl. 2), 319–326. doi: 10.1159/000213753

Triplett, J. C., Tramutola, A., Swomley, A., Kirk, J., Grimes, K., Lewis, K., et al. (2015). Age-related changes in the proteostasis network in the brain of the naked mole-rat: Implications promoting healthy longevity. *Biochim. Biophys. Acta* 1852, 2213–2224. doi: 10.1016/j.bbadis.2015.08.002

Ulgherait, M., Rana, A., Rera, M., Graniel, J., and Walker, D. W. (2014). AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. *Cell Rep.* 8, 1767–1780. doi: 10.1016/j.celrep.2014.08.006

Um, H. S., Kang, E. B., Leem, Y. H., Cho, I. H., Yang, C. H., Chae, K. R., et al. (2008). Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. *Int. J. Mol. Med.* 22, 529–539.

Unno, K., Asakura, H., Shibuya, Y., Kaiho, M., Okada, S., and Oku, N. (2000). Increase in basal level of Hsp70, consisting chiefly of constitutively expressed Hsp70 (Hsc70) in aged rat brain. *J. Gerontol. A Biol. Sci. Med. Sci.* 55, B329–B335. doi: 10.1093/gerona/55.7.b329

Vakifahmetoglu-Norberg, H., Kim, M., Xia, H. G., Iwanicki, M. P., Ofengeim, D., Coloff, J. L., et al. (2013). Chaperone-mediated autophagy degrades mutant p53. *Genes Dev.* 27, 1718–1730. doi: 10.1101/gad.220897.113

Vakifahmetoglu-Norberg, H., Xia, H. G., and Yuan, J. (2015). Pharmacologic agents targeting autophagy. *J. Clin. Invest.* 125, 5–13. doi: 10.1172/JCI73937

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A. L., Orosz, L., and Müller, F. (2003). Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* 426:620. doi: 10.1038/426620a

Vittorini, S., Paradiso, C., Donati, A., Cavallini, G., Masini, M., Gori, Z., et al. (1999). The age-related accumulation of protein carbonyl in rat liver correlates with the age-related decline in liver proteolytic activities. *J. Gerontol. A Biol. Sci. Med. Sci.* 54, B318–B323. doi: 10.1093/gerona/54.8.B318

Wang, Y., Martinez-Vicente, M., Krüger, U., Kaushik, S., Wong, E., Mandelkow, E. M., et al. (2009). Tau fragmentation, aggregation and clearance: the dual role

of lysosomal processing. *Hum. Mol. Genet.* 18, 4153–4170. doi: 10.1093/hmg/ddp367

Wang, Y., Martinez-Vicente, M., Kruger, U., Kaushik, S., Wong, E., Mandelkow, E. M., et al. (2010). Synergy and antagonism of macroautophagy and chaperone-mediated autophagy in a cell model of pathological tau aggregation. *Autophagy* 6, 182–183. doi: 10.4161/auto.6.1.10815

Wohlgemuth, S. E., Julian, D., Akin, D. E., Fried, J., Toscano, K., Leeuwenburgh, C., et al. (2007). Autophagy in the heart and liver during normal aging and calorie restriction. *Rejuvenation Res.* 10, 281–292. doi: 10.1089/rej.2006.0535

Woo, S. U., Sangai, T., Akcakanat, A., Chen, H., Wei, C., and Meric-Bernstam, F. (2017). Vertical inhibition of the PI3K/Akt/mTOR pathway is synergistic in breast cancer. *Oncogenesis* 6:e385. doi: 10.1038/oncsis.2017.86

Wu, H., Chen, S., Ammar, A. B., Xu, J., Wu, Q., Pan, K., et al. (2015). Crosstalk between macroautophagy and chaperone-mediated autophagy: implications for the treatment of neurological diseases. *Mol. Neurobiol.* 52, 1284–1296. doi: 10.1007/s12035-014-8933-0

Xie, Z., and Klionsky, D. J. (2007). Autophagosome formation: core machinery and adaptations. *Nat. Cell Biol.* 9, 1102–1109. doi: 10.1038/ncb1007-1102

Xilouri, M., and Stefanis, L. (2016). Chaperone mediated autophagy in aging: starve to prosper. *Ageing Res. Rev.* 32, 13–21. doi: 10.1016/j.arr.2016.07.001

Yang, D. S., Stavrides, P., Mohan, P. S., Kaushik, S., Kumar, A., Ohno, M., et al. (2011). Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. *Brain* 134, 258–277. doi: 10.1093/brain/awq341

Yang, Z., and Klionsky, D. J. (2010). Mammalian autophagy: core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* 22, 124–131. doi: 10.1016/j.ceb.2009.11.014

Ye, W., Xu, K., Huang, D., Liang, A., Peng, Y., Zhu, W., et al. (2011). Age-related increases of macroautophagy and chaperone-mediated autophagy in rat nucleus pulposus. *Connect. Tissue Res.* 52, 472–478. doi: 10.3109/03008207.2011.564336

Yu, Y., Feng, L., Li, J., Lan, X., A, L., Lv, X., et al. (2017). The alteration of autophagy and apoptosis in the hippocampus of rats with natural aging-dependent cognitive deficits. *Behav. Brain Res.* 334, 155–162. doi: 10.1016/j.bbr.2017.07.003

Yu, Z. Q., Ni, T., Hong, B., Wang, H. Y., Jiang, F. J., Zou, S., et al. (2012). Dual roles of Atg8-PE deconjugation by Atg4 in autophagy. *Autophagy* 8, 883–892. doi: 10.4161/auto.19652

Zhang, C., and Cuervo, A. M. (2008). Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat. Med.* 14, 959–965. doi: 10.1038/nm.1851

Zhang, X. J., Chen, S., Huang, K. X., and Le, W. D. (2013). Why should autophagic flux be assessed? *Acta Pharmacol. Sin.* 34, 595–599. doi: 10.1038/aps.2012.184

Zhang, Z., Fan, J., Becker, K. G., Graff, R. D., Lee, G. M., and Francomano, C. A. (2006). Comparaison of gene expression profile between human chondrons and chondrocytes: a cDNA microarray study. *Osteoarthr. Cartil.* 14, 449–459. doi: 10.1016/j.joca.2005.11.008

Zheng, Q., Huang, T., Zhang, L., Zhou, Y., Luo, H., Xu, H., et al. (2016). Dysregulation of ubiquitin-proteasome system in neurodegenerative diseases. *Front. Aging Neurosci.* 8:303. doi: 10.3389/fnagi.2016.00303

Zhou, J., Chong, S. Y., Lim, A., Singh, B. K., Sinha, R. A., Salmon, A. B., et al. (2017). Changes in macroautophagy, chaperone-mediated autophagy, and mitochondrial metabolism in murine skeletal and cardiac muscle during aging. *Aging* 9, 583–599. doi: 10.18632/aging.101181

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REVIEW
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The Gut-Brain Axis in Neurodegenerative Diseases and Relevance of the Canine Model: A Review

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Identifying appropriate animal models is critical in developing translatable *in vitro* and *in vivo* systems for therapeutic drug development and investigating disease pathophysiology. These animal models should have direct biological and translational relevance to the underlying disease they are supposed to mimic. Aging dogs not only naturally develop a cognitive decline in many aspects including learning and memory deficits, but they also exhibit human-like individual variability in the aging process. Neurodegenerative processes that can be observed in both human and canine brains include the progressive accumulation of β-amyloid (Aβ) found as diffuse plaques in the prefrontal cortex (PFC), including the *gyrus proreus (i.e., medial orbital PFC)*, as well as the hippocampus and the cerebral vasculature. Tau pathology, a marker of neurodegeneration and dementia progression, was also found in canine hippocampal synapses. Various epidemiological data show that human patients with neurodegenerative diseases have concurrent intestinal lesions, and histopathological changes in the gastrointestinal (GI) tract occurs decades before neurodegenerative changes. Gut microbiome alterations have also been reported in many neurodegenerative diseases including Alzheimer's (AD) and Parkinson's diseases, as well as inflammatory central nervous system (CNS) diseases. Interestingly, the dog gut microbiome more closely resembles human gut microbiome in composition and functional overlap compared to rodent models. This article reviews the physiology of the gut-brain axis (GBA) and its involvement with neurodegenerative diseases in humans. Additionally, we outline the advantages and weaknesses of current *in vitro* and *in vivo* models and discuss future research directions investigating major human neurodegenerative diseases such as AD and Parkinson's diseases using dogs.

Keywords: gut-brain axis, neurodegenerative disease, canine, translational, animal models, review

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INTRODUCTION

The gut-brain axis (GBA) is a highly complex interactive network between the gut and the brain, composed of endocrinological, immunological and neural mediators, as summarized in **Figure 1** (Rhee et al., 2009). The GBA is largely mediated by the central nervous system (CNS), the enteric nervous system (ENS), and the intestinal microbiota (Grenham et al., 2011). The extrinsic nerves of the gastrointestinal (GI) tract connect the gut to the brain through vagal and spinal afferent fibers, while the brain sends efferent sympathetic and parasympathetic fibers to the GI tract (Grenham et al., 2011; Browning and Travagli, 2014; Foster et al., 2017). The hypothalamic pituitary adrenal (HPA)-axis is known as the main modulator of the physiological stress response but it also modulates alimentary function during digestion (Tsigos and Chrousos, 2002) to facilitate gluconeogenesis. The hypothalamus releases corticotrophin-releasing factor (CRF) and different proteins within this family (e.g., CRF, urocortin 1–3) are also known to affect GI tract function, i.e., intestinal motility (Kihara et al., 2001), permeability (Zheng et al., 2013), and inflammation (Dinan et al., 2006). Specifically, changes in the GI motility induced by urocortin administration were noted in conscious rats, and this study also suggested that the vagal pathway could regulate the central action of urocortin (Kihara et al., 2001). Rats experiencing psychological stress showed decreased level of intestinal epithelial tight junction (TJ) proteins concurrent with increased intestinal permeability in the colon (Zheng et al., 2013). In addition, among patients with irritable bowel syndrome (IBS), the levels of proinflammatory cytokines including interleukin (IL)-6 and IL-8 were elevated as a result of adrenocorticotropic hormone (ACTH) stimulation (i.e., cortisol release; Dinan et al., 2006).

Various studies suggest that intestinal health has a significant impact on neurodegeneration despite the anatomical distance between the gut and the brain (Houser and Tansey, 2017; Zhang et al., 2018). Specifically, dysregulation of GBA cross-talk has been associated with metabolic syndrome (de Lartigue et al., 2011; Grasset et al., 2017) psychiatric disorders such as depression, anxiety, autism, as well as neurodegenerative diseases such as Parkinson's disease (PD), and Alzheimer's disease (AD; Sampson et al., 2016; Zhang et al., 2018). In reverse, these neurologic disorders are often times linked to altered intestinal health characterized by changes in the intestinal microbiota composition, which may disrupt the interplay between the gut and the brain (Esteve et al., 2011; O'Mahony et al., 2011). Many studies suggest that the intestinal microbiota contributes not only to modulating the communication and function of the GBA but also to modulating immune response through stimulation of cytokines and chemokines (Moloney et al., 2014). Similarly, the GBA interacts with intestinal cells and the ENS, as well as the CNS through neuroendocrine and metabolic pathways (Carabotti et al., 2015). Furthermore, ENS function can be influenced by the gut microbiota when they locally produce neurotransmitters, including γ-aminobutyric acid (GABA), amino-acid derivatives (e.g., serotonin, melatonin, and histamine) and fatty-acid derivatives (e.g., acetylcholine; Iyer et al., 2004) or biologically active catecholamines (i.e., dopamine and norepinephrine) in the gut lumen (Asano et al., 2012). The ENS is also targeted by bacterial metabolites such as short-chain fatty acids (SCFAs), including acetic acid, butyric acid, and propionic acid, which stimulate the sympathetic nervous system (Grider and Piland, 2007; Kimura et al., 2011), with downstream effects on learning and memory (Vecsey et al., 2007; Stefanko et al., 2009).

GBA IN NEURODEGENERATIVE DISEASES

Dysfunction of the GBA has been associated with psychiatric disorders including depression and anxiety, as well as neurodegenerative disorders including PD and AD (Sampson et al., 2016; Jiang et al., 2017). The following section will focus on recent findings of GBA involvement in PD and AD and their clinical features, as summarized in **Figure 2**.

Alzheimer's Disease

AD is a progressive neurodegenerative disease characterized by senile plaques consisting of misfolded β-amyloid (Aβ) fibrils and oligomers (Iadanza et al., 2018), as well as hyperphosphorylated tau protein in the various regions of the brain including cerebral cortex, locus coeruleus, and hippocampus (Llorens et al., 2017). Although such protein aggregation in the brain as well as non-neural tissues (i.e., the blood vessels, skin, subcutaneous tissue, and intestine) is a histological feature of AD, such deposition could simply be a consequence of various (epi) genetic alterations triggered by environmental exposures such as sociological, or medical nutritional stress (Lemche, 2018). In fact, synthetic Aß42 peptide aggregation has been reported in *Caenorhabditis elegans* aging models (Patel et al., 2017). Aβ fibrillar accumulation can coincide with clinical signs of cognitive dysfunction (Attems et al., 2005; Herzig et al., 2006; Pistollato et al., 2016), however, it is noteworthy that there is a high degree of variation in the extent of Aβ accumulation among patients with cognitive decline (Monsell et al., 2015). Although almost 100 years have passed since the very first diagnosis of AD, the exact pathogenesis of the disease is still largely unknown (Iadanza et al., 2018). Likewise, no effective therapy for modulation of AD is currently available.

One hypothesis for the involvement of the GBA in the pathophysiology of neurodegenerative diseases is microbial dysbiosis, which occurs as a result of antibiotic exposure (Vangay et al., 2015), dietary changes (Muegge et al., 2011), probiotics (Delzenne et al., 2011), or a variety of other disease conditions (Tilg and Moschen, 2014; Rosenfeld, 2015). Specifically, various studies have shown an association between gut microbiome dysbiosis and the aggregation of Aβ peptides in intestinal epithelial cells (Galloway et al., 2007, 2009) and the CNS (Nam et al., 2017; Lin et al., 2016) after high-fat diet feeding. Different components of the microbiota, such as bacteria, can excrete an immunogenic mixture of functional lipopolysaccharides (LPS), amyloid species, and exudates from their outer membranes into the local intestinal environment (Oli et al., 2012; Schwartz and Boles, 2013). Amyloid species and LPS are usually soluble, although they can polymerize and form insoluble fibrous protein aggregates,

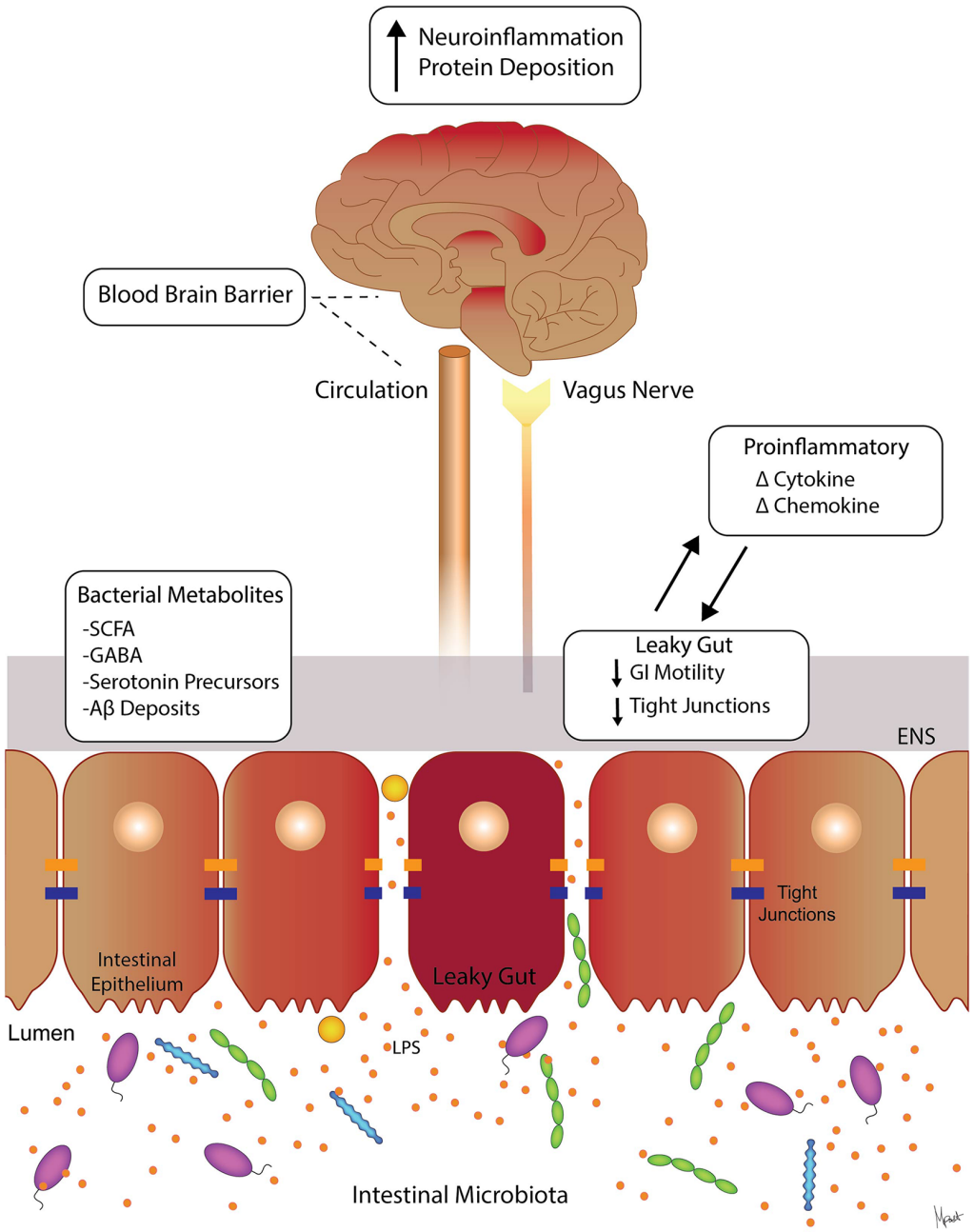


FIGURE 1 | Molecular pathways involved in gut-brain axis (GBA). Suggested signaling pathways and cross-talk between the intestinal microbiota, the intestinal barrier, immune modulators, and neural (brain, vagus, and ENS) systems. The intestinal microbiota can affect the levels of circulating local cytokines, cause "leaky gut" with increase GI permeability, and ultimately affect brain function (Moloney et al., 2014; Carabotti et al., 2015). Intestinal bacterial metabolites such as SCFA, GABA, and serotonin precursors are neuroactive and can affect ENS and the brain (Grider and Piland, 2007; Kimura et al., 2011). Abbreviations: ENS, enteric nervous system; SCFAs, short-chain fatty acids; GABA, γ-aminobutyric acid; LPS, lipopolysaccharide; GI, gastrointestinal.

leading to stimulation of oxidative stress and cross-seeding of further protein aggregation (Morales et al., 2013; Friedland, 2015; Iadanza et al., 2018). For example, the endotoxin from *Escherichia coli* was shown to enhance the Aβ fibril formation in an *in vitro* model (Asti and Gioglio, 2014). Also, co-incubation of Aβ peptide with LPS was shown to potentiate amyloids fibrillogenesis (Asti and Gioglio, 2014), and systemic injection of LPS in a transgenic AD mouse model resulted in severe amyloid deposition and tau pathology (Aziz et al., 2013; Mitew et al., 2013; Paula-Lima et al., 2013; Saulnier et al., 2013). Moreover, recent studies suggest that the structural overlap between bacterial amyloid proteins to human Aβ could induce *molecular mimicry*, an immune response against the self-antigens stimulated by a foreign

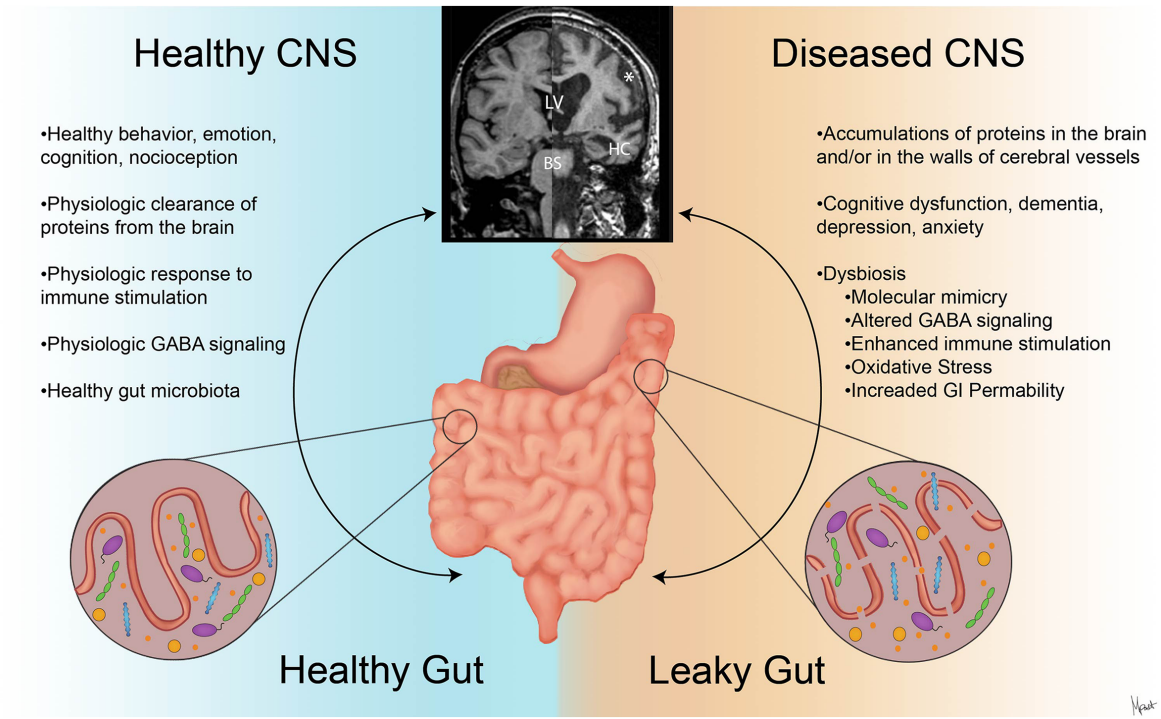


FIGURE 2 | The contrasts of clinical presentations on the GBA in health and neurodegenerative diseases. A stable intestinal microbiota is essential for healthy gut physiology and contributes to appropriate signaling along the GBA, promoting healthy physiologic status as well as central nervous system (CNS) status (*left*). Intestinal dysbiosis can negatively influence gut physiology and lead to abnormal GBA signaling (Friedland, 2015), resulting in accumulation of misfolded amyloid species (Galloway et al., 2007; de Lartigue et al., 2011). This can ultimately alter CNS functions and anatomy (Wu et al., 2017) as shown with magnetic resonance imaging (MRI) volumetric scans (*upper middle*). In the diseased CNS and gut state (*right*), cortical atrophy with the widening of the subarachnoid space (*), enlargement of the lateral ventricles (LV), hippocampus atrophy (HC), and brainstem (BS) volume reduction are seen with clinical and cognitive dysfunction (Johnson et al., 2012; Lee et al., 2015).

antigen sharing structural similarities, and ultimately causing greater inflammatory responses to cerebral Aβ due to altered gut microbiota (Delzenne et al., 2011; Muegge et al., 2011; Rosenfeld, 2015).

Another hypothesis for the pathogenesis of misfolded protein aggregation is the "Prion Concept." This hypothesis states that many neurodegenerative diseases exhibit accumulation of fibrillary, misfolded proteins similar to the propagation of prionopathies in the CNS (Goedert, 2015). Prionopathy also involves the GBA and the local immune system, where prions accumulate in dendritic cells in the Peyer's patches and other lymphoid follicles once entering the intestinal epithelium layer (Ano et al., 2009). Interestingly, earlier studies in a senescence-accelerated mouse model identified systemic senile amyloid proteins in Peyer's patches (Yoshioka et al., 1990). By interacting with dendritic cells, the misfolded protein might be transported to the ENS, and ultimately spread to the CNS compartment (Ano et al., 2009). A significant amount of functional amyloid protein was shown to be generated by certain bacteria, such as *E. coli*, *Bacillus subtilis*, *Salmonella enterica*, *Salmonella typhimurium*, and *Staphylococcus aureus*, and may contribute to the pathology of AD through the accumulation of misfolded Aβ oligomers and fibrils (Hufnagel et al., 2013; Schwartz and Boles, 2013). Some bacterial species, such as *Lactobacillus* spp. and *Bifidobacterium* spp. (both gram-positive bacteria) are known to possess the ability to metabolize glutamate, a well-known primary excitatory neurotransmitter, to produce GABA, a well-known primary inhibitory neurotransmitter (Paula-Lima et al., 2013). These observations suggest that alteration of the gut microbiota can compromise the endogenous production of GABA (Saulnier et al., 2013). In turn, alteration of GABA signaling in the brain has been linked to cognitive impairment, AD, anxiety, and depression (Aziz et al., 2013; Hornig, 2013; Mitew et al., 2013; Paula-Lima et al., 2013). Alternatively, gut bacteria can affect peripheral nerve functions through the production of neuromodulatory metabolites such as short-chain fatty acid (SCFAs; Kimura et al., 2011). SCFAs, i.e., acetic acid, butyric acid, and propionic acid, are produced by bacterial fermentation of dietary fiber in the colon (Kimura et al., 2011). SCFAs can stimulate the sympathetic nervous system to release serotonin, ultimately influencing the CNS cognitive processes such as learning and memory (Grider and Piland, 2007). Catabolism of SCFAs to ketone bodies may also provide an alternative source of ATP to the brain, which could be beneficial given that progressive glucose dysmetabolism has been reported in patients with AD (Sokoloff, 1973). Importantly, lower levels of SCFAs have also been shown to negatively affect immune responses,

epithelial cell growth, and possibly affect the function of both the central and peripheral nervous systems (Kimura et al., 2011; Bienenstock et al., 2015).

Parkinson's Disease

Patients with PD present with classic motor symptoms, such as asymmetric resting tremor, that are caused by progressive dopaminergic neuronal death in the substantia nigra pars compacta and loss of dopaminergic signaling (Houser and Tansey, 2017). The pathophysiology of neurodegeneration in PD has not been established. However, abundant evidence suggests that neuroinflammation and glial cell activation could play a significant role in PD etiopathogenesis (Rocha et al., 2015). Proinflammatory signaling molecules, including cytokines (i.e., IL-1β, IL-6, and TNF-α; Mogi et al., 1996) or enzymes [i.e., nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2); Prigione et al., 2009], and oxidative stress are considered major contributing factors to neurodegeneration and cell death in PD.

One of the leading hypotheses for the pathogenesis of PD is the abnormal accumulation of α-synuclein (αSYN; Wong and Krainc, 2017). This protein is present in various cell types in the body, and PD patients show increased expression of αSYN at presynaptic terminals of neurons and neurite projections (Wong and Krainc, 2017). This protein is highly soluble and regulates the presynaptic release of important neurotransmitters such as dopamine (Wong and Krainc, 2017). The αSYN protein is also expressed within the ENS and can be detected in intestinal submucosal neuronal structures from neurologically healthy individuals (Böttner et al., 2012; Shannon et al., 2012; Gold et al., 2013). However, through interactions with environmental factors and other proteins and small molecules (Hasegawa et al., 2002; Breydo et al., 2012), αSYN follows a β-sheet structure formation and loses its physiologic membrane-binding capacity, leading to the aggregation of misfolded proteins forming so-called Lewy neurites and Lewy bodies in dopaminergic neurons of substantia nigra and noradrenergic neurons of the locus coeruleus (Hasegawa et al., 2002). Aggregates of misfolded αSYN proteins decrease mitochondrial complex I activity, thus reducing the physiologic functions of mitochondria, which ultimately leads to oxidative stress in the neuron (Jenner, 2003; Prigione et al., 2009). Individuals with mutations in the αSYN gene *SNCA* or duplication of the wild-type *SNCA* allele are known to develop early-onset, rapidly-progressive PD (Klein and Westenberger, 2012). The spread of αSYN proteins from the ENS to the CNS by transsynaptic cell-to-cell transmission in both sympathetic and parasympathetic nervous systems (Danzer et al., 2012) is the foundation for the "Prion Concept" in PD pathophysiology (Brundin et al., 2016). Multiple studies have demonstrated the presence of αSYN aggregates in intestinal biopsies from clinically normal individuals who would develop PD later in their lives (Braak et al., 2006; Shannon et al., 2012; Hilton et al., 2014). This finding indicates that intestinal αSYN precedes sufficient CNS neurodegeneration to produce motor dysfunctions (Houser and Tansey, 2017). Various clinical GI signs or the characteristic PD ENS pathology often occur before brain functions are actually affected, with constipation being the most common GI complaint in PD (Sakakibara et al., 2003). This is likely due to an increased intestinal transit time both in the small and large intestines of PD patients (Sakakibara et al., 2003). In fact, it has been shown that constipation can be a pre-motor symptom of PD years before the patients present with the clinical signs consistent with CNS degeneration (Gao et al., 2011; Lesser, 2002). In addition, an increased intestinal permeability was shown in PD patients compared to healthy controls (Schwiertz et al., 2018). Other studies suggest that there is an increased risk of developing dementia (Chen et al., 2016) or PD (Lai et al., 2014) in patients with IBS.

Similar to the trend in AD research, the relationships between the intestinal microbiota and PD pathophysiology and their association with disturbed GI motility have been studied extensively and some of the reported differences include a decrease in fecal numbers of *Prevotella* spp. and *Clostridium* spp. in PD patients (Tan et al., 2014; Scheperjans et al., 2015). These intestinal bacteria are a major source of SCFAs, particularly butyrate, folate (vitamin B9), and thiamine (vitamin B1), which are critical for the long-term maintenance of the epithelial barrier function (Tan et al., 2014; Scheperjans et al., 2015). Interestingly, chronic exposure to these SCFAs has been associated with clinical improvement in patients with PD [i.e., decreased dopaminergic degeneration and disruption of blood-brain barrier (BBB)] and clinical symptoms (Luong and Nguyen, 2013; Scheperjans et al., 2015; Liu et al., 2017), possibly due to ketogenesis. Finally, there are a few anecdotal reports suggesting a role for the Tobacco Mosaic virus (TMV; Friedland, 2015) in the pathophysiology of PD, but these preliminary findings need to be consolidated by additional studies on the topic.

EXPERIMENTAL APPROACHES TO INVESTIGATING THE GBA

Both static and dynamic *in vitro* models have been utilized to advance the understanding of the role of the GBA in neurodegenerative diseases. In addition, novel primary intestinal stem cell (ISC) culture systems have been utilized to mimic both physiologic and pathophysiologic intestinal conditions *in vitro* contributing to defining gut-cross talk with local environment (Gonzalez et al., 2013; Sato and Clevers, 2013; Chandra et al., 2018). The benefits and disadvantages of two current *in vitro* models are summarized in **Figure 3**. Importantly, cognitive dysfunction is highly prevalent not only in AD patients but also in approximately one-third of patients with PD (herein referred to as "non-motor symptom" of PD; Chaudhuri et al., 2006). In the "*In Vitro* Models" section and "*In Vivo* Animal Models" section, our main focus will be on AD. However, findings from these *in vivo* models for investigating mechanisms of cognitive impairment would be relevant to PD as well. The similarities and differences of clinical and histological observations in humans, dogs, and rodents are further summarized in **Figure 4**.

In vitro Models

Static Systems

Development of translatable *in vitro* models is critical for elucidating disease pathophysiology and developing effective

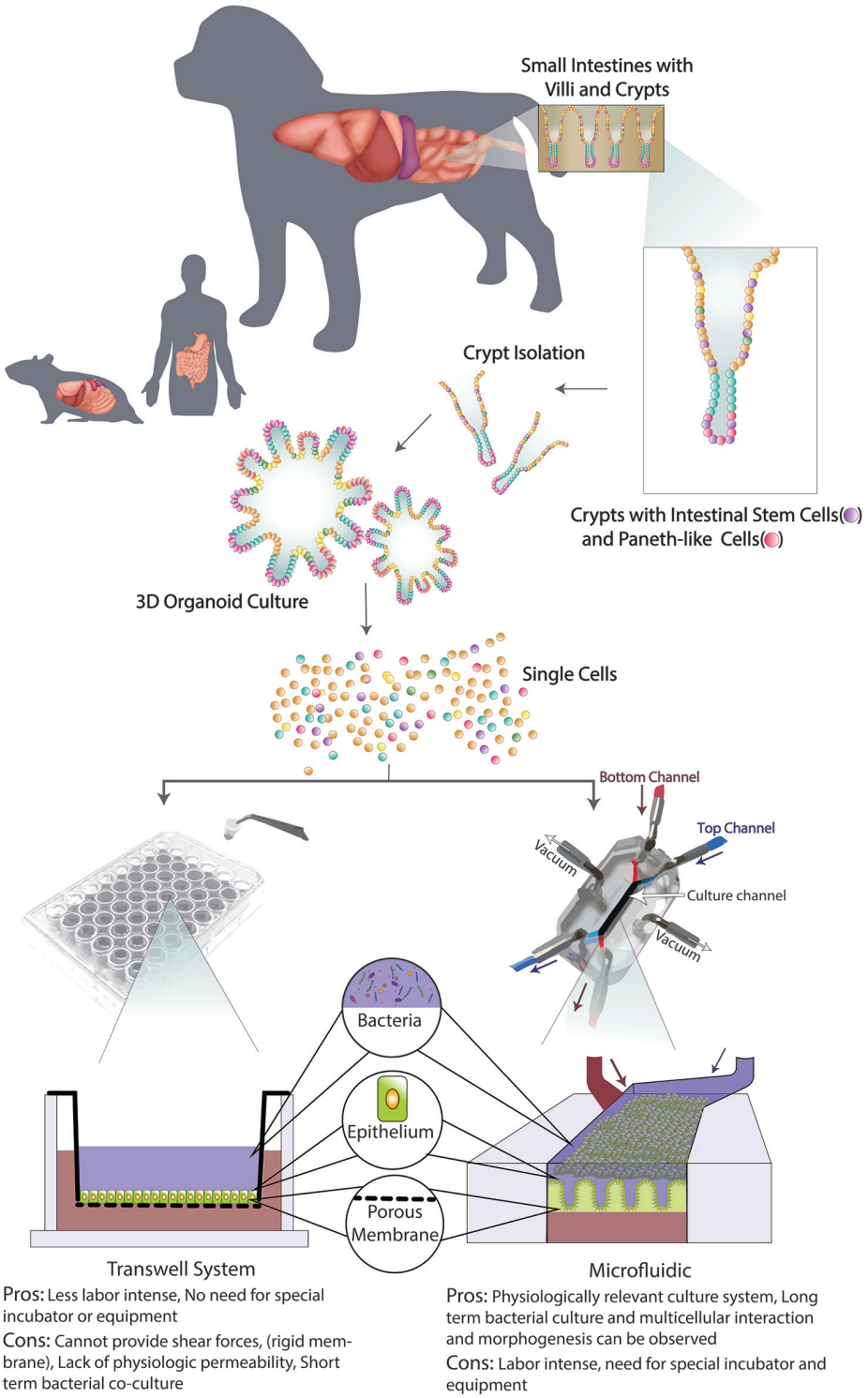


FIGURE 3 | Schematic of organoid 3D culture development and integration into Transwell and Microfluidic systems. First, the intestinal biopsy is obtained *via* endoscopically or surgically, then villi and crypts are isolated with intestinal stem cells (ISCs) and Paneth-like cells. When cultured in an extracellular matrix with appropriate microenvironment factors, long-term culture of 3D canine enteroids/colonoids (ENT/COL) is accomplished. Second, a single cell suspension from such 3D culture system will be integrated with Transwell (left) and microfluidic (right) systems. On the transwell insert, 3D ENT/COL is cultured on top of the porous membrane with culture medium in the apical (blue) side and then submerged in culture medium in the basolateral (red) wells. A schematic of a Gut-on-a-chip (GOAC) microdevice allows a closed system with microtubing. Arrows indicate the direction of the flow of culture medium in the apical (blue) and basolateral (red) microchannels.

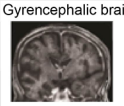
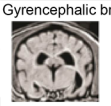
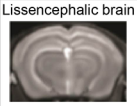
Feature	Human	Dog	Mouse
Onset	Age-associated disease	Age-associated disease	Transgenic mouse models with premature onset
Aβ pathology	Involves the grey matter, but also occurs in the walls of the parenchymal and leptomeningeal vessels	Involves the grey matter, but also occurs in the walls of the parenchymal and leptomeningeal vessels	Overexpression of non-modified Aβ and very low or absent expression of pyroglutamyl Aβ
Tau pathology	Within senile plaques and neurofibrillary tangles	Neurons and astrocytes in the cerebral cortex and hippocampus	Neurons in the hippocampus
Degenerative findings	Cortical atrophy and myelin degeneration	Cortical atrophy and myelin degeneration	Cortical degeneration in genetically modified mice
Anatomy	Gyrencephalic brain 	Gyrencephalic brain 	Lissencephalic brain 
Genetics	Heterogeneous populations of diverse genetic background	Heterogeneous populations of diverse genetic background	Genetic models with variable mutations, relatively homogenous populations

FIGURE 4 | Comparative features of neurodegenerative changes and anatomy in different mammalian species. Similarities and differences in the development of neurodegenerative diseases, such as Alzheimer's disease (AD), in human, dog, and mouse are listed.

therapies for neurodegenerative diseases. Currently, only about 7% of investigational compounds tested in phase III clinical trials progress on to the market in neurology (Kola and Landis, 2004). This is worse than the average of 11% success rate of drugs marketed for all disease categories (Kola and Landis, 2004; Adjei et al., 2009). The BBB, a unique interface between the peripheral vascular system and the CNS, is a unique feature of the GBA (Rubin et al., 1991). The critical roles of BBB include supplying nutrients to the CNS, allowing the removal of waste products (such as urea or potassium), and preventing blood-borne pathogens and toxic products from entering into the brain (Alcendor et al., 2012). The BBB consists of TJs between capillary endothelial cells without fenestrations, and therefore allows the BBB to maintain a low level of pinocytosis, which preserves the structural integrity of BBB (Alcendor et al., 2013).

Attempts to develop an *in vitro* model to recapitulate the complexity of the BBB have included brain microvascular endothelial cells and astrocytes in a Transwell culture (Ahmed et al., in press). Leveraging its similarity with conventional 2-dimensional (2D) culture systems and its relative simplicity, the Transwell BBB system has been widely used in a research setting (Rubin et al., 1991). However, the maintenance of TJ function requires the application of the shear forces which traditional static Transwell systems are not able to offer (Santaguida et al., 2006). These critical shortcomings, including lower transepithelial electrical resistance (TEER) and higher endothelial permeability than reported *in vivo*, typically lead to an overestimation of drug permeability across the BBB (Santaguida et al., 2006).

Additionally, current *in vitro* models does not replicate the close physiological cross-talk between pericytes and the capillary endothelium that comprise the neurovascular unit (Jamieson et al., 2017). Successful integration of intraluminal flow for the *in vitro* culture of astrocytes has resulted in more physiological endothelial cell polarity and strengthening of TJs (Cucullo et al., 2011).

Attempts were made to study the GBA using a Transwell culture system as well (Haller et al., 2000). However, this system included only a few components of the GBA, and it is important to note that Caco-2 cells, an immortalized cell line derived from human colorectal adenocarcinoma, are used to model the enteric epithelial cells in this system. Given these collective limitations, as well as the lack of integration of microbiome/ENS in the *in vitro* system, the results derived from these studies may not be readily indicative of translational efficacy.

Importantly, our group recently established canine primary enteroid and colonoid (ENT/COL) culture systems (Kingsbury et al., 2017; Mochel et al., 2017). This is a canine ISC culture system which closely mimics the physiologic structure and function of *in vivo* intestines from both healthy and diseased individuals (Chandra et al., 2018), and allows for the investigation of pathophysiology and treatment effects. Of note, canine cognitive dysfunction (CCD) is a well-studied clinical analog of AD (Kol et al., 2015; Schütt et al., 2015; Hoffman et al., 2018; Wang et al., 2018). Since dogs and humans share an anatomically and physiologically very similar GI tract and harbor a taxonomically and functionally largely overlapping microbiome, the dog provides unique features as a spontaneous model of disease (Coelho et al., 2018; Alessandri et al., 2019). Overall, this canine model may hold promise with its translational relevance for exploration of avenues of novel therapeutics for neurologic disease in the near future (Mochel et al., 2017).

Dynamic Model Systems Using Microfluidics

Only recently, a novel *ex vivo* model offering dynamic shear forces to mimic physiologic conditions called organ-on-a-chip (organ-OAC) has emerged (Kimura et al., 2008; Sung et al., 2011). This microfluidic device contains microtubing that allows for continuous flow of media and is comprised of multiple cell culture channels enabling co-culture of different cell types (Kim and Ingber, 2013; Kim et al., 2016a). Specifically, the Gut-on-a-chip (GOAC) models the complex human intestinal anatomy into a two-microchannel device where volumetric flow rate, mechanical deformations, and fluid shear stress can be adjusted to reproduce the *in vivo* physiology of

the gut (Kim and Ingber, 2013; Kim et al., 2016a). This biomimetic approach allows for the growth of the villous microarchitecture in Caco-2 cells, while proliferative cells from the intestinal crypt spontaneously migrate toward the villous tip similar to intestinal cells *in vivo* (Kim and Ingber, 2013). Also, differentiation of intestinal epithelial cell lines into four lineage-dependent subtypes (absorptive, mucus-secretory, enteroendocrine, and Paneth) is observed in this microfluidic system and presents a clear advantage over traditional 2D static culture systems (Kim and Ingber, 2013). When Caco-2 cells form 3-dimensional (3D) villi in the GOAC, they typically show enhanced epithelial barrier integrity, increased mucus production, and elevated drug-metabolizing P450 activity with augmented surface area and glucose reuptake, which are all relevant factors for modeling human intestinal physiology (Kim and Ingber, 2013). The 3D microarchitecture and increased mucus production are beneficial to grow live bacteria comprising the human gut microbiota using controlled flow and shear forces mimicking intestinal peristalsis (Kim et al., 2012; Kim et al., 2016a; Shin and Kim, 2018). Steady-state culture conditions inside the microchannels prevent the depletion of nutrients and the overgrowth of microbes (Kim et al., 2012). Critically, overgrowth of bacteria was seen only when manipulation to the shear stress was applied in the GOAC, which emulates the pathophysiological feature of the ileus (Kim et al., 2016a). By leveraging the innovative features of the GOAC, studies on the complex interactions between the host intestinal epithelium and the gut microbiome were also made possible (Kim et al., 2012; Kim et al., 2016b; Shin and Kim, 2018). For example, interactions between the intestinal epithelium, immune cell components, and intestinal bacteria (including non-pathogenic, pathogenic, or probiotic strains) were characterized by adding individual components one-at-a-time in a spatiotemporal manner (Kim et al., 2016a; Shin and Kim, 2018). This approach will enable researchers to evaluate the role of gut microbiome-brain axis in the development and progression of numerous intestinal diseases, such as inflammatory bowel disease (IBD) or colorectal cancer (CRC). Furthermore, the anoxic-oxic interface (AOI) of the oxygen gradient inside a modified GOAC was successfully recreated in a recent report, allowing for the co-culture of strict anaerobic intestinal bacteria and members of the fecal microbiome (Shin et al., 2019). This technology can be used to investigate the cross-talk between the gut microbiome and probiotics on intestinal health.

Recently, a BBB-OAC was established and showed physiological barrier functions (Wang et al., 2017), using ENS and enteroendocrine cells (EEC)-OAC combined to assess the GBA microenvironment (Ahmed et al., in press). Advancement in bioengineering techniques will allow incorporating multiple compartments in one *in vitro* system such as a GBA-OAC (Choe et al., 2017; Ahmed et al., in press; Lee and Sung, 2018). Despite the great promise of the Organ Chip technology, the transfer of cells from a macroscopic environment (e.g., well-plates) to a microfluidic system requires significant revision and optimization of cell culture protocols. Multiple factors differentiate microfluidic from macroscopic cell cultures. Microfluidic systems, for instance, harbor different culture channel surfaces and require fewer media volume as compared with macroscopic cultures (Sung and Shuler, 2009). Despite these limiting factors including the technology being labor-intensive, GOACs are a fast-growing model system that holds greater potential to investigate primary GI diseases. By extension, this system may be able to model GBA microenvironment and brain associations to better understand the role of enteric dysbiosis and neurodegenerative diseases.

In vivo Animal Models

While transgenic rodent models have been utilized to address targeted mechanistic questions relating to neuropathology and altered behavior (Hall and Roberson, 2012, 201), it is important to realize the inherent limitations of these *in vivo* models. Since mouse studies are used in the initial stages of drug discovery, the limitations in this animal model likely contribute to the poor success rate of AD drug discovery over the last 10 years (Kola and Landis, 2004; Adjei et al., 2009). One major limitation in studying the human GBA is a lack of an accurate animal model system that successfully replicates human ENS-microbiome interactions in health and disease. Investigation into the role of GBA with therapeutic interventions may require animal studies with tissues derived from animals that develop naturally occurring disease, including the dog. Since rodent diets differ substantially from that of humans, and diets are an important environmental factor shaping composition of the microbiome, comparing the effect of diet between species that harbor different microbial compositions (and likely functions) is difficult (Flint, 2011; Ravussin et al., 2012). For example, mice preferentially consume grains and cereals which contain relatively low ascorbic acid but have evolved their ability to synthesize this essential cofactor while humans have lost the ability to do so (Perlman, 2016) since they are omnivores. Different cytochrome P450 enzymes exist in mice compared to those in humans, thus each species has unique xenobiotic metabolism pathways that contribute to detoxification in each species (Martignoni et al., 2006; Anderson et al., 2009). These differing means of detoxification may be another reason why toxicology testing in mice has poor translatability to human toxicity (Olson et al., 2000).

Another factor explaining why rodent models do not mirror aspects of human pathophysiology is related to the limited tendency of some of these induced models to develop amyloidosis. As discussed before, AD is histologically characterized by the presence of Aβ aggregates in the walls of cerebral vessels (Attems, 2005; Herzig et al., 2006). Rodent models do not produce human sequence Aβ naturally (Shepherd et al., 2018), which limits their investigative utility as a translational model. Transgenic mouse models overexpressing mutant human amyloid precursor protein (APP) alone, or combined with transgenic presenilin 1 (PS1) and presenilin 2 (PS2), do have secondary Aβ plaque formation in the brain, histologically mimicking AD (Götz et al., 2008). However, these transgenic mouse models naturally have molecular and systemic resistance to Aβ pathology and therefore do not develop the extensive neuronal loss and clinical signs

seen in human AD patients (Martin et al., 2011). Lastly, there are fundamental differences in the anatomic folding of the cerebral cortex, with humans having a gyrencephalic brain and rodents having a lissencephalic brain (Sun and Hevner, 2014). A recent meta-analysis study demonstrates that various transgenic mouse models of AD show different characteristics compared to what have observed in the human AD (Hargis and Blalock, 2017). Specifically, the findings from spontaneous AD people were not consistent with those in transgenic AD mouse models, while human studies hold similar findings across different studies (Hargis and Blalock, 2017). The study also found that among the major transgenic AD mouse the findings were not similar to one another (Hargis and Blalock, 2017).

Accumulated data shows that the dog provides a superior model system to transgenic mouse models for investigating the influence of aging in the development and treatment of neurologic disease (Head, 2013). The dog is a more translationally relevant species because of the environmental, genomic, and intestinal physiologic features they share with humans (Cummings et al., 1996). Dogs are an ideal aging model since they show a parallel aging process to humans as evidenced by beagles between 5 and 9 years old showing cognitive dysfunction similar to humans between 40 and 60 years old (Patronek et al., 1997). In addition, brain vs. body size compares favorably between humans and dogs as compared with mice (Roth and Dicke, 2005), which is another advantage of using the dog as a disease animal model for neurologic diseases as canine brains undergo similar stress as humans (Roth and Dicke, 2005). Canine spontaneous disease models also offer additional predictive value for treatment efficacy before transitioning to human clinical trials (Kol et al., 2015; Schütt et al., 2016). Finally, dog genes have adapted to a starch-rich diet during domestication similar to humans, which suggests that studying such adaptations may improve our understanding of human evolution and disease (Axelsson et al., 2013).

Canine Models as Natural Models for Neurodegenerative Diseases: Similarities and Differences

Many human chronic disorders with a mixed genetic-environmental etiology (e.g., Diabetes Mellitus, IBD, CRC), including AD and PD, have well-studied clinical analogs in dogs (Kol et al., 2015; Schütt et al., 2016; Hoffman et al., 2018). Particularly relevant to AD, aged dogs with CCD spontaneously develop a progressive decline in cognitive function associated with advanced imaging abnormalities and histopathological features similar to AD (Davis and Head, 2014). For example, CCD dogs display progressive AD-like cortical atrophy (Rofina et al., 2006; Pugliese et al., 2010) in areas of the hippocampus that may be accompanied by ventricular enlargement (Su et al., 2005). Further, aged dog brains show other neuropathological and degenerative features similar to AD, including diffuse Aβ plaque deposition (Cummings et al., 1996; Borràs et al., 1999) with cortical amyloid angiopathy (CAA; Ishihara et al., 1991), neuronal loss in temporal regions first affected by AD (Colle et al., 2000), and dysfunction of neurotransmitter systems (Insua et al., 2012). Other neuropathological abnormalities shared between dogs and humans include hyperphosphorylated tau proteins in the brain (Yu et al., 2011; Böttner et al., 2012; Smolek et al., 2016) and increased plasma $A\beta_{1-42}$ levels, one of the biomarkers of AD (Schütt et al., 2015).

In addition to CCD as a model for AD, certain dog breeds are considered spontaneous models for PD. Canine multiple system degeneration (CMSD) is a fatal, inheritable movement disorder first described in Kerry Blue Terriers (deLahunta and Averill, 1976), then in Chinese Crested dogs (O'Brien et al., 2005), and these breeds are considered as natural models for PD. Dogs with CMSD are clinically normal until 3–6 months of age when they first develop the clinical signs of cerebellar ataxia (O'Brien et al., 2005). This progresses to akinesia (i.e., impairment in voluntary movement) and severe postural instability ultimately leading to euthanasia by 1–2 years of age due to a severe decline in quality of life (O'Brien et al., 2005). The histological hallmark of CMSD includes the loss of cerebellar Purkinje cells with degeneration of the olivary nucleus, substantia nigra, and caudate nucleus (deLahunta and Averill, 1976; Montgomery and Storts, 1983), areas of which are relevant to PD etiopathogenesis. Interestingly, the CMSD locus includes a segment that contains *PARK2*, the gene for parkin, and mutations in human *PARK2* is known to cause familial PD, which has clinical and pathological similarities to CMSD (O'Brien et al., 2005).

We acknowledge that there is no perfect animal model for investigating neurodegenerative disorders, and it should be recognized that the canine model also has limitations. For example, it has been recently shown that dogs lack aldehyde oxidases (AOXs) which catalyze the oxidation of aldehydes or N-heterocycles (Terao et al., 2006). This fact has physiological, pharmacological, and toxicological relevance since AOXs represent an important metabolic pathway that oxidizes numerous endogenous and exogenous substrates of biologic importance (Garattini et al., 2003). Also, humans and dogs have different CYP3A isoforms (i.e., canine CYP3A12 is equivalent to human CYP3A4) which impact species-specific differences in permeability, toxicity, and metabolism analysis between *in vitro* and *in vivo* systems (Zhang et al., 2001). A detailed assessment of drug transporters and metabolic enzymes expression *in vitro* is key to establish the predictive performance of these in systems recapitulating *in vivo* drug absorption and metabolism. Also, it is possible that differences in activity and substrate specificity/inhibitors and inducers are observed in the dog; therefore, utilizing *in vitro* systems from multiple different species would allow the supplementation of other *in vitro* systems that do not fully mimic human physiology on their own (Zhang et al., 2001).

CONCLUSION

Recent analyses suggest that one of the most expensive therapeutic areas having poor success rate in terms

of drug research and discovery (R&D) is neurology (Kola and Landis, 2004). One barrier to achieving lower attrition rates in neurology drug R&D is the lack of utilization of appropriate naturally occurring models of disease, such as CCD as a model for human AD. The dog is a particularly relevant species since it shares multiple epidemiologic features with humans, including similarities in diet and their intestinal microbiomes. Furthermore, CCD dogs can be used as a natural model for both AD, as well as PD, since clinical trials can be performed in dogs to assess the efficacy of novel treatments prior to human trials (i.e., reverse extrapolation). Importantly, since organoids are derived from individuals with different genotypes and environmental exposures, they are a highly relevant model system for *ex vivo* studies, and are of value in "precision medicine." Integration of organoid culture systems with GOAC technology will maintain patient-specific genetic and epigenetic disease characteristics influencing inter-patient drug screening during the early exploratory R&D phase. We predict that it will be possible to predict the outcome of novel therapeutics prior to human trials by combining data from GOAC models and clinical trials with dogs serving as a model for naturally occurring neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

YA and JM conceived the idea for the review. YA searched and reviewed the literature, drafted and revised the manuscript. AW further searched and reviewed the AD and PD literature and revised the manuscript. JM, DB, KA, AJ, AK, and HK reviewed and edited the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Adjei, A. A., Christian, M., and Ivy, P. (2009). Novel designs and end points for phase II clinical trials. *Clin. Cancer Res.* 15, 1866–1872. doi: 10.1158/1078-0432.ccr-08-2035

Ahmed, M., Puzan, M., and Koppes, D. A. (in press). Gut-brain-axis on a chip: a microfluidic model of the enteroendocrine-enteric nervous system interface:1. https://www.northeastern.edu/rise/presentations/gut-brain-axis-on-a-chip-a-microfluidic-model-of-the-enteroendocrine-enteric-nervous-system-interface/

Alcendor, D. J., Charest, A. M., Zhu, W. Q., Vigil, H. E., and Knobel, S. M. (2012). Infection and upregulation of proinflammatory cytokines in human brain vascular pericytes by human cytomegalovirus. *J. Neuroinflammation* 9:95. doi: 10.1186/1742-2094-9-95

Alcendor, D. J., Block, F. E. III., Cliffel, D. E., Daniels, J. S., Ellacott, K. L. J., Goodwin, C. R., et al. (2013). Neurovascular unit on a chip: implications for translational applications. *Stem Cell Res. Ther.* 4:S18. doi: 10.1186/scrt379

Alessandri, G., Milani, C., Mancabelli, L., Mangifesta, M., Lugli, G. A., Viappiani, A., et al. (2019). Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features. *Environ. Microbiol.* 21, 1331–1343. doi: 10.1111/1462-2920.14540

Ambrosini, Y., Borcherding, D., Kanthasamy, A., Kim, H. J., Jergens, A., Allenspach, K., et al. (2019). The role of the gut-brain axis in neurodegenerative diseases and relevance of the canine model: a review. *Preprints* 2019:2019010275. doi: 10.20944/preprints201901.0275.v1

Anderson, S., Luffer-Atlas, D., and Knadler, M. P. (2009). Predicting circulating human metabolites: how good are we? *Chem. Res. Toxicol.* 22, 243–256. doi: 10.1021/tx8004086

Ano, Y., Sakudo, A., Nakayama, H., and Onodera, T. (2009). Uptake and dynamics of infectious prion protein in the intestine. *Protein Pept. Lett.* 16, 247–255. doi: 10.2174/092986609787601642

Asano, Y., Hiramoto, T., Nishino, R., Aiba, Y., Kimura, T., Yoshihara, K., et al. (2012). Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303, G1288–G1295. doi: 10.1152/ajpgi.00341.2012

Asti, A., and Gioglio, L. (2014). Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? *J. Alzheimers Dis.* 39, 169–179. doi: 10.3233/jad-131394

Attems, J., Jellinger, K. A., and Lintner, F. (2005). Alzheimer's disease pathology influences severity and topographical distribution of cerebral amyloid angiopathy. *Acta Neuropathol.* 110, 222–231. doi: 10.1007/s00401-005-1064-y

Attems, J. (2005). Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathol.* 110, 345–359. doi: 10.1007/s00401-005-1074-9

Axelsson, E., Ratnakumar, A., Arendt, M.-L., Maqbool, K., Webster, M. T., Perloski, M., et al. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495, 360–364. doi: 10.1038/nature11837

Aziz, Q., Doré, J., Emmanuel, A., Guarner, F., and Quigley, E. M. M. (2013). Gut microbiota and gastrointestinal health: current concepts and future directions. *Neurogastroenterol. Motil.* 25, 4–15. doi: 10.1111/nmo.12046

Bienenstock, J., Kunze, W., and Forsythe, P. (2015). Microbiota and the gut-brain axis. *Nutr. Rev.* 73, 28–31. doi: 10.1093/nutrit/nuv019

Borràs, D., Ferrer, I., and Pumarola, M. (1999). Age-related changes in the brain of the dog. *Vet. Pathol.* 36, 202–211. doi: 10.1354/vp.36-3-202

Böttner, M., Zorenkov, D., Hellwig, I., Barrenschee, M., Harde, J., Fricke, T., et al. (2012). Expression pattern and localization of α-synuclein in the human enteric nervous system. *Neurobiol. Dis.* 48, 474–480. doi: 10.1016/j.nbd.2012.07.018

Braak, H., de Vos, R. A. I., Bohl, J., and Tredici, K. D. (2006). Gastric α-synuclein immunoreactive inclusions in meissner's and auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci. Lett.* 396, 67–72. doi: 10.1016/j.neulet.2005.11.012

Breydo, L., Wu, J. W., and Uversky, V. N. (2012). α-synuclein misfolding and Parkinson's disease. *Biochim. Biophys. Acta* 1822, 261–285. doi: 10.1016/j.bbadis.2011.10.002

Browning, K. N., and Travagli, R. A. (2014). Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. *Compr. Physiol.* 4, 1339–1368. doi: 10.1002/cphy.c130055

Brundin, P., Ma, J., and Kordower, J. H. (2016). How strong is the evidence that Parkinson's disease is a prion disorder? *Curr. Opin. Neurol.* 29, 459–466. doi: 10.1097/WCO.0000000000000349

Carabotti, M., Scirocco, A., Maselli, M. A., and Severi, C. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann. Gastroenterol.* 28, 203–209.

Chandra, L., Borcherding, D. C., Kingsbury, D., Atherly, T., Ambrosini, Y. M., Bourgois-Mochel, A., et al. (2018). Adult canine intestinal derived organoids: a novel *in vitro* system for translational research in comparative gastroenterology. *bioRxiv* [Preprint]. doi: 10.1101/466409

Chaudhuri, K. R., Healy, D. G., and Schapira, A. H. V. (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol.* 5, 235–245. doi: 10.1016/S1474-4422(06)70373-8

Chen, C.-H., Lin, C.-L., and Kao, C.-H. (2016). Irritable bowel syndrome is associated with an increased risk of dementia: a nationwide population-based study. *PLoS One* 11:e0144589. doi: 10.1371/journal.pone.0144589

Choe, A., Ha, S. K., Choi, I., Choi, N., and Sung, J. H. (2017). Microfluidic gut-liver chip for reproducing the first pass metabolism. *Biomed. Microdevices* 19:4. doi: 10.1007/s10544-016-0143-2

Coelho, L. P., Kultima, J. R., Costea, P. I., Fournier, C., Pan, Y., Czarnecki-Maulden, G., et al. (2018). Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome* 6:72. doi: 10.1186/s40168-018-0450-3

Colle, M.-A., Hauw, J.-J., Crespeau, F., Uchihara, T., Akiyama, H., Checler, F., et al. (2000). Vascular and parenchymal Aβ deposition in the aging dog: correlation with behavior. *Neurobiol. Aging* 21, 695–704. doi: 10.1016/S0197-4580(00)00113-5

Cucullo, L., Hossain, M., Puvenna, V., Marchi, N., and Janigro, D. (2011). The role of shear stress in blood-brain barrier endothelial physiology. *BMC Neurosci.* 12:40. doi: 10.1186/1471-2202-12-40

Cummings, B. J., Pike, C. J., Shankle, R., and Cotman, C. W. (1996). β-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. *Neurobiol. Aging* 17, 921–933. doi: 10.1016/s0197-4580(96)00170-4

Danzer, K. M., Kranich, L. R., Ruf, W. P., Cagsal-Getkin, O., Winslow, A. R., Zhu, L., et al. (2012). Exosomal cell-to-cell transmission of α synuclein oligomers. *Mol. Neurodegener.* 7:42. doi: 10.1186/1750-1326-7-42

Davis, P. R., and Head, E. (2014). Prevention approaches in a preclinical canine model of Alzheimer's disease: benefits and challenges. *Front. Pharmacol.* 5:47. doi: 10.3389/fphar.2014.00047

deLahunta, A., and Averill, D. R. Jr. (1976). Hereditary cerebellar cortical and extrapyramidal nuclear abiotrophy in kerry blue terriers. *J. Am. Vet. Med. Assoc.* 168, 1119–1124.

de Lartigue, G., de La Serre, C. B., and Raybould, H. E. (2011). Vagal afferent neurons in high fat diet-induced obesity; intestinal microflora, gut inflammation and cholecystokinin. *Physiol. Behav.* 105, 100–105. doi: 10.1016/j.physbeh.2011.02.040

Delzenne, N. M., Neyrinck, A. M., and Cani, P. D. (2011). Modulation of the gut microbiota by nutrients with prebiotic properties: consequences for host health in the context of obesity and metabolic syndrome. *Microb. Cell Fact.* 10:S10. doi: 10.1186/1475-2859-10-S1-S10

Dinan, T. G., Quigley, E. M. M., Ahmed, S. M. M., Scully, P., O'Brien, S., O'Mahony, L., et al. (2006). Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology* 130, 304–311. doi: 10.1053/j.gastro.2005.11.033

Esteve, E., Ricart, W., and Fernández-real, J.-M. (2011). Gut microbiota interactions with obesity, insulin resistance and type 2 diabetes: did gut microbiote co-evolve with insulin resistance? *Curr. Opin. Clin. Nutr. Metab. Care* 14, 483–490. doi: 10.1097/mco.0b013e328348c06d

Flint, H. J. (2011). Obesity and the gut microbiota. *J. Clin. Gastroenterol.* 45, S128–S132. doi: 10.1097/MCG.0b013e31821f44c4

Foster, J. A., Rinaman, L., and Cryan, J. F. (2017). Stress and the gut-brain axis: regulation by the microbiome. *Neurobiol. Stress* 7, 124–136. doi: 10.1016/j.ynstr.2017.03.001

Friedland, R. P. (2015). Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. *J. Alzheimers Dis.* 45, 349–362. doi: 10.3233/jad-142841

Galloway, S., Jian, L., Johnsen, R., Chew, S., and Mamo, J. C. L. (2007). β-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. *J. Nutr. Biochem.* 18, 279–284. doi: 10.1016/j.jnutbio.2006.07.003

Galloway, S., Takechi, R., Pallebage-Gamarallage, M. M. S., Dhaliwal, S. S., and Mamo, J. C. L. (2009). Amyloid-β colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* 8:46. doi: 10.1186/1476-511X-8-46

Gao, X., Chen, H., Schwarzschild, M. A., and Ascherio, A. (2011). A prospective study of bowel movement frequency and risk of Parkinson's disease. *Am. J. Epidemiol.* 174, 546–551. doi: 10.1093/aje/kwr119

Garattini, E., Mendel, R., Romão, M. J., Wright, R., and Terao, M. (2003). Mammalian molybdo-flavoenzymes, an expanding family of proteins: structure, genetics, regulation, function and pathophysiology. *Biochem. J.* 372, 15–32. doi: 10.1042/bj20030121

Goedert, M. (2015). Alzheimer's and Parkinson's diseases: the prion concept in relation to assembled Aβ, tau, and α-synuclein. *Science* 349:1255555. doi: 10.1126/science.1255555

Gold, A., Turkalp, Z. T., and Munoz, D. G. (2013). Enteric α-synuclein expression is increased in Parkinson's disease but not Alzheimer's disease. *Mov. Disord.* 28, 237–240. doi: 10.1002/mds.25298

Gonzalez, L. M., Williamson, I., Piedrahita, J. A., Blikslager, A. T., and Magness, S. T. (2013). Cell lineage identification and stem cell culture in a porcine model for the study of intestinal epithelial regeneration. *PLoS One* 8:e66465. doi: 10.1371/journal.pone.0066465

Götz, J., Ittner, L. M., Schonrock, N., and Cappai, R. (2008). An update on the toxicity of Aβ in Alzheimer's disease. *Neuropsychiatr. Dis. Treat.* 4, 1033–1042. doi: 10.2147/ndt.s3016

Grasset, E., Puel, A., Charpentier, J., Collet, X., Christensen, J. E., Tercé, F., et al. (2017). A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. *Cell Metab.* 25, 1075.e5–1090.e5. doi: 10.1016/j.cmet.2017.04.013

Grenham, S., Clarke, G., Cryan, J. F., and Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Front. Physiol.* 2:94. doi: 10.3389/fphys.2011.00094

Grider, J. R., and Piland, B. E. (2007). The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292, G429–G437. doi: 10.1152/ajpgi.00376.2006

Hall, A. M., and Roberson, E. D. (2012). Mouse models of Alzheimer's disease. *Brain Res. Bull.* 88, 3–12. doi: 10.1016/j.brainresbull.2011.11.017

Haller, D., Bode, C., Hammes, W. P., Pfeifer, A. M. A., Schiffrin, E. J., and Blum, S. (2000). Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. *Gut* 47, 79–87. doi: 10.1136/gut.47.1.79

Hargis, K. E., and Blalock, E. M. (2017). Transcriptional signatures of brain aging and Alzheimer's disease: what are our rodent models telling us? *Behav. Brain Res.* 322, 311–328. doi: 10.1016/j.bbr.2016.05.007

Hasegawa, M., Fujiwara, H., Nonaka, T., Wakabayashi, K., Takahashi, H., Lee, V. M.-Y., et al. (2002). Phosphorylated α-synuclein is ubiquitinated in α-synucleinopathy lesions. *J. Biol. Chem.* 277, 49071–49076. doi: 10.1074/jbc.M208046200

Head, E. (2013). A canine model of human aging and Alzheimer's disease. *Biochim. Biophys. Acta* 1832, 1384–1389. doi: 10.1016/j.bbadis.2013.03.016

Herzig, M. C., van Nostrand, W. E., and Jucker, M. (2006). Mechanism of cerebral β-amyloid angiopathy: murine and cellular models. *Brain Pathol.* 16, 40–54. doi: 10.1111/j.1750-3639.2006.tb00560.x

Hilton, D., Stephens, M., Kirk, L., Edwards, P., Potter, R., Zajicek, J., et al. (2014). Accumulation of α-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. *Acta Neuropathol.* 127, 235–241. doi: 10.1007/s00401-013-1214-6

Hoffman, J. M., Creevy, K. E., Franks, A., O'Neill, D. G., and Promislow, D. E. L. (2018). The companion dog as a model for human aging and mortality. *Aging Cell* 17:e12737. doi: 10.1111/acel.12737

Hornig, M. (2013). The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness. *Curr. Opin. Rheumatol.* 25, 488–795. doi: 10.1097/bor.0b013e32836208de

Houser, M. C., and Tansey, M. G. (2017). The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinsons Dis.* 3:3. doi: 10.1038/s41531-016-0002-0

Hufnagel, D. A., Tükel, Ç., and Chapman, M. R. (2013). Disease to dirt: the biology of microbial amyloids. *PLoS Pathog.* 9:e1003740. doi: 10.1371/journal.ppat.1003740

Iadanza, M. G., Jackson, M. P., Hewitt, E. W., Ranson, N. A., and Radford, S. E. (2018). A new era for understanding amyloid structures and disease. *Nat. Rev. Mol. Cell Biol.* 19, 755–773. doi: 10.1038/s41580-018-0060-8

Insua, D., Corredoira, A., González-Martínez, Á., Suárez, M.-L., Santamarina, G., Sarasa, M., et al. (2012). Expression of P75 NTR, a marker for basal forebrain cholinergic neurons, in young and aged dogs with or without cognitive dysfunction syndrome. *J. Alzheimers Dis.* 28, 291–296. doi: 10.3233/jad-2011-110905

Ishihara, T., Gondo, T., Takahashi, M., Uchino, F., Ikeda, S.-I., Allsop, D., et al. (1991). Immunohistochemical and immunoelectron microscopical characterization of cerebrovascular and senile plaque amyloid in aged dogs' brains. *Brain Res.* 548, 196–205. doi: 10.1016/0006-8993(91)91122-h

Iyer, L. M., Aravind, L., Coon, S. L., Klein, D. C., and Koonin, E. V. (2004). Evolution of cell-cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet.* 20, 292–299. doi: 10.1016/j.tig.2004.05.007

Jamieson, J. J., Searson, P. C., and Gerecht, S. (2017). Engineering the human blood-brain barrier *in vitro*. *J. Biol. Eng.* 11:37. doi: 10.1186/s13036-017-0076-1

Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Ann. Neurol.* 53, S26–S38. doi: 10.1002/ana.10483

Jiang, C., Li, G., Huang, P., Liu, Z., and Zhao, B. (2017). The gut microbiota and Alzheimer's disease. *J. Alzheimers Dis.* 58, 1–15. doi: 10.3233/JAD-161141

Johnson, K. A., Fox, N. C., Sperling, R. A., and Klunk, W. E. (2012). Brain imaging in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* 2:a006213. doi: 10.1101/cshperspect.a006213

Kihara, N., Fujimura, M., Yamamoto, I., Itoh, E., Inui, A., and Fujimiya, M. (2001). Effects of central and peripheral urocortin on fed and fasted gastroduodenal motor activity in conscious rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280, G406–G419. doi: 10.1152/ajpgi.2001.280.3.g406

Kim, H. J., Huh, D., Hamilton, G., and Ingber, D. E. (2012). Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* 12, 2165–2174. doi: 10.1039/c2lc40074j

Kim, H. J., and Ingber, D. E. (2013). Gut-on-a-chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integr. Biol.* 5, 1130–1140. doi: 10.1039/c3ib40126j

Kim, H. J., Li, H., Collins, J. J., and Ingber, D. E. (2016a). Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc. Natl. Acad. Sci. U S A* 113, E7–E15. doi: 10.1073/pnas.1522193112

Kim, H. J., Lee, J., Choi, J.-H., Bahinski, A., and Ingber, D. E. (2016b). Co-culture of living microbiome with microengineered human intestinal villi in a gut-on-a-chip microfluidic device. *J. Vis. Exp.* 114:e54344. doi: 10.3791/54344

Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., et al. (2011). Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc. Natl. Acad. Sci. U S A* 108, 8030–8035. doi: 10.1073/pnas.1016088108

Kimura, H., Yamamoto, T., Sakai, H., Sakai, Y., and Fujii, T. (2008). An integrated microfluidic system for long-term perfusion culture and on-line monitoring of intestinal tissue models. *Lab Chip* 8, 741–746. doi: 10.1039/b717091b

Kingsbury, D. D., Sun, L., Qi, Y., Fredericks, J., Wang, Q., M Wannemuehler, J., et al. (2017). Optimizing the development and characterization of canine small intestinal crypt organoids as a research model. *Gastroenterology* 152:S353. doi: 10.1016/s0016-5085(17)31441-5

Klein, C., and Westenberger, A. (2012). Genetics of Parkinson's disease. *Cold Spring Harb. Perspect. Med.* 2:a008888. doi: 10.1101/cshperspect.a008888

Kol, A., Arzi, B., Athanasiou, K. A., Farmer, D. L., Nolta, J. A., Rebhun, R. B., et al. (2015). Companion animals: translational scientist's new best friends. *Sci. Transl. Med.* 7:308ps21. doi: 10.1126/scitranslmed.aaa9116

Kola, I., and Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3, 711–716. doi: 10.1038/nrd1470

Lai, S.-W., Liao, K.-F., Lin, C.-L., and Sung, F.-C. (2014). Irritable bowel syndrome correlates with increased risk of Parkinson's disease in taiwan. *Eur. J. Epidemiol.* 29, 57–62. doi: 10.1007/s10654-014-9878-3

Lee, J. H., Ryan, J., Andreescu, C., Aizenstein, H., and Lim, H. K. (2015). Brainstem morphological changes in Alzheimer's disease. *Neuroreport* 26, 411–415. doi: 10.1097/wnr.0000000000000362

Lee, S. Y., and Sung, J. H. (2018). Gut-liver on a chip toward an *in vitro* model of hepatic steatosis. *Biotechnol. Bioeng.* 115, 2817–2827. doi: 10.1002/bit.26793

Lemche, E. (2018). Early life stress and epigenetics in late-Onset Alzheimer's dementia: a systematic review. *Curr. Genomics* 19, 522–602. doi: 10.2174/1389202919666171229145156

Lesser, G. T. (2002). Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology* 58:838. doi: 10.1212/wnl.58.5.838-a

Lin, B., Hasegawa, Y., Takane, K., Koibuchi, N., Cao, C., and Kim-Mitsuyama, S. (2016). High-fat-diet intake enhances cerebral amyloid angiopathy and cognitive impairment in a mouse model of Alzheimer's disease, independently of metabolic disorders. *J. Am. Heart Assoc.* 5:e003154. doi: 10.1161/JAHA.115.003154

Liu, J., Wang, F., Liu, S., Du, J., Hu, X., Xiong, J., et al. (2017). Sodium butyrate exerts protective effect against Parkinson's disease in mice via stimulation of glucagon like peptide-1. *J. Neurol. Sci.* 381, 176–181. doi: 10.1016/j.jns.2017.08.3235

Llorens, F., Thüne, K., Andrés-Benito, P., Tahir, W., Ansoleaga, B., Hernández-Ortega, K., et al. (2017). MicroRNA expression in the locus coeruleus, entorhinal cortex, and hippocampus at early and middle stages of braak neurofibrillary tangle pathology. *J. Mol. Neurosci.* 63, 206–215. doi: 10.1007/s12031-017-0971-4

Luong, K. V. Q., and Nguyen, L. T. H. (2013). The beneficial role of thiamine in parkinson disease. *CNS Neurosci. Ther.* 19, 461–468. doi: 10.1111/cns.12078

Martignoni, M., Groothuis, G. M. M., and de Kanter, R. (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin. Drug Metab. Toxicol.* 2, 875–894. doi: 10.1517/17425255.2.6.875

Martin, S. B., Dowling, A. L. S., and Head, E. (2011). Therapeutic interventions targeting β amyloid pathogenesis in an aging dog model. *Curr. Neuropharmacol.* 9, 651–661. doi: 10.2174/157015911798376217

Mitew, S., Kirkcaldie, M. T. K., Dickson, T. C., and Vickers, J. C. (2013). Altered synapses and gliotransmission in Alzheimer's disease and AD model mice. *Neurobiol. Aging* 34, 2341–2351. doi: 10.1016/j.neurobiolaging.2013.04.010

Mochel, J. P., Jergens, A. E., Kingsbury, D., Kim, H. J., Martín, M. G., and Allenspach, K. (2017). Intestinal stem cells to advance drug development, precision, and regenerative medicine: a paradigm shift in translational research. *AAPS J.* 20:17. doi: 10.1208/s12248-017-0178-1

Mogi, M., Harada, M., Narabayashi, H., Inagaki, H., Minami, M., and Nagatsu, T. (1996). Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile Parkinsonism and Parkinson's disease. *Neurosci. Lett.* 211, 13–16. doi: 10.1016/0304-3940(96)12706-3

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., and Cryan, J. F. (2014). The microbiome: stress, health and disease. *Mamm. Genome* 25, 49–74. doi: 10.1007/s00335-013-9488-5

Monsell, S. E., Kukull, W. A., Roher, A. E., Maarouf, C. L., Serrano, G., Beach, T. G., et al. (2015). Characterizing apolipoprotein E ε4 carriers and noncarriers with the clinical diagnosis of mild to moderate Alzheimer dementia and minimal β-amyloid peptide plaques. *JAMA Neurol.* 72, 1124–1131. doi: 10.1001/jamaneurol.2015.1721

Montgomery, D. L., and Storts, R. W. (1983). Hereditary striatonigral and cerebello-olivary degeneration of the kerry blue terrier. I. Gross and light microscopic central nervous system lesions. *Vet. Pathol.* 20, 143–159. doi: 10.1177/030098588302000202

Morales, R., Moreno-Gonzalez, I., and Soto, C. (2013). Cross-seeding of misfolded proteins: implications for etiology and pathogenesis of protein misfolding diseases. *PLoS Pathog.* 9:e1003537. doi: 10.1371/journal.ppat.1003537

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana, L., et al. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 332, 970–974. doi: 10.1126/science.1198719

Nam, K. N., Mounier, A., Wolfe, C. M., Fitz, N. F., Carter, A. Y., Castranio, E. L., et al. (2017). Effect of high fat diet on phenotype, brain transcriptome and lipidome in Alzheimer's model mice. *Sci. Rep.* 7:4307. doi: 10.1038/s41598-017-04412-2

O'Brien, D. P., Johnson, G. S., Schnabel, R. D., Khan, S., Coates, J. R., Johnson, G. C., et al. (2005). Genetic mapping of canine multiple system degeneration and ectodermal dysplasia loci. *J. Hered.* 96, 727–734. doi: 10.1093/jhered/esi086

O'Mahony, S. M., Hyland, N. P., Dinan, T. G., and Cryan, J. F. (2011). Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology* 214, 71–88. doi: 10.1007/s00213-010-2010-9

Oli, M. W., Otoo, H. N., Crowley, P. J., Heim, K. P., Nascimento, M. M., Ramsook, C. B., et al. (2012). Functional amyloid formation by streptococcus mutans. *Microbiology* 158, 2903–2916. doi: 10.1099/mic.0.060855-0

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., et al. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.* 32, 56–67. doi: 10.1006/rtph.2000.1399

Patel, A., Malinovska, L., Saha, S., Wang, J., Alberti, S., Krishnan, Y., et al. (2017). ATP as a biological hydrotrope. *Science* 356, 753–756. doi: 10.1126/science.aaf6846

Patronek, G. J., Waters, D. J., and Glickman, L. T. (1997). Comparative longevity of pet dogs and humans: implications for gerontology research. *J. Gerontol. A Biol. Sci. Med. Sci.* 52, B171–B178. doi: 10.1093/gerona/52a.3.b171

Paula-Lima, A. C., Brito-Moreira, J., and Ferreira, S. T. (2013). Deregulation of excitatory neurotransmission underlying synapse failure in Alzheimer's disease. *J. Neurochem.* 126, 191–202. doi: 10.1111/jnc.12304

Perlman, R. L. (2016). Mouse models of human disease. *Evol. Med. Public Health* 2016, 170–176. doi: 10.1093/emph/eow014

Pistollato, F., Cano, S. S., Elio, I., Vergara, M. M., Giampieri, F., and Battino, M. (2016). Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. *Nutr. Rev.* 74, 624–634. doi: 10.1093/nutrit/nuw023

Prigione, A., Isaias, I. U., Galbussera, A., Brighina, L., Begni, B., Andreoni, S., et al. (2009). Increased oxidative stress in lymphocytes from untreated Parkinson's disease patients. *Parkinsonism Relat. Disord.* 15, 327–328. doi: 10.1016/j.parkreldis.2008.05.013

Pugliese, M., Carrasco, J. L., Gomez-Anson, B., Andrade, C., Zamora, A., Rodríguez, M. J., et al. (2010). Magnetic resonance imaging of cerebral involutional changes in dogs as markers of aging: an innovative tool adapted from a human visual rating scale. *Vet. J.* 186, 166–171. doi: 10.1016/j.tvjl.2009.08.009

Ravussin, Y., Koren, O., Spor, A., LeDuc, C., Gutman, R., Stombaugh, J., et al. (2012). Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity Silver Spring* 20, 738–747. doi: 10.1038/oby.2011.111

Rhee, S. H., Pothoulakis, C., and Mayer, E. A. (2009). Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat. Rev. Gastroenterol. Hepatol.* 6, 306–314. doi: 10.1038/nrgastro.2009.35

Rocha, N. P., de Miranda, A. S., and Teixeira, A. L. (2015). Insights into neuroinflammation in Parkinson's disease: from biomarkers to anti-inflammatory based therapies. *Biomed Res. Int.* 2015:628192. doi: 10.1155/2015/628192

Rofina, J. E., van Ederen, A. M., Toussaint, M. J. M., Secrève, M., van der Spek, A., van der Meer, I., et al. (2006). Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease. *Brain Res.* 1069, 216–226. doi: 10.1016/j.brainres.2005.11.021

Rosenfeld, C. S. (2015). Microbiome disturbances and autism spectrum disorders. *Drug Metab. Dispos.* 43, 1557–1571. doi: 10.1124/dmd.115.063826

Roth, G., and Dicke, U. (2005). Evolution of the brain and intelligence. *Trends Cogn. Sci.* 9, 250–257. doi: 10.1016/j.tics.2005.03.005

Rubin, L. L., Hall, D. E., Porter, S., Barbu, K., Cannon, C., Horner, H. C., et al. (1991). A cell culture model of the blood-brain barrier. *J. Cell Biol.* 115, 1725–1735. doi: 10.1083/jcb.115.6.1725

Sakakibara, R., Odaka, T., Uchiyama, T., Asahina, M., Yamaguchi, K., Yamaguchi, T., et al. (2003). Colonic transit time and rectoanal videomanometry in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 74, 268–272. doi: 10.1136/jnnp.74.2.268

Sampson, T. R., Debelius, J. W., Thron, T., Janssen, S., Shastri, G. G., Ilhan, Z. E., et al. (2016). Gut Microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 167, 1469.e12–1480.e12. doi: 10.1016/j.cell.2016.11.018

Santaguida, S., Janigro, D., Hossain, M., Oby, E., Rapp, E., and Cucullo, L. (2006). Side by side comparison between dynamic versus static models of blood-brain barrier *in vitro*: a permeability study. *Brain Res.* 1109, 1–13. doi: 10.1016/j.brainres.2006.06.027

Sato, T., and Clevers, H. (2013). Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 340, 1190–1194. doi: 10.1126/science.1234852

Saulnier, D. M., Ringel, Y., Heyman, M. B., Foster, J. A., Bercik, P., Shulman, R. J., et al. (2013). The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes* 4, 17–27. doi: 10.4161/gmic.22973

Scheperjans, F., Aho, V., Pereira, P. A. B., Koskinen, K., Paulin, L., Pekkonen, E., et al. (2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov. Disord.* 30, 350–358. doi: 10.1002/mds.26069

Schütt, T., Helboe, L., Pedersen, L. Ø., Waldemar, G., Berendt, M., and Pedersen, J. T. (2016). Dogs with cognitive dysfunction as a spontaneous model for early Alzheimer's disease: a translational study of neuropathological and inflammatory markers. *J. Alzheimers Dis.* 52, 433–449. doi: 10.3233/JAD-151085

Schütt, T., Toft, N., and Berendt, M. (2015). Cognitive function, progression of age-related behavioral changes, biomarkers, and survival in dogs more than 8 years old. *J. Vet. Intern. Med.* 29, 1569–1577. doi: 10.1111/jvim.13633

Schwartz, K., and Boles, B. R. (2013). Microbial amyloids—functions and interactions within the host. *Curr. Opin. Microbiol.* 16, 93–99. doi: 10.1016/j.mib.2012.12.001

Schwiertz, A., Spiegel, J., Dillmann, U., Grundmann, D., Bürmann, J., Faßbender, K., et al. (2018). Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. *Parkinsonism Relat. Disord.* 50, 104–107. doi: 10.1016/j.parkreldis.2018.02.022

Shannon, K. M., Keshavarzian, A., Mutlu, E., Dodiya, H. B., Daian, D., Jaglin, J. A., et al. (2012). α-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov. Disord.* 27, 709–715. doi: 10.1002/mds.23838

Shepherd, A., Zhang, T. D., Zeleznikow-Johnston, A. M., Hannan, A. J., and Burrows, E. L. (2018). Transgenic mouse models as tools for understanding how increased cognitive and physical stimulation can improve cognition in Alzheimer's disease. *Brain Plast.* 4, 127–150. doi: 10.3233/bpl-180076

Shin, W., Wu, A., Massidda, M. W., Foster, C., Thomas, N., Lee, D.-W., et al. (2019). A robust longitudinal co-culture of obligate anaerobic gut microbiome with human intestinal epithelium in an anoxic-oxic interface-on-a-chip. *Front. Bioeng. Biotechnol.* 7:13. doi: 10.3389/fbioe.2019.00013

Shin, W., and Kim, H. J. (2018). Intestinal barrier dysfunction orchestrates the onset of inflammatory host-microbiome cross-talk in a human gut inflammation-on-a-chip. *Proc. Natl. Acad. Sci. U S A* 115, E10539–E10547. doi: 10.1073/pnas.1810819115

Smolek, T., Madari, A., Farbakova, J., Kandrac, O., Jadhav, S., Cente, M., et al. (2016). Tau hyperphosphorylation in synaptosomes and neuroinflammation are associated with canine cognitive impairment. *J. Comp. Neurol.* 524, 874–895. doi: 10.1002/cne.23877

Sokoloff, L. (1973). Metabolism of ketone bodies by the brain. *Annu. Rev. Med.* 24, 271–280. doi: 10.1146/annurev.me.24.020173.001415

Stefanko, D. P., Barrett, R. M., Ly, A. R., Reolon, G. K., and Wood, M. A. (2009). Modulation of long-term memory for object recognition via HDAC inhibition. *Proc. Natl. Acad. Sci. U S A* 106, 9447–9452. doi: 10.1073/pnas.0903964106

Sun, T., and Hevner, R. F. (2014). Growth and folding of the mammalian cerebral cortex: from molecules to malformations. *Nat. Rev. Neurosci.* 15, 217–232. doi: 10.1038/nrn3707

Su, M.-Y., Tapp, P. D., Vu, L., Chen, Y.-F., Chu, Y., Muggenburg, B., et al. (2005). A longitudinal study of brain morphometrics using serial magnetic resonance imaging analysis in a canine model of aging. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 29, 389–397. doi: 10.1016/j.pnpbp.2004.12.005

Sung, J. H., and Shuler, M. L. (2009). Prevention of air bubble formation in a microfluidic perfusion cell culture system using a microscale bubble trap. *Biomed. Microdevices* 11, 731–738. doi: 10.1007/s10544-009-9286-8

Sung, J. H., Yu, J., Luo, D., Shuler, M. L., and March, J. C. (2011). Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model. *Lab Chip* 11, 389–392. doi: 10.1039/c0lc00273a

Tan, A. H., Mahadeva, S., Thalha, A. M., Gibson, P. R., Kiew, C. K., Yeat, C. M., et al. (2014). Small intestinal bacterial overgrowth in Parkinson's

disease. *Parkinsonism Relat. Disord.* 20, 535–540. doi: 10.1016/j.parkreldis.2014.02.019

Terao, M., Kurosaki, M., Barzago, M. M., Varasano, E., Boldetti, A., Bastone, A., et al. (2006). Avian and canine aldehyde oxidases. Novel insights into the biology and evolution of molybdo-flavoenzymes. *J. Biol. Chem.* 281, 19748–19761. doi: 10.1074/jbc.m600850200

Tilg, H., and Moschen, A. R. (2014). Microbiota and diabetes: an evolving relationship. *Gut* 63, 1513–1521. doi: 10.1136/gutjnl-2014-306928

Tsigos, C., and Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J. Psychosom. Res.* 53, 865–871. doi: 10.1016/S0022-3999(02)00429-4

Vangay, P., Ward, T., Gerber, J. S., and Knights, D. (2015). Antibiotics, pediatric dysbiosis, and disease. *Cell Host Microbe* 17, 553–564. doi: 10.1016/j.chom.2015.04.006

Vecsey, C. G., Hawk, J. D., Lattal, K. M., Stein, J. M., Fabian, S. A., Attner, M. A., et al. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity *via* CREB: CBP-dependent transcriptional activation. *J. Neurosci.* 27, 6128–6140. doi: 10.1523/jneurosci.0296-07.2007

Wang, Y. I., Abaci, H. E., and Shuler, M. L. (2017). Microfluidic blood-brain barrier model provides *in vivo*-like barrier properties for drug permeability screening. *Biotechnol. Bioeng.* 114, 184–194. doi: 10.1002/bit.26045

Wong, Y. C., and Krainc, D. (2017). α-synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. *Nat. Med.* 23, 1–13. doi: 10.1038/nm.4269

Wang, J., Wang, T., Sun, Y., Feng, Y., Kisseberth, W. C., Henry, C. J., et al. (2018). Proliferative and invasive colorectal tumors in pet dogs provide unique insights into human colorectal cancer. *Cancers* 10:E330. doi: 10.3390/cancers10090330

Wu, S.-C., Cao, Z.-S., Chang, K.-M., and Juang, J.-L. (2017). Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in *Drosophila*. *Nat. Commun.* 8:24. doi: 10.1038/s41467-017-00040-6

Yoshioka, H., Takeda, T., Higuchi, K., Ohshio, G., Miyake, T., Sugiyama, T., et al. (1990). Immunohistochemical examination of Peyer's patches in senescence-accelerated mice. *Autoimmunity* 8, 25–35. doi: 10.3109/08916939008998429

Yu, C.-H., Song, G.-S., Yhee, J.-Y., Kim, J.-H., Im, K.-S., Nho, W.-G., et al. (2011). Histopathological and immunohistochemical comparison of the brain of human patients with Alzheimer's disease and the brain of aged dogs with cognitive dysfunction. *J. Comp. Pathol.* 145, 45–58. doi: 10.1016/j.jcpa.2010.11.004

Zhang, L., Fitzloff, J. F., Engel, L. C., and Cook, C. S. (2001). Species difference in stereoselective involvement of CYP3A in the mono-N-dealkylation of disopyramide. *Xenobiotica* 31, 73–83. doi: 10.1080/00498250110037488

Zhang, T., Han, Y., Wang, J., Hou, D., Deng, H., Deng, Y. L., et al. (2018). Comparative epidemiological investigation of Alzheimer's disease and colorectal cancer: the possible role of gastrointestinal conditions in the pathogenesis of AD. *Front. Aging Neurosci.* 10:176. doi: 10.3389/fnagi.2018.00176

Zheng, G., Wu, S.-P., Hu, Y., Smith, D. E., Wiley, J. W., and Hong, S. (2013). Corticosterone mediates stress-related increased intestinal permeability in a region-specific manner. *Neurogastroenterol. Motil.* 25, e127–e139. doi: 10.1111/nmo.12066

Conflict of Interest Statement: JM, AJ, KA, and HK are founders of a company, 3D Health Solutions. JM, AJ, and KA are founders of a company, LEAH (Life Engine Animal Health, Inc.) located in Rochester, MN, USA. AK has an equity interest in PK Biosciences Corporation located in Ames, IA, USA. The terms of this arrangement have been reviewed and approved by Iowa State University in accordance with its conflict of interest policies.

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White Matter Microstructural Damage as an Early Sign of Subjective Cognitive Decline

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Background and Objective: Subjective cognitive decline (SCD) is considered a preclinical state of Alzheimer's disease (AD) and may represent a more advanced preclinical status than amnestic mild cognitive impairment (aMCI). Our aim was to explore changes in the white matter (WM) microstructure and their correlation with cognitive function in these AD-spectrum patients.

Methods: Diffusion tensor images from 43 individuals with normal cognition (NC), 38 SCD patients, and 36 aMCI patients were compared using an atlas-based segmentation strategy. The correlation between diffusion parameters and cognitive function was further analyzed.

Results: The anatomical pattern of WM impairment was generally similar between SCD and aMCI patients. However, aMCI patients showed significantly lower fractional anisotropy (i.e., corpus callosum forceps major and forceps minor) and increased mean diffusivity [i.e., bilateral anterior thalamic radiation (ATR), left corticospinal tract (CST), forceps minor, left cingulum (cingulate gyrus), left cingulum hippocampus, and left inferior fronto-occipital fasciculus (IFO)] in some tracts than did SCD subjects, indicating a disruption in WM microstructural integrity in the aMCI. Individuals with microstructural disruption in forceps minor, left cingulum (cingulate gyrus), and left cingulum hippocampus tracts performed worse in general cognition and memory function tests, as indicated by line regression analysis.

Conclusion: SCD individuals had extensive WM microstructural damage in a pattern similar to that seen in aMCI, although presenting a cognitive performance comparable with that of cognitively healthy individuals. Our results suggest that WM integrity might precede objectively measurable memory decline and may be a potential early biomarker for AD.

Keywords: Alzheimer's disease, subjective cognitive decline, amnestic mild cognitive impairment, diffusion tensor imaging, white matter pathway

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment and the loss of daily living abilities, and its pathological processes are thought to occur decades before clinical symptoms were detectable. Amnestic mild cognitive impairment (aMCI), a transitional zone between normal ageing and clinically probable AD, has a dementia conversion rate as high as 10–15% per year (Petersen, 2004). Threatened by the lack of curative treatments for AD and aMCI, advancing the time window for intervention would offer an alternative opportunity to slow down or reverse the deterioration.

Subjective cognitive decline (SCD) refers to the subjective experience of cognitive decline in memory and/or other cognitive functions, without any objective evidence of cognitive impairment (Jessen et al., 2014). Given that elderly people with AD usually experience SCD prior to the onset of dementia, SCD has been regarded as the earliest stage in the continuous progression to AD (Reisberg et al., 2008; Hafkemeijer et al., 2013). Subjective decline in memory vs. in other cognitive domains could result in a higher risk of conversion to subsequent aMCI or AD (Rönnlund et al., 2015; Buckley et al., 2016). A recent review has shown that compared with age-matched controls, individuals with SCD suffered a 4.5-fold higher risk of progressing to subsequent MCI, and the risk of eventual progression to AD increased by 6.5-fold (Lin et al., 2019). Another meta-analysis has suggested that approximately 25% of elderly people with SCD will develop MCI due to AD in the next 4 years (Mitchell et al., 2014). Because, SCD may be the first symptom in the pathological cascade of AD, it is of great value to identify AD-related pathological markers in the SCD population.

Previous studies using cerebrospinal fluid (CSF), structural or functional magnetic resonance imaging (MRI), fluorodeoxyglucose positron emission tomography (FDG-PET), and Pittsburgh compound B-positron emission tomography (PiB-PET) have provided substantial evidence that SCD is linked to underlying AD pathology (Sun et al., 2015; Cheng et al., 2017). For example, decreased amyloid beta protein 42 (Aβ-42) and elevated tau level in CSF were found in patients with SCD (Visser et al., 2009). SCD patients also tend to present AD/aMCI-like gray matter atrophy, decreased metabolism, and increased amyloid load, especially in the hippocampus (Vannini et al., 2017; Zhao et al., 2019).

Although less studied, white matter (WM) degeneration has also been demonstrated as an important feature in the spectrum of AD, as identified by histological examination and neuroimaging studies (Caso et al., 2016). A meta-analysis of diffusion tensor imaging (DTI) research revealed that WM microstructural alterations in aMCI and AD were widespread throughout the brain (Sexton et al., 2011), particularly in direct connections from the ventromedial frontal cortex to the medial temporal lobe (MTL) and from the precuneus to the MTL by the way of retrosplenial cortex (Salat et al., 2010). To a certain extent, these regional tissue changes resembled the anatomic connectivity of MTL structures, which have almost equal effects on memory processes, whereas the executive function alterations are related to the frontal-temporal (especially MTL) WM microstructural damage (Sui and Rajapakse, 2018). Cingulum bundle and fornix are the core WM tracts connecting directly to the MTL. The cingulum bundle is a prominent tract that interconnects the frontal, parietal, and medial temporal sites while also connecting the subcortical nuclei to the cingulate gyrus (Bubb et al., 2018). It is mainly responsible for communications between components of the limbic system, which is vital for normal cognition (Bürgel et al., 2006). Impaired integrity of the cingulum microstructure was detected in aMCI and AD, which correlated with memory, attention/executive function, language, and visuo-spatial function impairment (Choo et al., 2010; Wang et al., 2012; Kantarci et al., 2017). Fornix projects from the hippocampus to other brain regions and is also responsible for episodic memory loss in older adults with AD and aMCI (Copenhaver et al., 2006). Actually, the pattern of WM alterations seen in DTI was also anatomically concordant with that of gray matter atrophy, initially starting from the limbic bundles (i.e., MTL) and gradually progressing to the temporal lobe, parietal lobe, and frontal lobe WM tracts (Nowrangi et al., 2013; Konukoglu et al., 2016) as the clinical AD disease progresses. Moreover, this pattern of diffusion abnormalities is associated with the neurofibrillary tangle pathology stage, as well as clinical disease severity (Kantarci et al., 2017). Regarding the association between SCD and preclinical AD dementia, we speculated that WM microstructural changes may occur as early as SCD, yet the characteristics remain to be further investigated.

DTI is an advanced MRI quantitative technique for studying the microstructure of WM by utilizing the diffusion motion characteristics of water molecule interactions in tissues. It can sensitively detect the destruction of the WM microstructure such as demyelination, axonal injury, edema, or necrosis (Shaikh et al., 2018). Fractional anisotropy (FA) and mean diffusivity (MD) are two commonly employed diffusion parameters in the analysis of DTI data. FA reflects the directional variation of water molecule diffusion measured in the different directions (Soares et al., 2013; Shaikh et al., 2018), whereas MD represents the average diffusivity (Soares et al., 2013; Shaikh et al., 2018). The FA decrease and MD increase are parallel to the loss of myelin and axonal membranes that restrict random motion of water molecules along the WM tracts. Together, alterations in FA and MD indicate disruption in WM microstructural integrity. AD subjects had lower FA and higher MD within the temporal, parietal, and prefrontal lobes than had cognitively healthy aging adults in a large body of studies (Sexton et al., 2011; Acosta-Cabronero and Nestor, 2014). Therefore, we expect a similar pattern to be found in aMCI and SCD. The purpose of this study was to explore the differences in WM integrity among cognitively normal elderly, aMCI, and SCD and to analyze the correlation between WM integrity and cognitive function.

MATERIALS AND METHODS

Participants

A total of 117 right-handed Chinese Han subjects (43 normal controls and 38 SCD and 36 aMCI patients) were enrolled

in this study at the Department of Neurology of the Nanjing Drum Tower Hospital of Nanjing University Medical School from June 2016 to May 2019. This study was approved by the ethics committees of the Nanjing Drum Tower Hospital of Nanjing University Medical School (clinical trial government identifier: NCT01364246), and written informed consent was obtained from each subject prior to participation. All subjects underwent a comprehensive neuropsychological test and 3.0-T whole brain MRI scanning, a routine blood test, and a general medical examination by an experienced neurologist. The cognitive functions of all the subjects were evaluated by an experienced neuropsychologist using the Chinese version of the Mini-Mental State Examination (MMSE) and the Beijing version of Montreal Cognitive Assessment (MoCA; Lu et al., 2011) as general cognitive function screening; the auditory verbal learning test Huashan version (AVLT) as an episodic memory function assessment (Zhao et al., 2012); and the Clinical Dementia Rating (CDR) scale, activities of daily living (ADL) assessment, Hamilton Depression Rating Scale (HAMD), and Hamilton Anxiety Rating Scale (HAMA). The inclusion criteria for normal control group were as follows: (1) no reported cognition complaints; (2) normal MMSE, MoCA, and AVLT scores adjusted for age and education; and (3) CDR score = 0 and ADL score = 8. Patients with SCD were diagnosed based on the research criteria for pre-MCI SCD (Jessen et al., 2014): (1) self-reported experience of continuous decline in memory (within the last 5 years); (2) MMSE, MoCA, and AVLT test scores all within the normal range after adjusting for age and years of education; and (3) CDR score = 0 and ADL score = 8. The Petersen criteria were applied in the diagnosis of aMCI (Petersen, 2004): (1) subjective memory complaint confirmed by an informant; (2) objective memory impairment detected by the MoCA or AVLT (at least 1.5 standard deviations below normative values for age and/or education); (3) preserved general cognitive function (MMSE $\geq$ 24); (4) CDR score = 0.5; (5) ADL score = 8; and (6) failure to meet the criteria for dementia according to National Institute on Aging and the Alzheimer's Association (NIA-AA) criteria. Exclusion criteria for all the subjects were as follows: (1) age less than 50 years old; (2) a history of stroke; (3) central nervous system diseases that could cause cognitive decline, including vascular dementia, Parkinson's disease, epilepsy, central nervous system infection, subarachnoid hemorrhage, or multiple sclerosis; (4) the presence of WM lesions equal to or higher than Fazekas grade II (Wahlund et al., 2001); (5) severe depression (HAMD $\geq$ 17), anxiety (HAMA $\geq$ 21), schizophrenia or other mental illness; (6) severe systemic diseases such as heart failure and kidney dysfunction; (7) history of drugs or alcohol abuse; (8) intolerance of MRI examination or inability to complete neuropsychological testing; and (9) other diseases that may affect cognition.

Image Acquisition

All MRI scans were acquired using 3.0-T scanner [Achieva 3.0 T TX (eight-channel head coil) or 3.0 T Ingenia (32-channel head coil), Philips, Eindhoven, Netherlands] at Nanjing Drum Tower Hospital. Subjects were placed in the supine position. Sagittal T_1-weighted MR images were performed by a three-dimensional turbo fast echo acquisition with the following parameters: repetition time (TR) = 9.8 ms, echo time (TE) = 4.6 ms, inversion time (TI) = 900 ms, flip angle = 8°, field of view (FOV) = 256 × 256 mm, matrix = 256 × 256, number of slices = 192, and slice thickness = 1 mm. DTI data were obtained using an echo planar imaging (EPI) sequence with the following parameters: in 32 non-collinear directions diffusion encoding (b = 1,000 s/mm^2 for each direction) and one image with no diffusion weighting (b = 0 s/mm^2), TR = 9,154 ms, TE = 55 ms, flip angle = 90°, matrix size = 112 × 112, FOV = 224 × 224 mm, and slice thickness = 2.5 mm. The total scan lasted 13 min. Additionally, axial T_2-weighted, diffusion-weighted imaging (DWI) sequence, and fluid-attenuated inversion recovery (FLAIR) sequence were collected to detect acute or subacute infarctions and visible WM damage.

Diffusion Tensor Imaging Processing

The atlas-based segmentation strategy was employed to investigate diffusion abnormalities in this study. The processing of DTI data was carried out with PANDA software, following the default pipeline setting[1] (Cui et al., 2013). PANDA is a toolbox to perform analyses and calculations of brain diffusion images, which integrates FSL[2], Diffusion Tool kit[3], and MRIcron[4]. The major steps include the following: (1) converting original data (DICOM) to the NIFIT format; (2) removing non-brain tissue; (3) correcting eddy current and head motion; (4) adjusting the diffusion gradient direction; and (5) calculating the diffusion tensor metrics, including FA, MD, radial diffusivity, and axial diffusivity. Finally, the FA images in the native space were nonlinearly registered to the FA standard template in the Montreal Neurological Institute (MNI) space using FSL's FNIRT command. To observe changes in the major WM tracts, all DTI metrics were registered to the JHU WM Tractography Atlas (Hua et al., 2008). Under the present research, only FA and MD diffusion tensor metrics were discussed. The WM fiber pathways of interest were as follows (**Figure 1**): anterior thalamic radiation (ATR); CST; cingulum (cingulate gyrus; CgC); cingulum (hippocampus; CgH); corpus callosum (forceps major); corpus callosum (forceps minor); inferior fronto-occipital fasciculus (IFO); inferior longitudinal fasciculus (ILF); superior longitudinal fasciculus (SLF); uncinate fasciculus (UF); and superior longitudinal fasciculus-temporal part (tSLF). All of the tracts were evaluated in both hemispheres, except for the corpus callosum (forceps major) and corpus callosum (forceps minor).

[1] http://www.nitrc.org/projects/panda/
[2] https://fsl.fmrib.ox.ac.uk/fsl/fslwiki
[3] http://www.trackvis.org/dtk/
[4] https://www.nitrc.org/projects/mricron

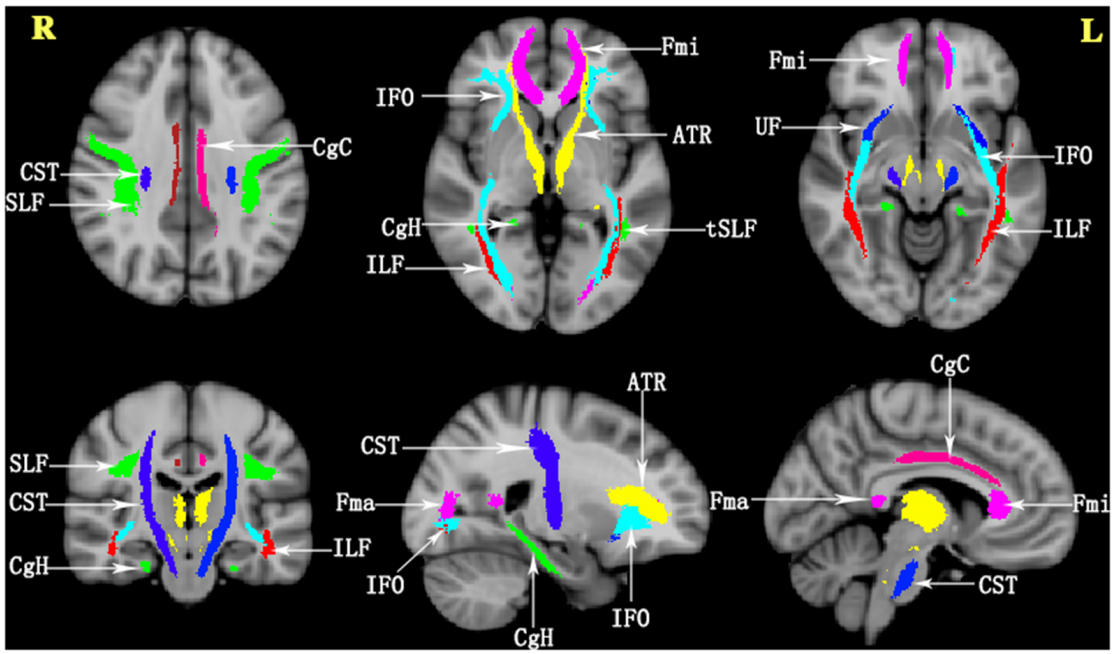

FIGURE 1 | The details of the tracts in the JHU White Matter (WM) Tractography Atlas in the present study. ATR, anterior thalamic radiation; CST, corticospinal tract; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); Fma, forceps major; Fmi, forceps minor; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus; tSLF, superior longitudinal fasciculus-temporal part; L, left; R, right.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Science (SPSS, v20.0)[5]. The categorization of demographic variables was expressed in terms of frequency and percentage (%), and differences were assessed using the chi-square test. Continuous demographic variables are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the differences in the studied variables (i.e., the results of ADL, HAMA, HAMD, and demographic information) across groups. If the ADL, HAMA, HAMD, and demographic information had significant difference across three groups, *Post hoc* analyses were performed to further explore the details of these group differences using least significant difference (LSD) pairwise comparisons. Furthermore, analysis of covariance (ANCOVA) was used to analyze the results of the cognitive evaluations (MMSE, MoCA, and AVLT) and the diffusion metrics of FA and MD values difference in 20 WM tracts of interest among three groups, with age, gender, and years of education as covariates. Bonferroni correction was conducted to adjust the false-positive rate ($P < 0.05/20$), and significant results further underwent pairwise comparison. To investigate the relationship between the cognitive test scores (MMSE, MoCA, AVLT-immediate recall, AVLT-delayed recall, and AVLT-recognition) and the diffusion parameters of FA and MD, the Pearson correlation analysis was initially performed. The tracts with significance were further included in the stepwise multiple linear regression model, with cognitive scores as dependent variables; FA and MD as independent variables; and age, gender, and years of education as covariates. $P < 0.05$ was considered statistically significant.

[5]http://www-01.ibm.com/software/analytics/spss

RESULTS

Demographic and Neuropsychological Characteristics

The demographic information and neuropsychological performance of the three groups are shown in **Table 1**. There were no significant differences in years of education, gender, vascular risk factors, family history of dementia, and HAMD and HAMA scores among the three groups. However, the NC group was younger overall than the aMCI and SCD groups (it should be noted that the age effect was removed in all subsequent DTI analyses). The comparisons between SCD and NC groups revealed no significant differences in MMSE, MoCA, AVLT-immediate recall, AVLT-delayed recall, or AVLT-recognition scores. General cognitive function (i.e., MMSE and MoCA scores) and memory ability (i.e., AVLT-immediate recall, AVLT-delayed recall, and AVLT-recognition scores) were significantly lower in the aMCI group than in the SCD and NC groups.

Group Comparisons of Atlas-Based Tracts

In general, the diffusion metrics FA and MD in the SCD group were between those of the NC and aMCI groups, as shown in **Tables 2**, **3**. ANOVA revealed that FA values in the forceps

TABLE 1 | Demographic, clinical, and neuropsychological data.

Variables	NC group (*n* = 43)	SCD group (*n* = 38)	aMCI group (*n* = 36)	*F* or χ^2 value	*P*-value
Gender (F/M)	20/23	21/17	21/15	1.216	0.544
Age (years)	61.91 ± 6.358	66.42 ± 6.685	67.58 ± 6.792	8.342	<0.001[a,b]
Education (years)	12.58 ± 3.417	12.24 ± 3.412	10.86 ± 3.531	2.643	0.076
ADL	8 ± 0	8 ± 0	8 ± 0		
Vascular risk factors, n (%)					
Hypertension	19 (44.2%)	9 (23.7%)	12 (33.3%)	3.785	0.151
Diabetes mellitus	6 (14.0%)	5 (13.2%)	11 (30.6%)	4.712	0.095
Hyperlipidemia	7 (16.3%)	8 (21.1%)	4 (11.1%)	1.343	0.511
Smoking	4 (9.3%)	2 (13.2%)	2 (5.6%)	1.255	0.534
Drinking	4 (9.3%)	2 (5.3%)	3 (8.3%)	0.494	0.781
Family history of dementia	2 (4.7%)	4 (10.5%)	3 (8.3%)	1.011	0.603
Mental status					
HAMD	5.95 ± 4.731	4.66 ± 3.787	5.17 ± 4.45	0.913	0.404
HAMA	8.58 ± 6.318	7.13 ± 6.095	6.08 ± 6.04	1.164	0.198
General cognition					
MMSE[#]	28.93 ± 1.183	28.76 ± 1.46	27.47 ± 1.859	4.837	<0.001[a,b]
MoCA[#]	26.7 ± 1.897	26.45 ± 2.076	20.22 ± 2.508	47.641	<0.001[a,b]
Episodic memory					
AVLT-IR[#]	17.81 ± 3.756	17.58 ± 4.104	11.94 ± 4.42	10.049	<0.001[a,b]
AVLT-DR[#]	6.09 ± 1.757	5.21 ± 2.183	1.72 ± 1.907	22.531	<0.001[a,b]
AVLT-R[#]	20.98 ± 2.345	20.58 ± 2.815	17.64 ± 3.081	6.985	<0.001[a,b]

Note. Values are mean ± SD; NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment; AVLT-IR, auditory verbal learning test immediate recall; AVLT-DR, auditory verbal learning test delayed recall; AVLT-R, auditory verbal learning test recognition; ADL, activities of daily living; HAMD, Hamilton Depression Rating Scale; HAMA, Hamilton Anxiety Rating Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment. [a]Post hoc paired comparisons showed significant group differences between NC and aMCI. [b]Post hoc paired comparisons showed significant group differences between SCD and aMCI. [#]Analysis of covariance (ANCOVA) using age, gender, and education as covariates.

major and forceps minor, as well as MD values in the bilateral ATR, left CST, left CgC, left CgH, forceps minor, and left IFO, were significantly different among the three groups after Bonferroni correction.

Post hoc comparisons showed that the FA values in the forceps major and forceps minor were significantly decreased in both the SCD group and aMCI group relative to those in the NC group (**Figure 2**; **Table 4**), whereas MD values were significantly higher in more extensive regions, including in the bilateral ATR, left CST, left CgC, left CgH, forceps minor, and left IFO (**Figure 3**; **Table 4**). However, the SCD group, when compared with the aMCI group, presented significantly

TABLE 2 | Analysis of covariance of atlas-based tract FA values among the three groups.

Tracts	NC	SCD	aMCI	*F*-value	*P*-value
ATR.L	0.36 ± 0.016	0.343 ± 0.022	0.337 ± 0.031	5.007	0.008**
ATR.R	0.347 ± 0.015	0.327 ± 0.021	0.325 ± 0.028	6.197	0.003**
CTR.L	0.52 ± 0.017	0.508 ± 0.022	0.501 ± 0.025	4.499	0.013*
CTR.R	0.523 ± 0.02	0.512 ± 0.022	0.5 ± 0.027	5.457	0.005**
CgC.L	0.488 ± 0.021	0.47 ± 0.033	0.456 ± 0.035	4.233	0.017*
CgC.R	0.433 ± 0.029	0.414 ± 0.037	0.397 ± 0.04	4.038	0.02*
CgH.L	0.355 ± 0.019	0.341 ± 0.027	0.333 ± 0.029	3.271	0.042*
CgH.R	0.336 ± 0.029	0.325 ± 0.033	0.318 ± 0.033	1.288	0.28
Fma	0.535 ± 0.018	0.512 ± 0.025	0.511 ± 0.03	7.519	<0.001***
Fmi	0.398 ± 0.019	0.377 ± 0.02	0.374 ± 0.025	7.419	<0.001***
IFO.L	0.406 ± 0.017	0.393 ± 0.021	0.387 ± 0.027	3.263	0.042*
IFO.R	0.405 ± 0.018	0.392 ± 0.022	0.388 ± 0.03	2.51	0.086
ILF.L	0.404 ± 0.017	0.391 ± 0.019	0.387 ± 0.023	4.691	0.011*
ILF.R	0.418 ± 0.02	0.409 ± 0.023	0.405 ± 0.023	1.411	0.248
SLF.L	0.36 ± 0.016	0.351 ± 0.017	0.351 ± 0.021	1.538	0.219
SLF.R	0.369 ± 0.018	0.362 ± 0.019	0.359 ± 0.021	0.406	0.667
UF.L	0.367 ± 0.019	0.36 ± 0.027	0.352 ± 0.024	1.208	0.303
UF.R	0.364 ± 0.02	0.358 ± 0.024	0.349 ± 0.028	1.213	0.301
tSLF.L	0.446 ± 0.035	0.447 ± 0.034	0.44 ± 0.039	0.514	0.6
tSLF.R	0.498 ± 0.038	0.494 ± 0.036	0.5 ± 0.042	0.168	0.846

*Note. Values are mean ± SD. Analysis of covariance (ANCOVA) using age, gender, and education as covariates. NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment. *P < 0.05; **P < 0.01; ***P < 0.05 after Bonferroni correction (P < 0.05/20). ATR, anterior thalamic radiation; CST, corticospinal tract; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); Fma, forceps major; Fmi, forceps minor; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus; tSLF, superior longitudinal fasciculus-temporal part; L, left; R, right; FA, fractional anisotropy.*

TABLE 3 | Analysis of covariance of atlas-based tract MD values among the three groups.

Tracts	NC	SCD	aMCI	*F*-value	*P*-value
ATR.L	0.836 ± 0.061	0.924 ± 0.127	0.971 ± 0.149	7.658	0.001***
ATR.R	0.843 ± 0.059	0.959 ± 0.132	0.975 ± 0.173	7.899	0.001***
CTR.L	0.774 ± 0.019	0.8 ± 0.033	0.803 ± 0.038	7.103	0.001***
CTR.R	0.779 ± 0.023	0.8 ± 0.033	0.8 ± 0.042	1.738	0.181
CgC.L	0.747 ± 0.024	0.773 ± 0.038	0.781 ± 0.037	6.805	0.002***
CgC.R	0.739 ± 0.024	0.758 ± 0.034	0.761 ± 0.041	1.781	0.173
CgH.L	0.76 ± 0.03	0.793 ± 0.041	0.818 ± 0.072	8.088	0.001***
CgH.R	0.783 ± 0.048	0.821 ± 0.057	0.844 ± 0.135	2.458	0.090
Fma	0.872 ± 0.063	0.925 ± 0.089	0.9 ± 0.099	2.275	0.108
Fmi	0.842 ± 0.035	0.886 ± 0.047	0.909 ± 0.059	16.472	<0.001***
IFO.L	0.779 ± 0.021	0.809 ± 0.04	0.819 ± 0.047	6.589	0.002***
IFO.R	0.788 ± 0.024	0.814 ± 0.043	0.814 ± 0.055	1.728	0.182
ILF.L	0.788 ± 0.022	0.806 ± 0.028	0.814 ± 0.065	1.552	0.216
ILF.R	0.71 ± 0.231	0.726 ± 0.218	0.762 ± 0.141	0.093	0.911
SLF.L	0.721 ± 0.235	0.761 ± 0.229	0.807 ± 0.145	0.217	0.805
SLF.R	0.714 ± 0.232	0.748 ± 0.225	0.788 ± 0.144	0.123	0.884
UF.L	0.737 ± 0.241	0.767 ± 0.232	0.849 ± 0.189	0.817	0.444
UF.R	0.738 ± 0.247	0.771 ± 0.236	0.844 ± 0.201	0.527	0.592
tSLF.L	0.699 ± 0.228	0.708 ± 0.213	0.748 ± 0.133	0.131	0.877
tSLF.R	0.706 ± 0.231	0.713 ± 0.217	0.731 ± 0.135	0.124	0.883

*Note. Values are mean ± SD reflecting the MD values × 10^{-3} mm^2 s^{-1}. Analysis of covariance (ANCOVA) using age, gender, and education as covariates. NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment. ***P < 0.05 after Bonferroni correction (P < 0.05/20). ATR, anterior thalamic radiation; CST, corticospinal tract; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); Fma, forceps major; Fmi, forceps minor; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus; tSLF, superior longitudinal fasciculus-temporal part; L, left; R, right; MD, mean diffusivity.*

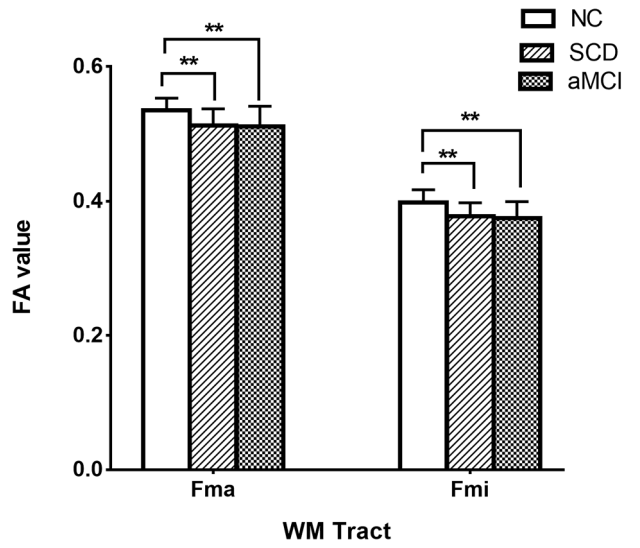

FIGURE 2 | *Post hoc* analysis of FA values for tracts that remained significant after Bonferroni correction in the three groups. NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment; Fma, forceps major; Fmi, forceps minor; WM, white matter; FA, fractional anisotropy; **$P < 0.01$.

lower MD values in the left CgH and forceps minor (**Figure 3**; **Table 4**).

Relationship Between Diffusion Metrics and Neuropsychological Scores

The linear regression analysis results between cognitive function scores and FA values in the overall sample are presented in **Table 5**. In our study, the FA value in the left CgH was independently positively correlated with MMSE (β = 0.335, $P < 0.001$) and AVLT-immediate recall (β = 0.275, $P = 0.002$) scores; the FA values in the left CgC were independently correlated with performance on MoCA (β = 0.295, $P < 0.001$), AVLT-delayed recall (β = 0.339, $P < 0.001$), and AVLT-recognition (β = 0.296, $P < 0.001$) assessments.

In addition, the results of the regression analysis of the cognitive function scores and the MD values within the entire sample are shown in **Table 6**. MD values in the left CgH were independently negatively correlated with MMSE (β = −1.89, $P = 0.04$) and MoCA ($\beta = -0.227$, $P = 0.015$) scores. MD values in the left CgC were negatively correlated with MMSE ($\beta = -0.193$, $P = 0.037$) and AVLT-recognition ($\beta = -0.377$, $P < 0.001$) scores. MD values in the forceps minor were independently correlated with MoCA ($\beta = -0.245$, $P = 0.009$), AVLT-immediate recall ($\beta = -0.337$, $P < 0.001$), and AVLT-delayed recall ($\beta = -0.439$, $P < 0.001$) scores.

DISCUSSION

In the present study, changes in the microstructural integrity of WM in AD-spectrum patients were revealed using DTI metrics. The anatomical pattern of WM impairment was similar in both SCD and aMCI patients, and the damage was less severe in SCD patients than in aMCI patients. In addition, significant correlations between diffusion metrics and cognition suggested that those individuals with WM microstructural disruption performed worse in general cognition and memory function tests. These results supported the previous theory that the DTI index can be a sensitive measure for detecting changes of WM microstructural in AD degenerative

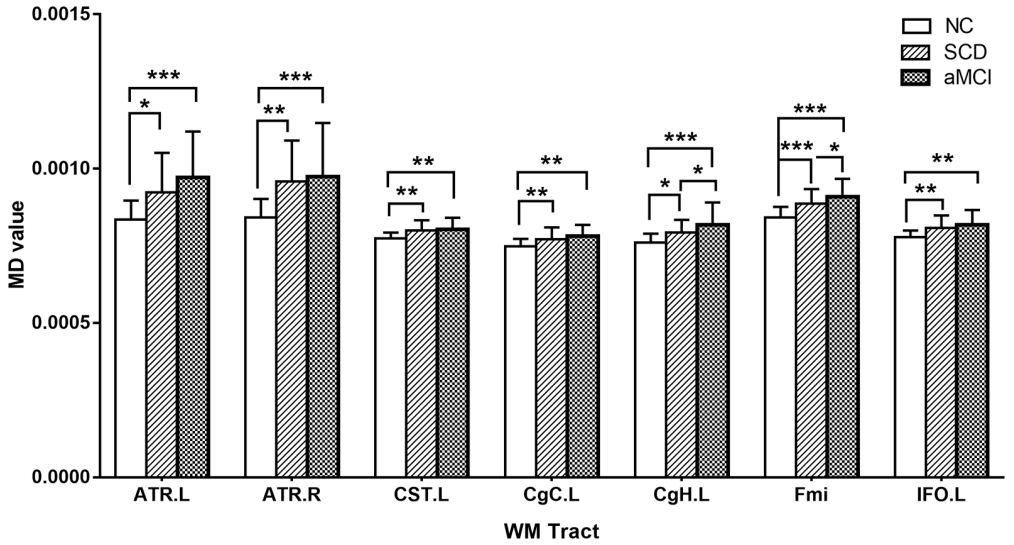

FIGURE 3 | *Post hoc* analysis of MD values for tracts that remained significant after Bonferroni correction in the three groups. NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment; ATR, anterior thalamic radiation; CST, corticospinal tract; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); Fmi, forceps minor; IFO, inferior fronto-occipital fasciculus; MD, mean diffusivity; L, left; R, right; WM, white matter; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

TABLE 4 | *Post hoc* analysis of the significant tracts surviving Bonferroni correction in the three groups.

White tracts	Group			ANCOVA		*Post hoc*	*P*-value	
	NC	SCD	aMCI	*F*-value	*P*-value	SCD vs. NC	aMCI vs. NC	SCD vs. aMCI
ATR.L								
MD	0.836 ± 0.061	0.924 ± 0.127	0.971 ± 0.149	7.658	**0.001**	**0.014**	**<0.001**	NS
ATR.R								
MD	0.843 ± 0.059	0.959 ± 0.132	0.975 ± 0.173	7.899	**0.001**	**0.001**	**<0.001**	NS
CST.L								
MD	0.774 ± 0.019	0.8 ± 0.033	0.803 ± 0.038	7.103	**0.001**	**0.002**	**0.001**	NS
CgC.L								
MD	0.747 ± 0.024	0.773 ± 0.038	0.781 ± 0.037	6.805	**0.002**	**0.008**	**0.001**	NS
CgH.L								
MD	0.76 ± 0.03	0.793 ± 0.041	0.818 ± 0.072	8.088	**0.001**	**0.034**	**<0.001**	**0.04**
Fma								
FA	0.535 ± 0.018	0.512 ± 0.025	0.511 ± 0.03	7.519	**<0.001**	**0.001**	**0.002**	NS
Fmi								
FA	0.398 ± 0.019	0.377 ± 0.02	0.374 ± 0.025	7.419	**<0.001**	**0.001**	**0.001**	NS
MD	0.842 ± 0.035	0.886 ± 0.047	0.909 ± 0.059	16.47	**<0.001**	**<0.001**	**<0.001**	**0.018**
IFO.L								
MD	0.779 ± 0.021	0.809 ± 0.04	0.819 ± 0.047	6.589	**0.002**	**0.009**	**0.001**	NS

Note. Data are represented as mean ± SD; the MD data equal to MD values × 10^{-3} mm^2 s^{-1}. P-values in bold indicated meeting statistical significance. NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment; ATR, anterior thalamic radiation; CST, corticospinal tract; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); Fma, forceps major; Fmi, forceps minor; IFO, inferior fronto-occipital fasciculus; L, left; R, right; FA, fractional anisotropy; MD, mean diffusivity; NS, no significance.

processes and can offer a new insight into AD pathophysiology (Caso et al., 2016).

Research interest is shifting to increasingly earlier stages, as the origin of AD and keys to treatment probably lie in the prevention of progression to a fully fledged disease (Slot et al., 2018). It should be noted that SCD has been shown to be the first clinical manifestation of AD, and there is increasing interest in the study of individuals meeting SCD criteria to assess the progression of this disease in the subjects who have a higher risk of cognitive decline (Sánchez-Benavides et al., 2018). Therefore, it is essential to evaluate SCD participant characteristics related to cognitive complaints. In the present study, SCD subjects exhibited significantly lower FA and significantly higher MD across extensive regions, as expected from previous AD-spectrum studies (Caso et al., 2016). Specifically, individuals with SCD showed an intermediate level of microstructure changes between those seen in NC and aMCI patients, whereas the damaging patterns of WM fibers were similar to those seen in aMCI. These brain regions showing compromised WM integrity were parallel to the

TABLE 5 | Multiple linear regression models for different cognitive scores and correlated FA variables.

Dependent variable	Variables included in the model	Unstandardized coefficients *B*	Standardized coefficients β	*P*-value	95% confidence interval
MMSE	Constant	19.204		<0.001	14.735–23.672
	CgH.L	26.186	0.335	**<0.001**	13.157–39.214
MoCA	Constant	3.345		0.480	−6.009 to 12.698
	CgC.L	35.868	0.295	**<0.001**	16.231–55.506
	Education	0.335	0.286	0.001	0.146–0.525
AVLT-IR	Constant	−2.519		0.658	−13.764 to 8.726
	CgH.L	53.077	0.275	**0.002**	20.292–85.863
AVLT-DR	Constant	−8.791		0.008	−15.226 to −2.357
	CgC.L	27.843	0.339	**<0.001**	14.167–41.520
AVLT-R	Constant	5.449		0.184	−2.630 to 13.528
	CgC.L	30.015	0.296	**<0.001**	12.843–47.187

Note. First, all FA values of all atlas-based tracts were correlated with neuropsychological scores, and only variables correlated with neuropsychological score at P < 0.05 were used in subsequent stepwise multiple linear regressions controlling for age, gender, and years of education. P-values in bold indicated that the corresponding tracts were significantly correlated with cognitive function. AVLT-IR, auditory verbal learning test immediate recall; AVLT-DR, auditory verbal learning test delayed recall; AVLT-R, auditory verbal learning test recognition; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); L, left; R, right; FA, fractional anisotropy.

TABLE 6 | Multiple linear regression models for different cognitive scores and correlated MD variables.

Dependent variable	Variables included in the model	Unstandardized coefficients *B*	Standardized coefficients β	*P*-value	95% confidence interval
MMSE	Constant	40.099		<0.001	32.876–47.323
	CgH.L	−7,055.505	−1.89	**0.040**	−13,771.278 to −339.732
	Education	0.121	0.2	0.02	0.02–0.223
	CgC.L	−10,117.516	−0.193	**0.037**	−19,596.560 to −638.47
MoCA	Constant	47.183		<0.001	36.827–57.540
	CgH.L	−16,324.704	−0.227	**0.015**	−29,484.453 to −3,164.955
	Education	0.381	0.325	<0.001	0.2–0.562
	Fmi	−16,525.910	−0.245	**0.009**	−28,933.617 to −4,118.203
AVLT-IR	Constant	37.190		<0.001	24.546–49.834
	Fmi	−29,064.516	−0.337	**<0.001**	−43,295.34 to −14,833.68
	Education	0.345	0.231	0.006	0.099–0.592
AVLT-DR	Constant	21.965		<0.001	15.543–28.387
	Fmi	−20,011.718	−0.439	**<0.001**	−27,260.017 to −12,763.420
AVLT-R	Constant	44.019		<0.001	33.349–54.69
	CgC.L	−31,774.021	−0.377	**<0.001**	−45,602.9 to −17,945.13753

Note. First, all MD values of all atlas-based tracts were correlated with neuropsychological scores, and only variables correlated with neuropsychological score at P < 0.05 were used in subsequent stepwise multiple linear regressions controlling for age, gender, and years of education. P-values in bold indicated that the corresponding tracts were significantly correlated with cognitive function. AVLT-IR, auditory verbal learning test immediate recall; AVLT-DR, auditory verbal learning test delayed recall; AVLT-R, auditory verbal learning test recognition; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; CgC, cingulum (cingulate gyrus); CgH, cingulum (hippocampus); Fmi, forceps minor; L, left; R, right; MD, mean diffusivity.

results of a recent SCD study by Ohlhauser et al., who also discovered widespread significant impairment in superior and inferior longitudinal fasciculi, fronto-occipital fasciculi, and corpus callosum (Ohlhauser et al., 2019). Some previous studies have detected impaired WM integrity in the MTL in SCD patients than in normal controls using TBSS analysis, which also provided additional evidence for our research results (Wang et al., 2012; Selnes et al., 2013). Our findings demonstrate that distinctive WM integrity alterations can occur early in SCD, although neuropsychological test results of these patients are comparable to those of healthy controls. Thus, DTI indexes can be a sensitive and reliable imaging technique to detect changes in WM microstructure, even in this very early stage of AD. Moreover, the present approach may contribute to the development of strategies to stratify SCD subjects with different risk levels for AD.

The impaired WM pathways (including the ATR, forceps minor, forceps major, CgC, CgH, and IFO) were the tracts connecting earliest affected gray matter structures in AD (i.e., hippocampus, cingulate gyrus, medial prefrontal cortex, and posterior cingulate cortex). These findings are in line with previous studies reporting decreased FDG metabolism, increased Aβ deposition, and pathological tau accumulation in these regions in SCD, which closely coincided with the imaging features found in early AD patients (Snitz et al., 2015; Buckley et al., 2017; Vannini et al., 2017). In that sense, our findings showing altered WM tracts connecting these main cortical hubs of cognitive brain networks may provide novel

insights into the pathological mechanisms of AD from the WM neurodegeneration perspective. Furthermore, most of the mentioned tracts link brain areas belonging to the default mode network, which has consistently been found to be affected across the development of AD (Zhang et al., 2010). Regions in the default mode network, such as the medial prefrontal cortex, are related to working memory, whereas MTL regions are involved in sustaining long-term memory. Many studies have demonstrated that individuals with SCD show abnormal activity in the default mode network, as assessed by functional MRI (Rodda et al., 2009, 2011). Thus, the damaged WM tracts linking the default mode network could be partly responsible for these functional changes.

In addition, the present aMCI group showed significantly higher MD values in the left CgH and forceps minor than in those of SCD patients. These results were in line with the findings by Parra et al., whose participants were young presymptomatic presenilin 1 mutation carriers. These young hereditary AD carriers showed a significant increase in MD values in bilateral CgH and forceps minor (Parra et al., 2015). Jung et al. (2015) also found that in the aMCI group, compared with the SCD group, only the MD value in the left CgH was significantly increased. Therefore, we speculate that the destruction of the WM integrity in the left CgH and forceps minor may be crucial for progressive cognitive impairment. A significant of numerical increases was also observed in MD of the left CgH and forceps minor in SCD and aMCI groups compared with the NC.

The multiple general linear regression model analysis showed that after age, gender, and years of education were adjusted, FA and/or MD values in the left CgC, left CgH, and forceps minor were independently correlated with overall cognitive function and memory function. The lower the FA value and the higher the MD value were, the worse the cognitive performance. The cingulum, as the core structure in the Papez circuit of the cholinergic system, is an important pathway to maintain communication among the limbic system, which plays a role in perception, executive control, episodic memory, and understanding (Bubb et al., 2018). The disruption of the cingulum can lead to the disruption of communication from the hippocampus and cingulate gyrus to the cerebral cortex, subsequently impairing memory and other cognitive domains. The forceps minor connects the bilateral prefrontal cortex, where the encoding and extraction of episodic memory occur (Jeong et al., 2015). Grambaite et al. (2010) noticed in SCD and aMCI subjects who had decreased FA values in the forceps minor were related to verbal memory. Parra's study found that an increase in the MD values in the forceps minor and left CgH could predict poor short-term memory task performance (Parra et al., 2015), which agreed with our results. Hence, we can infer that WM microstructural impairment may be an important predictor of overall cognitive and memory function and an early marker of the development of AD.

There are still some limitations to this study. First, this study is cross-sectional and is insufficient for predicting the clinical outcomes of SCD and aMCI patients. Second, the present sample of SCD and aMCI participants was recruited based on clinical criteria, which may not have displayed the underlying AD pathology. Future studies should add biomarker information to better characterize the findings. Therefore, these data should be interpreted with caution. Third, WM microstructures may be different owing to the diverse stages of SCD, but currently, there are no clear criteria to classify the severity of SCD. In addition, our study focused only on the changes in the WM microstructure of SCD patients. It is unclear whether these changes are related to gray matter atrophy, functional changes, or CSF pathological indicators. Finally, we only focused on the most commonly used metrics (FA and MD), but other metrics in DTI, such as radial and axial diffusivity, will be investigated subsequently. The longitudinal change pattern of WM microstructure from preclinical to clinical AD needs to be confirmed by large sample longitudinal studies.

In summary, SCD individuals had extensive WM microstructural damage in a pattern similar to that seen in aMCI. DTI-based diffusion parameters could be useful imaging biomarkers for the early diagnosis and stage monitoring of AD. Considering that individuals with SCD are at high risk for aMCI and AD, future studies are needed to characterize individuals with SCD longitudinally to identify individuals who may eventually develop AD, enabling more timely interventions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committees of the Affiliated Drum Tower Hospital of Nanjing University Medical School (clinical trials government identifier: NCT01364246). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HZ and FB designed the study, collected the data, and edited the manuscript. YX designed the study and edited the manuscript. CL collected the data, wrote and edited the manuscript, and performed the statistics. RQ wrote and edited the manuscript. ML, DY, LH, and RL collected the data. HC validated the statistics.

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REFERENCES

Acosta-Cabronero, J., and Nestor, P. J. (2014). Diffusion tensor imaging in Alzheimer's disease: insights into the limbic-diencephalic network and methodological considerations. *Front. Aging Neurosci.* 6:266. doi: 10.3389/fnagi.2014.00266

Bubb, E. J., Metzler-Baddeley, C., and Aggleton, J. P. (2018). The cingulum bundle: anatomy, function, and dysfunction. *Neurosci. Biobehav. Rev.* 92, 104–127. doi: 10.1016/j.neubiorev.2018.05.008

Buckley, R. F., Hanseeuw, B., Schultz, A. P., Vannini, P., Aghjayan, S. L., Properzi, M. J., et al. (2017). Region-specific association of subjective cognitive decline with tauopathy independent of global β-amyloid burden. *JAMA Neurol.* 74, 1455–1463. doi: 10.1001/jamaneurol.2017.2216

Buckley, R. F., Maruff, P., Ames, D., Bourgeat, P., Martins, R. N., Masters, C. L., et al. (2016). Subjective memory decline predicts greater rates of clinical progression in preclinical Alzheimer's disease. *Alzheimers Dement.* 12, 796–804. doi: 10.1016/j.jalz.2015.12.013

Bürgel, U., Amunts, K., Hoemke, L., Mohlberg, H., Gilsbach, J. M. and Zilles, K. (2006). White matter fiber tracts of the human brain: three-dimensional mapping at microscopic resolution, topography and intersubject variability. *Neuroimage* 29, 1092–1105. doi: 10.1016/j.neuroimage.2005.08.040

Caso, F., Agosta, F., and Filippi, M. (2016). Insights into white matter damage in Alzheimer's disease: from postmortem to *in vivo* diffusion tensor MRI studies. *Neurodegener. Dis.* 16, 26–33. doi: 10.1159/000441422

Cheng, Y. W., Chen, T. F., and Chiu, M. J. (2017). From mild cognitive impairment to subjective cognitive decline: conceptual and methodological evolution. *Neuropsychiatr. Dis. Treat.* 13, 491–498. doi: 10.2147/ndt.s123428

Choo, I. H., Lee, D. Y., Oh, J. S., Lee, J. S., Lee, D. S., Song, I. C., et al. (2010). Posterior cingulate cortex atrophy and regional cingulum disruption in mild cognitive impairment and Alzheimer's disease. *Neurobiol. Aging* 31, 772–779. doi: 10.1016/j.neurobiolaging.2008.06.015

Copenhaver, B. R., Rabin, L. A., Saykin, A. J., Roth, R. M., Wishart, H. A., Flashman, L. A., et al. (2006). The fornix and mammillary bodies in older adults with Alzheimer's disease, mild cognitive impairment, and cognitive complaints: a volumetric MRI study. *Psychiatry Res.* 147, 93–103. doi: 10.1016/j.pscychresns.2006.01.015

Cui, Z., Zhong, S., Xu, P., He, Y., and Gong, G. (2013). PANDA: a pipeline toolbox for analyzing brain diffusion images. *Front. Hum. Neurosci.* 7:42. doi: 10.3389/fnhum.2013.00042

Grambaite, R., Stenset, V., Reinvang, I., Walhovd, K. B., Fjell, A. M., and Fladby, T. (2010). White matter diffusivity predicts memory in patients with subjective and mild cognitive impairment and normal CSF total tau levels. *J. Int. Neuropsychol. Soc.* 16, 58–69. doi: 10.1017/s1355617709990932

Hafkemeijer, A., Altmann-Schneider, I., Oleksik, A. M., van de Wiel, L., Middelkoop, H. A., van Buchem, M. A., et al. (2013). Increased functional connectivity and brain atrophy in elderly with subjective memory complaints. *Brain Connect.* 3, 353–362. doi: 10.1089/brain.2013.0144

Hua, K., Zhang, J., Wakana, S., Jiang, H., Li, X., Reich, D. S., et al. (2008). Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. *NeuroImage* 39, 336–347. doi: 10.1016/j.neuroimage.2007.07.053

Jeong, W., Chung, C. K., and Kim, J. S. (2015). Episodic memory in aspects of large-scale brain networks. *Front. Hum. Neurosci.* 9:454. doi: 10.3389/fnhum.2015.00454

Jessen, F., Amariglio, R. E., van Boxtel, M., Breteler, M., Ceccaldi, M., Chetelat, G., et al. (2014). A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimers Dement.* 10, 844–852. doi: 10.1016/j.jalz.2014.01.001

Jung, W. B., Lee, Y. M., Kim, Y. H., and Mun, C. W. (2015). Automated classification to predict the progression of Alzheimer's disease using whole-brain volumetry and DTI. *Psychiatry Investig.* 12, 92–102. doi: 10.4306/pi.2015.12.1.92

Kantarci, K., Murray, M. E., Schwarz, C. G., Reid, R. I., Przybelski, S. A., Lesnick, T., et al. (2017). White-matter integrity on DTI and the pathologic staging of Alzheimer's disease. *Neurobiol. Aging* 56, 172–179. doi: 10.1016/j.neurobiolaging.2017.04.024

Konukoglu, E., Coutu, J. P., Salat, D. H., and Fischl, B. (2016). Multivariate statistical analysis of diffusion imaging parameters using partial least squares: application to white matter variations in Alzheimer's disease. *NeuroImage* 134, 573–586. doi: 10.1016/j.neuroimage.2016.04.038

Lin, Y., Shan, P. Y., Jiang, W. J., Sheng, C., and Ma, L. (2019). Subjective cognitive decline: preclinical manifestation of Alzheimer's disease. *Neurol. Sci.* 40, 41–49. doi: 10.1007/s10072-018-3620-y

Lu, J., Li, D., Li, F., Zhou, A., Wang, F., Zuo, X., et al. (2011). Montreal cognitive assessment in detecting cognitive impairment in Chinese elderly individuals: a population-based study. *J. Geriatr. Psychiatry Neurol.* 24, 184–190. doi: 10.1177/0891988711422528

Mitchell, A. J., Beaumont, H., Ferguson, D., Yadegarfar, M., and Stubbs, B. (2014). Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. *Acta Psychiatr. Scand.* 130, 439–451. doi: 10.1111/acps.12336

Nowrangi, M. A., Lyketsos, C. G., Leoutsakos, J. M., Oishi, K., Albert, M., Mori, S., et al. (2013). Longitudinal, region-specific course of diffusion tensor imaging measures in mild cognitive impairment and Alzheimer's disease. *Alzheimers Dement.* 9, 519–528. doi: 10.1016/j.jalz.2012.05.2186

Ohlhauser, L., Parker, A. F., Smart, C. M., and Gawryluk, J. R. (2019). White matter and its relationship with cognition in subjective cognitive decline. *Alzheimers Dement.* 11, 28–35. doi: 10.1016/j.dadm.2018.10.008

Parra, M. A., Saarimaki, H., Bastin, M. E., Londono, A. C., Pettit, L., Lopera, F., et al. (2015). Memory binding and white matter integrity in familial Alzheimer's disease. *Brain* 138, 1355–1369. doi: 10.1093/brain/awv048

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. *J. Intern. Med.* 256, 183–194. doi: 10.1111/j.1365-2796.2004.01388.x

Reisberg, B., Prichep, L., Mosconi, L., John, E. R., Glodzik-Sobanska, L., Boksay, I., et al. (2008). The pre-mild cognitive impairment, subjective cognitive impairment stage of Alzheimer's disease. *Alzheimers Dement.* 4, S98–S108. doi: 10.1016/j.jalz.2007.11.017

Rodda, J. E., Dannhauser, T. M., Cutinha, D. J., Shergill, S. S., and Walker, Z. (2009). Subjective cognitive impairment: increased prefrontal cortex activation compared to controls during an encoding task. *Int. J. Geriatr. Psychiatry* 24, 865–874. doi: 10.1002/gps.2207

Rodda, J., Dannhauser, T., Cutinha, D. J., Shergill, S. S., and Walker, Z. (2011). Subjective cognitive impairment: functional MRI during a divided attention task. *Eur. Psychiatry* 26, 457–462. doi: 10.1016/j.eurpsy.2010.07.003

Rönnlund, M., Sundström, A., Adolfsson, R., and Nilsson, L. G. (2015). Self-reported memory failures: associations with future dementia in a population-based study with long-term follow-up. *J. Am. Geriatr. Soc.* 63, 1766–1773. doi: 10.1111/jgs.13611

Salat, D. H., Tuch, D. S., van der Kouwe, A. J., Greve, D. N., Pappu, V., Lee, S. Y., et al. (2010). White matter pathology isolates the hippocampal formation in Alzheimer's disease. *Neurobiol. Aging* 31, 244–256. doi: 10.1016/j.neurobiolaging.2008.03.013

Sánchez-Benavides, G., Grau-Rivera, O., Suárez-Calvet, M., Minguillon, C., Cacciaglia, R., Gramunt, N., et al. (2018). Brain and cognitive correlates of subjective cognitive decline-plus features in a population-based cohort. *Alzheimers Res. Ther.* 10:123. doi: 10.1186/s13195-018-0449-9

Selnes, P., Aarsland, D., Bjornerud, A., Gjerstad, L., Wallin, A., Hessen, E., et al. (2013). Diffusion tensor imaging surpasses cerebrospinal fluid as predictor of cognitive decline and medial temporal lobe atrophy in subjective cognitive impairment and mild cognitive impairment. *J. Alzheimers Dis.* 33, 723–736. doi: 10.3233/jad-2012-121603

Sexton, C. E., Kalu, U. G., Filippini, N., Mackay, C. E., and Ebmeier, K. P. (2011). A meta-analysis of diffusion tensor imaging in mild cognitive impairment and Alzheimer's disease. *Neurobiol. Aging* 32, 2322.e5–2322.e18. doi: 10.1016/j.neurobiolaging.2010.05.019

Shaikh, S., Kumar, A., and Bansal, A. (2018). Diffusion tensor imaging: An overview. *Neurol. India* 66, 1603–1611. doi: 10.4103/0028-3886.246233

Slot, R. E. R., Verfaillie, S. C. J., Overbeek, J. M., Timmers, T., Wesselman, L. M. P., Teunissen, C. E., et al. (2018). Subjective cognitive impairment cohort (SCIENCe): study design and first results. *Alzheimers Res. Ther.* 10:76. doi: 10.1186/s13195-018-0390-y

Snitz, B. E., Lopez, O. L., McDade, E., Becker, J. T., Cohen, A. D., Price, J. C., et al. (2015). Amyloid-β imaging in older adults presenting to a memory clinic with subjective cognitive decline: a pilot study. *J. Alzheimers Dis.* 48, S151–S159. doi: 10.3233/jad-150113

Soares, J. M., Marques, P., Alves, V., and Sousa, N. (2013). A hitchhiker's guide to diffusion tensor imaging. *Front. Neurosci.* 7:31. doi: 10.3389/fnins.2013.00031

Sui, X. and Rajapakse, J. C. (2018). Profiling heterogeneity of Alzheimer's disease using white-matter impairment factors. *Neuroimage Clin.* 20, 1222–1232. doi: 10.1016/j.nicl.2018.10.026

Sun, Y., Yang, F. C., Lin, C. P., and Han, Y. (2015). Biochemical and neuroimaging studies in subjective cognitive decline: progress and perspectives. *CNS Neurosci. Ther.* 21, 768–775. doi: 10.1111/cns.12395

Vannini, P., Hanseeuw, B., Munro, C. E., Amariglio, R. E., Marshall, G. A., Rentz, D. M., et al. (2017). Hippocampal hypometabolism in older adults with memory complaints and increased amyloid burden. *Neurology* 88, 1759–1767. doi: 10.1212/wnl.0000000000003889

Visser, P. J., Verhey, F., Knol, D. L., Scheltens, P., Wahlund, L. O., Freund-Levi, Y., et al. (2009). Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study. *Lancet Neurol.* 8, 619–627. doi: 10.1016/S1474-4422(09)70139-5

Wahlund, L. O., Barkhof, F., Fazekas, F., Bronge, L., Augustin, M., Sjogren, M., et al. (2001). A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 32, 1318–1322. doi: 10.1161/01.str.32.6.1318

Wang, Y., West, J. D., Flashman, L. A., Wishart, H. A., Santulli, R. B., Rabin, L. A., et al. (2012). Selective changes in white matter integrity in MCI and older adults with cognitive complaints. *Biochim. Biophys. Acta* 1822, 423–430. doi: 10.1016/j.bbadis.2011.08.002

Zhang, H. Y., Wang, S. J., Liu, B., Ma, Z. L., Yang, M., Zhang, Z. J., et al. (2010). Resting brain connectivity: changes during the progress of Alzheimer disease. *Radiology* 256, 598–606. doi: 10.1148/radiol.10091701

Zhao, Q., Lv, Y., Zhou, Y., Hong, Z., and Guo, Q. (2012). Short-term delayed recall of auditory verbal learning test is equivalent to long-term delayed recall for identifying amnestic mild cognitive impairment. *PLoS One* 7:e51157. doi: 10.1371/journal.pone.0051157

Zhao, W., Wang, X., Yin, C., He, M., Li, S., and Han, Y. (2019). Trajectories of the hippocampal subfields atrophy in the Alzheimer's disease: a structural imaging study. *Front. Neuroinform.* 13:13. doi: 10.3389/fninf.2019.00013

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Depression, Anxiety, and Apathy in Mild Cognitive Impairment: Current Perspectives

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Objective: Mild cognitive impairment (MCI) is an important risk state for dementia, particularly Alzheimer's disease (AD). Depression, anxiety, and apathy are commonly observed neuropsychiatric features in MCI, which have been linked to cognitive and functional decline in daily activities, as well as disease progression. Accordingly, the study's objective is to review the prevalence, neuropsychological characteristics, and conversion rates to dementia between MCI patients with and without depression, anxiety, and apathy.

Methods: A PubMed search and critical review were performed relating to studies of MCI, depression, anxiety, and apathy.

Results: MCI patients have a high prevalence of depression/anxiety/apathy; furthermore, patients with MCI and concomitant depression/anxiety/apathy have more pronounced cognitive deficits and progress more often to dementia than MCI patients without depression/anxiety/apathy.

Conclusions and Implications: Depression, anxiety, and apathy are common in MCI and represent possible risk factors for cognitive decline and progression to dementia. Further studies are needed to better understand the role and neurobiology of depression, anxiety, and apathy in MCI.

Keywords: mild cognitive impairment, depression, anxiety, apathy, dementia

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INTRODUCTION

Mild cognitive impairment (MCI) represents a transitional stage between healthy aging and dementia. Subjects with MCI complain about cognitive impairments, have documented cognitive deficits relative to age- and education-matched controls—although they are less impaired than patients with dementia—and have largely intact activities of daily living (Sanford, 2017). Depending on the inclusion criteria, the prevalence of MCI in the general older population has been estimated between 5.0%–36.7% (Sachdev et al., 2015) and 11%–33% of subjects with MCI develop dementia within 2 years (Luis et al., 2003; Bruscoli and Lovestone, 2004). MCI is thus regarded as an important risk state for dementia.

However, longitudinal studies showed that up to 50% of patients with MCI at their first doctor visit returned to normal at follow-up examinations (Larrieu et al., 2002; Ganguli et al., 2004), particularly in population-based cohorts. Apart from neurodegenerative disorders, many factors affect cognitive performance in older populations, including age, gender, education, vascular risk factors, genetic background, hormonal changes, and comorbidities (Yaffe, 2018). Additionally, studies of MCI differed greatly as to their inclusion criteria, study settings, sample characteristics, classification and subtype of MCI, observation period, and evaluation of cognitive and other relevant features. Combined with the biological heterogeneity of the MCI syndrome, these factors are the reason why the prognosis of cognitive deficits in the older adults and in MCI varies greatly across studies. Furthermore, a lot of MCI patients revert to a normal stage. This could be due to lack of biomarkers at the time of the diagnoses, which makes it difficult to draw inferences on the nature of psychiatric symptoms. Alzheimer's disease (AD) is defined by its underlying pathologic processes that can be documented by postmortem examination or *in vivo* by biomarkers in the National Institute on Aging and Alzheimer's Association Research Framework in Jack et al. (2018). In the new framework, the diagnosis of AD is not based on the symptoms or signs but a biological construct.

SEARCH CRITERIA

We included original studies from tertiary referrals and population-based studies written in English that examined populations with MCI, depression, anxiety and apathy. A systematic literature search was performed using the PubMed database. Search terms were *depression, apathy, anxiety, neuropsychology, cognitive, conversion, progression, or prognosis* in combination with *MCI or CIND*. Articles were included if they had primary data derived from cross-sectional or longitudinal studies and were published prior to May 2018. Studies was excluded if they: (a) presented a reanalysis of subpopulations already included in other studies; (b) reported a patient population of less than 10 patients; or (c) were commentaries, technical notes, or review articles summarizing the results of previous studies.

Psychological Symptoms in MCI

Behavioral disorders and psychological symptoms often accompany MCI and were reported to affect its presentation and course. Psychopathology, such as depression, anxiety, or apathy, are frequent in pre-dementia stages, dementia, and also in normal aging; prevalence rates for depression in normal older subjects range between 14.6% and 53% (Wada et al., 2004; Wen et al., 2010; Zivin et al., 2010; Vadla et al., 2013; Qadir et al., 2014), for anxiety between 3.7% and 43% (Bryant et al., 2008; Vadla et al., 2013; Arbus et al., 2014; Katzman et al., 2014), and for apathy between 2% and 75.2% (Adams, 2001; Lyketsos et al., 2002; Onyike et al., 2007). Cognitive impairment in major depression is well documented and is part of depression and its DSM criteria; the deterioration of processing speed and executive, attentional, and amnestic functions are frequent findings (Christensen et al., 1997; Lee R. S. C. et al., 2012; Rock et al., 2014). Cognitive deficits were found more often in older than younger depressed adults (Thomas et al., 2009; van den Kommer et al., 2012) and are more pronounced if depression was combined with anxiety (Basso et al., 2007; Rosenberg et al., 2011). Moreover, several studies have identified depression as a risk factor for disease progression and the development of dementia (Moon et al., 2017; Sugarman et al., 2018). Depressive symptoms may affect balance in MCI patients, potentially increasing the risk of falls (Pieruccini-Faria et al., 2018). Apathy, characterized by reduced motivation, reduced goal-directed behavior, and a flattening of affect (Steffens et al., 2000; Delrieu et al., 2015), may overlap with, but can be differentiated from, depression. Apathy also deteriorates cognitive functions in patients with normal aging, MCI, and dementia (Brodaty et al., 2010; Delrieu et al., 2015) and is a potential risk factor for dementia (Palmer et al., 2010; Richard et al., 2012). Similarly, anxiety—defined as anxious behavior or abnormal fear—had a reported impact on conversion from MCI to AD, directly or indirectly *via* depression (Palmer et al., 2007; Potvin et al., 2011).

In sum, depression, anxiety, and apathy are common in MCI, and a conjoint influence of psychopathology and MCI on cognitive abilities and the development of dementia has been reported (Palmer et al., 2010; Delrieu et al., 2015). However, studies of MCI differed grossly as to their methodology, such as patient selection, diagnostic criteria, assessment, and other features. Thus, findings regarding the neuropsychological and prognostic features of apathy and depression in MCI were inconsistent, and the overall picture is therefore cloudy. It also remains unclear which subtypes or characteristics of geriatric depression deteriorate cognition (Dillon et al., 2014).

MCI Patients Have Higher Rates of Depression

The reported prevalence of depression in MCI patients ranged between 16.9%–55%, whereas only 11%–30% of older adults presented significant depressive symptoms; (Kivelä et al., 1988; Gallo and Lebowitz, 1999; Steffens et al., 2000; Copeland et al., 2004; Lee and Shinkai, 2005) this indicates that MCI patients had higher rates of depression than normal adults, and those rates were independent from age, race, gender, and study type. The different MCI definitions, depression instruments, and criteria lead to the wide range of prevalence of depression in MCI patients. A meta-analysis showed the prevalence of depression in patients with MCI is 32% (Ismail et al., 2017), but the use of anti-depressive drugs was not shown to be a protective factor of dementia (Chan et al., 2019). Other studies have shown that subjects with depression have a higher incidence of MCI (Muller et al., 2007; Ng et al., 2009). Depressive patients have more amyloid abnormalities than non-depressive patients (Donovan et al., 2018) MCI with Aβ burden of the brain is associated with an increased risk of having neuropsychiatric symptoms (Krell-Roesch et al., 2019). Individuals with MCI are at an increased risk of progression to more severe cognitive impairment (Mitchell and Shiri-Feshki, 2009) and can have subtle impairments in everyday functioning (Hughes et al., 2012) and co-occurring depressive symptoms (Byers and Yaffe,

2011). A study found the prevalence of depression in AD and multidomain-MCI was 49.6% and 44.1%, respectively (Di Iulio et al., 2010). There is a high prevalence of neuropsychiatric disturbances in patients with MCI, including depression, apathy, anxiety, aggression, and agitation (Apostolova and Cummings, 2008; Monastero et al., 2009). Neuropsychiatric symptoms containing depression and apathy as well as subjective cognitive decline may be among the symptoms of preclinical stages of AD (Rosenberg et al., 2013; Vogel et al., 2017; Jessen, 2019), and they are early manifestations of AD symptomatology and possible predictors of progression from MCI to dementia (Palmer et al., 2007, 2010; Monastero et al., 2009; Kim et al., 2013).

Depressive symptoms were commonly associated with MCI, and anxiety-depression was found to be a significant risk factor (Rodríguez-Sánchez et al., 2011; Juarez-Cedillo et al., 2012; Moretti et al., 2013). The reported prevalence of depression in MCI patients is sensitive to the criteria used to diagnose MCI and its subtypes (Sasaki et al., 2009). Subjects with MCI are more likely to develop depression compared with those with normal cognitive function, especially with those with amnestic MCI (aMCI; Hidaka et al., 2012; Shahnawaz et al., 2013). However, some studies found that there is no difference in rates of depression between aMCI and non-aMCI groups (Brown et al., 2014). MCI patients with depressive symptoms showed more severe behavioral symptoms and verbally agitated behavior (Van der Mussele et al., 2013), and some studies reported an increased AD risk in MCI patients with depression (Modrego and Ferrández, 2004), whereas other studies reported no effect (Rozzini et al., 2005; Palmer et al., 2007).

MCI Patients With Depression Have More Cognitive Deficits

Patients with depression and MCI scored worse in memory function (Modrego and Ferrández, 2004; Brunet et al., 2011; Yoon et al., 2017), and the executive function, dementia screening, flexibility, and lexico-semantic function were significantly worse in patients with MCI with stable depression than MCI patients without depression (Lee G. J. et al., 2012). Though not every study used the same instruments, the results were consistent. The cognitive function of patients with both MCI and depression was worse than patients without depression in most studies, but there was no difference in visuoconstructional abilities, visuoperceptual abilities, and results on the Boston Naming Test in some studies between patients with MCI and those without MCI (Brunet et al., 2011; Steenland et al., 2012).

Depressed individuals tend to have lower processing speeds; (Nebes et al., 2000; Sheline et al., 2006) exhibit worse performances during tasks involving selective attention, response inhibition, and performance monitoring; and lower acquisition and retrieval of new information than non-depressed individuals (Beats et al., 1996). Late-life depression is related to deficits in short-term memory. In older subjects without dementia, depressive symptoms are associated with memory complaints (Zandi, 2004) and worse cognitive performance (Sheline et al., 2006), such as executive functions (Elderkin-Thompson et al., 2006; Sheline et al., 2006), attention, and processing speed (Elderkin-Thompson et al., 2006). MCI patients with depression also have significantly lower scores on immediate memory and delayed memory indices than MCI patients without depression (Johnson et al., 2013).

Depressive symptomatology might precede the development of cognitive decline by a decade or more (Geda et al., 2004) and is a clinical correlate of memory awareness in patients with AD dementia (O'Connell et al., 2014). Depression exacerbates pre-existing cognitive impairment by depleting cognitive reserve or otherwise lowering the threshold for the clinical manifestation of dementia (Jorm, 2001). Neuropsychiatric syndromes of apathy and depression may represent earlier signs of neurodegeneration than cognitive or functional impairments, and these behavioral prodromes may also predict different cognitive and functional trajectories (Zahodne and Tremont, 2013). Semantic deficits in aMCI are somewhat associated with the presence of concomitant depressive symptoms, but depression alone cannot account solely for the semantic deficits (Brunet et al., 2011). The presence of functional impairment was excluded in early definitions of MCI, but some recent studies have reported varying degrees of functional impairment associated with MCI (Farias et al., 2006). Furthermore, functional impairment is a defining feature of MCI and is partially dependent on the degree of cognitive impairment, and functional ability seems to be more related to depression (Bombin et al., 2012). Executive functions are independently related to anxiety disorders in MCI patients (Rozzini et al., 2009).

MCI Patients With Depression Have Higher Conversion Rates to Dementia

The reported annual conversion rate of MCI to dementia was between 25% and 28% in the population of MCI patients with depression; MCI patients with stable depression demonstrated a significantly higher rate of conversion to AD (31%) compared to MCI patients without depression (13.5%; Lee G. J. et al., 2012). The results of a log-rank test were consistent with those findings (Modrego and Ferrández, 2004). Previous studies have found that the annual conversion rate from MCI to dementia is 4.2% in the general population and 10%–15% in high-risk clinical samples (Mitchell and Shiri-Feshki, 2009; Petersen et al., 2009). Depression is a major risk factor for incidence of dementia and is associated with greater atrophy in AD-affected regions; thus depression in individuals with MCI may be associated with underlying neuropathological changes, and depression may be a potentially useful clinical marker for identifying MCI patients who are most likely to progress to AD (Lee G. J. et al., 2012). Late-life depression is a strong risk factor for normal subjects progressing to MCI. The "always depressed" have only a modest increased risk of progression from MCI to AD, but there is no effect of prior depression (Steenland et al., 2012). A Geriatric Depression Scale (GDS) score of 6 or higher is independently associated with a much greater likelihood of developing MCI after adjusting for age, education, alcohol use, benzodiazepine use, and study site, which indicates that elevated depressive symptoms are an important risk factor for cognitive disorders and lower cognitive performance among women living to their ninth and tenth

decades (Spira et al., 2012). The association between depression and cognition decline is more pronounced in MCI than AD (Lee et al., 2019). Disease progression in AD can be measured in different ways such as everyday cognition and instrumental activities of daily living (IADL; Weintraub et al., 2018). However, different types of neuropsychiatric symptoms predict different measures of AD disease progression, e.g., Affective syndromes characterized by depressive symptoms are associated with faster functional decline whereas Manic syndromes are better at predicting cognitive decline (Palmer et al., 2011). Individuals with greater symptoms of the hyperactivity and mood items on the Neuropsychiatric Inventory-Questionnaire (NPI-Q) and the presence of depressive symptoms in patients with amyloid-positive MCI are more associated with progression to AD dementia (Moon et al., 2017; Sugarman et al., 2018). A recent meta-analysis showed depressive symptoms in MCI predicted dementia in community-based studies (RR = 1.69; Tan et al., 2019).

However, there is research that demonstrated contrary conclusions. A 3-year prospective study of MCI outpatients demonstrated no increased risk of AD in patients with symptoms of depression (Palmer et al., 2010). One study found a strong negative influence of depression on conversion to AD in MCI patients (Defrancesco et al., 2017), while another study showed that depressive symptoms are not associated with the rate of progression to dementia in MCI patients and that gender moderated the association between depressive symptoms and conversion to dementia (Panza et al., 2008). Other studies found that the increased endorsement of memory problems is the only significant predictor of conversion to dementia, which likely represents insight into cognitive problems more than depressive symptomatology in MCI individuals (Mackin et al., 2012). The different results about depression in MCI may be because studies did not account for length of depression, incident depression, if it was treated, etc., since depression is not a stable state and the clinical characteristics of the depression may play a role. One study found that persistent or incident depression worsens cognitive outcome while no or recovered depression does not affect it in early AD patients (Spalletta et al., 2012). Further studies on the type and clinical characteristics of depression in MCI patients are needed.

Vascular factors play an important role in depression within preclinical dementia. In MCI patients, new onset of depression was associated with deep subcortical cerebral white matter hyperintensity severity (Kim et al., 2016). Another study showed that the cognitive decline was associated with vascular burden (white matter hyperintensity) in remitted geriatric depression patients but neurodegeneration (left hippocampal volume) in aMCI patients (Ye et al., 2017). A cohort with 35,791 participants that were followed up for 13 years showed that individuals with depression had a higher risk of dementia, and that, furthermore, depression had a more significant effect on participants with incident stroke or newly diagnosed hypertension, which indicated that targeting vascular disorders might lower dementia risk (Köhler et al., 2015). Magnetic resonance imaging (MRI) signs are independent risk factors for dementia and MCI; in a cohort study of 1,553 participants, vascular changes (subcortical microhemorrhages and infarcts) were more important in the development of MCI than in its progression to dementia, while AD signature region volume was important in both stages (Wu et al., 2019).

Apathy: A More Important Indicator

The reported prevalence of apathy in MCI is between 10.7% and 44.8% (Palmer et al., 2010; Chan et al., 2011; Richard et al., 2012; Pink et al., 2015). Studies report differences in apathy prevalence depending on MCI type: 6.9% in amnestic-MCI and 14.7% in multidomain-MCI (Di Iulio et al., 2010). Apathy is defined as diminished motivation for at least 4 weeks and accompanied by any two of the following: reduced goal-directed behavior, reduced goal-directed cognitive activity, and reduced emotions (Robert et al., 2009). Apathy may be more likely to occur in those with frontal lobe deficits (Brodaty et al., 2005). Increased apathy is found to mediate the relationship between cognition and depression (Funes et al., 2018). Apathy is the most common and persistent neuropsychiatric symptom in AD patients, occurring in 55% of dementia patients (Aalten et al., 2007), which is a prevalent neuropsychiatric manifestation in individuals with AD. It can exert a greater impact on daily functioning than depression, which increases reliance on caregivers (Zahodne and Tremont, 2013).

Patients with MCI with apathy have an increased risk of dementia, independent of depression. A systematic review found that apathy was associated with an approximately two-fold increased risk of dementia in memory clinic patients (van Dalen et al., 2018). Robert et al. (2006) found that MCI patients with apathy develop AD more than MCI patients without apathy. After adjusting for the baseline diagnosis of apathy, the risk of progressing to AD in MCI patients with apathy is more than several times higher than in MCI patients without apathy, while there is no increased risk of progressing to AD for MCI patients with depression compared to MCI patients without depression (Palmer et al., 2010). Furthermore, Robert et al. (2008) subdivided the symptoms of apathy and found that the risk of conversion to AD was significantly higher for patients presenting a lack of interest, even after using Cox's analyses that controls for age, gender, and education. To determine whether apathy is a more important indicator than depression and anxiety for converting MCI to dementia, further studies are needed.

Anxiety: Another Important Symptom

Anxiety symptoms have been studied less than depression, and the relationship between anxiety and cognition is complex. The reported prevalence of anxiety in MCI patients ranged between 9.9%–52% (Lyketsos et al., 2002; Palmer et al., 2007; Chan et al., 2011; Gallagher et al., 2011). A meta-analysis showed that the prevalence of anxiety in patients with MCI was 14.3% in community-wide samples and was 31.2% in clinic-based samples (Chen et al., 2018). Such inconsistencies in reported prevalence may be attributed to differences in recruitment strategies and methodology (Gallagher et al., 2011). There is a high rate of comorbid depressive disorders in MCI patients with anxiety, confirming a positive correlation

between the two neuropsychiatric disturbances in both demented and non-demented older people (Porter et al., 2003; Rozzini et al., 2009). Anxiety symptoms have been found to have a strict interaction with executive functions in MCI, and thus they may be a marker of incipient cognitive decline in MCI (Rozzini et al., 2009).

Generalized anxiety disorder is the main anxiety disorder associated with poor global cognitive functioning, and this association is moderated by sex but not by the presence of depressive episodes (Potvin et al., 2011). Anxiety symptoms are a risk factor for AD in the older adults with MCI in population-based samples (Palmer et al., 2007) but not in clinical samples (Devier et al., 2009). Biringer et al. (2005) observed that a high anxiety level was related to cognitive functioning only when it occurred with depressive symptoms, whereas Paterniti et al. (1999) found that a high anxiety level was associated with poor global cognitive functioning in non-depressed men. Some results demonstrated that high anxiety levels were negatively associated with cognitive performance (Bierman et al., 2005), while others suggested that co-morbid depressive symptoms accounted for this association (Biringer et al., 2005). Anxiety symptoms improved the predictive validity of MCI for identifying future AD, suggesting that mood-related depressive symptoms in preclinical AD may be related to the neuropathologic mechanism (Palmer et al., 2007).

Anticipatory anxiety is significantly associated with earlier conversion to AD, but this association does not remain significant following an adjustment for cognitive status at the baseline; anxiety for upcoming events and purposeless activity frequently co-occur, which indicates anticipatory anxiety may be a marker of severity rather than an independent predictor of disease progression (Gallagher et al., 2011). Devier showed that different risk profiles have been described for state vs. trait anxiety: state anxiety was not a significant predictor of future conversion to AD, but higher trait anxiety predicted a lower risk of future conversion to AD (Devier et al., 2009).

Other investigators have failed to find an association between anxiety symptoms in patients with MCI and an increased risk of conversion to AD (Robert et al., 2008; Devier et al., 2009). Anxiety level is not predictive of cognitive performance on four assessments in a 9-years follow-up (Bierman et al., 2008). and the cross-sectional association between a high level of anxiety and poor cognitive functioning is temporary (Bierman et al., 2005, 2008). Further research with long-term follow-up in larger samples is needed to clarify the role of anxiety in predicting MCI conversion to AD.

CONCLUSIONS AND FUTURE PERSPECTIVES

Depression, anxiety, and apathy are common in MCI patients and are important indicators in the progression to dementia in MCI patients, which emphasizes the importance of assessing depressive symptoms as well as anxiety and apathy in the early stages of cognitive impairment. Further studies are needed to better understand the role and neurobiology of depression, anxiety, and apathy in MCI. Indeed, further studies on observation of larger patient populations and long follow-up are needed.

AUTHOR CONTRIBUTIONS

LM designed and wrote the manuscript.

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REFERENCES

Aalten, P., Verhey, F. R. J., Boziki, M., Bullock, R., Byrne, E. J., Camus, V., et al. (2007). Neuropsychiatric syndromes in dementia. Results from the European Alzheimer Disease Consortium: part I. *Dement. Geriatr. Cogn. Disord.* 24, 457–463. doi: 10.1159/000110738

Adams, K. B. (2001). Depressive symptoms, depletion, or developmental change? Withdrawal, apathy, and lack of vigor in the geriatric depression scale. *Gerontologist* 41, 768–777. doi: 10.1093/geront/41.6.768

Apostolova, L. G., and Cummings, J. L. (2008). Neuropsychiatric manifestations in mild cognitive impairment: a systematic review of the literature. *Dement. Geriatr. Cogn. Disord.* 25, 115–126. doi: 10.1159/000112509

Arbus, C., Hergueta, T., Duburcq, A., Saleh, A., Le Guern, M. E., Robert, P., et al. (2014). Adjustment disorder with anxiety in old age: comparing prevalence and clinical management in primary care and mental health care. *Eur. Psychiatry* 29, 233–238. doi: 10.1016/j.eurpsy.2013.04.002

Basso, M. R., Lowery, N., Ghormley, C., Combs, D., Purdie, R., Neel, J., et al. (2007). Comorbid anxiety corresponds with neuropsychological dysfunction in unipolar depression. *Cogn. Neuropsychiatry* 12, 437–456. doi: 10.1080/13546800701446517

Beats, B. C., Sahakian, B. J., and Levy, R. (1996). Cognitive performance in tests sensitive to frontal lobe dysfunction in the elderly depressed. *Psychol. Med.* 26, 591–603. doi: 10.1017/s0033291700035662

Bierman, E. J. M., Comijs, H. C., Jonker, C., and Beekman, A. T. F. (2005). Effects of anxiety versus depression on cognition in later life. *Am. J. Geriatr. Psychiatry* 13, 686–693. doi: 10.1176/appi.ajgp.13.8.686

Bierman, E. J. M., Comijs, H. C., Rijmen, F., Jonker, C., and Beekman, A. T. F. (2008). Anxiety symptoms and cognitive performance in later life: results from the longitudinal aging study Amsterdam. *Aging Ment. Health* 12, 517–523. doi: 10.1080/13607860802224276

Biringer, E., Mykletun, A., Dahl, A. A., Smith, A. D., Engedal, K., Nygaard, H. A., et al. (2005). The association between depression, anxiety, and cognitive function in the elderly general population—the Hordaland Health study. *Int. J. Geriatr. Psychiatry* 20, 989–997. doi: 10.1002/gps.1390

Bombin, I., Santiago-Ramajo, S., Garolera, M., Vega-González, E. M., Cerulla, N., Caracuel, A., et al. (2012). Functional impairment as a defining feature of: amnestic MCI cognitive, emotional, and demographic correlates. *Int. Psychogeriatr.* 24, 1494–1504. doi: 10.1017/S1041610212000622

Brodaty, H., Altendorf, A., Withall, A., and Sachdev, P. (2010). Do people become more apathetic as they grow older? A longitudinal study in healthy individuals. *Int. Psychogeriatr.* 22, 426–436. doi: 10.1017/s1041610209991335

Brodaty, H., Sachdev, P. S., Withall, A., Altendorf, A., Valenzuela, M. J., and Lorentz, L. (2005). Frequency and clinical, neuropsychological and neuroimaging correlates of apathy following stroke—the Sydney Stroke study. *Psychol. Med.* 35, 1707–1716. doi: 10.1017/s0033291705006173

Brown, P. J., Sneed, J. R., Rutherford, B. R., Devanand, D. P., and Roose, S. P. (2014). The nuances of cognition and depression in older adults: the need for a comprehensive assessment. *Int. J. Geriatr. Psychiatry* 29, 506–514. doi: 10.1002/gps.4033

Brunet, J., Hudon, C., Macoir, J., Belleville, S., Rousseau, F., Bouchard, R. W., et al. (2011). The relation between depressive symptoms and semantic memory in amnestic mild cognitive impairment and in late-life depression. *J. Int. Neuropsychol. Soc.* 17, 865–874. doi: 10.1017/s1355617711000877

Bruscoli, M., and Lovestone, S. (2004). Is MCI really just early dementia? A systematic review of conversion studies. *Int. Psychogeriatr.* 16, 129–140. doi: 10.1017/s1041610204000092

Bryant, C., Jackson, H., and Ames, D. (2008). The prevalence of anxiety in older adults: methodological issues and a review of the literature. *J. Affect. Disord.* 109, 233–250. doi: 10.1016/j.jad.2007.11.008

Byers, A. L., and Yaffe, K. (2011). Depression and risk of developing dementia. *Nat. Rev. Neurol.* 7, 323–331. doi: 10.1038/nrneurol.2011.60

Chan, W. C., Lam, L. C., Tam, C. W., Lui, V. W., Leung, G. T., Lee, A. T., et al. (2011). Neuropsychiatric symptoms are associated with increased risks of progression to dementia: a 2-year prospective study of 321 Chinese older persons with mild cognitive impairment. *Age Ageing* 40, 30–35. doi: 10.1093/ageing/afq151

Chan, J. Y. C., Yiu, K. K. L., Kwok, T. C. Y., Wong, S. Y. S., and Tsoi, K. K. F. (2019). Depression and antidepressants as potential risk factors in dementia: a systematic review and meta-analysis of 18 longitudinal studies. *J. Am. Med. Dir. Assoc.* 20, 279.e1–286.e1. doi: 10.1016/j.jamda.2018.12.004

Chen, C., Hu, Z., Jiang, Z., and Zhou, F. (2018). Prevalence of anxiety in patients with mild cognitive impairment: a systematic review and meta-analysis. *J. Affect. Disord.* 236, 211–221. doi: 10.1016/j.jad.2018.04.110

Christensen, H., Griffiths, K., Mackinnon, A., and Jacomb, P. (1997). A quantitative review of cognitive deficits in depression and Alzheimer-type dementia. *J. Int. Neuropsychol. Soc.* 3, 631–651. doi: 10.1017/s1355617797006310

Copeland, J. R. M., Beekman, A. T. F., Braam, A. W., Dewey, M. E., Delespaul, P., Fuhrer, R., et al. (2004). Depression among older people in Europe: the EURODEP studies. *World Psychiatry* 3, 45–49.

Defrancesco, M., Marksteiner, J., Kemmler, G., Fleischhacker, W. W., Blasko, I., and Deisenhammer, E. A. (2017). Severity of depression impacts imminent conversion from mild cognitive impairment to Alzheimer's disease. *J. Alzheimers Dis.* 59, 1439–1448. doi: 10.3233/jad-161135

Delrieu, J., Desmidt, T., Camus, V., Sourdet, S., Boutoleau-Bretonnière, C., Mullin, E., et al. (2015). Apathy as a feature of prodromal Alzheimer's disease: an FDG-PET ADNI study. *Int. J. Geriatr. Psychiatry* 30, 470–477. doi: 10.1002/gps.4161

Devier, D. J., Pelton, G. H., Tabert, M. H., Liu, X., Cuasay, K., Eisenstadt, R., et al. (2009). The impact of anxiety on conversion from mild cognitive impairment to Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 24, 1335–1342. doi: 10.1002/gps.2263

Di Iulio, F., Palmer, K., Blundo, C., Casini, A. R., Gianni, W., Caltagirone, C., et al. (2010). Occurrence of neuropsychiatric symptoms and psychiatric disorders in mild Alzheimer's disease and mild cognitive impairment subtypes. *Int. Psychogeriatr.* 22, 629–640. doi: 10.1017/S1041610210000281

Dillon, C., Tartaglini, M. F., Stefani, D., Salgado, P., Taragano, F. E., and Allegri, R. F. (2014). Geriatric depression and its relation with cognitive impairment and dementia. *Arch. Gerontol. Geriatr.* 59, 450–456. doi: 10.1016/j.archger.2014.04.006

Donovan, N. J., Locascio, J. J., Marshall, G. A., Gatchel, J., Hanseeuw, B. J., Rentz, D. M., et al. (2018). Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults. *Am. J. Psychiatry* 175, 530–537. doi: 10.1176/appi.ajp.2017.17040442

Elderkin-Thompson, V., Mintz, J., Haroon, E., Lavretsky, H., and Kumar, A. (2006). Executive dysfunction and memory in older patients with major and minor depression. *Arch. Clin. Neuropsychol.* 21, 669–676. doi: 10.1016/j.acn.2006.05.011

Farias, S. T., Mungas, D., Reed, B. R., Harvey, D., Cahn-Weiner, D., and DeCarli, C. (2006). MCI is associated with deficits in everyday functioning. *Alzheimer Dis. Assoc. Disord.* 20, 217–223. doi: 10.1097/01.wad.0000213849.51495.d9

Funes, C. M., Lavretsky, H., Ercoli, L., St Cyr, N., and Siddarth, P. (2018). Apathy mediates cognitive difficulties in geriatric depression. *Am. J. Geriatr. Psychiatry* 26, 100–106. doi: 10.1016/j.jagp.2017.06.012

Gallagher, D., Coen, R., Kilroy, D., Belinski, K., Bruce, I., Coakley, D., et al. (2011). Anxiety and behavioural disturbance as markers of prodromal Alzheimer's disease in patients with mild cognitive impairment. *Int. J. Geriatr. Psychiatry* 26, 166–172. doi: 10.1002/gps.2509

Gallo, J. J., and Lebowitz, B. D. (1999). The epidemiology of common late-life mental disorders in the community: themes for the new century. *Psychiatr. Serv.* 50, 1158–1166. doi: 10.1176/ps.50.9.1158

Ganguli, M., Dodge, H. H., Shen, C., and DeKosky, S. T. (2004). Mild cognitive impairment, amnestic type: an epidemiologic study. *Neurology* 63, 115–121. doi: 10.1212/01.wnl.0000132523.27540.81

Geda, Y. E., Smith, G. E., Knopman, D. S., Boeve, B. F., Tangalos, E. G., Ivnik, R. J., et al. (2004). *De novo* genesis of neuropsychiatric symptoms in mild cognitive impairment (MCI). *Int. Psychogeriatr.* 16, 51–60. doi: 10.1017/s1041610204000067

Hidaka, S., Ikejima, C., Kodama, C., Nose, M., Yamashita, F., Sasaki, M., et al. (2012). Prevalence of depression and depressive symptoms among older Japanese people: comorbidity of mild cognitive impairment and depression. *Int. J. Geriatr. Psychiatry* 27, 271–279. doi: 10.1002/gps.2715

Hughes, T. F., Chang, C.-C. H., Bilt, J. V., Snitz, B. E., and Ganguli, M. (2012). Mild cognitive deficits and everyday functioning among older adults in the community: the Monongahela-Youghiogheny Healthy Aging Team study. *Am. J. Geriatr. Psychiatry* 20, 836–844. doi: 10.1097/jgp.0b013e3182423961

Ismail, Z., Elbayoumi, H., Fischer, C. E., Hogan, D. B., Millikin, C. P., Schweizer, T., et al. (2017). Prevalence of depression in patients with mild cognitive impairment. *JAMA Psychiatry* 74, 58–67. doi: 10.1001/jamapsychiatry.2016.3162

Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., et al. (2018). NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 14, 535–562. doi: 10.1016/j.jalz.2018.02.018

Jessen, F. (2019). What are we trying to prevent in Alzheimer disease? *Dialogues Clin. Neurosci.* 21, 27–34.

Johnson, L. A., Mauer, C., Jahn, D., Song, M., Wyshywaniuk, L., Hall, J. R., et al. (2013). Cognitive differences among depressed and non-depressed MCI participants: a project FRONTIER study. *Int. J. Geriatr. Psychiatry* 28, 377–382. doi: 10.1002/gps.3835

Jorm, A. F. (2001). History of depression as a risk factor for dementia: an updated review. *Aust. N. Z. J. Psychiatry* 35, 776–781. doi: 10.1046/j.1440-1614.2001.00967.x

Juarez-Cedillo, T., Sanchez-Arenas, R., Sanchez-Garcia, S., Garcia-Peña, C., Hsiung, G. Y., Sepehry, A. A., et al. (2012). Prevalence of mild cognitive impairment and its subtypes in the Mexican population. *Dement. Geriatr. Cogn. Disord.* 34, 271–281. doi: 10.1159/000345251

Katzman, M. A., Bleau, P., Blier, P., Chokka, P., Kjernisted, K., Van Ameringen, M., et al. (2014). Canadian clinical practice guidelines for the management of anxiety, posttraumatic stress and obsessive-compulsive disorders. *BMC Psychiatry* 14:S1. doi: 10.1186/1471-244X-14-S1-S1

Kim, S. H., Kang, H. S., Kim, H. J., Ryu, H. J., Kim, M. Y., Seo, S. W., et al. (2013). Neuropsychiatric predictors of conversion to dementia both in patients with amnestic mild cognitive impairment and those with subcortical vascular MCI. *Clin. Neurol. Neurosurg.* 115, 1264–1270. doi: 10.1016/j.clineuro.2012.11.029

Kim, S., Woo, S. Y., Kang, H. S., Lim, S. W., Choi, S. H., Myung, W., et al. (2016). Factors related to prevalence, persistence and incidence of depressive symptoms in mild cognitive impairment: vascular depression construct. *Int. J. Geriatr. Psychiatry* 31, 818–826. doi: 10.1002/gps.4400

Kivelä, S. L., Pahkala, K., and Laippala, P. (1988). Prevalence of depression in an elderly population in Finland. *Acta Psychiatr. Scand.* 78, 401–413. doi: 10.1111/j.1600-0447.1988.tb06358.x

Köhler, S., Buntinx, F., Palmer, K., and van den Akker, M. (2015). Depression, vascular factors, and risk of dementia in primary care: a retrospective cohort study. *J. Am. Geriatr. Soc.* 63, 692–698. doi: 10.1111/jgs.13357

Krell-Roesch, J., Vassilaki, M., Mielke, M. M., Kremers, W. K., Lowe, V. J., Vemuri, P., et al. (2019). Cortical β-amyloid burden, neuropsychiatric symptoms, and cognitive status: the mayo clinic study of aging. *Transl. Psychiatry* 9:123. doi: 10.1038/s41398-019-0456-z

Larrieu, S., Letenneur, L., Orgogozo, J. M., Fabrigoule, C., Amieva, H., Le Carret, N., et al. (2002). Incidence and outcome of mild cognitive impairment in a population-based prospective cohort. *Neurology* 59, 1594–1599. doi: 10.1212/01.wnl.0000034176.07159.f8

Lee, R. S. C., Hermens, D. F., Porter, M. A., and Redoblado-Hodge, M. A. (2012). A meta-analysis of cognitive deficits in first-episode major depressive disorder. *J. Affect. Disord.* 140, 113–124. doi: 10.1016/j.jad.2011.10.023

Lee, C. H., Kim, D. H., and Moon, Y. S. (2019). Differential associations between depression and cognitive function in MCI and AD: a cross-sectional study. *Int. Psychogeriatr.* 31, 1151–1158. doi: 10.1017/s1041610218001527

Lee, G. J., Lu, P. H., Hua, X., Lee, S., Wu, S., Nguyen, K., et al. (2012). Depressive symptoms in mild cognitive impairment predict greater atrophy in Alzheimer's disease-related regions. *Biol. Psychiatry* 71, 814–821. doi: 10.1016/j.biopsych.2011.12.024

Lee, Y., and Shinkai, S. (2005). Correlates of cognitive impairment and depressive symptoms among older adults in Korea and Japan. *Int. J. Geriatr. Psychiatry* 20, 576–586. doi: 10.1002/gps.1313

Luis, C. A., Loewenstein, D. A., Acevedo, A., Barker, W. W., and Duara, R. (2003). Mild cognitive impairment: directions for future research. *Neurology* 61, 438–444. doi: 10.1212/01.wnl.0000080366.90234.7f

Lyketsos, C. G., Lopez, O., Jones, B., Fitzpatrick, A. L., Breitner, J., and DeKosky, S. (2002). Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA* 288, 1475–1483. doi: 10.1001/jama.288.12.1475

Mackin, R. S., Insel, P., Aisen, P. S., Geda, Y. E., Weiner, M. W., and Alzheimer's Disease Neuroimaging Initiative. (2012). Longitudinal stability of subsyndromal symptoms of depression in individuals with mild cognitive impairment: relationship to conversion to dementia after 3 years. *Int. J. Geriatr. Psychiatry* 27, 355–363. doi: 10.1002/gps.2713

Mitchell, A. J., and Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. *Acta Psychiatr. Scand.* 119, 252–265. doi: 10.1111/j.1600-0447.2008.01326.x

Modrego, P. J., and Ferrández, J. (2004). Depression in patients with mild cognitive impairment increases the risk of developing dementia of Alzheimer type: a prospective cohort study. *Arch. Neurol.* 61, 1290–1293. doi: 10.1001/archneur.61.8.1290

Monastero, R., Mangialasche, F., Camarda, C., Ercolani, S., and Camarda, R. (2009). A systematic review of neuropsychiatric symptoms in mild cognitive impairment. *J. Alzheimers Dis.* 18, 11–30. doi: 10.3233/JAD-2009-1120

Moon, B., Kim, S., Park, Y. H., Lim, J. S., Youn, Y. C., Kim, S., et al. (2017). Depressive symptoms are associated with progression to dementia in patients with amyloid-positive mild cognitive impairment. *J. Alzheimers Dis.* 58, 1255–1264. doi: 10.3233/jad-170225

Moretti, F., De Ronchi, D., Palmer, K., Forlani, C., Morini, V., Ferrari, B., et al. (2013). Prevalence and characteristics of mild cognitive impairment in the general population. Data from an Italian population-based study: the Faenza project. *Aging Ment. Health* 17, 267–275. doi: 10.1080/13607863.2012.732034

Muller, M., Tang, M.-X., Schupf, N., Manly, J. J., Mayeux, R., and Luchsinger, J. A. (2007). Metabolic syndrome and dementia risk in a multiethnic elderly cohort. *Dement. Geriatr. Cogn. Disord.* 24, 185–192. doi: 10.1159/000105927

Nebes, R. D., Butters, M. A., Mulsant, B. H., Pollock, B. G., Zmuda, M. D., Houck, P. R., et al. (2000). Decreased working memory and processing speed mediate cognitive impairment in geriatric depression. *Psychol. Med.* 30, 679–691. doi: 10.1017/s0033291799001968

Ng, T. P., Niti, M., Zaw, M. H., and Kua, E. H. (2009). Depressive symptoms and incident cognitive impairment in cognitively well-functioning older men and women. *J. Am. Geriatr. Soc.* 57, 1058–1063. doi: 10.1111/j.1532-5415.2009.02262.x

O'Connell, M. E., Dal Bello-Haas, V., Crossley, M., and Morgan, D. (2014). Clinical correlates of awareness for balance, function and memory: evidence for the modality specificity of awareness. *J. Aging Res.* 2014:674716. doi: 10.1155/2014/674716

Onyike, C. U., Sheppard, J.-M. E., Tschanz, J. T., Norton, M. C., Green, R. C., Steinberg, M., et al. (2007). Epidemiology of apathy in older adults: the Cache County study. *Am. J. Geriatr. Psychiatry* 15, 365–375. doi: 10.1097/01.jgp.0000235689.42910.0d

Palmer, K., Berger, A. K., Monastero, R., Winblad, B., Backman, L., and Fratiglioni, L. (2007). Predictors of progression from mild cognitive impairment to Alzheimer disease. *Neurology* 68, 1596–1602. doi: 10.1212/01.wnl.0000260968.92345.3f

Palmer, K., Di Iulio, F., Varsi, A. E., Gianni, W., Sancesario, G., Caltagirone, C., et al. (2010). Neuropsychiatric predictors of progression from amnestic-mild cognitive impairment to Alzheimer's disease: the role of depression and apathy. *J. Alzheimers Dis.* 20, 175–183. doi: 10.3233/JAD-2010-1352

Palmer, K., Lupo, F., Perri, R., Salamone, G., Fadda, L., Caltagirone, C., et al. (2011). Predicting disease progression in alzheimer's disease: the role of neuropsychiatric syndromes on functional and cognitive decline. *J. Alzheimers Dis.* 24, 35–45. doi: 10.3233/JAD-2010-101836

Panza, F., Capurso, C., D'Introno, A., Colacicco, A. M., Zenzola, A., Menga, R., et al. (2008). Impact of depressive symptoms on the rate of progression to dementia in patients affected by mild cognitive impairment. The Italian longitudinal study on aging. *Int. J. Geriatr. Psychiatry* 23, 726–734. doi: 10.1002/gps.1967

Paterniti, S., Dufouil, C., Bisserbe, J. C., and Alpérovitch, A. (1999). Anxiety, depression, psychotropic drug use and cognitive impairment. *Psychol. Med.* 29, 421–428. doi: 10.1017/s0033291798008010

Petersen, R. C., Roberts, R. O., Knopman, D. S., Boeve, B. F., Geda, Y. E., Ivnik, R. J., et al. (2009). Mild cognitive impairment: ten years later. *Arch. Neurol.* 66, 1447–1455. doi: 10.1080/13607863.2012.732034

Pieruccini-Faria, F., Muir-Hunter, S. W., and Montero-Odasso, M. (2018). Do depressive symptoms affect balance in older adults with mild cognitive impairment? Results from the "gait and brain study". *Exp. Gerontol.* 108, 106–111. doi: 10.1016/j.exger.2018.04.004

Pink, A., Stokin, G. B., Bartley, M. M., Roberts, R. O., Sochor, O., Machulda, M. M., et al. (2015). Neuropsychiatric symptoms, APOE ϵ4 and the risk of incident dementia: a population-based study. *Neurology* 84, 935–943. doi: 10.1212/WNL.0000000000001307

Porter, V. R., Buxton, W. G., Fairbanks, L. A., Strickland, T., O'Connor, S. M., Rosenberg-Thompson, S., et al. (2003). Frequency and characteristics of anxiety among patients with Alzheimer's disease and related dementias. *J. Neuropsychiatry Clin. Neurosci.* 15, 180–186. doi: 10.1176/jnp.15.2.180

Potvin, O., Forget, H., Grenier, S., Préville, M., and Hudon, C. (2011). Anxiety, depression and 1-year incident cognitive impairment in community-dwelling older adults. *J. Am. Geriatr. Soc.* 59, 1421–1428. doi: 10.1111/j.1532-5415.2011.03521.x

Qadir, F., Haqqani, S., Khalid, A., Huma, Z., and Medhin, G. (2014). A pilot study of depression among older people in Rawalpindi, Pakistan. *BMC Res. Notes* 7:409. doi: 10.1186/1756-0500-7-409

Richard, E., Schmand, B., Eikelenboom, P., Yang, S. C., Ligthart, S. A., Moll van Charante, E. P., et al. (2012). Symptoms of apathy are associated with progression from mild cognitive impairment to Alzheimer's disease in non-depressed subjects. *Dement. Geriatr. Cogn. Disord.* 33, 204–209. doi: 10.1159/000338239

Robert, P. H., Berr, C., Volteau, M., Bertogliati, C., Benoit, M., Sarazin, M., et al. (2006). Apathy in patients with mild cognitive impairment and the risk of developing dementia of Alzheimer's disease: a one-year follow-up study. *Clin. Neurol. Neurosurg.* 108, 733–736. doi: 10.1016/j.clineuro.2006.02.003

Robert, P. H., Berr, C., Volteau, M., Bertogliati-Fileau, C., Benoit, M., Guerin, O., et al. (2008). Importance of lack of interest in patients with mild cognitive impairment. *Am. J. Geriatr. Psychiatry* 16, 770–776. doi: 10.1097/jgp.0b013e31817e73db

Robert, P., Onyike, C. U., Leentjens, A. F., Dujardin, K., Aalten, P., Starkstein, S., et al. (2009). Proposed diagnostic criteria for apathy in Alzheimer's disease and other neuropsychiatric disorders. *Eur. Psychiatry* 24, 98–104. doi: 10.1016/j.eurpsy.2008.09.001

Rock, P. L., Roiser, J. P., Riedel, W. J., and Blackwell, A. D. (2014). Cognitive impairment in depression: a systematic review and meta-analysis. *Psychol. Med.* 44, 2029–2040. doi: 10.1017/S0033291713002535

Rodríguez-Sánchez, E., Mora-Simón, S., Patino-Alonso, M. C., García-García, R., Escribano-Hernández, A., García-Ortiz, L., et al. (2011). Prevalence of cognitive impairment in individuals aged over 65 in an urban area: DERIVA study. *BMC Neurol.* 11:147. doi: 10.1186/1471-2377-11-147

Rosenberg, P. B., Mielke, M. M., Appleby, B. S., Oh, E. S., Geda, Y. E., and Lyketsos, C. G. (2013). The association of neuropsychiatric symptoms in MCI with incident dementia and alzheimer disease. *Am. J. Geriatr. Psychiatry* 21, 685–695. doi: 10.1016/j.jagp.2013.01.006

Rosenberg, P. B., Mielke, M. M., Appleby, B., Oh, E., Leoutsakos, J.-M., and Lyketsos, C. G. (2011). Neuropsychiatric symptoms in MCI subtypes: the importance of executive dysfunction. *Int. J. Geriatr. Psychiatry* 26, 364–372. doi: 10.1002/gps.2535

Rozzini, L., Chilovi, B. V., Peli, M., Conti, M., Rozzini, R., Trabucchi, M., et al. (2009). Anxiety symptoms in mild cognitive impairment. *Int. J. Geriatr. Psychiatry* 24, 300–305. doi: 10.1002/gps.2106

Rozzini, L., Chilovi, B. V., Trabucchi, M., and Padovani, A. (2005). Depression is unrelated to conversion to dementia in patients with mild cognitive impairment. *Arch. Neurol.* 62:505; author reply 505–506. doi: 10.1001/archneur.62.3.505-a

Sachdev, P. S., Lipnicki, D. M., Kochan, N. A., Crawford, J., Thalamuthu, A., Andrews, G., et al. (2015). The prevalence of mild cognitive impairment in diverse geographical and ethnocultural regions: the COSMIC collaboration. *PLoS One* 10:e0142388. doi: 10.1371/journal.pone.0142388

Sanford, A. M. (2017). Mild cognitive impairment. *Clin. Geriatr. Med.* 33, 325–337. doi: 10.1016/j.cger.2017.02.005

Sasaki, M., Kodama, C., Hidaka, S., Yamashita, F., Kinoshita, T., Nemoto, K., et al. (2009). Prevalence of four subtypes of mild cognitive impairment and APOE in a Japanese community. *Int. J. Geriatr. Psychiatry* 24, 1119–1126. doi: 10.1002/gps.2234

Shahnawaz, Z., Reppermund, S., Brodaty, H., Crawford, J. D., Draper, B., Trollor, J. N., et al. (2013). Prevalence and characteristics of depression in mild cognitive impairment: the Sydney memory and ageing study. *Acta Psychiatr. Scand.* 127, 394–402. doi: 10.1111/acps.12008

Sheline, Y. I., Barch, D. M., Garcia, K., Gersing, K., Pieper, C., Welsh-Bohmer, K., et al. (2006). Cognitive function in late life depression: relationships to depression severity, cerebrovascular risk factors and processing speed. *Biol. Psychiatry* 60, 58–65. doi: 10.1016/j.biopsych.2005.09.019

Spalletta, G., Caltagirone, C., Girardi, P., Gianni, W., Casini, A. R., and Palmer, K. (2012). The role of persistent and incident major depression on rate of cognitive deterioration in newly diagnosed Alzheimer's disease patients. *Psychiatry Res.* 198, 263–268. doi: 10.1016/j.psychres.2011.11.018

Spira, A. P., Rebok, G. W., Stone, K. L., Kramer, J. H., and Yaffe, K. (2012). Depressive symptoms in oldest-old women: risk of mild cognitive impairment and dementia. *Am. J. Geriatr. Psychiatry* 20, 1006–1015. doi: 10.1097/jgp.0b013e318235b611

Steenland, K., Karnes, C., Seals, R., Carnevale, C., Hermida, A., and Levey, A. (2012). Late-life depression as a risk factor for mild cognitive impairment or Alzheimer's disease in 30 US Alzheimer's disease centers. *J. Alzheimers Dis.* 31, 265–275. doi: 10.3233/JAD-2012-111922

Steffens, D. C., Skoog, I., Norton, M. C., Hart, A. D., Tschanz, J. T., Plassman, B. L., et al. (2000). Prevalence of depression and its treatment in an elderly population: the Cache County study. *Arch. Gen. Psychiatry* 57, 601–607. doi: 10.1001/archpsyc.57.6.601

Sugarman, M. A., Alosco, M. L., Tripodis, Y., Steinberg, E. G., and Stern, R. A. (2018). Neuropsychiatric symptoms and the diagnostic stability of mild cognitive impairment. *J. Alzheimers Dis.* 62, 1841–1855. doi: 10.3233/JAD-170527

Tan, E. Y. L., Köhler, S., Hamel, R. E. G., Muñoz-Sánchez, J. L., Verhey, F. R. J., and Ramakers, I. H. G. B. (2019). Depressive symptoms in mild cognitive impairment and the risk of dementia: a systematic review and comparative meta-analysis of clinical and community-based studies. *J. Alzheimers Dis.* 67, 1319–1329. doi: 10.3233/jad-180513

Thomas, A. J., Gallagher, P., Robinson, L. J., Porter, R. J., Young, A. H., Ferrier, I. N., et al. (2009). A comparison of neurocognitive impairment in younger and older adults with major depression. *Psychol. Med.* 39, 725–733. doi: 10.1017/s0033291708004042

Vadla, D., Bozikov, J., Blazeković-Milaković, S., and Kovacić, L. (2013). [Anxiety and depression in elderly - prevalence and association with health care]. *Lijec. Vjesn.* 135, 134–138.

van Dalen, J. W., van Wanrooij, L. L., Moll van Charante, E. P., Brayne, C., van Gool, W. A., and Richard, E. (2018). Association of apathy with risk of incident dementia: a systematic review and meta-analysis. *JAMA Psychiatry* 75, 1012–1021. doi: 10.1001/jamapsychiatry.2018.1877

van den Kommer, T. N., Dik, M. G., Comijs, H. C., Jonker, C., and Deeg, D. J. H. (2012). The role of lipoproteins and inflammation in cognitive decline: do they interact? *Neurobiol. Aging* 33, 196.e1–196.e12. doi: 10.1016/j.neurobiolaging.2010.05.024

Van der Mussele, S., Bekelaar, K., Le Bastard, N., Vermeiren, Y., Saerens, J., Somers, N., et al. (2013). Prevalence and associated behavioral symptoms of depression in mild cognitive impairment and dementia due to Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 28, 947–958. doi: 10.1002/gps.3909

Vogel, J. W., Doležalová, M. V., La Joie, R., Marks, S. M., Schwimmer, H. D., Landau, S. M., et al. (2017). Subjective cognitive decline and β-amyloid burden predict cognitive change in healthy elderly. *Neurology* 89, 2002–2009. doi: 10.1212/WNL.0000000000004627

Wada, T., Ishine, M., Sakagami, T., Okumiya, K., Fujisawa, M., Murakami, S., et al. (2004). Depression in Japanese community-dwelling elderly—prevalence and association with ADL and QOL. *Arch. Gerontol. Geriatr.* 39, 15–23. doi: 10.1016/j.archger.2003.12.003

Weintraub, S., Carrillo, M. C., Farias, S. T., Goldberg, T. E., Hendrix, J. A., Jaeger, J., et al. (2018). Measuring cognition and function in the preclinical stage of Alzheimer's disease. *Alzheimers Dement.* 4, 64–75. doi: 10.1016/j.trci.2018.01.003

Wen, Z., Bi-Rong, D., Chang-Quan, H., Zhen-Chan, L., Yuan, Z., Hong-Mei, W., et al. (2010). Depression and hypertension among Chinese nonagenarians and centenarians. *Int. J. Geriatr. Psychiatry* 25, 554–561. doi: 10.1002/gps.2364

Wu, A., Sharrett, A. R., Gottesman, R. F., Power, M. C., Mosley, T. H. Jr., Jack, C. R. Jr., et al. (2019). Association of brain magnetic resonance imaging signs with cognitive outcomes in persons with nonimpaired cognition and mild cognitive impairment. *JAMA Netw. Open* 2:e193359. doi: 10.1001/jamanetworkopen.2019.3359

Yaffe, K. (2018). Modifiable risk factors and prevention of dementia what is the latest evidence? *JAMA Intern. Med.* 178, 281–282. doi: 10.1001/jamainternmed.2017.7299

Ye, Q., Su, F., Gong, L., Shu, H., Liao, W., Xie, C., et al. (2017). Divergent roles of vascular burden and neurodegeneration in the cognitive decline of geriatric depression patients and mild cognitive impairment patients. *Front. Aging Neurosci.* 9:288. doi: 10.3389/fnagi.2017.00288

Yoon, S., Shin, C., and Han, C. (2017). Depression and cognitive function in mild cognitive impairment: a 1-year follow-up study. *J. Geriatr. Psychiatry Neurol.* 30, 280–288. doi: 10.1177/0891988717723741

Zahodne, L. B., and Tremont, G. (2013). Unique effects of apathy and depression signs on cognition and function in amnestic mild cognitive impairment. *Int. J. Geriatr. Psychiatry* 28, 50–56. doi: 10.1002/gps.3789

Zandi, T. (2004). Relationship between subjective memory complaints, objective memory performance, and depression among older adults. *Am. J. Alzheimers Dis. Other Demen.* 19, 353–360. doi: 10.1177/153331750401900610

Zivin, K., Llewellyn, D. J., Lang, I. A., Vijan, S., Kabeto, M. U., Miller, E. M., et al. (2010). Depression among older adults in the United States and England. *Am. J. Geriatr. Psychiatry* 18, 1036–1044. doi: 10.1097/JGP.0b013e3181dba6d2

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Low-Density Lipoprotein Cholesterol and Alzheimer's Disease: A Systematic Review and Meta-Analysis

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Objective: To assess the association between low-density lipoprotein cholesterol (LDL-c) and risk of Alzheimer's disease (AD).

Methods: Embase, Pubmed, and Web of Science were searched until June 2019. Standard mean difference (SMD) with 95% confidence intervals (CI) was estimated using random-effects models.

Results: Our meta-analysis of 26 studies revealed higher levels of LDL-c in AD than that of non-dementia controls (SMD = 0.35, 95% CI 0.12–0.58, $p < 0.01$). The meta-regression analysis on confounders showed that age ($p < 0.01$, Adj R-squared = 92.41%) and cardiovascular disease ($p = 0.01$, Adj R-squared = 85.21%), but not the body mass index, education, smoking, hypertension and diabetes mellitus, exerted an impact on the relationship between LDL-c and risk of ICH. Further subgroup analysis of age showed LDL-c levels in AD patients aged 60–70 were higher than that of non-dementia (60 ≤ age < 70: SMD = 0.80, 95% CI 0.23–1.37, $p < 0.01$); but no association between the SMD of AD in LDL-c and age over 70 was noted across the studies (70 ≤ age < 77: SMD = −0.02, 95% CI −0.39∼0.34, $p = 9.0$; 77 ≤ age < 80: SMD = 0.15, 95% CI −0.17∼0.47, $p = 0.35$; ≥80: SMD = 0.53, 95% CI −0.04∼1.11, $p = 0.07$). The concentrations of LDL-c during the quintile interval of 3∼4 were positively associated with AD (121 ≤ concentration < 137: SMD = 0.98, 95% CI 0.13∼1.82, $p = 0.02$; ≥137: SMD = 0.62, 95% CI 0.18∼1.06, $p < 0.01$); whereas there was no correlation between AD and LDL-c within the quintile interval of 1∼2 (103.9 ≤ concentration < 112: SMD = 0.08, 95% CI −0.20∼0.35, $p = 0.59$; 112 ≤ concentration < 121: SMD = −0.26, 95% CI −0.58∼0.06, $p = 0.11$).

Conclusions: Elevated concentration of LDL-c (>121 mg/dl) may be a potential risk factor for AD. This association is strong in patients aged 60–70 years, but vanishes with advancing age.

Keywords: LDL-c, Alzheimer's disease, risk factor, meta-analysis, association

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive and irreversible decline in cognition (Kapogiannis et al., 2019). It accounts for approximate two-thirds of all dementias with an increasing morbidity (Prince et al., 2013) and heavy burden of finance (Reitz and Mayeux, 2014). Recognizing that disease-modifying interventions have the greatest chance of success, the emphasis has shifted to controlling underlying risk factors such as diabetes mellitus (Martinez-Valbuena et al., 2019), hypertension (Barnes and Yaffe, 2011), smoking (Durazzo et al., 2014), sleep disturbances (Sindi et al., 2018), and low educational attainment (Barnes and Yaffe, 2011). Moreover, it is reported that APOE4 affects the pathology of AD by multifaceted mechanisms, including abnormal lipid metabolism, inflammatory alterations, and impairment of astrocyte- and microglia-mediated Aβ clearance (Lin et al., 2018; Jeong et al., 2019). Dyslipidemia mainly high level of low-density lipoprotein cholesterol (LDL-c) is thought to have vascular and neurotoxic effects and is implicated in the pathogenesis of AD (Whitmer et al., 2005).

LDL-c, which is synthesized in the blood vessels and degraded in the liver, is a type of lipoprotein particle that carries cholesterol into cells of peripheral tissue. LDL-c causes atherosclerotic cardiovascular disease (Ference et al., 2017), and lowering LDL-c level has been demonstrated to reduce myocardial infarction and stroke in high-risk populations (Schaefer, 2014; Sabatine et al., 2015). However, whether elevated LDL-c level is related to the risk of AD remains unconfirmed. Several studies reported that patients with AD exhibited higher level of LDL-c when compared with normal controls (Lesser et al., 2009; Wingo et al., 2019). In contrast, some of the studies detected no significant difference in LDL-c level between AD patients and healthy controls (Davidson et al., 2012; Li et al., 2017). The patients included in the above studies did not exclude the use of cholesterol-lowering drugs, which played vague role in pathogenesis of AD and might not represent the true LDL-c level of AD patients. Given these uncertainties and contradictions, it prompted us to conduct a meta-analysis of existing studies without the interference of cholesterol-lowering drugs to elucidate a more precise association between LDL-c and AD than individual studies, with the expectation of an aggregate estimate of AD risk for specified changes in serum LDL-c.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria

Studies were included if they met the criteria as follows: (1) the diagnosis of AD is based on the validated diagnostic criteria. Specifically, the Diagnostic and Statistical Manual of Mental Disorders (DSM) -III, -IV, or -V criteria (American Psychiatric Association, 1980, 1987, 1994, 2013), International Classification of Diseases (ICD)−10 criteria, and the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984) criteria were used for the diagnosis of AD. Six papers that used other diagnostic criteria but were substantially consistent with those we specified were also included (Caramelli et al., 1999; Lesser et al., 2001; Solfrizzi et al., 2002; Macesic et al., 2017; Chen et al., 2019); (2) a measure of association was described for serum LDL-c to incident AD; (2) the mean levels of LDL-c in AD and non-dementia were recorded; (3) study design was case-control study; (4) the report with large sample was used if subjects came from one center. The exclusion criteria were: (1) duplicated publications (2) studies with overlapping data; (3) studies recorded participants receiving statins or other cholesterol-lowering drugs; (4) the complications of severe liver dysfunctions, heart failure and malignant diseases; (5) case reports, animal studies, letters to editor, reviews, and comments.

Literature Search

We sought available studies on the relationship between serum LDL-c and Alzheimer's disease using a retrieval of pubmed (until June 2019), Embase (until June 2019) and Web of Science (1950 to June 2019) databases. Search terms used for the studies were "Alzheimer," "dementia," "cognitive," "amentia," and "low density lipoprotein." Reference lists of involved reviews were also checked for additional articles in the original literature search, limited to English language studies on human subjects.

Data Extraction and Collation

Two investigators independently abstracted all search data and any discrepancies were resolved by group discussion. The general characteristics of included studies were as follows: first author, publication year, country, detection method, male subjects, age, and LDL-c levels (**Table 1**). Other baseline characteristics included body mass index, education, and vascular risk factors such as smoking, hypertension, diabetes mellitus and cardiovascular disease (CVD), as shown in **Supplementary Table 2**. The pooled data on baseline characteristics of included studies were extracted and summarized (**Table 2**). The developed guidelines of preferred reporting items for systematic reviews and meta-analyses (PRISMA) (protocol number: PROSPERO CRD42019127818) were listed (**Supplementary Table 1**) (Moher et al., 2009).

Statistical Analysis

Data analyses were conducted by using the software STATA version 15.0 and Review Manager 5.3. Effect size of standard mean difference (SMD) for continuous variables, or odds ratio (OR) for binary variables, with 95% confidence intervals (CI) were calculated to compare the differences in LDL-c level between AD and non-dementia group. The pooled SMD was assessed by the *Z*-test and the inter-study heterogeneity was estimated by the I^2 test (25, 50, and 75% representing low, moderate, and high degrees of heterogeneity, respectively; Higgins et al., 2003). Fixed effects models were applied for the evidence of statistical heterogeneity ($I^2 < 50\%$, and $p \geq 0.05$); otherwise, random effects models were adopted (Higgins and Thompson, 2002; Higgins et al., 2003). To further assess the sources of heterogeneity, meta-regression analyses were utilized to evaluate the effects of confounding factors on the association between LDL-c levels and AD. A key factor considered was

TABLE 1 | General characteristics of the included studies.

References	Country	Detecting methods	Alzheimer's disease			Controls		
			Male/n	LDL-c, mg/dl	Age, Years	Male/n	LDL-c, mg/dl	Age, Years
Ban et al. (2009)	Japan	Precipitation	79/197	123 ± 2	80 ± 1	29/47	121 ± 4	75 ± 1
Cacabelos et al. (2003)	Spain	NR	NR/147	155.69 ± 39.72	71.73 ± 9.61	NR/109	155.22 ± 43.5	50.20 ± 12.06
Caramelli et al. (1999)	Brazil	NR	24-Nov	131.23 ± 35.53	67.2 ± 10.6	13/32	126.47 ± 31.07	68.2 ± 10.6
Chen et al. (2019)	China	Enzymatic	56/117	130.67 ± 34.73	67.64 ± 6.65	44/117	95.25 ± 23.46	66.06 ± 6.00
Hoshino et al. (2002)	Japan	Precipitation	23/82	119.1 ± 27.7	77.0 ± 6.8	13/40	110 ± 24.4	84.2 ± 3.1
Kouzuki et al. (2018)	Japan	NR	16/42	110.8 ± 39.4	80.5 ± 5.7	5/18	119.2 ± 35.7	75.6 ± 5.5
Kuo et al. (1998)	America	Chromatography	NR/64	124 ± 7	81.6 ± 0.9	NR/36	95.5 ± 5	78.7 ± 1.3
Lehtonen and Luutonen (1986)	Finland	Precipitation	0/22	138.46 ± 51.92	≥90	0/23	114.23 ± 28.85	≥90
Lesser et al. (2001)	America	Precipitation	NR/44	132.5 ± 40.5	87.0 ± 8.5	NR/22	119.5 ± 38	82.0 ± 7
Macesic et al. (2017)	Serbia	Friedewald	18/62	165.38 ± 38.46	73.1 ± 5.8	20/40	126.92 ± 30.77	68.4 ± 5.5
Mamo et al. (2008)	Australia	Centrifugation	NR/10	117.31 ± 10.77	79.2 ± 1.8	NR/10	118.85 ± 7.69	80.5 ± 1.5
Moroney et al. (1999)	America	Friedewald	63/225	111.54 ± 33.46	77.7 ± 6.3	248/764	120 ± 34.23	74.1 ± 5.5
Panza et al. (2003)	Italy	Friedewald	15/49	119.23 ± 34.62	71.6 ± 9.3	13/45	142.31 ± 38.46	65.8 ± 11.6
Paragh et al. (2002)	Hungary	Friedewald	10/30	147.69 ± 23.08	64.3 ± 11.7	14/40	100 ± 23.08	72.3 ± 9.6
Reitz et al. (2004)	America	Friedewald	55/244	120.11 ± 35.8	82.85 ± 7.3	760/2226	120.16 ± 34.3	76.42 ± 6.3
Ryglewicz et al. (2002)	Poland	Enzymatic	NR/26	149 ± 38	67 ± 8.4	NR/46	138 ± 38.2	67.5 ± 6.9
Scacchi et al. (1998)	Italy	Friedewald	23/80	113.08 ± 38.08	83.5 ± 5.9	36/155	132.69 ± 45.38	78.3 ± 7.0
Shafagoj et al. (2018)	Jordan	Enzymatic	14/38	103.9 ± 32.7	74.2 ± 5.4	11/33	113.6 ± 26.4	72.4 ± 6.3
Solfrizzi et al. (2002)	Italy	Friedewald	12/49	117.31 ± 32.69	71.6 ± 9.3	13/45	141.92 ± 37.69	65.8 ± 11.6
Tang et al. (2019)	China	Chromatography	78/143	109.95 ± 25.11	62.89 ± 8.38	75/140	100.63 ± 23	64.10 ± 9.49
Warren et al. (2012)	America	NR	45/150	106.8 ± 36.5	79.5 ± 6.17	61/197	88.3 ± 37.17	70 ± 6.33
Watanabe et al. (2005)	Japan	Friedewald	NR/106	106 ± 34	79 ± 7	NR/227	100 ± 37	76 ± 10
Wolf et al. (2004)	Sweden	Enzymatic	9/25	153.85 ± 38.46	77.9 ± 3.0	8/26	146.15 ± 38.46	78.5 ± 3.0
Yamamoto et al. (2005)	Japan	Friedewald	24/61	108 ± 36	80 ± 6	17/32	105 ± 38	77 ± 5
Yavuz et al. (2008)	Turkey	Enzymatic	49/132	125 ± 37.43	74.1 ± 7.4	52/158	125.6 ± 34.43	74.5 ± 6.3
Wehr et al. (2006)	Poland	Enzymatic	33/97	141.5 ± 40.7	71.8 ± 7.9	65/139	125.6 ± 46.6	70.5 ± 8.8

n, number; LDL-c, low-density lipoprotein cholesterol; NR, not reported.

the adjustment for age, given its modifying effect on LDL-c for the incidence of AD. Subgroup analysis based on age (quartile: $60 \leq$ age < 70, $70 \leq$ age < 77, $77 \leq$ age < 80, and ≥ 80), concentration (quartile: $103.9 \leq$ concentration < 112, $112 \leq$ concentration < 121, $121 \leq$ concentration < 137, and ≥ 137), and sample size (< 50 and more) in a series of studies were performed in LDL-c for the risk estimates of AD. Sensitivity analysis was carried out by removing any one of the studies each time to examine its impact on the pooled risk estimates. Publication bias was evaluated by Egger's weighted regression test, and $p < 0.05$ indicated a possible risk of publication bias (Egger et al., 1997).

RESULTS

Study Selection and Characteristics

The preliminary retrieval generated 1,388 articles, which reduced to 124 by reviewing title and abstract. After inspection of the full text, 98 articles were further excluded. Subsequently, 26 eligible articles including a hand search of citations in the reports of published studies or reviews were selected into the meta-analysis (for detailed steps, see **Figure 1**; Lehtonen and Luutonen, 1986; Kuo et al., 1998; Scacchi et al., 1998; Caramelli et al., 1999; Moroney et al., 1999; Lesser et al., 2001; Hoshino et al., 2002; Paragh et al., 2002; Ryglewicz et al., 2002; Solfrizzi et al., 2002; Cacabelos et al., 2003; Panza et al., 2003; Reitz et al., 2004; Wolf et al., 2004; Watanabe et al., 2005; Yamamoto et al., 2005; Wehr et al., 2006; Mamo

TABLE 2 | Pooled weighted characteristics.

	Alzheimer's disease vs. control arm		*p*-Value
	SMD	95% CI	
Age	0.62	(0.28, 0.95)	< 0.001
Body mass index	−0.31	(−0.48, −0.13)	0.001
Education	0.26	(−0.78, 1.30)	0.626
	Odds ratio	**95% CI**	***p*-Value**
Male gender	0.86	(0.71, 1.04)	0.112
Smoking	1.33	(0.71, 2.47)	0.376
Hypertension	0.91	(0.62, 1.35)	0.639
Diabetes mellitus	1.02	(0.82, 1.26)	0.884
Cardiovascular disease	1.28	(0.61, 2.70)	0.513

SMD, standard mean difference; CI, confidence interval.

et al., 2008; Yavuz et al., 2008; Ban et al., 2009; Warren et al., 2012; Macesic et al., 2017; Kouzuki et al., 2018; Shafagoj et al., 2018; Chen et al., 2019; Tang et al., 2019). **Table 1** showed general characteristics of twenty-six studies involving 2,266 AD patients and 4,767 non-dementia controls. **Table 2** gives details of included studies that provided pooled data on baseline characteristics between AD patients and non-dementia controls. **Figure 2** revealed the standard mean difference of AD in serum LDL-c in each study and the summary SMD for all studies combined. **Table 3** showed the association between LDL-c and AD according to category of age (quartile interval), LDL-c concentration (quartile interval), and sample size ($n < 50$ and more). Meta-regression analyses of age, body mass index (BMI), education, smoking, hypertension, diabetes mellitus (**Figure 3**) and CVD (**Supplementary Figure 1**) were conducted to assess the effects of these confounding factors on the association of LDL-c levels with AD. Stroke data from AD patients and non-dementia controls were insufficient for meta-regression analysis.

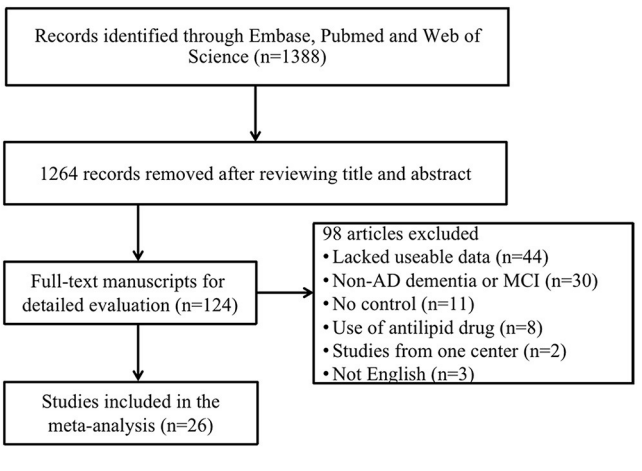

FIGURE 1 | Flow chart of study selection in the meta-analysis. AD, Alzheimer's disease; MCI, mild cognitive impairment.

Meta-Analysis

Random effects models were prespecified to combine estimates from different studies based on existence of high heterogeneity ($I^2 = 92.8\%$, $p < 0.01$). Results from the meta-analysis of 26 studies revealed higher levels of LDL-c in AD than that of non-dementia controls (SMD = 0.35, 95% CI 0.12∼0.58, $p < 0.01$; **Figure 2**), which was consistent with the results of the fixed-effect model (SMD = 0.16, 95%CI 0.10∼0.22, $p < 0.01$; **Supplementary Figure 2**). The meta-regression of confounding factors showed that age ($p < 0.01$, Adj R-squared = 92.41%; **Figure 3A**) and CVD ($p = 0.01$, Adj R-squared = 85.21%; **Supplementary Figure 1**) exerted an effect on the association of LDL-c with AD; whereas other parameters including BMI ($p = 0.063$, Adj R-squared = −6.53%; **Figure 3B**), education ($p = 0.50$, Adj R-squared = −11.58%; **Figure 3C**), smoking ($p = 0.10$, Adj R-squared = 43.90%; **Figure 3D**), hypertension ($p = 0.98$, Adj R-squared = −22.11%; **Figure 3E**) and diabetes mellitus ($p = 0.57$, Adj R-squared = −13.04%; **Figure 3F**) had no impact on the outcomes. As shown in **Table 2**, we found no statistic differences of the pooled weighted characteristics on male gender (OR = 0.86, 95% CI 0.71∼1.04, $p = 0.11$), education (SMD = 0.26, 95% CI−0.78∼1.30, $p < 0.63$), smoking (OR = 1.33, 95% CI 0.71∼2.47, $p = 0.38$), hypertension (OR = 0.91, 95% CI 0.62∼1.35, $p = 0.64$), diabetes mellitus (OR = 1.02, 95% CI

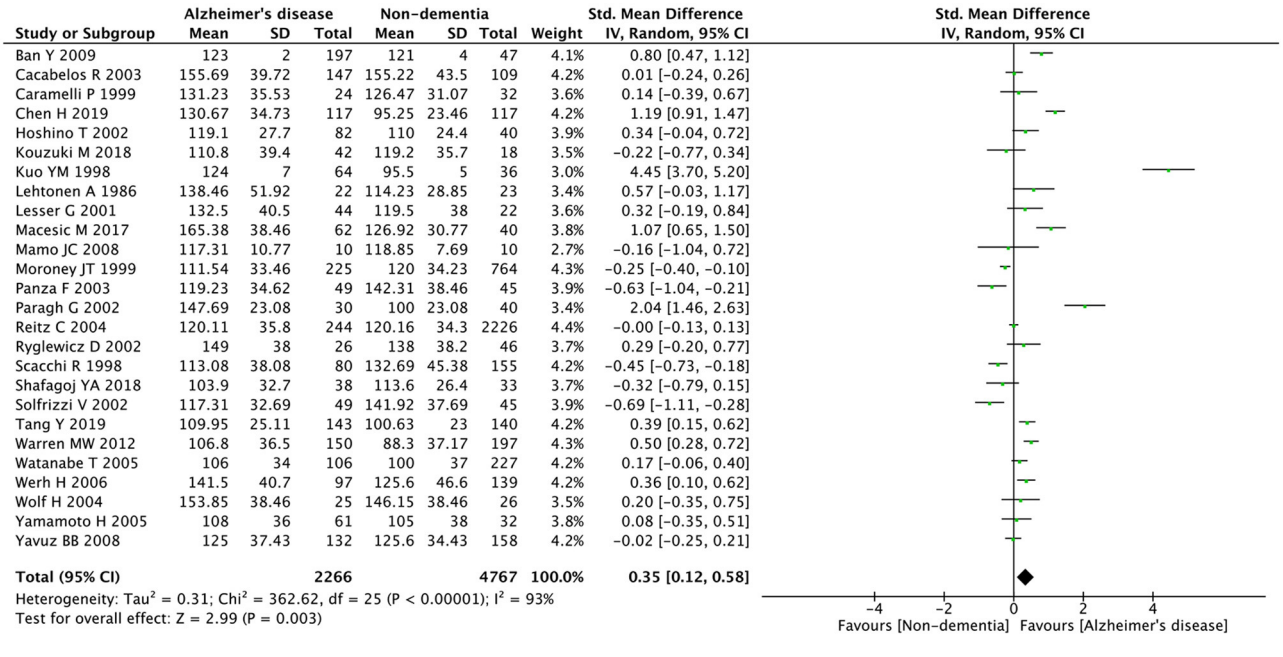

FIGURE 2 | Forest plots of the comparisons in relation to LDL-c levels between Alzheimer's disease and non-dementia. CI, confidence interval.

TABLE 3 | Results of subgroup analysis on age, dose of LDL-c and sample size.

Analyte	Studies	*n* (cases/control)	Stratification	Interval	AD vs. control arm		*p*-value
					SMD	95% CI	
Age (yrs)	5 (Caramelli et al., 1999; Paragh et al., 2002; Reitz et al., 2004; Kouzuki et al., 2018; Chen et al., 2019)	340/375	Quartile1	60–70	0.80	(0.23, 1.37)	< 0.01
	7 (Solfrizzi et al., 2002; Cacabelos et al., 2003; Panza et al., 2003; Wehr et al., 2006; Yavuz et al., 2008; Macesic et al., 2017; Shafagoj et al., 2018)	574/569	Quartile2	70–77	−0.02	(−0.39, 0.34)	0.90
	6 (Moroney et al., 1999; Hoshino et al., 2002; Wolf et al., 2004; Watanabe et al., 2005; Mamo et al., 2008; Warren et al., 2012)	598/1264	Quartile3	77–80	0.15	(−0.17, 0.47)	0.35
	8 (Lehtonen and Luutonen, 1986; Kuo et al., 1998; Scacchi et al., 1998; Lesser et al., 2001; Reitz et al., 2004; Yamamoto et al., 2005; Ban et al., 2009; Kouzuki et al., 2018)	754/2559	Quartile4	≥80	0.53	(−0.04, 1.11)	0.07
Concentration (mg/dl)	7(Moroney et al., 1999; Watanabe et al., 2005; Yamamoto et al., 2005; Warren et al., 2012; Kouzuki et al., 2018; Shafagoj et al., 2018; Tang et al., 2019)	765/1411	Quartile1	103.9–112	0.08	(−0.20, 0.35)	0.59
	6 (Scacchi et al., 1998; Hoshino et al., 2002; Solfrizzi et al., 2002; Panza et al., 2003; Reitz et al., 2004; Mamo et al., 2008)	514/2521	Quartile2	112–121	−0.26	(−0.58, 0.06)	0.11
	6 (Kuo et al., 1998; Caramelli et al., 1999; Lesser et al., 2001; Yavuz et al., 2008; Ban et al., 2009; Chen et al., 2019)	578/412	Quartile3	121–137	0.98	(0.13, 1.82)	0.02
	7 (Lehtonen and Luutonen, 1986; Paragh et al., 2002; Ryglewicz et al., 2002; Cacabelos et al., 2003; Wolf et al., 2004; Wehr et al., 2006; Macesic et al., 2017)	409/423	Quartile4	≥137	0.62	(0.18, 1.06)	< 0.01
Sample size (n)	11 (Lehtonen and Luutonen, 1986; Caramelli et al., 1999; Lesser et al., 2001; Paragh et al., 2002; Ryglewicz et al., 2002; Solfrizzi et al., 2002; Panza et al., 2003; Wolf et al., 2004; Mamo et al., 2008; Kouzuki et al., 2018; Shafagoj et al., 2018)	359/340	Small	< 50	0.13	(−0.30, 0.56)	0.56
	15 (Kuo et al., 1998; Scacchi et al., 1998; Moroney et al., 1999; Hoshino et al., 2002; Cacabelos et al., 2003; Reitz et al., 2004; Watanabe et al., 2005; Yamamoto et al., 2005; Wehr et al., 2006; Yavuz et al., 2008; Ban et al., 2009; Warren et al., 2012; Macesic et al., 2017; Chen et al., 2019; Tang et al., 2019)	1907/4427	Large	≥50	0.44	(0.16, 0.72)	< 0.01

yrs, years; SMD, standard mean difference; CI, confidence interval.

0.82∼1.26, $p = 0.88$) and CVD (OR = 1.28, 95% CI 0.61∼2.70, $p = 0.51$) between AD and non-dementia controls; whilst there was a positive correlation of age (SMD = 0.62, 95% CI 0.28∼0.95, $p < 0.01$) and a inverse correlation of BMI (SMD = −0.31, 95% CI −0.48∼-0.13, $p < 0.01$) between AD and controls. Subgroup analysis on age showed LDL-c levels in AD patients aged 60 to 70 were higher than that of non-dementia (60 ≤ age < 70: SMD = 0.8, 95% CI 0.23∼1.37, $p < 0.01$); but no association between the SMD of AD in LDL-c and age over 70 was noted across the studies (70 ≤ age < 77: SMD = −0.02, 95% CI −0.39∼0.34, $p =$ 9.0; 77 ≤ age < 80: SMD = 0.15, 95% CI −0.17∼0.47, $p = 0.35$; ≥80: SMD = 0.53, 95% CI −0.04∼1.11, $p = 0.07$; **Table 3**). The concentrations of LDL-c during the quintile interval of 3∼4 were positively associated with AD (121 ≤ concentration < 137: SMD = 0.98, 95% CI 0.13∼1.82, $p = 0.02$; ≥137: SMD = 0.62, 95%CI 0.18∼1.06, $p < 0.01$); however, there was no correlation between AD and LDL-c within the quintile interval of 1∼2 (103.9 ≤ concentration < 112: SMD = 0.08, 95% CI −0.20∼0.35, $p = 0.59$; 112 ≤ concentration < 121: SMD = −0.26, 95% CI −0.58∼0.06, $p = 0.11$; **Table 3**). We found an association between LDL-c levels and AD in studies with large sample size (≥50: SMD = 0.44, 95% CI 0.16∼0.72, $p < 0.01$); whilst no association was found in studies with small sample size (<50: SMD = 0.13, 95% CI −0.30∼0.56, $p = 0.56$; **Table 3**).

Sensitivity Analysis and Publication Bias

Sensitivity analyses showed that no single study exerted substantial influence on the pooled effect size after sequentially omitting a study (**Figure 4**). As shown in **Figure 5**, there was no significant evidence of publication bias according to the results of Egger's test ($p = 0.084$).

DISCUSSION

In our comprehensive meta-analysis, 26 eligible studies involving 7,033 participants were summarized to estimate the impact of serum LDL-c on the incident of Alzheimer's disease. To our best acknowledgment, this is the first systematic overview that reported an assessment of LDL-c for AD risk in the absence of cholesterol-lowering drugs and vascular risk factors (e.g., smoking, hypertension, diabetes mellitus, and CVD). Although the heterogeneity across the included studies indicated conflicting views of previous evidence, the pooled effect size exhibited a significant increase in risk of AD for individuals with higher levels of LDL-c. Furthermore, we conducted stratified analyses to explore the underlying relationship between serum LDL-c and AD incidence in a more in-depth way, and meanwhile, tried

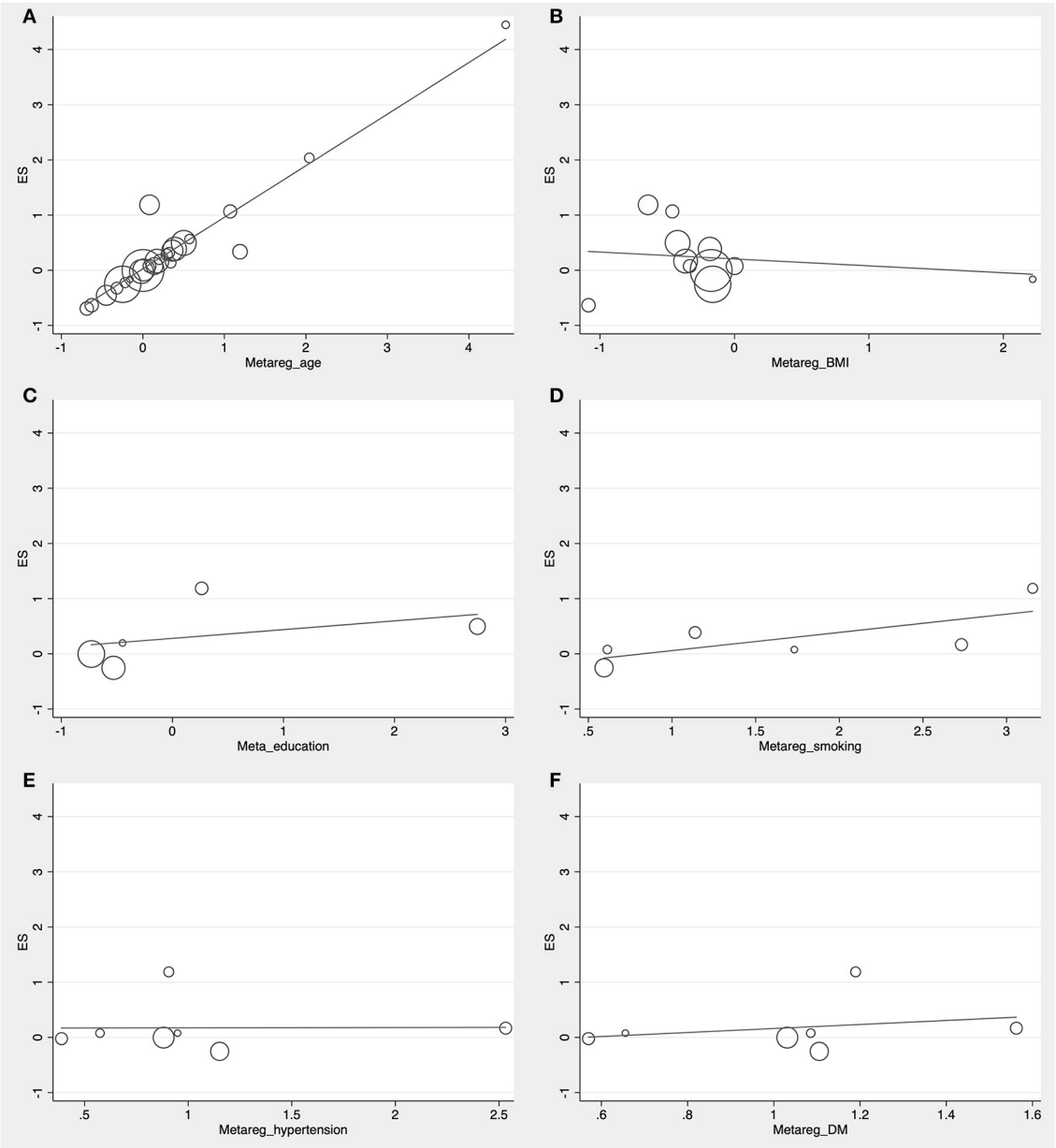

FIGURE 3 | Forest plots of the meta-regression analyses on age **(A)**, body mass index **(B)**, education **(C)**, smoking **(D)**, hypertension **(E)**, and diabetes mellitus **(F)** in relation to LDL-c levels between Alzheimer's disease and non-dementia. ES, effect size.

to find out the factors affecting its correlation by meta-regression analysis.

The results emerging from this meta-analysis revealed that there were higher levels of LDL-c in patients with AD than that of non-dementia controls, implying serum LDL-c likely to be a risk factor for AD. Consistent with our results, an observational study showed that the higher LDL-c level measured before the diagnosis of dementia, the faster the memory loss of AD patients (Helzner et al., 2009). Epidemiologic and experimental data demonstrated that serum LDL-c was involved in the development of Alzheimer amyloid pathology (Pappolla et al., 2003). In practice, however, lipoprotein-bound cholesterol does not flow directly from the bloodstream into the brain, but instead ACTS through an intermediate metabolite linking LDL-c closely to the onset of AD. The neurotoxic oxysterol 27-hydroxycholesterol (27-OHC) is such an extra-cerebral metabolite of cholesterol that crosses the blood-brain barrier. Evidence from AD patients and APP/PS1 mice confirmed that excessive flux of 27-OHC entering the brain led to enhanced deposition of β-amyloid (Zhang et al., 2019) and

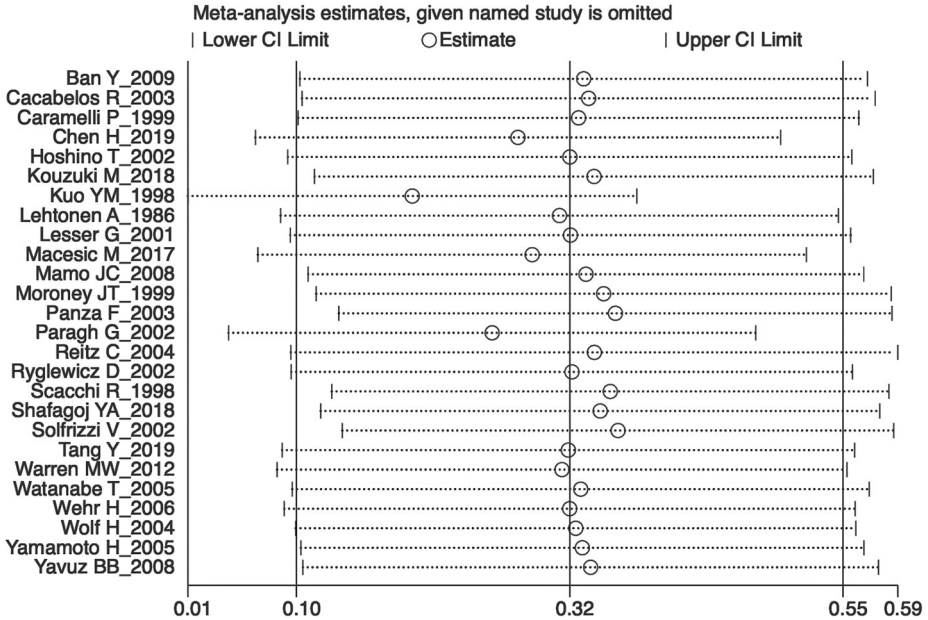

FIGURE 4 | The plot in the sensitivity analysis of current meta-analysis (given named study was omitted).

reduced brain glucose uptake (Ismail et al., 2015). In primary cultures of rat hippocampal cells, 27-OHC decreased expression of the "memory protein" Arc (activity regulated cytoskeleton associated protein), and thus to accelerating the process of neurodegeneration such as AD (Björkhem et al., 2009; Heverin et al., 2015). Additionally, a population-based autopsy study revealed an accumulation of 27-OHC in brains of AD patients, which partially supported its role as a major pathogenetic factor (Shafaati et al., 2011). This accumulation was a subsequent consequence of elevated LDL-c level in the circulation; in turn, lowering LDL-c level was supposed to have a causal effect on the reduction of AD risk, as validated by a large-scale Mendelian randomization study of 111,194 individuals (Benn et al., 2017).

Qualitative determination of the association between AD risk and elevated LDL-c level is not sufficient; moreover, quantifying the impact of alterations in LDL-c concentration on the incidence of AD appears to be more meaningful. After the exclusion of differences in relation to vascular risk factors (e.g., smoking, hypertension, diabetes mellitus, and CVD) between AD patients and non-dementia controls, subgroup analysis on concentration showed that LDL-c level above 121 mg/dl was positively related to AD; whereas no association was found when LDL-c level dropped to 103.9–121 mg/dl. Due to the lack of relevant data in the selected studies, we do not certain whether LDL-c level below 103.9 mg/dl has implications on AD. Previous study showed that reduction of LDL-c level by mutations in PCSK9 and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) exerted no causal effect on high risk of AD (Benn et al., 2017). It can be argued that probably a small amounts of AD patients with PCSK9 and HMGCR variants were recruited in the eligible studies examined in the current meta-analysis, which may partially offset our findings. If that's the case, elevated LDL-c level is more strongly related to risk of AD. However, it has been suggested that extremely low levels of cholesterol are potentially detrimental to neurocognitive function. The reason may be that cholesterol accounting for 85% of the brain is an essential component for the synaptogenesis of myelin axons (Björkhem and Meaney, 2004; Krakowski and Czobor, 2011). Evidence from the Framingham Heart Study demonstrated that normal cognitive performance required a certain level of cholesterol to maintain (Elias et al., 2005), so the reduction of LDL-c to different levels is associated with either cognitive impairment or improvement (Rojas-Fernandez et al., 2014). Even though there is some volatility in LDL-c level due to the average data extracted, the results of subgroup analysis would provide certain guiding significance for the treatment of AD with LDL-c lowering; more specifically, it is reasonable to assume that regulation of LDL-c levels between 103.9 and 121 mg/dl might reduce or eliminate the adverse effect of LDL-c on the pathogenesis of AD.

Furthermore, confounding factors that possibly influence the association between LDL-c and AD needed to be investigated due to the high heterogeneity among studies. The data of included studies were sorted out for gender, age, BMI, education, and those except four took account of vascular risk factors such as smoking, hypertension, diabetes mellitus, and CVD. Among these baseline characteristics, both age and BMI showed statistical differences between AD patients and non-dementia controls; that is, AD was positively correlated with age and negatively related to BMI, which was in line with previous results (Helzner et al., 2009; Nordestgaard et al., 2017). However, low BMI was

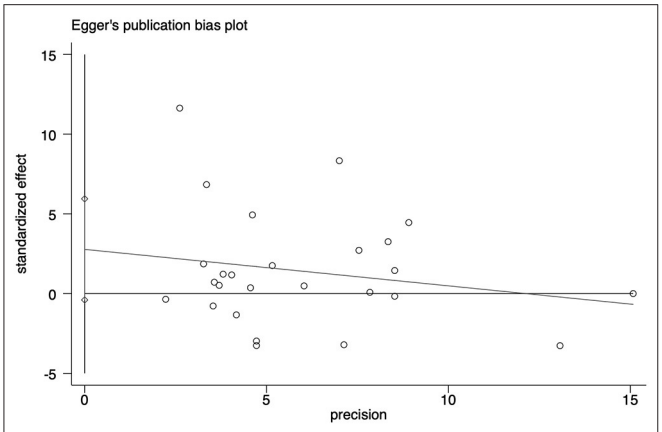

FIGURE 5 | Egger's funnel plot to detect risk of publication bias in the meta-analysis.

not a causal risk factor for AD and that the corresponding observational relationship were possibly attributed to reverse causation or confounding (Nordestgaard et al., 2017). Further meta-regression analysis revealed that not BMI and other confounders including education, smoking, hypertension, and diabetes mellitus, but the age and CVD exerted an impact on the relationship between LDL-c and risk of AD. Consequently, only age had both positive results and was considered more of an effect modifier than a confounder, which might explain 92.41% of the variance seen in this type of meta-analysis. Age imposes the greatest risk for dementia and mortality (Vermunt et al., 2019), and inhibition interventions of aging are possibly linked to LDL-c. Mice treated with metformin, for example, enjoyed an extended span of health and longevity as well as reduction in LDL-c (Martin-Montalvo et al., 2013). In current meta-analysis, subgroup analysis on age showed LDL-c levels higher in AD patients aged 60–70 than that of non-dementia, but no association of AD with LDL-c in patients over the age of 70, indicating that the neurotoxic role of LDL-c in AD may only apply to individuals aged 60–70 and gradually subsides with advancing age. These results were consistent with the Washington Heights/Inwood Columbia Aging Project (Helzner et al., 2009), presumably that enzymatic activity and mRNA level of pancreatic lipase decreased with advancing age (Yamamoto et al., 2014), so did lipid ingestion and absorption, and thus to abnormal LDL-c metabolism. Cardiovascular disease contributes to AD, and both of them mutually affect respective pathological processes (Liu et al., 2014; Bleckwenn et al., 2017), which is consistent with our findings of meta-regression. Previous studies demonstrated that patients with AD are prone to arteriosclerotic microangiopathy, whilst the amounts of senile plaques in brains of patients with CVD are much higher (Sparks et al., 1990; Casserly and Topol, 2004). In addition, subgroup analysis of large sample studies revealed a positive association of LDL-c with AD risk, but no correlation was in subgroup analysis of small sample studies, implying that sample size-related differences had an implication on its correlation. As the precision of summary estimate improves with the increase of sample size, large sample studies more accurately represent the reliability of the relationship between LDL-c and AD. Further studies with LDL-c below 103.9 mg/dl at baseline or after statins therapy in large sample cohorts are required to replenish the association of AD incident with LDL-c.

Limitations

There exist noteworthy limitations on our study. Variability in diagnostic criteria of AD between data sets may affect our results. Moreover, vascular dementia might misclassify as AD due to the overlaps in symptomatology, pathophysiology and risk factors, and approximately one-third of cases diagnosed with AD while alive have no pathological evidence of the disease at autopsy. Although statins may have a medication-specific effect on AD, there is possibly a bias to exclude a large number of studies on the use of statins. The statistical heterogeneity was evident across the individual studies, which might be related to differences in age, concentration of LDL-c and sample size. The results of subgroup analyses were not absolutely conclusive and should be interpreted with caution, as data on age and LDL-c concentration were obtained from the mean value of cases in each study. Differences in general characteristics (e.g., age, CVD) and genetic factors (e.g., APOE4 allele, variants in PCSK9 and HMGCR) between AD patients and non-dementia controls may affect outcomes. Other sources of heterogeneity may be derived from differences in detection methods, cut-off value of LDL-c, dietary intake and exercise habits among various studies. Although Egger's test implied no publication bias in the meta-analysis, systematic reviews in favor of positive findings may lead to potential bias.

CONCLUSIONS

Considering the results of this study, we may resumptively claim that elevated concentration of LDL-c (>121 mg/dl) is a potential risk factor for AD. This strong association is significant in patients with AD aged 60–70 years, but vanishes with increasing age. The present meta-analysis provides a promising strategy for reducing the risk of AD in patients with hyperlipidemia, which may be achieved by regulating LDL-c concentration between 103.9 and 121 mg/dl with statins. Prospective studies that exclude potential confounders, more scientific design, and adequate long-term follow-up are needed to validate this hypothesis.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

The study was conceived by CZ and ZZ. Literature search and selection were conducted by XZ and YL The data were extracted and analyzed by KK, RZ, JX, and CL. The rough manuscript was drafted by ZZ, MZ, and CZ. All authors

corrected and approved the final version of the manuscript after review.

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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.2020.00005/full#supplementary-material

Supplementary Table 1 | The developed guidelines of preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Supplementary Table 2 | Other supplementary baseline characteristics of included studies. n, number; BMI, body mass index; HBP, high blood pressure; CVD, cardiovascular disease; NR, Not reported.

Supplementary Figure 1 | Forest plots of the meta-regression analyses on CVD in relation to LDL-c levels between Alzheimer's disease and non-dementia. CVD, cardiovascular disease; ES, effect size.

Supplementary Figure 2 | Forest plots of the comparisons using the fixed-effect model in relation to LDL-c levels between Alzheimer's disease and non-dementia. CI, confidence interval.

REFERENCES

American Psychiatric Association (1980). *Diagnostic and Statistical Manual of Mental Disorders*, *3rd Edn*. Washington, DC: American Psychiatric Association.

American Psychiatric Association (1987). *Diagnostic and Statistical Manual of Mental Disorders*, *3rd Edn*. Washington, DC: American Psychiatric Association.

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*, *4th Edn*. Washington, DC: American Psychiatric Association.

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders, DSM-5*, *5th Edn*. Washington, DC: American Psychiatric Association. doi: 10.1176/appi.books.9780890425596

Ban, Y., Watanabe, T., Suguro, T., Matsuyama, T. A., Iso, Y., Sakai, T., et al. (2009). Increased plasma urotensin-II and carotid atherosclerosis are associated with vascular dementia. *J. Atheroscler. Thromb.* 16, 179–187. doi: 10.5551/jat.E608

Barnes, D. E., and Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.* 10, 819–828. doi: 10.1016/S1474-4422(11)70072-2

Benn, M., Nordestgaard, B. G., Frikke-Schmidt, R., Tybjærg-Hansen, A. (2017). Low LDL cholesterol, PCSK9 and HMGCR genetic variation, and risk of Alzheimer's disease and Parkinson's disease: Mendelian randomisation study. *BMJ* 357:j1648. doi: 10.1136/bmj.j1648

Björkhem, I., Cedazo-Minguez, A., Leoni, V., and Meaney, S. (2009). Oxysterols and neurodegenerative diseases. *Mol. Aspects Med.* 30, 171–179. doi: 10.1016/j.mam.2009.02.001

Björkhem, I., and Meaney, S. (2004). Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc. Biol.* 24, 806–815. doi: 10.1161/01.ATV.0000120374.59826.1b

Bleckwenn, M., Kleineidam, L., Wagner, M., Jessen, F., Weyerer, S., Werle, J., et al. (2017). Impact of coronary heart disease on cognitive decline in Alzheimer's disease: a prospective longitudinal cohort study in primary care. *Br. J. Gen. Pract.* 67, e111–e117. doi: 10.3399/bjgp16X688813

Cacabelos, R., Fernández-Novoa, L., Lombardi, V., Corzo, L., Pichel, V., and Kubota, Y. (2003). Cerebrovascular risk factors in Alzheimer's disease: brain hemodynamics and pharmacogenomic implications. *Neurol Res.* 25, 567–580. doi: 10.1179/016164103101202002

Caramelli, P., Nitrini, R., Maranhão, R., Lourenço, A. C., Damasceno, M. C., Vinagre, C., et al. (1999). Increased apolipoprotein B serum concentration in Alzheimer's disease. *Acta Neurol. Scand.* 100, 61–63. doi: 10.1111/j.1600-0404.1999.tb00724.x

Casserly, I., and Topol, E. J. (2004). Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. *Lancet* 363, 1139–1146. doi: 10.1016/S0140-6736(04)15900-X

Chen, H., Du, Y., Liu, S., Ge, B., Ji, Y., and Huang, G. (2019). Association between serum cholesterol levels and Alzheimer's disease in China a case-control study. *Int. J. Food Sci. Nutr.* 70, 405–411. doi: 10.1080/09637486.2018.1508426

Davidson, J. E., Lockhart, A., Amos, L., Stirnadel-Farrant, H. A., Mooser, V., Sollberger, M., et al. (2012). Plasma lipoprotein-associated phospholipase A2 activity in Alzheimer's disease, amnestic mild cognitive impairment, and cognitively healthy elderly subjects a cross-sectional study. *Alzheimers Res. Ther.* 4:51. doi: 10.1186/alzrt154

Durazzo, T. C., Mattsson, N., Weiner, M. W., and Alzheimer's Disease Neuroimaging Initiative. (2014). Smoking and increased Alzheimer's disease risk: a review of potential mechanisms. *Alzheimers Dement.* 10, S122–S145. doi: 10.1016/j.jalz.2014.04.009

Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315, 629–634. doi: 10.1136/bmj.315.7109.629

Elias, P. K., Elias, M. F., D'Agostino, R. B., Sullivan, L. M., and Wolf, P. A. (2005). Serum cholesterol and cognitive performance in the Framingham Heart Study. *Psychosom. Med.* 67, 24–30. doi: 10.1097/01.psy.0000151745.67285.c2

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., et al. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur. Heart J.* 38, 2459–2472. doi: 10.1093/eurheartj/ehx144

Helzner, E. P., Luchsinger, J. A., Scarmeas, N., Cosentino, S., Brickman, A. M., Glymour, M. M., et al. (2009). Contribution of vascular risk factors to the progression in Alzheimer disease. *Arch. Neurol.* 66, 343–348. doi: 10.1001/archneur.66.3.343

Heverin, M., Maioli, S., Pham, T., Mateos, L., Camporesi, E., Ali, Z., et al. (2015). 27-Hydroxycholesterol mediates negative effects of dietary cholesterol on cognition in mice. *Behav. Brain. Res.* 278, 356–359. doi: 10.1016/j.bbr.2014.10.018

Higgins, J. P., and Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Stat Med.* 21, 1539–1558. doi: 10.1002/sim.1186

Higgins, J. P., Thompson, S. G., Deeks, J. J., and Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ* 327, 557–560. doi: 10.1136/bmj.327.7414.557

Hoshino, T., Kamino, K., and Matsumoto, M. (2002). Gene dose effect of the APOE-epsilon 4 allele on plasma HDL cholesterol level in patients with Alzheimer's disease. *Neurobiol. Aging* 23, 41–45. doi: 10.1016/S0197-4580(01)00252-4

Ismail, M. A., Mateos, L., Maioli, S., Merino-Serrais, P., Ali, Z., Lodeiro, M., et al. (2015). 27-Hydroxycholesterol impairs neuronal glucose uptake through an IRAP/GLUT4 system dysregulation. *J. Exp. Med.* 214, 699–717. doi: 10.1084/jem.20160534

Jeong, W., Lee, H., Cho, S., and Seo, J. (2019). ApoE4-induced cholesterol dysregulation and its brain cell type-specific implications in the pathogenesis of Alzheimer's disease. *Mol. Cells* 42, 739–746. doi: 10.14348/molcells.2019.0200

Kapogiannis, D., Mustapic, M., Shardell, M. D., Berkowitz, S. T., Diehl, T. C., Spangler, R. D., et al. (2019). Association of extracellular vesicle biomarkers

with Alzheimer disease in the Baltimore Longitudinal Study of Aging. *JAMA Neurol.* 76, 1340–1351. doi: 10.1001/jamaneurol.2019.2462

Kouzuki, M., Nagano, M., Suzuki, T., Katsumata, Y., Nakamura, S., Takamura, A., et al. (2018). Cerebrospinal fluid biomarkers of Alzheimer's disease are associatedwith carotid plaque score and hemodynamics in intra- and extra-cranialarteries on ultrasonography. *J. Clin. Neurosci.* 49, 32–36. doi: 10.1016/j.jocn.2017.12.006

Krakowski, M., and Czobor, P. (2011). Cholesterol and cognition in schizophrenia: a double-blind study of patients randomized to clozapine, olanzapine and haloperidol. *Schizophr Res.* 130, 27–33. doi: 10.1016/j.schres.2011.04.005

Kuo, Y. M., Emmerling, M. R., Bisgaier, C. L., Essenburg, A. D., Lampert, H. C., Drumm, D., et al. (1998). Elevated low-density lipoprotein in Alzheimer's disease correlates with brain abeta 1–42 levels. *Biochem. Biophys. Res. Commun.* 252, 711–715. doi: 10.1006/bbrc.1998.9652

Lehtonen, A., and Luutonen, S. (1986). High-density lipoprotein cholesterol levels of very old people in the diagnosis of dementia. *Age Ageing* 15, 267–270. doi: 10.1093/ageing/15.5.267

Lesser, G., Kandiah, K., Libow, L. S., Likourezos, A., Breuer, B., Marin, D., et al. (2001). Elevated serum total and LDL cholesterol in very old patients with Alzheimer's disease. *Dement Geriatr. Cogn. Disord.* 12, 138–145. doi: 10.1159/000051248

Lesser, G. T., Haroutunian, V., Purohit, D. P., Schnaider Beeri, M., Schmeidler, J., Honkanen, L., et al. (2009). Serum lipids are related to Alzheimer's pathology in nursing home residents. *Dement Geriatr. Cogn. Disord.* 27, 42–49. doi: 10.1159/000189268

Li, H., Zhou, J., Yue, Z., Feng, L., Luo, Z., Chen, S., et al. (2017). A complex association between ABCA7 genotypes and blood lipid levels in Southern Chinese Han patients of sporadic Alzheimer's disease. *J. Neurol. Sci.* 382, 13–17. doi: 10.1016/j.jns.2017.09.016

Lin, Y. T., Seo, J., Gao, F., Feldman, H. M., Wen, H. L., Penney, J., et al. (2018). APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron* 98, 1141–1154.e7. doi: 10.1016/j.neuron.2018.05.008

Liu, G., Yao, L., Liu, J., Jiang, Y., Ma, G., Genetic and Environmental Risk for Alzheimer's disease (GERAD1) Consortium, et al. (2014). Cardiovascular disease contributes to Alzheimer's disease: evidence from large-scale genome-wide association studies. *Neurobiol Aging* 35, 786–792. doi: 10.1016/j.neurobiolaging.2013.10.084

Macesic, M., Lalic, N. M., Kostic, V. S., Jotic, A., Lalic, K., Stefanova, E., et al. (2017). Impaired insulin sensitivity and secretion in patients with Alzheimer's disease: the relationship with other atherosclerosis risk factors. *Curr. Vasc. Pharmacol.* 15, 158–166. doi: 10.2174/1570161114666160905170644

Mamo, J. C., Jian, L., James, A. P., Flicker, L., Esselmann, H., Wiltfang, J., et al. (2008). Plasma lipoprotein beta-amyloid in subjects with Alzheimer's disease or mild cognitive impairment. *Ann. Clin. Biochem.* 45(Pt 4), 395–403. doi: 10.1258/acb.2008.007214

Martinez-Valbuena, I., Valenti-Azcarate, R., Amat-Villegas, I., Riverol, M., Marcilla, I., de Andrea, C. E., et al. (2019). Amylin as a potential link between type 2 diabetes and Alzheimer's disease. *Ann. Neurol.* 86, 539–551. doi: 10.1002/ana.25570

Martin-Montalvo, A., Mercken, E. M., Mitchell, S. J., Palacios, H. H., Mote, P. L., Scheibye-Knudsen, M., et al. (2013). Metformin improves healthspan and lifespan in mice. *Nat. Commun.* 4:2192. doi: 10.1038/ncomms3192

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34, 939–944. doi: 10.1212/WNL.34.7.939

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., and PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 6:e1000097. doi: 10.1371/journal.pmed.1000097

Moroney, J. T., Tang, M. X., Berglund, L., Small, S., Merchant, C., Bell, K., et al. (1999). Low-density lipoprotein cholesterol and the risk of dementia with stroke. *JAMA* 282, 254–260. doi: 10.1001/jama.282.3.254

Nordestgaard, L. T., Tybjærg-Hansen, A., Nordestgaard, B. G., and Frikke-Schmidt, R. (2017). Body mass index and risk of Alzheimer's disease: a Mendelian randomization study of 399,536 individuals. *J. Clin. Endocrinol. Metab.* 102, 2310–2320. doi: 10.1210/jc.2017-00195

Panza, F., Solfrizzi, V., Colacicco, A. M., Basile, A. M., D'Introno, A., Capurso, C., et al. (2003). Apolipoprotein E (APOE) polymorphism influences serum APOE levels in Alzheimer's disease patients and centenarians. *Neuroreport* 14, 605–608. doi: 10.1097/00001756-200303240-00016

Pappolla, M. A., Bryant-Thomas, T. K., Herbert, D., Pacheco, J., Fabra Garcia, M., Manjon, M., et al. (2003). Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. *Neurology* 61, 199–205. doi: 10.1212/01.WNL.0000070182.02537.84

Paragh, G., Balla, P., Katona, E., Seres, I., Egerházi, A., and Degrell, I. (2002). Serum paraoxonase activity changes in patients with Alzheimer's disease and vascular dementia. *Eur. Arch. Psychiatry. Clin. Neurosci.* 252, 63–67. doi: 10.1007/s004060200013

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., and Ferri, C. P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement.* 9, 63–75.e2. doi: 10.1016/j.jalz.2012.11.007

Reitz, C., and Mayeux, R. (2014). Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem Pharmacol.* 88, 640–651. doi: 10.1016/j.bcp.2013.12.024

Reitz, C., Tang, M. X., Luchsinger, J., and Mayeux, R. (2004). Relation of plasma lipids to Alzheimer disease and vascular dementia. *Arch. Neurol.* 61, 705–714. doi: 10.1001/archneur.61.5.705

Rojas-Fernandez, C. H., Goldstein, L. B., Levey, A. I., Taylor, B. A., Bittner, V., and The National Lipid Association's Safety Task Force. (2014). An assessment by the Statin Cognitive Safety Task Force: 2014 update. *J. Clin. Lipidol.* 8, S5–16. doi: 10.1016/j.jacl.2014.02.013

Ryglewicz, D., Rodo, M., Kunicki, P. K., Bednarska-Makaruk, M., Graban, A., Lojkowska, W., et al. (2002). Plasma antioxidant activity and vascular dementia. *J. Neurol. Sci.* 203, 195–197. doi: 10.1016/S0022-510X(02)00290-3

Sabatine, M. S., Giugliano, R. P., Wiviott, S. D., Raal, F. J., Blom, D. J., Robinson, J., et al. (2015). Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N. Engl. J. Med.* 372, 1500–1509. doi: 10.1056/NEJMoa1500858

Scacchi, R., De Bernardini, L., Mantuano, E., Vilardo, T., Donini, L. M., Ruggeri, M., et al. (1998). DNA polymorphisms of apolipoprotein B and angiotensin I-converting enzyme genes and relationships with lipid levels in Italian patients with vascular dementia or Alzheimer's disease. *Dement Geriatr. Cogn. Disord.* 9, 186–190. doi: 10.1159/000017045

Schaefer, S. (2014). ACP Journal Club. Review: in older patients without CVD, statins reduce MI and stroke but not all-cause mortality. *Ann. Intern. Med.* 160:JC8. doi: 10.7326/0003-4819-160-10-201405200-02008

Shafaati, M., Marutle, A., Pettersson, H., Lövgren-Sandblom, A., Olin, M., Pikuleva, I., et al. (2011). Marked accumulation of 27-hydroxycholesterol in the brains of Alzheimer's patients with the Swedish APP 670/671 mutation. *J. Lipid. Res.* 52, 1004–1010. doi: 10.1194/jlr.M014548

Shafagoj, Y. A., Naffa, R. G., El-Khateeb, M. S., Abdulla, Y. L., Al-Qaddoumi, A. A., Khatib, F. A., et al. (2018). APOE Gene polymorphism among Jordanian Alzheimer's patients with relation to lipid profile. *Neurosciences* 23, 29–34. doi: 10.17712/nsj.2018.1.20170169

Sindi, S., Kåreholt, I., Johansson, L., Skoog, J., Sjöberg, L., Wang, H. X., et al. (2018). Sleep disturbances and dementia risk: a multicenter study. *Alzheimers Dement.* 14, 1235–1242. doi: 10.1016/j.jalz.2018.05.012

Solfrizzi, V., Panza, F., Colacicco, A. M., D'introno, A., Capurso, C., Gatti, G., et al. (2002). Serum apolipoprotein E levels in Alzheimer's disease and extreme longevity. *Arch. Gerontol. Geriatr.* 35, 345–352. doi: 10.1016/S0167-4943(02)00126-7

Sparks, D. L., Hunsaker, J. C., Scheff, S. W., Kryscio, R. J., Henson, J. L., and Markesbery, W. R. (1990). Cortical senile plaques in coronary artery disease, aging and Alzheimer's disease. *Neurobiol Aging* 11, 601–607. doi: 10.1016/0197-4580(90)90024-T

Tang, Y., Li, Y. M., Zhang, M., Chen, Y. Q., and Sun, Q. (2019). ε3/4 genotype of the apolipoprotein E is associated with higher risk of Alzheimer's disease in patients with type 2 diabetes mellitus. *Gene* 703, 65–70. doi: 10.1016/j.gene.2019.03.024

Vermunt, L., Sikkes, S. A. M., van den Hout, A., Handels, R., Bos, I., van der Flier, W. M., et al. (2019). Duration of preclinical, prodromal, and dementia stages of Alzheimer's disease in relation to age, sex, and APOE genotype. *Alzheimers Dement.* 15, 888–898. doi: 10.1016/j.jalz.2019.04.001

Warren, M. W., Hynan, L. S., and Weiner, M. F. (2012). Lipids and Adipokines as risk factors for Alzheimer's disease. *J. Alzheimers Dis.* 29, 151–157. doi: 10.3233/JAD-2012-111385

Watanabe, T., Miyazaki, A., Katagiri, T., Yamamoto, H., Idei, T., and Iguchi, T. (2005). Relationship between serum insulin-like growth factor-1 levels and Alzheimer's disease and vascular dementia. *J. Am. Geriatr. Soc.* 53, 1748–1753. doi: 10.1111/j.1532-5415.2005.53524.x

Wehr, H., Bednarska-Makaruk, M., Łojkowska, W., Graban, A., Hoffman-Zacharska, D., Kuczynska-Zardzewiały, A., et al. (2006). Differences in risk factors for dementia with neurodegenerative traits and for vascular dementia. *Dement Geriatr. Cogn. Disord.* 22, 1–7. doi: 10.1159/000092845

Whitmer, R. A., Sidney, S., Selby, J., Johnston, S. C., and Yaffe, K. (2005). Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology* 64, 277–281. doi: 10.1212/01.WNL.0000149519.47454.F2

Wingo, T. S., Cutler, D. J., Wingo, A. P., Le, N. A., Rabinovici, G. D., Miller, B. L., et al. (2019). Association of early-onset Alzheimer disease with elevated low-density lipoprotein cholesterol levels and rare genetic coding variants of APOB. *JAMA Neurol.* 76, 809–817. doi: 10.1001/jamaneurol.2019.0648

Wolf, H., Hensel, A., Arendt, T., Kivipelto, M., Winblad, B., and Gertz, H. J. (2004). Serum lipids and hippocampal volume the link to Alzheimer's disease. *Ann. Neurol.* 56, 745–748. doi: 10.1002/ana.20289

Yamamoto, H., Watanabe, T., Miyazaki, A., Katagiri, T., Idei, T., Iguchi, T., et al. (2005). High prevalence of Chlamydia pneumoniae antibodies and increased high-sensitive C-reactive protein in patients with vascular dementia. *J. Am. Geriatr. Soc.* 53, 583–589. doi: 10.1111/j.1532-5415.2005.53204.x

Yamamoto, K., Kitano, Y., Shuang, E., Hatakeyama, Y., Sakamoto, Y., Honma, T., et al. (2014). Decreased lipid absorption due to reduced pancreatic lipase activity in aging male mice. *Biogerontology* 15, 463–473. doi: 10.1007/s10522-014-9512-5

Yavuz, B. B., Yavuz, B., Halil, M., Cankurtaran, M., Ulger, Z., Cankurtaran, E. S., et al. (2008). Serum elevated gamma glutamyltransferase levels may be a marker for oxidative stress in Alzheimer's disease. *Int. Psychogeriatr.* 20, 815–823. doi: 10.1017/S1041610208006790

Zhang, X., Xi, Y., Yu, H., An, Y., Wang, Y., Tao, L., et al. (2019). 27-hydroxycholesterol promotes Aβ accumulation via altering Aβ metabolism in mild cognitive impairment patients and APP/PS1 mice. *Brain Pathol.* 29, 558–573. doi: 10.1111/bpa.12698

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frontiers in Aging Neuroscience

REVIEW
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The Human Body as a Super Network: Digital Methods to Analyze the Propagation of Aging

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Biological aging is a complex process involving multiple biological processes. These can be understood theoretically though considering them as individual networks—e.g., epigenetic networks, cell-cell networks (such as astroglial networks), and population genetics. Mathematical modeling allows the combination of such networks so that they may be studied in unison, to better understand how the so-called "seven pillars of aging" combine and to generate hypothesis for treating aging as a condition at relatively early biological ages. In this review, we consider how recent progression in mathematical modeling can be utilized to investigate aging, particularly in, but not exclusive to, the context of degenerative neuronal disease. We also consider how the latest techniques for generating biomarker models for disease prediction, such as longitudinal analysis and parenclitic analysis can be applied to as both biomarker platforms for aging, as well as to better understand the inescapable condition. This review is written by a highly diverse and multi-disciplinary team of scientists from across the globe and calls for greater collaboration between diverse fields of research.

Keywords: propagation of aging, network analysis, digital medicine, aging, inflammaging

INTRODUCTION

Aging is the inescapable consequence of life that is common to all. However, the impact of aging on individuals can be very different, where some people live to a high age whilst maintaining excellent physical/mental health yet others may accumulate detrimental symptoms of aging relatively young. This leads to the distinction between "chronological" and "biological" age, where chronological age is an unwavering constant, biological age is a consequence of genetics, environmental exposure, and

lifestyle and may be used as a metric to predict health risks. Unlike chronological aging, the rate of biological aging can change—the potential to distinguish biological from chronological aging, to treat or even reverse it, is the ambition of modern medicine.

Aging is a complex phenomenon in which the combination of genetic, environmental, and stochastic factors leads to highly personalized age-phenotypes. In the past years, researchers have attempted to identify the key tenants of the aging process (López-Otín et al., 2013; Kennedy et al., 2014; **Figure 1**). Despite some differences in the proposed hallmarks of aging, both the studies underline their large interconnectedness. These pillars are not discrete processes and an impact in any area can be propagated through all the other pillars.

Interestingly, the impairment of several (if not all) hallmarks/pillars of aging results in the accumulation of damaged and/or misplaced self-molecules that fuel inflammatory responses, promoting a status of chronic, low-grade and sterile (that is, occurring in the absence of infections) inflammation, that has been termed "inflamm-aging" (Franceschi et al., 2006). Although inflammaging is usually referred to as a systematic proinflammatory status, characterized by an increase in circulating levels of pro-inflammatory cytokines (such as IL-6, CRP), it should be taken into account that (1) inflammaging derives from a balance between pro-inflammatory and anti-inflammatory molecules; and (2) systemic inflammation is the result of the sum of multiple local inflammation events, occurring at the level of specific tissues, organs, and systems (Franceschi et al., 2017a). Inflammaging has been recognized as one of the main triggers of age-related diseases (Franceschi and Campisi, 2014; Furman et al., 2019). In turn, age-related diseases can promote a pro-inflammatory status, thus establishing a vicious circle between inflammaging and age-related diseases (Vitale et al., 2013; Franceschi et al., 2018).

To further compound the complexity, the mechanistic processes of aging occur on many levels, from the molecular (epigenetic, somatic mutation, metabolomic, etc.) in individual cells, tissues and organs, to the population level (genetic). At each strata, the rate and processes of aging and their contribution to inflammaging can be different, leading to a truly chimeric condition in both individuals and the greater population. Several studies suggest, for example, that the liver can age successfully, compared to other organs/systems (Bacalini et al., 2018; Morsiani et al., 2019), and yet this ability changes with age. Genome sequencing has demonstrated that cirrhotic livers have a higher mutational burden compared to normal livers (Brunner et al., 2019). As a consequence, the aging trajectories of an individual are the result of the interaction between organs/systems, each of which, in turn, derives from the combination of specific developmental programs, environmental exposures (i.e., biographies) and genetic backgrounds (Grignolio et al., 2014; Franceschi et al., 2017b, 2019).

Whilst it has been shown that analysis of serum metabolites can distinguish between different organ systems and nutrition (Sato et al., 2018), their variability and sensitivity to change make it challenging to correlate these to disease or age. However, the chemical environment of a cell is not only determined by its extracellular environment, but also by its genomic signature.

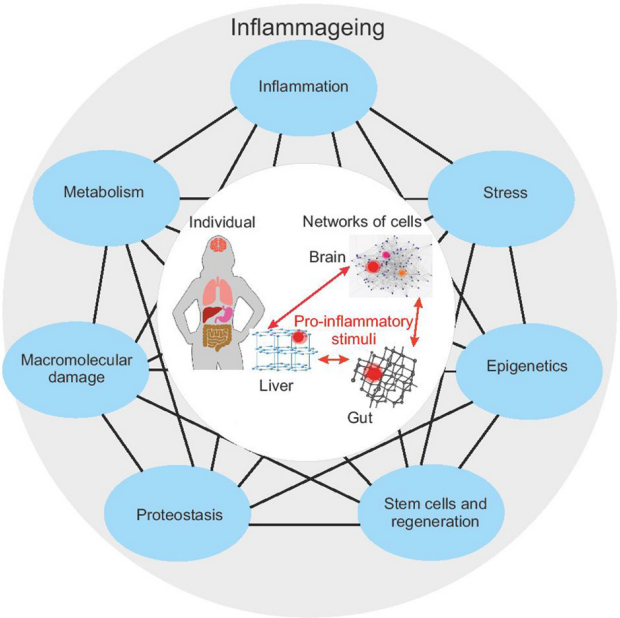

FIGURE 1 | The seven pillars of aging according to Kennedy et al. (2014; blue outer nodes) are connected, such that perturbation in one pillar can affect each other pillar. The impairment of one or more pillars results in a chronic pro-inflammatory status—inflammaging. Inflammaging in a single part of the body can have distal effects on other systems (central circle) and thus can propagate aging. Studying the interaction of all these systems requires a systems biology approach. Given that each pillar or biological process can be represented by its network, this requires a network-of-networks solution.

Several genomic loci have been associated with metabolites (reviewed in Suhre and Gieger, 2012) and a genome-wide association study, reported associations of 400 metabolites with 145 genomic loci (Shin et al., 2014). Analysis of single nucleotide polymorphisms (SNPs) associated with aging and longevity has identified genes in insulin-like growth factor signaling, DNA repair/telomerase maintenance and reactive oxygen species scavenging pathways. Dato et al. (2018) highlight that a single SNP can affect the resultant phenotype by its transmission through multiple genes, therefore to associate SNPs with aging requires the analysis of SNP-SNP interactions. Furthermore, we think this should be extended to links between SNPs and other genetic factors such as epigenetic marks. Analysis of the association between SNPs and aging underpins the genetic contribution to longevity. On one hand, this variation is relatively static and thus their analysis is not affected by chronological age or circadian rhythms. On the other hand, whilst SNPs can be associated with various phenotypic traits, variation in gene expression during aging as a consequence of environment (disease, diet, UV-exposure, etc.) is due to other regulatory pathways, such as epigenetic reprogramming.

Therefore, to fully understand aging, we should consider all age-related processes in unison. This is a challenging

problem, not only from an experimental design perspective but also from the point of data analysis and mathematical modeling. Indeed, it is only now that such an approach may be conceivable using multi-'omic technologies, big data analysis techniques, and super-computing. Significant advancement in the field of medical statistics has been made in the last decade with peak computing performance of 1EFLOP being achieved (Top500.org, 2020). With the corresponding technologies and frameworks, scientists and engineers have unprecedented opportunities to prototype and test various architectures of complex multilevel artificial neural networks and other deep learning techniques capable of analyzing such big data. Indeed, deep neural networks have been used to predict a person's age using a basic blood test (Putin et al., 2017). In general, recent achievements of scientists show that modern systems, including those built on deep neural networks and supercomputer computations, open up new perspectives in the early diagnosis and treatment of several diseases.

This review, aimed towards researchers both in the field of biological aging as well as mathematical modeling, considers the use of multiplexed networks and longitudinal analysis to study the problem of aging, with examples drawn from the study of astroglial cell networks and epigenetics studies of the "biological clock." We also consider approaches to study aging as a longitudinal, continuous phenomenon, rather than in a discrete-ordinal manner. Finally, we comment on the increasing complexity of this field, looking at the future directions, moving from population-level data to generating personalized aging profiles and treatments. The review calls for greater multi-disciplinary research to exploit modern and future capabilities for the study of aging and longevity.

EPIGENETIC AGING AND BIOLOGICAL CLOCK

Epigenetic modifications include a wide range of molecular mechanisms that play a pivotal role in the regulation of gene expression and genomic architecture. Among them, one of the best characterized is DNA methylation, a covalent modification of DNA that occurs preferentially at cytosines in a CpG dinucleotide. DNA methylation patterns are established early during development and can be stably maintained during cell divisions (Jones and Liang, 2009). Besides being relatively stable from a biological point of view, DNA methylation marks are well maintained during DNA and chromatin precipitation. This consideration, combined with the availability of several approaches to measure DNA methylation at a gene-targeted, genome-wide, and whole-genome level, makes this epigenetic modification an ideal candidate to identify longevity biomarkers. Indeed, DNA methylation is dynamically remodeled during several physiological and pathological conditions (Luo et al., 2018) including aging (Bacalini et al., 2017; Ciccarone et al., 2018; Unnikrishnan et al., 2019). Different types of changes to DNA methylation occurs during aging:

(1) Reproducible directional changes (prevalently hypermethylation, but also hypomethylation) of specific CpG sites (Hannum et al., 2013; Horvath, 2013);
(2) Hypomethylation of CpG sites within repetitive regions (Cardelli, 2018);
(3) Increase in the variability of methylation levels of a certain CpG position, considering a general population of individuals (Slieker et al., 2016);
(4) Increase in stochastic epi-mutations, that is, changes in DNA methylation levels of a certain CpG site that are not shared among the individuals of a general population.

So far, attention has been mainly focused on directional age-associated changes in DNA methylation and several CpG sites with tissue-specific age-dependent methylation levels have been described (Hannum et al., 2013). Unfortunately, it is often difficult to establish a causative link between DNA methylation remodeling and aging phenotype. In the studies that assessed methylation and gene expression from the same tissue, only a minor subset of genes with age-associated correlations between DNA methylation and gene expression was identified (Reynolds et al., 2014; Tserel et al., 2015). On the contrary, most of the loci showing hyper- or hypomethylation during aging were associated with genes with low transcription or without age-dependent expression changes (Reynolds et al., 2014; Tserel et al., 2015; Bacalini et al., 2018). Despite this, interesting hints resulted from the analysis of the pathways/ontologies enriched in loci with differential methylation during aging. Several of these studies were performed in whole blood or isolated blood cell types, and accordingly, pathways related to the regulation of immune functions were reproducibly enriched (Wang et al., 2016; Li et al., 2019). Other pathways enriched in loci with age-dependent methylation levels are linked to functions of the extracellular matrix (Wang et al., 2016; Li et al., 2017) and neurotransmission (Ong and Holbrook, 2014). Finally, it is worth noting that multiple studies reported that loci showing hyper- or hypomethylation with aging are enriched in bivalent chromatin domains, usually located in the promoters of developmentally regulated genes.

In recent years researchers have exploited the increased knowledge of age-associated directional changes by developing epigenetic clocks, which are mathematical models that combine the methylation of specific CpG sites (usually below 600) to provide an estimate of the epigenetic age of an individual (Bartlett et al., 2014). Several epigenetic clocks, differing in both the included CpG sites and the human tissues on which they have been validated, have been proposed (Hannum et al., 2013; Horvath, 2013; Weidner et al., 2014; Horvath et al., 2018; Levine et al., 2018). Although with some differences, these clocks have been comprehensively shown to detect age acceleration effects associated to different age-related conditions, spanning from neurodegenerative diseases to cancer and also prospectively reviewed in (Field et al., 2018; Horvath and Raj, 2018). Despite these successful results, much has still to be done in this sense. In particular, the use of appropriate mathematical approaches will likely permit us to develop epigenetic clocks based not only on directional changes in DNA methylation, but also on the other aspects of age-related DNA methylation

remodeling (hypomethylation of repetitive elements, increase in variability and epimutations), thus improving the performance of predictors and broadening the spectra of age-related diseases that could benefit from early diagnosis.

NETWORK MODELING

Mathematical modeling aims to reduce complex problems into defined parameters; adjusting the parameters of the models to provide insight into real systems. Networks present a simple framework to model complex systems that comprise of a large number of interacting elements. The network for any biological system can be represented by nodes (vertices) and links (edges). For example, biomolecules may be represented by vertices and their intermolecular interactions by edges. In this way, all biological systems can be studied in a single framework. Network spectra (eigenvalues) are known to provide rich information on the topological structure and diffusion of signals within them (Sarkar and Jalan, 2018), providing an indirect blueprint of complex systems. With age-related diseases, cancer has received the most attention, from a network theory perspective. For example, network spectra provide a comprehensive approach to analyzing proteomic data for breast, oral, ovarian, cervical, lung, colon, and prostate cancer (Rai et al., 2017). This analysis demonstrated that the protein-protein interaction networks of the normal and cancerous tissues associated with the seven cancers have overall similar topological and spectral properties but some changes in the complexity were unique to different cancers under their study. Similarly, network spectra have been successfully used in many other instances to classify disease states from healthy states of a tissue (Jalan et al., 2015; Rai et al., 2015). Importantly, analysis of common proteins in all cancer networks have helped to reveal proteins which not only occupied significant positions in all the layers, but are also directly involved in causing cancer (Rai et al., 2017). The prediction and analysis of micro-RNAs targeting these proteins provide a hint towards their possible roles in tumorigenesis. This novel approach of network spectra should help in understanding cancer at the fundamental level and provide a clue to develop promising single-drug therapy for multiple diseases as well as personalized medicine.

Biological age acceleration, expressed in epigenetic biomarkers, has not been explicitly related to network signatures of cancer or other age-related diseases. The first step to address this was made by Krivonosov et al. (2020), where parenclitic network analysis (Zanin et al., 2014) was employed to characterize differential DNA methylation of mothers and siblings of Down Syndrome patients. Network indices revealed age and group dependence, and the constructed networks as a whole suggested some associated molecular functions, according to Gene Ontology analysis. The developed approach is a promising tool to access the other cases of accelerated and decelerated aging.

Simplifications are necessary and unavoidable to build a meaningful mathematical model to identify the major biological mechanisms. Finding the right balance between a detailed description and a deeper understanding is an enduring challenge. Already very strong simplifications can lead to unexpected and barely understood behavior as soon as large networks of interacting players are involved. The mammalian brain with its network of spiking neurons is probably one of the most prominent examples in biology. The individual neurons and the synaptic communication amongst them are quite well understood but the orchestrated function as a whole is still puzzling. Mathematical modeling offers an approach to bridge gaps in understanding. For example, at rest, the neurons in the brain are far from being inactive but generate spontaneous firing activity. Detailed functional magnetic resonance imaging (fMRI) of the spontaneous activity has been used as a baseline to classify task-related activation in cognitive studies. These have shown that resting-state activity, first considered as simple noise, contains much more structure and information in a complex non-Gaussian activity pattern, than previously the information contained can be used to reveal functional connections (DeWeese and Zador, 2006; Murphy et al., 2009; Harris and Thiele, 2011; Foster et al., 2016). Invasive and non-invasive electrophysiological recordings and fMRI reveal a remarkable correspondence between spontaneous and task-based parcellations of large-scale functional brain networks across many spatiotemporal scales. This demonstrates that structural properties of neural networks and their functional repertoire can be inferred by the spontaneous neural activity, with clinical applications (Fox and Greicius, 2010). However, the use of fMRI and its variants (e.g., time-varying functional connectivity fMRI) for studying neuronal connectivity remains somewhat controversial owing to the difficulty in suitable controls and a need for better statistical models (Lurie et al., 2020).

Substantial effort has been made to develop simple models of excitatory and inhibitory spiking neurons, aiming to mimic the cortical activity (Gutkin and Ermentrout, 1998; Rauch et al., 2003; Jolivet et al., 2004, 2006; Shlizerman and Holmes, 2012). A review by Gerstner and Kistler (2002) gives some guidelines for extracting relevant dynamical features of networks of integrate-and-fire neuron models to connect these with real measurements (Gerstner and Kistler, 2002). The spontaneous activity or the persistent, selective delay activity are examples of *in vivo* neuron properties that can be linked to simple integrate-and-fire neuron models.

Collective Irregular Dynamics (CID) in so-called balanced networks of spiking neurons can act as a mathematical testbed for the background activity at rest. Balanced networks are such that the excitatory and inhibitory activities compensate each other (Vogels et al., 2005). The CID is a dynamic phenomenon known from dynamic system theory and we propose to transfer the concept to spontaneous background activities observed in the brain. It is a macroscopically observable phenomenon that originated with an orchestrated interplay of individual neurons (Ullner et al., 2018). The considered neuronal networks of spiking neurons are random (Brunel, 2000; Ostojic, 2014). The network is free of any external driving or input and so the resulting complex behavior is fully self-generated. Although the setup of the mathematical model seems simple, the joint activity is far from being trivial. The overall scenario of CID in the

balanced spiking network is reminiscent of the background activity of the brain at rest state.

How can such a paradigmatic model help the medicine to achieve healthy aging? The brain represents one of the target organs of damage for several diseases and undergoes structural and functional changes over its life span. For instance, classical galactosemia is a rare genetic metabolic disorder that impairs the ability to metabolize the sugar galactose. It results in chronic deterioration with a significant influence on the quality of life and general cognitive performance, including alterations to rest-state behavior (van Erven et al., 2017). In another recent example, fMRI or echocardiogram measurements pointed to a possible connection between the modulations of intrinsic resting-state and chronic migraines of female patients (Androulakis et al., 2017). These results demonstrated an overall decrease in resting-state functional connectivity of the default mode network, the salience network, and the central executive network in women with chronic migraines. The connections between the CID phenomenon, the brain's background activity at rest, and age-related diseases are a speculative proposal at an early stage to illustrate the benefits and challenges of such a cross-disciplinary approach. However, mathematical models bridge gaps in knowledge and can be used as a hypothesis testbed to address critical conditions. Brain dynamics at rest might reveal early precursors before changes on cellular or organ levels are detectable. These findings, in turn, inform molecular biology to identify the underlying molecular mechanisms or to understand malfunctions on tissue or organ strata. The real benefit of mathematical modeling unfurls if neuronal data related to diseases are available. The mathematical model could be used to identify the critical network parameter that generates a malfunction. The continuous path from the healthy rest state to pathological behavior in the mathematical model might reveal early precursors to intervene the progression in patients.

Mathematical modeling is a powerful bottom-up tool if developed in close interaction with the biological and medical progress. Aging, understood as a dynamic system, has the potential to change the paradigm from a vain endeavor to fight a disease to a journey in a complicated and diversified landscape with many possible tracks (Hedden and Gabrieli, 2004; Grady, 2012).

Applications to Age-Related Diseases

Aging of the brain is associated with neurodegenerative disorders, the most prevalent of which is Alzheimer's and Parkinson's diseases. These are the most common causes of dementia in the elderly, affecting over 10% of the population over the age of 65 in the United States (Querfurth and LaFerla, 2010). Despite significant research progress, the pathogenesis of Alzheimer's and Parkinson's diseases remain fragmentarily understood, partly due to the extremely complex intercellular cross-talks taking place throughout the aging process (Henstridge and Spires-Jones, 2018; Jagust, 2018; Styr and Slutsky, 2018). Considering the complexity of cellular and molecular interactions, mathematical modeling provides a unique opportunity to further understand the pathogenetic mechanisms of age-related neurodegenerative disorders. There are two recent reviews about mathematical modeling efforts on the whole in neurodegenerative diseases (Lloret-Villas et al., 2017) and in particular in Parkinson's disease (Bakshi et al., 2019). Noteworthy are several mathematical models of the pathogenesis of Alzheimer's disease (AD) which describe the dynamic cross-talks that occur among microglia, astroglia, neurons, and amyloid-β (Aβ; **Figure 2**). Kyrtsos and Baras (2015) proposed a model to study the role of the glymphatic system induced clearance of Aβ from the brain via the perivascular space surrounding cerebral blood vessels in AD.

Experiments have shown that astrocytes play an important role not only in the process of elimination of soluble proteins and metabolites from the central nervous system (CNS; Rasmussen et al., 2018) but also in regulating cellular functions and information transmission in the nervous system (Perea and Araque, 2010; Araque et al., 2014). In contrast with neuronal cells, astrocytes do not generate electrical excitations (action potentials). However, their intracellular dynamics have shown similar excitable properties for changes in calcium concentration (Nadkarni and Jung, 2003; Semyanov, 2019). These signals can affect neuronal excitability and the efficiency of synaptic transmission between neurons by Ca^{2+}-dependent release of gliotransmitters (e.g., glutamate, D-serine, ATP;

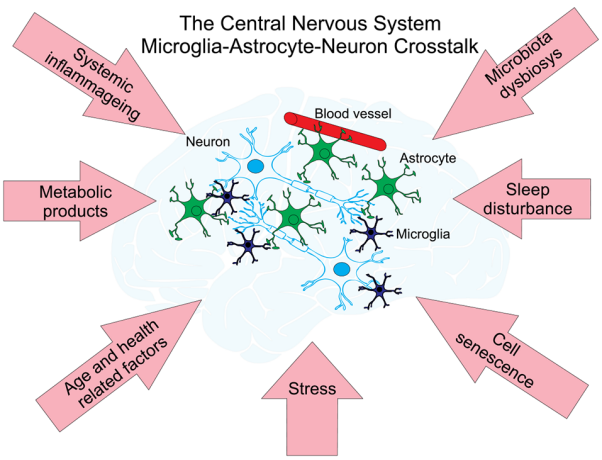

FIGURE 2 | Neuronal networks—cell types and factors interacting with each other affect age-related diseases. There are three main types of cells within neural cellular networks. A neuron or nerve cell is an electrically excitable cell that communicates with other neurons *via* specialized connections called synapses. They are the basic (functional and structural) unit of nervous tissue and the central nervous system (CNS). Astrocytes support neuronal function by providing essential structural and nutritional support, neurotransmitter trafficking and recycling and may also contribute to brain information processing. Astrocytes function as versatile metabolic sensors of CNS milieu and play an important role in the maintenance of brain metabolic homeostasis (for a recent review see Marina et al., 2018). Microglia are the only immune cells that permanently reside in the CNS. In the past decade, studies on microglia have expanded from investigating their function as resident macrophages of the brain and mediators of injury, neuroinflammation and neurodegeneration (reviewed in Salter and Stevens, 2017; Tay et al., 2017) to understanding their origins and non-immunological roles in the CNS. Networks of these cells are under the influence of different factors affecting the development of age-related diseases (pink arrows) and are supporting their metabolism by the interchange of metabolic products with blood vessels.

Savtchouk and Volterra, 2018). It has emerged that astrocytes are interconnected into networks by gap junction channels. Networks of astrocytes accompanying neuronal cells generate collective activity patterns that can regulate neuronal signaling by facilitating or by suppressing synaptic transmission (Perea and Araque, 2010; Araque et al., 2014; De Pittà et al., 2016).

Despite efforts in recent years to model the role of astrocytes in information processing in the CNS (Oschmann et al., 2018; Kanakov et al., 2019), only a few computational models are investigating the role of astrocytes in neurodegenerative diseases. The most popular pathological behavior of astrocytes investigated by modeling is epilepsy (Ullah et al., 2009; Volman et al., 2012; Amiri et al., 2013; Tewari and Parpura, 2013) and AD (Lenk et al., 2016). These computational studies describe the abnormal astrocyte regulation of synaptic transmission and pathological release of gliotransmitters from astrocytes. There has been no model developed to study the age-induced changes in the morphology of astrocytes. However, in experimental studies, it was shown that pathology astrocytes undergo morphological and functional remodeling that is dependent on an injury, neurodegenerative disease, and aging processes (Dossi et al., 2018; Verkhratsky, 2019; Verkhratsky et al., 2019). Such models can be developed based on existing models that take into account realistic cell morphology (Savtchenko et al., 2018; Gordleeva et al., 2019; Wu et al., 2019). The role of astrocytes in neurodegenerative diseases and the aging process requires further investigation. Biophysical models of astrocytic regulation of synaptic transmission in neuronal circuits both at the level of individual cells and at the network level should be developed and investigated for aging based on experimental data. Simulation experiments in large-scale neuron-glial networks reproducing the signaling observed in experiments with aging and neurodegenerative diseases are expected. The need for such studies is related to the identification of targets for the effects of pharmacological agents in the treatment of neurodegenerative diseases caused by violations of neuronal signaling.

Further, there has been no model developed to investigate the role of cellular senescence and the propagation of senescent associated secretory phenotype (SASP) molecules through brain tissue in aging and age-related diseases (Baker and Petersen, 2018). This idea has been conceptualized as inflammageing linked to garb-aging (Franceschi et al., 2018) and is based on a hypothesis that the progressive accumulation of senescent cells (and their pro-inflammatory SASP phenotype) in all organs and tissues contribute to aging/inflammaging and this state can propagate through the tissue or brain network. It will be very interesting to develop a network model describing the interaction between healthy and senescent microglia and astrocytes, the concentration of garbage accumulated during neuronal activity (cellular and molecular garbage: cell debris, resulting from cell death, misplaced/altered/oxidized molecules, gut microbiota products, internal exposome, among others) and cleaned by healthy glial cells via the glymphatic system (Benveniste et al., 2019), and propagation of the signaling SASP molecules in some volume of the brain tissue. Models of such type can help to understand the mechanisms of the inflammageing propagation through the brain network resulting in aging and age-related diseases.

On a molecular level, computational modeling could be a useful way to study AD by handling numerous parameters related to ion channels and electrophysiology. We have noted 10 models are published in ModelDB with the software NEURON (Markaki et al., 2005; Ferrante et al., 2008; Morse et al., 2010; Bhattacharya et al., 2011; Culmone and Migliore, 2012; Romani et al., 2013; Bianchi et al., 2014; Rowan et al., 2014; Coskren et al., 2015; Rumbell et al., 2016; **Table 1**). Here, neural networks are introduced and interfaced with amyloid effect and chemical or electrical stimulation. So far, different channels, chemical agents, synapses, and morphological properties have been modeled for AD. As we understand more about the mechanisms modulating the excitability of AD neurons to a greater extend, modeling brings insights into how to mediate the ongoing damage of AD by chemicals or low-intensity electrostimulation. However, comprehensive modeling of the neural environment, e.g., the role of glial cell-networks during AD is missing.

In addition to the amyloid hypothesis, brain inflammation (increased microglia and astrocyte activation) has been increasingly recognized as a potential mechanism of AD pathogenesis (Heppner et al., 2015; Parbo et al., 2017; Sawikr et al., 2017). Evident changes have been found in microglia and astroglia in the post-mortem brains of AD patients (Heneka et al., 2015). Also, genome-wide analysis suggests that several genes increasing the risk of AD modulate the glial clearance of misfolded proteins and inflammation. The understanding of immune/inflammatory pathways in AD and their regulatory mechanisms should offer opportunities for drug development targeting neuroinflammation (Fu et al., 2019). However, to date, most of the anti-inflammatory drug candidates undergoing clinical trials have failed. Thus, a systems approach to studying AD by combining detailed morphological reconstruction and advanced neural network modeling to cover both neurons and glia of the AD brain may highlight new therapeutic opportunities. The quantitative and systems thinking will provide a big picture for probing AD and effective treatment approaches in the future.

EMERGING STRATEGIES FOR EARLY DIAGNOSIS OF AGE-RELATED DISEASES: BAYESIAN ESTIMATION, NEURAL NETWORKS AND PARENCLITIC ANALYSIS

It has been shown that many of the molecular and cellular mechanisms involved in aging are closely related to those driving the appearance and development of cancerous tumors, either because they are shared or because they are divergent (Finkel et al., 2007; Aunan et al., 2017). Such mechanisms include the role of genomic instability, telomere attrition, epigenetic changes, loss of proteostasis, decreased nutrient sensing and altered metabolism, cellular senescence, and stem cell function (Maslov and Vijg, 2009; Campisi, 2013; Hou et al., 2015). As a consequence, the recent exploration and progress of new technologies to detect early signs of oncological disorders should

TABLE 1 | Summary of 10 computational models on AD study.

Model type	Cell type	Working mechanisms	Main results	Reference
Multi-compartment	CA1 pyramidal neuron	Incorporate different calcium channels	Decreased excitability of aged CA1 cells	Markaki et al. (2005)
Multi-compartment	CA1 pyramidal neuron	Endogenous and exogenous chemical modulation on membrane	Explore the effect of chemicals on neural diseases	Ferrante et al. (2008)
Multi-compartment	CA1 pyramidal neuron	Blocking A-type K^+ currents	Back-propagating action potentials in the dendrites induce hyperexcitability	Morse et al. (2010)
Neural network	Non-specific cells in thalamus and cortex	Excitatory and inhibitory connectivity	Active synapses in the thalamus decrease alpha-band EEG wave	Bhattacharya et al. (2011)
Multi-compartment	CA1 pyramidal neuron	Aβ-peptides progressive accumulation	Multi mechanisms modulate excitability	Culmone and Migliore (2012)
Single neuron with synapses input	CA1 pyramidal neuron	Aβ-induced enhancement of release probability	Alter the spike probability of CA1 pyramidal neurons	Romani et al. (2013)
CA1 network	CA1 pyramidal neurons with interneurons	Increasing the cAMP Response Element Binding protein	CREB-based therapies for AD	Bianchi et al. (2014)
Neural network	Cell populations in one column	Low-intensity electrostimulation	Raise activity and break ongoing damage	Rowan et al. (2014)
Multi-compartment	Pyramidal neuron in monkey cortex	Combining morphology and ion channels	Membrane resistance and changed morphology affect excitability	Coskren et al. (2015)
Multi-compartment	Pyramidal neuron in monkey cortex	Automated parameter optimization	Get many parameters fitting the model	Rumbell et al. (2016)

Abbreviations in the table: Electroencephalogram (EEG); cAMP Response Element Binding Protein (CREB); Alzheimer's disease (AD).

also be relevant for the assessment of significant mismatches between chronological and biological age.

One of the main trends of modern healthcare is directed towards personalized medicine. All individuals differ in genotype and phenotype and thus should be managed differently for disease prevention, detection, and treatment. Modern 'omics technologies are capable of acquiring large amounts of quantitative, or semi-quantitative data (mass spectrometry, quantitative PCR, microarrays, etc.) relatively cheaply, thus there is a large potential to delivering truly personalized medicine based on an individual's molecular profile.

Significant improvements in screening procedures for early cancer detection can be attained by using quantitative tools for the analysis of longitudinal biomarkers—instead of simple cut-off values (McIntosh et al., 2002). This has been recently shown, e.g., for the case of invasive epithelial ovarian cancer, where the use of a single threshold rule is the current norm for interpretation of serum Cancer Antigen 125 (CA125) as a first-line test in ovarian screening (Blyuss et al., 2018). It was demonstrated in the recent United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS; Menon et al., 2015), that it is not an individuals' CA125 measurement that indicates cancer development, rather a deviation from personal baseline. Therefore, recent approaches in ovarian cancer are directed towards constructing personalized baselines based on patients' serial measurements with analyzing further sequential measurements from the perspective of previous history (Whitwell et al., 2020). For example, three approaches have been applied to longitudinal serological data from ovarian cancer: (1) the methods of mean trends (MMT) algorithm (Blyuss et al., 2018) which evaluates the dynamics of longitudinal markers using weighted derivatives of marker changes as well as the average area under the time series, coefficient of variation and "center of mass" as predictors in logistic regression; (2) The Risk of Ovarian Cancer Algorithm (ROCA), that fits Bayesian hierarchical change-point model on CA125 serial data (Skates et al., 2001); and (3) Parametric Empirical Bayes (PEB) that evaluates deviation from normality based on population characteristics such as the population mean and within-subject and between-subject variances. They significantly outperform single CA125 cut-offs, demonstrating the effectiveness of the personalized approach, both in terms of area under the receiver operating curve (AUC) and in terms of sensitivity at a fixed, clinically relevant, specificity.

Sophisticated procedures for early detection of oncological diseases, such as Bayesian computation methods or deep learning techniques (Goodfellow et al., 2016), involving more than one biomarker can further reduce human intervention in the diagnostic process. In particular, it has been recently shown (Mariño et al., 2017) that the combined analysis of a group of specific biomarkers (namely CA125 and Human Epididymis Protein 4 or Glycodelin) improves the detection of change-points (from personal baseline to deviance) in multiple time series data (compared to the analysis of CA125 alone) which, in turn, can be associated with the development of tumors. Similar processes related to the loss of proteostasis play a key role in biological

aging and they can be detected at an early stage employing the same class of quantitative analysis techniques.

Although not as straightforward to interpret, from a clinical point of view, as Bayesian models, deep learning techniques are currently attracting attention in many biomedical applications. In particular, recurrent neural networks can integrate information of multiple biomarkers without the need to construct explicit probabilistic models, as opposed to Bayesian analysis methods. This has been recently shown in (Vázquez et al., 2018), where a quantitative performance study of these two approaches for the diagnosis of ovarian cancer from longitudinal biomarker data has been carried out.

The challenge with large, multi-omic data sets is in analyzing them in a biologically meaningful manner. The difficulty in interpreting large scale data sets is due to the non-linearity of molecular pathways; i.e., for each pathway, there are multiple branching points and multiple levels of regulation such that a perturbation of a single analyte (mRNA, protein, metabolite) may have a cellular effect that is not immediately obvious (Haas et al., 2017). Therefore, taking large data sets and analyzing fold-change of single analytes (mRNA, protein, metabolites, etc.) without taking into account everything else, lacks biological context. To overcome this, it is possible to use pre-defined annotations (gene ontology, pathways) to identify biological patterns. However, this requires prior knowledge regarding the analytes function, currently (November 2019) Swissprot, a manually curated database of proteins contains 561, 356 annotated proteins, whereas TrEMBL, a related database comprised of computationally curated annotations contains 181, 787, 788 proteins—highlighting the enormous black hole that exists in experimentally verified annotations (Bateman et al., 2017), a crucial limitation in these approaches.

One way to address this issue is to use techniques that require no *a priori* knowledge of the analytes. Parenclitic networks, first published by Zanin et al. (2014), identify global changes between two data sets through graph-based analysis, where nodes (vertices) represent analytes and edges between nodes are present if that pair of analytes differ between the two data sets. Thus, analytes that are changing become well connected within the network whereas those that change only a little or not at all are weakly connected. Since the construction of these networks is not based on fold change or p-value (α-value) they are not affected by the inherent bias of these commonly used statistics. The seminal parenclitic article analyzed transcript data from Arabidopsis, and since then have been applied to DNA methylation data (Karsakov et al., 2017), proteomic data (Whitwell et al., 2018) and credit card fraud detection (Zanin et al., 2018). A full description of how to construct parenclitic networks are presented in Whitwell et al. (2018), in which multiple approaches to network construction and the integration of categorical variables into the network are also discussed. When applied to ovarian cancer, the networks provide two levels of information. First logistic regression models of the network topologies were able to distinguish case/control, and second, analysis of individual nodes suggested granzyme H and fibroblast growth factor-binding protein 1 as changing as early as 34 months pre-diagnosis.

The critical feature of longitudinal analysis is to detect, in an individual, when a marker is changing, thus overcoming natural variation of an analyte within a population that may mask diagnosis using simple threshold-based diagnosis. Of course, whilst it is trivial to include new biomarkers in logistic regression models, the lack of available biomarkers hampers the application of this approach. Combining longitudinal analysis with holistic techniques, such as parenclitic networks, that can exploit personalized 'omics screening (e.g., routine transcriptomic, proteomic analysis) could be an important advancement in this field.

SUMMARY AND FUTURE PERSPECTIVE

Without any doubt, the rapid development of artificial intelligence will lead to a new generation of personalized patient tools. Each patient will be associated with a digital profile, analyzed by an algorithm, which will recommend a personalized treatment based on previous learning, data mining, and even communication with other artificial intelligence algorithms through the worldwide web.

It remains that the practical utilization of neural networks for the analysis of medical data is a challenging problem. Recently there has been explosive-like progress in the development of artificial intelligence machine learning methods for pattern recognition of different kinds. These results have included deep learning convolutional neural networks, generative adversarial networks, and state-of-the-art architectures of recurrent neural networks including Long Short Time Memory and Gated Recurrent Unit networks. However, in contrast to image processing, except for some rare examples (Angermueller et al., 2017; Putin et al., 2017), the application of deep learning neural network for the early diagnosis of cancer (and thus applications for aging), based on the analysis of proteomic and epigenetic data has not progressed a lot. The major challenge is the application of deep neural networks for an analysis of high dimension low sample size data vital for diagnostics of age-related diseases. Feature selection methods, such as Lasso, have been suggested to solve this problem. However, Lasso ignored the nonlinearity and interactions among features. More efficient methods have included Hilbert-Schmidt Independence Criterion Lasso (HSIC-Lasso) and Least Angle Nonlinear Distributed feature selection (LAND) methods (Yamada et al., 2016), and did not require training with a large sample size. The same advantage was implemented in the Deep Neural Pursuit networks (Liu et al., 2017) and deep feature selection. The efficiency and usability of these new methodologies seem to be very promising and are under active investigation now. This approach should be also definitely linked with other network methods because network biology can provide insightful models for genetic phenomena such as penetrance, epistasis, and modes of inheritance, all of which are integral aspects of Mendelian and complex diseases (Furlong, 2013). In particular, it looks very promising to link deep learning networks with recently developed parenclitic network analysis (Whitwell et al., 2018). The advantage of this approach is the possibility to represent data in the form of a connected

graph, even in the cases when no known interactions between parameters are available. The outcomes of this representation can be then used for training the deep neural network. This approach will, however, require a detailed investigation of this methodology and comparison with other abovementioned methods and with well-established machine learning algorithms such as feature vector machines, random forest, or other sparse methods.

Effective data analysis will be impossible without an understanding of underlying biological mechanisms and, hence, we should work on the integration of data analysis and mathematical modeling. The most challenging problem here is the automatic integration of experimental data with mathematical models. Many fundamental principles governing brain functioning are unclear: What are its properties? How do these properties change over time? How to integrate realistic morphological data into computational modeling of aging-related neural diseases?

Through this review, we have highlighted and discussed several analytical tools and modeling approaches that can be applied to the field of personalized medicine and aging. The very realistic future of personalized medicine and understanding of the complex biological super-network underpinning aging lies in the conflation of these ideas.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. CF established this research collaboration. CF, AZ, SG, MI and HW conceived the manuscript. All authors contributed to writing the manuscript. SG created the figures. HW compiled and edited the manuscript.

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REFERENCES

Amiri, M., Hosseinmardi, N., Bahrami, F., and Janahmadi, M. (2013). Astrocyte-neuron interaction as a mechanism responsible for generation of neural synchrony: a study based on modeling and experiments. *J. Comput. Neurosci.* 34, 489–504. doi: 10.1007/s10827-012-0432-6

Androulakis, X. M., Krebs, K., Peterlin, B. L., Zhang, T., Maleki, N., Sen, S., et al. (2017). Modulation of intrinsic resting-state fMRI networks in women with chronic migraine. *Neurology* 89, 163–169. doi: 10.1212/wnl.0000000000004089

Angermueller, C., Lee, H. J., Reik, W., and Stegle, O. (2017). DeepCpG: accurate prediction of single-cell DNA methylation states using deep learning. *Genome Biol.* 18:67. doi: 10.1186/s13059-017-1189-z

Araque, A., Carmignoto, G., Haydon, P. G., Oliet, S. H. R., Robitaille, R., and Volterra, A. (2014). Gliotransmitters travel in time and space. *Neuron* 81, 728–739. doi: 10.1016/j.neuron.2014.02.007

Aunan, J. R., Cho, W. C., and Søreide, K. (2017). The biology of aging and cancer: a brief overview of shared and divergent molecular hallmarks. *Aging Dis.* 8, 628–642. doi: 10.14336/ad.2017.0103

Bacalini, M. G., D'Aquila, P., Marasco, E., Nardini, C., Montesanto, A., Franceschi, C., et al. (2017). The methylation of nuclear and mitochondrial DNA in ageing phenotypes and longevity. *Mech. Ageing Dev.* 165, 156–161. doi: 10.1016/j.mad.2017.01.006

Bacalini, M. G., Franceschi, C., Gentilini, D., Ravaioli, F., Zhou, X., Remondini, D., et al. (2018). Molecular aging of human liver: an epigenetic/transcriptomic signature. *J. Gerontol. A Biol. Sci. Med. Sci.* 74, 1–8. doi: 10.1093/gerona/gly048

Baker, D. J., and Petersen, R. C. (2018). Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives. *J. Clin. Invest.* 128, 1208–1216. doi: 10.1172/jci95145

Bakshi, S., Chelliah, V., Chen, C., and van der Graaf, P. H. (2019). Mathematical biology models of Parkinson's disease. *CPT Pharmacometrics Syst. Pharmacol.* 8, 77–86. doi: 10.1002/psp4.12362

Bartlett, T. E., Olhede, S. C., and Zaikin, A. (2014). A DNA methylation network interaction measure and detection of network oncomarkers. *PLoS One* 9:e84573. doi: 10.1371/journal.pone.0084573

Bateman, A., Martin, M. J., O'Donovan, C., Magrane, M., Alpi, E., Antunes, R., et al. (2017). UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* 45, D158–D169. doi: 10.1093/nar/gkw1099

Benveniste, H., Liu, X., Koundal, S., Sanggaard, S., Lee, H., and Wardlaw, J. (2019). The glymphatic system and waste clearance with brain aging: a review. *Gerontology* 65, 106–119. doi: 10.1159/000490349

Bhattacharya, B. S., Coyle, D., and Maguire, L. P. (2011). A thalamo-cortico-thalamic neural mass model to study α rhythms in Alzheimer's disease. *Neural Netw.* 24, 631–645. doi: 10.1016/j.neunet.2011.02.009

Bianchi, D., De Michele, P., Marchetti, C., Tirozzi, B., Cuomo, S., Marie, H., et al. (2014). Effects of increasing CREB-dependent transcription on the storage and recall processes in a hippocampal CA1 microcircuit. *Hippocampus* 24, 165–177. doi: 10.1002/hipo.22212

Blyuss, O., Burnell, M., Ryan, A., Gentry-Maharaj, A., Mariño, I. P., Kalsi, J., et al. (2018). Comparison of longitudinal CA125 algorithms as a first-line screen for ovarian cancer in the general population. *Clin. Cancer Res.* 24, 4726–4733. doi: 10.1158/1078-0432.CCR-18-0208

Brunel, N. (2000). Dynamics of sparsely connected networks of excitatory and inhibitory spiking neurons. *J. Comput. Neurosci.* 8, 183–208. doi: 10.1023/a:1008925309027

Brunner, S. F., Roberts, N. D., Wylie, L. A., Moore, L., Aitken, S. J., Davies, S. E., et al. (2019). Somatic mutations and clonal dynamics in healthy and cirrhotic human liver. *Nature* 574, 538–542. doi: 10.1038/s41586-019-1670-9

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

Cardelli, M. (2018). The epigenetic alterations of endogenous retroelements in aging. *Mech. Ageing Dev.* 174, 30–46. doi: 10.1016/j.mad.2018.02.002

Ciccarone, F., Tagliatesta, S., Caiafa, P., and Zampieri, M. (2018). DNA methylation dynamics in aging: how far are we from understanding the mechanisms? *Mech. Ageing Dev.* 174, 3–17. doi: 10.1016/j.mad.2017.12.002

Coskren, P. J., Luebke, J. I., Kabaso, D., Wearne, S. L., Yadav, A., Rumbell, T., et al. (2015). Functional consequences of age-related morphologic changes to pyramidal neurons of the rhesus monkey prefrontal cortex. *J. Comput. Neurosci.* 38, 263–283. doi: 10.1007/s10827-014-0541-5

Culmone, V., and Migliore, M. (2012). Progressive effect of β amyloid peptides accumulation on CA1 pyramidal neurons: a model study suggesting possible treatments. *Front. Comput. Neurosci.* 6:52. doi: 10.3389/fncom.2012.00052

Dato, S., Soerensen, M., De Rango, F., Rose, G., Christensen, K., Christiansen, L., et al. (2018). The genetic component of human longevity: new insights from the analysis of pathway-based SNP-SNP interactions. *Aging Cell* 17:e12755. doi: 10.1111/acel.12755

De Pittà, M., Brunel, N., and Volterra, A. (2016). Astrocytes: orchestrating synaptic plasticity? *Neuroscience* 323, 43–61. doi: 10.1016/j.neuroscience.2015.04.001

DeWeese, M. R., and Zador, A. M. (2006). Non-gaussian membrane potential dynamics imply sparse, synchronous activity in auditory cortex. *J. Neurosci.* 26, 12206–12218. doi: 10.1523/JNEUROSCI.2813-06.2006

Dossi, E., Vasile, F., and Rouach, N. (2018). Human astrocytes in the diseased brain. *Brain Res. Bull.* 136, 139–156. doi: 10.1016/j.brainresbull.2017.02.001

Ferrante, M., Blackwell, K., Migliore, M., and Ascoli, G. (2008). Computational models of neuronal biophysics and the characterization of potential neuropharmacological targets. *Curr. Med. Chem.* 15, 2456–2471. doi: 10.2174/092986708785909094

Field, A. E., Robertson, N. A., Wang, T., Havas, A., Ideker, T., and Adams, P. D. (2018). DNA methylation clocks in aging: categories, causes, and consequences. *Mol. Cell* 71, 882–895. doi: 10.1016/j.molcel.2018.08.008

Finkel, T., Serrano, M., and Blasco, M. A. (2007). The common biology of cancer and ageing. *Nature* 448, 767–774. doi: 10.1038/nature05985

Foster, B. L., He, B. J., Honey, C. J., Jerbi, K., Maier, A., and Saalmann, Y. B. (2016). Spontaneous neural dynamics and multi-scale network organization. *Front. Syst. Neurosci.* 10:7. doi: 10.3389/fnsys.2016.00007

Fox, M. D., and Greicius, M. (2010). Clinical applications of resting state functional connectivity. *Front. Syst. Neurosci.* 4:19. doi: 10.3389/fnsys.2010.00019

Franceschi, C., and Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *Biol. Sci. Cite J. as J. Gerontol. A Biol. Sci. Med. Sci.* 69, S4–S9. doi: 10.1093/gerona/glu057

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., et al. (2006). Inflamm-aging: an evolutionary perspective on immunosenescence. *Ann. N Y Acad. Sci.* 908, 244–254. doi: 10.1111/j.1749-6632.2000.tb06651.x

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., and Santoro, A. (2018). Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14, 576–590. doi: 10.1038/s41574-018-0059-4

Franceschi, C., Garagnani, P., Vitale, G., Capri, M., and Salvioli, S. (2017a). Inflammaging and 'Garb-aging'. *Trends Endocrinol. Metab.* 28, 199–212. doi: 10.1016/j.tem.2016.09.005

Franceschi, C., Salvioli, S., Garagnani, P., de Eguileor, M., Monti, D., and Capri, M. (2017b). Immunobiography and the heterogeneity of immune responses in the elderly: a focus on inflammaging and trained immunity. *Front. Immunol.* 8:982. doi: 10.3389/fimmu.2017.00982

Franceschi, C., Ostan, R., Mariotti, S., Monti, D., and Vitale, G. (2019). The aging thyroid: a reappraisal within the geroscience integrated perspective. *Endocr. Rev.* 40, 1250–1270. doi: 10.1210/er.2018-00170

Fu, W.-Y., Wang, X., and Ip, N. Y. (2019). Targeting neuroinflammation as a therapeutic strategy for Alzheimer's disease: mechanisms, drug candidates, and new opportunities. *ACS Chem. Neurosci.* 10, 872–879. doi: 10.1021/acschemneuro.8b00402

Furlong, L. I. (2013). Human diseases through the lens of network biology. *Trends Genet.* 29, 150–159. doi: 10.1016/j.tig.2012.11.004

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., et al. (2019). Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 25, 1822–1832. doi: 10.1038/s41591-019-0675-0

Gerstner, W., and Kistler, W. M. (2002). *Spiking Neuron Models: Single Neurons, Populations, Plasticity*. Cambridge, MA: Cambridge University Press. Available online at: https://www.cambridge.org/vi/academic/subjects/computer-science/computational-biology-and-bioinformatics/spiking-neuron-models-single-neurons-populations-plasticity?format=PB. Accessed November 15, 2019.

Goodfellow, I., Bengio, Y., and Courville, A. (2016). *Deep Learning*. MIT Press.

Gordleeva, S. Y., Ermolaeva, A. V., Kastalskiy, I. A., and Kazantsev, V. B. (2019). Astrocyte as spatiotemporal integrating detector of neuronal activity. *Front. Physiol.* 10:294. doi: 10.3389/fphys.2019.00294

Grady, C. (2012). The cognitive neuroscience of ageing. *Nat. Rev. Neurosci.* 13, 491–505. doi: 10.1038/nrn3256

Grignolio, A., Mishto, M., Caetano Faria, A. M., Garagnani, P., Franceschi, C., and Tieri, P. (2014). Towards a liquid self: how time, geography, and life experiences reshape the biological identity. *Front. Immunol.* 5:153. doi: 10.3389/fimmu.2014.00153

Gutkin, B. S., and Ermentrout, G. B. (1998). Dynamics of membrane excitability determine interspike interval variability: a link between spike generation mechanisms and cortical spike train statistics. *Neural Comput.* 10, 1047–1065. doi: 10.1162/089976698300017331

Haas, R., Zelezniak, A., Iacovacci, J., Kamrad, S., Townsend, S. J., and Ralser, M. (2017). Designing and interpreting "multi-omic" experiments that may change our understanding of biology. *Curr. Opin. Syst. Biol.* 6, 37–45. doi: 10.1016/j.coisb.2017.08.009

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., et al. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol. Cell* 49, 359–367. doi: 10.1016/j.molcel.2012.10.016

Harris, K. D., and Thiele, A. (2011). Cortical state and attention. *Nat. Rev. Neurosci.* 12, 509–523. doi: 10.1038/nrn308

Hedden, T., and Gabrieli, J. D. E. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nat. Rev. Neurosci.* 5, 87–96. doi: 10.1038/nrn1323

Heneka, M. T., Carson, M. J., El Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., et al. (2015). Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 14, 388–405. doi: 10.1016/S1474-4422(15)70016-5

Henstridge, C. M., and Spires-Jones, T. L. (2018). Modeling Alzheimer's disease brains *in vitro*. *Nat. Neurosci.* 21, 899–900. doi: 10.1038/s41593-018-0177-2

Heppner, F. L., Ransohoff, R. M., and Becher, B. (2015). Immune attack: the role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci.* 16, 358–372. doi: 10.1038/nrn3880

Horvath, S. (2013). DNA methylation age of human tissues and cell types. *Genome Biol.* 14:R115. doi: 10.1186/gb-2013-14-10-r115

Horvath, S., and Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* 19, 371–384. doi: 10.1038/s41576-018-0004-3

Horvath, S., Oshima, J., Martin, G. M., Lu, A. T., Quach, A., Cohen, H., et al. (2018). Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and *ex vivo* studies. *Aging* 10, 1758–1775. doi: 10.18632/aging.101508

Hou, L., Joyce, B. T., Gao, T., Liu, L., Zheng, Y., Penedo, F. J., et al. (2015). Blood telomere length attrition and cancer development in the normative aging study cohort. *EBioMedicine* 2, 591–596. doi: 10.1016/j.ebiom.2015.04.008

Jagust, W. (2018). Following the pathway to Alzheimer's disease. *Nat. Neurosci.* 21, 306–308. doi: 10.1038/s41593-018-0085-5

Jalan, S., Kanhaiya, K., Rai, A., Bandapalli, O. R., and Yadav, A. (2015). Network topologies decoding cervical cancer. *PLoS One* 10:e0135183. doi: 10.1371/journal.pone.0135183

Jolivet, R., Lewis, T. J., and Gerstner, W. (2004). Generalized integrate-and-fire models of neuronal activity approximate spike trains of a detailed model to a high degree of accuracy. *J. Neurophysiol.* 92, 959–976. doi: 10.1152/jn.00190.2004

Jolivet, R., Rauch, A., Lüscher, H.-R., and Gerstner, W. (2006). Predicting spike timing of neocortical pyramidal neurons by simple threshold models. *J. Comput. Neurosci.* 21, 35–49. doi: 10.1007/s10827-006-7074-5

Jones, P. A., and Liang, G. (2009). Rethinking how DNA methylation patterns are maintained. *Nat. Rev. Genet.* 10, 805–811. doi: 10.1038/nrg2651

Kanakov, O., Gordleeva, S., Ermolaeva, A., Jalan, S., and Zaikin, A. (2019). Astrocyte-induced positive integrated information in neuron-astrocyte ensembles. *Phys. Rev. E* 99:012418. doi: 10.1103/physreve.99.012418

Karsakov, A., Bartlett, T., Ryblov, A., Meyerov, I., Ivanchenko, M., and Zaikin, A. (2017). Parenclitic network analysis of methylation data for cancer identification. *PLoS One* 12:e0169661. doi: 10.1371/journal.pone.0169661

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., et al. (2014). Geroscience: linking aging to chronic disease. *Cell* 159, 709–713. doi: 10.1016/j.cell.2014.10.039

Krivonosov, M., Nazarenko, T., Bacalini, M. G., Franceschi, C., Zaikin, A., and Ivanchenko, M. (2020). Age-dependent DNA methylation parenclitic networks in family-based cohort patients with down syndrome. *bioRxiv* [Preprint]. doi: 10.1101/2020.03.10.986505

Kyrtsos, C. R., and Baras, J. S. (2015). Modeling the role of the glymphatic pathway and cerebral blood vessel properties in Alzheimer's disease pathogenesis. *PLoS One* 10:e0139574. doi: 10.1371/journal.pone.0139574

Lenk, K., Raisanen, E., and Hyttinen, J. A. K. (2016). "Understanding the role of astrocytic GABA in simulated neural networks," in *Proceedings of the 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)* (Orlando, FL, USA: IEEE), 6121–6124.

Levine, M. E., Lu, A. T., Quach, A., Chen, B. H., Assimes, T. L., Bandinelli, S., et al. (2018). An epigenetic biomarker of aging for lifespan and healthspan. *Aging* 10, 573–591. doi: 10.18632/aging.101414

Li, G., Liu, K.-Y., and Qiu, Z.-P. (2019). An integrative module analysis of DNA methylation landscape in aging. *Exp. Ther. Med.* 17, 3411–3416. doi: 10.3892/etm.2019.7334

Li, S., Christiansen, L., Christensen, K., Kruse, T. A., Redmond, P., Marioni, R. E., et al. (2017). Identification, replication and characterization of epigenetic remodelling in the aging genome: a cross population analysis. *Sci. Rep.* 7:8183. doi: 10.1038/s41598-017-08346-7

Liu, B., Wei, Y., Zhang, Y., and Yang, Q. (2017). "Deep neural networks for high dimension, low sample size data," in *Proceedings of the Twenty-Sixth International Joint Conference on Artificial Intelligence* (California, MA: International Joint Conferences on Artificial Intelligence Organization), 2287–2293.

Lloret-Villas, A., Varusai, T. M., Juty, N., Laibe, C., Le NovÈre, N., Hermjakob, H., et al. (2017). The impact of mathematical modeling in understanding the mechanisms underlying neurodegeneration: evolving dimensions and future directions. *CPT pharmacometrics Syst. Pharmacol.* 6, 73–86. doi: 10.1002/psp4.12155

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039

Luo, C., Hajkova, P., and Ecker, J. R. (2018). Dynamic DNA methylation: in the right place at the right time. *Science* 361, 1336–1340. doi: 10.1126/science.aat6806

Lurie, D. J., Kessler, D., Bassett, D. S., Betzel, R. F., Breakspear, M., Kheilholz, S., et al. (2020). Questions and controversies in the study of time-varying functional connectivity in resting fMRI. *Netw. Neurosci.* 4, 30–69. doi: 10.1162/netn_a_00116

Marina, N., Turovsky, E., Christie, I. N., Hosford, P. S., Hadjihambi, A., Korsak, A., et al. (2018). Brain metabolic sensing and metabolic signaling at the level of an astrocyte. *Glia* 66, 1185–1199. doi: 10.1002/glia.23283

Mariño, I. P., Blyuss, O., Ryan, A., Gentry-Maharaj, A., Timms, J. F., Dawnay, A., et al. (2017). Change-point of multiple biomarkers in women with ovarian cancer. *Biomed. Signal Process. Control* 33, 169–177. doi: 10.1016/j.bspc.2016.11.015

Markaki, M., Orphanoudakis, S., and Poirazi, P. (2005). Modelling reduced excitability in aged CA1 neurons as a calcium-dependent process. *Neurocomputing* 65–66, 305–314. doi: 10.1016/j.neucom.2004.10.023

Maslov, A. Y., and Vijg, J. (2009). Genome instability, cancer and aging. *Biochim. Biophys. Acta* 1790, 963–969. doi: 10.1016/j.bbagen.2009.03.020

McIntosh, M. W., Urban, N., and Karlan, B. (2002). Generating longitudinal screening algorithms using novel biomarkers for disease. *Cancer Epidemiol. Biomarkers Prev.* 11, 159–166.

Menon, U., Ryan, A., Kalsi, J., Gentry-Maharaj, A., Dawnay, A., Habib, M., et al. (2015). Risk algorithm using serial biomarker measurements doubles the number of screen-detected cancers compared with a single-threshold rule in the united kingdom collaborative trial of ovarian cancer screening. *J. Clin. Oncol.* 33, 2062–2071. doi: 10.1200/jco.2014.59.4945

Morse, T. M., Carnevale, N. T., Mutalik, P. G., Migliore, M., and Shepherd, G. M. (2010). Abnormal excitability of oblique dendrites implicated in early Alzheimer's: a computational study. *Front. Neural Circuits* 4:16. doi: 10.3389/fncir.2010.00016

Morsiani, C., Bacalini, M. G., Santoro, A., Garagnani, P., Collura, S., D'Errico, A., et al. (2019). The peculiar aging of human liver: a geroscience perspective within transplant context. *Ageing Res. Rev.* 51, 24–34. doi: 10.1016/j.arr.2019.02.002

Murphy, K., Birn, R. M., Handwerker, D. A., Jones, T. B., and Bandettini, P. A. (2009). The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *NeuroImage* 44, 893–905. doi: 10.1016/j.neuroimage.2008.09.036

Nadkarni, S., and Jung, P. (2003). Spontaneous oscillations of dressed neurons: a new mechanism for epilepsy? *Phys. Rev. Lett.* 91:268101. doi: 10.1103/physrevlett.91.268101

Ong, M.-L., and Holbrook, J. D. (2014). Novel region discovery method for Infinium 450K DNA methylation data reveals changes associated with aging in muscle and neuronal pathways. *Aging Cell* 13, 142–155. doi: 10.1111/acel.12159

Oschmann, F., Berry, H., Obermayer, K., and Lenk, K. (2018). From in silico astrocyte cell models to neuron-astrocyte network models: a review. *Brain Res. Bull.* 136, 76–84. doi: 10.1016/j.brainresbull.2017.01.027

Ostojic, S. (2014). Two types of asynchronous activity in networks of excitatory and inhibitory spiking neurons. *Nat. Neurosci.* 17, 594–600. doi: 10.1038/nn.3658

Parbo, P., Ismail, R., Hansen, K. V., Amidi, A., Mårup, F. H., Gottrup, H., et al. (2017). Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. *Brain* 140, 2002–2011. doi: 10.1093/brain/awx120

Perea, G., and Araque, A. (2010). GLIA modulates synaptic transmission. *Brain Res. Rev.* 63, 93–102. doi: 10.1016/j.brainresrev.2009.10.005

Putin, E., Mamoshina, P., Aliper, A., Korzinkin, M., Moskalev, A., Kolosov, A., et al. (2017). Deep biomarkers of human aging: application of deep neural networks to biomarker development. *Aging* 8, 1021–1033. doi: 10.18632/aging.100968

Querfurth, H. W., and LaFerla, F. M. (2010). Alzheimer's disease. *N. Engl. J. Med.* 362, 329–344. doi: 10.1056/NEJMra0909142

Rai, A., Menon, A. V., and Jalan, S. (2015). Randomness and preserved patterns in cancer network. *Sci. Rep.* 4:6368. doi: 10.1038/srep06368

Rai, A., Pradhan, P., Nagraj, J., Lohitesh, K., Chowdhury, R., and Jalan, S. (2017). Understanding cancer complexome using networks, spectral graph theory and multilayer framework. *Sci. Rep.* 7:41676. doi: 10.1038/srep41676

Rasmussen, M. K., Mestre, H., and Nedergaard, M. (2018). The glymphatic pathway in neurological disorders. *Lancet Neurol.* 17, 1016–1024. doi: 10.1016/s1474-4422(18)30318-1

Rauch, A., La Camera, G., Lüscher, H.-R., Senn, W., and Fusi, S. (2003). Neocortical pyramidal cells respond as integrate-and-fire neurons to *in vivo*-like input currents. *J. Neurophysiol.* 90, 1598–1612. doi: 10.1152/jn.00293.2003

Reynolds, L. M., Taylor, J. R., Ding, J., Lohman, K., Johnson, C., Siscovick, D., et al. (2014). Age-related variations in the methylome associated with gene expression in human monocytes and T cells. *Nat. Commun.* 5:5366. doi: 10.1038/ncomms6366

Romani, A., Marchetti, C., Bianchi, D., Leinekugel, X., Poirazi, P., Migliore, M., et al. (2013). Computational modeling of the effects of amyloid-β on release probability at hippocampal synapses. *Front. Comput. Neurosci.* 7:1. doi: 10.3389/fncom.2013.00001

Rowan, M. S., Neymotin, S. A., and Lytton, W. W. (2014). Electrostimulation to reduce synaptic scaling driven progression of Alzheimer's disease. *Front. Comput. Neurosci.* 8:39. doi: 10.3389/fncom.2014.00039

Rumbell, T. H., Draguljić, D., Yadav, A., Hof, P. R., Luebke, J. I., and Weaver, C. M. (2016). Automated evolutionary optimization of ion channel conductances and kinetics in models of young and aged rhesus monkey pyramidal neurons. *J. Comput. Neurosci.* 41, 65–90. doi: 10.1007/s10827-016-0605-9

Salter, M. W., and Stevens, B. (2017). Microglia emerge as central players in brain disease. *Nat. Med.* 23, 1018–1027. doi: 10.1038/nm.4397

Sarkar, C., and Jalan, S. (2018). Spectral properties of complex networks. *Chaos* 28:102101. doi: 10.1063/1.5040897

Sato, S., Parr, E. B., Devlin, B. L., Hawley, J. A., and Sassone-Corsi, P. (2018). Human metabolomics reveal daily variations under nutritional challenges specific to serum and skeletal muscle. *Mol. Metab.* 16, 1–11. doi: 10.1016/j.molmet.2018.06.008

Savtchenko, L. P., Bard, L., Jensen, T. P., Reynolds, J. P., Kraev, I., Medvedev, N., et al. (2018). Disentangling astroglial physiology with a realistic cell model in silico. *Nat. Commun.* 9:3554. doi: 10.1038/s41467-018-05896-w

Savtchouk, I., and Volterra, A. (2018). Gliotransmission: beyond black-and-white. *J. Neurosci.* 38, 14–25. doi: 10.1523/JNEUROSCI.0017-17.2017

Sawikr, Y., Yarla, N. S., Peluso, I., Kamal, M. A., Aliev, G., and Bishayee, A. (2017). Neuroinflammation in Alzheimer's disease: the preventive and therapeutic potential of polyphenolic nutraceuticals. *Adv. Protein Chem. Struct. Biol.* 108, 33–57. doi: 10.1016/bs.apcsb.2017.02.001

Semyanov, A. (2019). Spatiotemporal pattern of calcium activity in astrocytic network. *Cell Calcium* 78, 15–25. doi: 10.1016/j.ceca.2018.12.007

Shin, S.-Y., Fauman, E. B., Petersen, A.-K., Krumsiek, J., Santos, R., Huang, J., et al. (2014). An atlas of genetic influences on human blood metabolites. *Nat. Genet.* 46, 543–550. doi: 10.1038/ng.2982

Shlizerman, E., and Holmes, P. (2012). Neural dynamics, bifurcations and firing rates in a quadratic integrate-and-fire model with a recovery variable. I: deterministic behavior. *Neural Comput.* 24, 2078–2118. doi: 10.1162/neco_a_00308

Skates, S. J., Pauler, D. K., and Jacobs, I. J. (2001). Screening based on the risk of cancer calculation from bayesian hierarchical changepoint and mixture models of longitudinal markers. *J. Am. Stat. Assoc.* 96, 429–439. doi: 10.1198/016214501753168145

Slieker, R. C., van Iterson, M., Luijk, R., Beekman, M., Zhernakova, D. V., Moed, M. H., et al. (2016). Age-related accrual of methylomic variability is linked to fundamental ageing mechanisms. *Genome Biol.* 17:191. doi: 10.1186/s13059-016-1053-6

Styr, B., and Slutsky, I. (2018). Imbalance between firing homeostasis and synaptic plasticity drives early-phase Alzheimer's disease. *Nat. Neurosci.* 21, 463–473. doi: 10.1038/s41593-018-0080-x

Suhre, K., and Gieger, C. (2012). Genetic variation in metabolic phenotypes: study designs and applications. *Nat. Rev. Genet.* 13, 759–769. doi: 10.1038/nrg3314

Tay, T. L., Savage, J. C., Hui, C. W., Bisht, K., and Tremblay, M.-È. (2017). Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. *J. Physiol.* 595, 1929–1945. doi: 10.1113/jp272134

Tewari, S., and Parpura, V. (2013). A possible role of astrocytes in contextual memory retrieval: an analysis obtained using a quantitative framework. *Front. Comput. Neurosci.* 7:145. doi: 10.3389/fncom.2013.00145

Top500.org. (2020). *The TOP500 Project.* Available online at: https://www.top500.org/lists/2018/11/. Accessed April 29, 2020

Tserel, L., Kolde, R., Limbach, M., Tretyakov, K., Kasela, S., Kisand, K., et al. (2015). Age-related profiling of DNA methylation in CD8+ T cells reveals changes in immune response and transcriptional regulator genes. *Sci. Rep.* 5:13107. doi: 10.1038/srep13107

Ullah, G., Cressman, J. R. Jr., Barreto, E., and Schiff, S. J. (2009). The influence of sodium and potassium dynamics on excitability, seizures, and the stability of persistent states: II. Network and glial dynamics. *J. Comput. Neurosci.* 26, 171–183. doi: 10.1007/s10827-008-0130-6

Ullner, E., Politi, A., and Torcini, A. (2018). Ubiquity of collective irregular dynamics in balanced networks of spiking neurons. *Chaos* 28:081106. doi: 10.1063/1.5049902

Unnikrishnan, A., Freeman, W. M., Jackson, J., Wren, J. D., Porter, H., and Richardson, A. (2019). The role of DNA methylation in epigenetics of aging. *Pharmacol. Ther.* 195, 172–185. doi: 10.1016/j.pharmthera.2018.11.001

van Erven, B., Jansma, B. M., Rubio-Gozalbo, M. E., and Timmers, I. (2017). Exploration of the brain in rest: resting-state functional MRI abnormalities in patients with classic galactosemia. *Sci. Rep.* 7:9095. doi: 10.1038/s41598-017-09242-w

Vázquez, M. A., Mariño, I. P., Blyuss, O., Ryan, A., Gentry-Maharaj, A., Kalsi, J., et al. (2018). A quantitative performance study of two automatic methods for the diagnosis of ovarian cancer. *Biomed. Signal Process. Control* 46, 86–93. doi: 10.1016/j.bspc.2018.07.001

Verkhratsky, A. (2019). Astroglial calcium signaling in aging and Alzheimer's disease. *Cold Spring Harb. Perspect. Biol.* 11:a035188. doi: 10.1101/cshperspect.a035188

Verkhratsky, A., Rodrigues, J. J., Pivoriunas, A., Zorec, R., and Semyanov, A. (2019). Astroglial atrophy in Alzheimer's disease. *Pflugers Arch.* 471, 1247–1261. doi: 10.1007/s00424-019-02310-2

Vitale, G., Salvioli, S., and Franceschi, C. (2013). Oxidative stress and the ageing endocrine system. *Nat. Rev. Endocrinol.* 9, 228–240. doi: 10.1038/nrendo.2013.29

Vogels, T. P., Rajan, K., and Abbott, L. F. (2005). Neural network dynamics. *Annu. Rev. Neurosci.* 28, 357–376. doi: 10.1146/annurev.neuro.28.061604.135637

Volman, V., Bazhenov, M., and Sejnowski, T. J. (2012). Computational models of neuron-astrocyte interaction in epilepsy. *Front. Comput. Neurosci.* 6:58. doi: 10.3389/fncom.2012.00058

Wang, Y., Huang, T., Xie, L., and Liu, L. (2016). Integrative analysis of methylation and transcriptional profiles to predict aging and construct aging specific cross-tissue networks. *BMC Syst. Biol.* 10:132. doi: 10.1186/s12918-016-0354-4

Weidner, C., Lin, Q., Koch, C., Eisele, L., Beier, F., Ziegler, P., et al. (2014). Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol.* 15:R24. doi: 10.1186/gb-2014-15-2-r24

Whitwell, H. J., Blyuss, O., Menon, U., Timms, J. F., and Zaikin, A. (2018). Parenclitic networks for predicting ovarian cancer. *Oncotarget* 9, 22717–22726. doi: 10.18632/oncotarget.25216

Whitwell, H. J., Worthington, J., Blyuss, O., Gentry-Maharaj, A., Ryan, A., Gunu, R., et al. (2020). Improved early detection of ovarian cancer using longitudinal multimarker models. *Br. J. Cancer* 122, 847–856. doi: 10.1038/s41416-019-0718-9

Wu, Y.-W., Gordleeva, S., Tang, X., Shih, P.-Y., Dembitskaya, Y., and Semyanov, A. (2019). Morphological profile determines the frequency of spontaneous calcium events in astrocytic processes. *Glia* 67, 246–262. doi: 10.1002/glia.23537

Yamada, M., Tang, J., Lugo-Martinez, J., Hodzic, E., Shrestha, R., Saha, A., et al. (2016). *Ultra High-Dimensional Nonlinear Feature Selection for Big Biological Data.* Available online at: http://arxiv.org/abs/1608.04048. Accessed November 15, 2019.

Zanin, M., Alcazar, J. M., Carbajosa, J. V., Paez, M. G., Papo, D., Sousa, P., et al. (2014). Parenclitic networks: uncovering new functions in biological data. *Sci. Rep.* 4:5112. doi: 10.1038/srep05112

Zanin, M., Romance, M., Moral, S., and Criado, R. (2018). Credit card fraud detection through parenclitic network analysis. *Complexity* 2018, 1–9. doi: 10.1155/2018/5764370

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frontiers in Aging Neuroscience

REVIEW
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Age-Related Olfactory Dysfunction: Epidemiology, Pathophysiology, and Clinical Management

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Like other sensory systems, olfactory function deteriorates with age. Epidemiological studies have revealed that the incidence of olfactory dysfunction increases at the age of 60 and older and males are more affected than females. Moreover, smoking, heavy alcohol use, sinonasal diseases, and Down's syndrome are associated with an increased incidence of olfactory dysfunction. Although the pathophysiology of olfactory dysfunction in humans remains largely unknown, studies in laboratory animals have demonstrated that both the peripheral and central olfactory nervous systems are affected by aging. Aged olfactory neuroepithelium in the nasal cavity shows the loss of mature olfactory neurons, replacement of olfactory neuroepithelium by respiratory epithelium, and a decrease in basal cell proliferation both in the normal state and after injury. In the central olfactory pathway, a decrease in the turnover of interneurons in the olfactory bulb (OB) and reduced activity in the olfactory cortex under olfactory stimulation is observed. Recently, the association between olfactory impairment and neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), has gained attention. Evidence-based pharmacotherapy to suppress or improve age-related olfactory dysfunction has not yet been established, but preliminary results suggest that olfactory training using odorants may be useful to improve some aspects of age-related olfactory impairment.

Keywords: aging, olfactory receptor neurons, basal cells, regeneration, olfactory bulb, olfactory cortex, neurodegenerative diseases

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INTRODUCTION

Olfaction is a sense that allows the detection of odors in the surrounding environment. Olfaction is necessary to identify food, predators, and sexual partners for most of the wildlife, being indispensable for species survival. Moreover, in humans, olfaction not only guarantees greater safety by allowing the detection of fire, gas leakage, and spoiled foods but also a greater quality of life through the appreciation of food, wine, and pleasant smells. Nonetheless, olfactory impairment has traditionally received less attention compared to visual and auditory impairment. Thus, it may even be difficult for healthy individuals to understand the inconvenience of olfactory

impairment. However, patients with olfactory impairment face a series of daily-life problems (Miwa et al., 2001; Brämerson et al., 2007; Gopinath et al., 2012a; Croy et al., 2014) as well as psychological issues including depression, anxiety, and other negative emotions (Croy et al., 2014).

Several pathoetiologies, including chronic rhinosinusitis, viral infection, head trauma, and intake of toxic drugs are associated with the development of olfactory dysfunction (Hummel et al., 2017). Along with these pathologies, age is one of the most important factors associated with human olfactory dysfunction (Schiffman, 1997; Brämerson et al., 2007; Doty and Kamath, 2014; Mobley et al., 2014; Attems et al., 2015). As other sensory functions such as hearing and vision, olfactory ability deteriorates with aging, and the majority of the patients with the complaint of olfactory impairment are middle-aged and elderly patients. In line with the rapid growth of the geriatric population in developed countries, the number of individuals with olfactory impairment is also expected to rapidly grow. For elderly people with decreased physical and social activity, food may be a source of joy in their daily life (Markovic et al., 2007), consequently, olfactory dysfunction may lead to impairing their quality of life. Recent studies have also demonstrated that patients with age-related neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), develop olfactory dysfunction from the early stages of the diseases (Kovács, 2004; Barresi et al., 2012; Doty, 2017). More recent studies in the general population have also demonstrated that olfactory dysfunction is an independent risk factor for mortality (Gopinath et al., 2012b; Pinto et al., 2014; Devanand et al., 2015; Schubert et al., 2017; Liu et al., 2019), suggesting that olfaction may serve as a biomarker for systemic life activity. Effective day-to-day management of olfactory dysfunction as well as the development of new treatment strategies for age-related olfactory dysfunction are therefore important goals to support healthy, successful aging.

While there has been rapid progress in understanding the molecular mechanisms mediating olfaction (Ihara et al., 2013; Takeuchi and Sakano, 2014), the pathogenetic processes underlying age-related human olfactory dysfunction, and, in particular, the changes in the olfactory neural system, remain largely unknown. This review article, therefore, aimed to shed light on age-related changes occurring in the olfactory neural system during olfactory deterioration, both within the context of the normal aging process and under pathological conditions.

OVERVIEW OF THE ANATOMY OF OLFACTORY NEURAL PATHWAYS

The human olfactory mucosa is located in the superior part of the olfactory cleft, overlying the cribriform plate as well as the superior part of the nasal septum and the middle and superior turbinates. In many animal species, including mice, rats, and dogs, the olfactory mucosa covers a large proportion of the nasal cavity (Mery et al., 1994; Kavoi et al., 2010). In contrast, in humans, the relative surface of olfactory mucosa is very small, covering only a few cm^2 in each nostril (Holbrook et al., 2011).

The olfactory mucosa is composed of the neuroepithelium and underlying lamina propria. The neuroepithelium is a pseudostratified epithelium, with the basal cells residing at the bottom of the epithelium above the basement membrane (**Figure 1A**). There are two distinct types of basal cells, horizontal basal cells (HBCs), and globose basal cells (GBCs). Above the basal cells is a layer of neural cells with various differentiation status, ranging from the immature (basal) to the mature (apical) status (**Figure 1A**). Olfactory receptor neurons (ORNs) are bipolar neurons, whose dendrites extend to the surface of the epithelium and axons extend to the olfactory bulb (OB). The end of ORN dendrites forms a knob-like structure, known as the olfactory vesicle, from which several olfactory cilia emanate. The most apical zone of the olfactory neuroepithelium is occupied by the nuclei and/or cytoplasm of the supporting cells (Moran et al., 1982; Morrison and Costanzo, 1990; Jafek et al., 2002). The lamina propria beneath the basement membrane contains vessels, olfactory nerve bundles, and Bowman's glands. The function of Bowman's glands remains unclear, but it is speculated that they contribute to: (1) the protection of olfactory cilia; (2) the transport of odorants; (3) the prevention of mucosal infection through the secretion of antimicrobial proteins; and (4) the biochemical detoxification of chemicals through biotransformation enzymes (Getchell and Getchell, 1991; Mellert et al., 1992; Matarazzo et al., 2002; Ling et al., 2004).

Because olfaction is a chemical sensor that detects evaporated chemicals, olfactory receptors need to be exposed to the external environment. The olfactory mucosa is part of the airway mucosa. External exposure is a distinct feature of the olfactory sensory system from the visual and auditory systems, which perceive physical stimuli made of light and sound, respectively, have their receptors protected inside the body. The peripheral olfactory organ is therefore always at risk of being injured by extrinsic pathogens and chemicals. Conversely, olfaction plays an indispensable role in survival, contributing to food detection, predator avoidance, and mating in the wildlife. To meet these diverse needs, the mammalian olfactory neural system has a unique regenerative capacity compared to other sensory systems. The most distinct feature of such regenerative capacity is the continuous proliferation of basal cells in the neuroepithelium. GBCs are a type of neural stem cells, which continuously undergo cell division even in undamaged conditions and give rise to new ORNs (**Figure 1A**). When the neuroepithelium is injured, such proliferative activity is upregulated so the neuroepithelium is regenerated rapidly (Matulionis, 1975; Graziadei and Graziadei, 1979; Hurtt et al., 1988; Schwob et al., 1992, 1995; Genter et al., 1995; Bergman et al., 2002; Ducray et al., 2002; Schwob, 2002; Suzukawa et al., 2011). Although it is unknown how long it takes for the human olfactory neuroepithelium to recover from damage, in rats and mice the olfactory neuroepithelium morphologically recovers from experimentally-induced mucosal injury within one month (Graziadei and Graziadei, 1979; Schwob, 2002; Suzukawa et al., 2011). In undamaged conditions, HBCs are quiescent cells, but in the event of severe mucosal damage, HBCs function as stem cells and proliferate to give rise to each cell type in the neuroepithelium (Farbman, 1990; Schwob et al., 1992, 2017; Schwob, 2002;

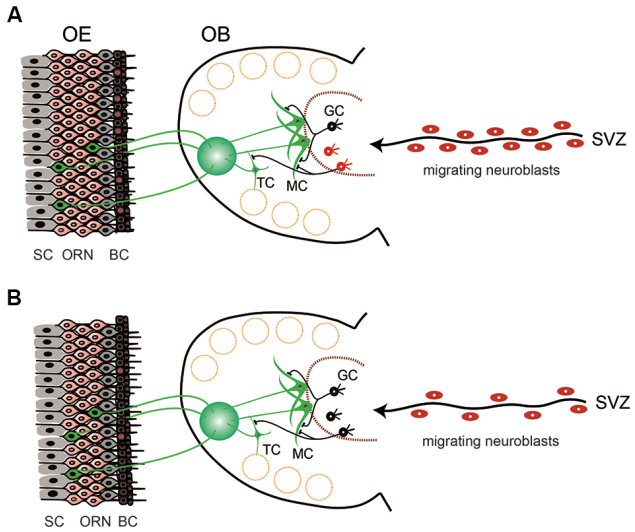

FIGURE 1 | Schematic view of the structure and cell dynamics of the olfactory neuroepithelium (OE) and olfactory bulb (OB) in young **(A)** and aged **(B)** mice. **(A)** OE is composed of layers of supporting cells (SC), olfactory receptor neurons (ORN), and basal cells (BC). BCs continuously undergo cell division to give rise to new neuronal cells (BCs with red nuclei). ORNs extend axons to the glomerulus of the OB, where they make synaptic connections with the neurites of mitral cells (MC) and tufted cells (TC). There is also continuous cell generation in the stem cell population in the subventricular zone (SVZ). Generated neuroblasts then migrate to reach the OB and differentiate into the interneurons [mostly into granule cells (GC)] and contribute to the modulation of olfactory information. **(B)** In aged animals, the cell division in the BCs is decreased and the number of ORNs is also reduced. Cell division in the SVZ is reduced, as well as the number of neuroblasts migrating to the OB. However, the number of granule cells in the OB is almost stable, suggesting that the turnover of granule cells is reduced and the lifespan of the cells is prolonged (black GCs).

Beites et al., 2005; Brann and Firestein, 2014). This regenerative ability is retained until old age, though its efficacy decreases (Morrison and Costanzo, 1995; Hahn et al., 2005; Suzukawa et al., 2011; Brann and Firestein, 2014).

Olfactory signals from ORNs relay to second-order neurons, namely the mitral cells and tufted cells in the OB. ORNs and mitral/tufted cells make synapses, forming signal-processing modules named glomerulus (**Figure 1A**). In mice, each ORN expresses only one of more than 1,000 types of olfactory receptors. The axons of ORNs expressing the same olfactory receptors converge on only a few glomeruli. Functional studies have demonstrated that an odorant receptor can be activated by multiple odorant molecules, whereas an odorant molecule can activate multiple odorant receptors. Therefore, an odorant is identified as a unique combination of olfactory receptor responses, which eventually leads to a unique activation profile of olfactory glomeruli in the OB. This mechanism is thought to enable the olfactory system to discriminate thousands of odors (Ressler et al., 1994; Mori et al., 1999). These second-order neurons extend their axons along the lateral olfactory tract toward the structure of the primary olfactory cortex, such as the anterior olfactory nucleus, piriform cortex, and entorhinal cortex. Odor processing may also involve other central brain areas, including the hippocampus, amygdala, and orbitofrontal cortex (Gottfried, 2010; **Figure 2**).

Although the mitral cells and tufted cells are generated only during the embryonic period, there is a continuous turnover of interneurons in the OB, as in the neuroepithelium. Continuous cell generation occurs in the stem cell population located in the subventricular zone (SVZ). The generated neuroblasts migrate to the OB, where they differentiate into interneurons (mostly into granule cells; Whitman and Greer, 2009; Lazarini and Lledo, 2011; Mobley et al., 2014) and contribute to the reorganization of the olfactory neural pathways (**Figure 1A**).

AGE-RELATED CHANGES IN HUMAN AND ANIMAL OLFACTORY FUNCTION IN PHYSIOLOGICAL CONDITIONS

Many studies have revealed that the human olfactory function, including the ability to detect, differentiate, and identify odors, declines with age. Doty et al. (1984) investigated the olfactory identification ability in a variety of age generations using the Pennsylvania smell identification test and found that significant olfactory deterioration starts from the age in 60 in males and 70 in females, with females maintaining a superior ability in each generation. Hummel et al. (2007) examined more than 3,000 normal subjects using Sniffin' Sticks, a battery of olfactory tests, and reported that the sum of threshold, discrimination, and identification scores (TDI scores) declined with age, with odor thresholds declining most dramatically compared to odor discrimination and odor identification. In Japan, Saito et al. (2006) demonstrated using the Japanese olfactory identification test (OSIT-J) that olfactory identification ability declined from the age of 50 onward.

Konstantinidis et al. (2006) reported that the identification of unpleasant odors is independent of age, suggesting that the decline in olfactory sensitivity may depend on the type of odorants. Conversely, Sinding et al. (2014) reported that age-related loss in olfactory sensitivity is similar for light and heavy molecules. It was also reported that the detection thresholds for lavandin oil (a highly complex substance) and n-butanol (a single compound) are elevated to a similar extent in subjects over 70 years compared to controls under 30 (Stevens and Spencer, 1994).

In laboratory animal studies, behavioral tests have also demonstrated that several aspects of olfactory function decline with age. For example, olfactory perceptual learning, an ongoing process whereby animals learn to discriminate odorants, is impaired with aging (Moreno et al., 2014). Additionally, older mice require more training sessions and make more errors than younger mice for olfactory discrimination and have significantly higher detection thresholds for ethyl acetate vapor (Patel and Larson, 2009). Psychophysical experiments in rats found the highest olfactory sensitivity to occur at 13 months of age and the lowest sensitivity at 25 months of age and older. However, aged rats did not show any age-related deterioration in learning ability in an olfactory discrimination task compared to young adults (Kraemer and Apfelbach, 2004).

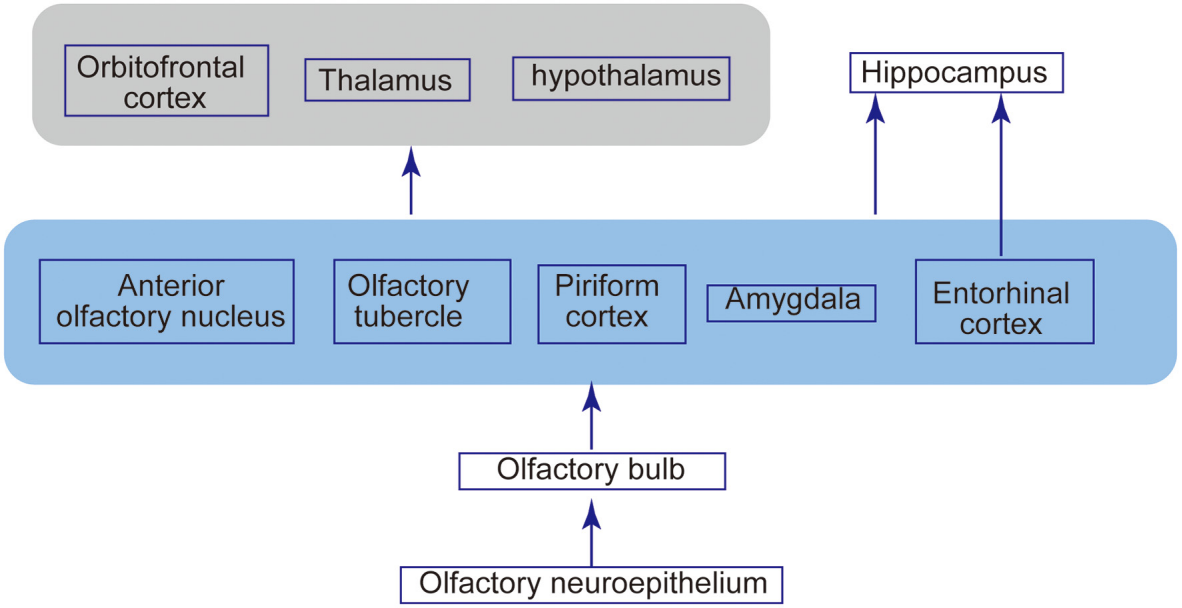

FIGURE 2 | Olfactory structures and neural connections. Odor molecules are perceived by odorant receptors expressed on the olfactory receptor neurons in the neuroepithelium. Olfactory signals are then sent intracranially to the OB and relayed to the second-order neurons, mitral cells, and tufted cells. These second-order neurons extend their axons along the lateral olfactory tract toward the structure of the primary olfactory cortex, such as the anterior olfactory nucleus, piriform cortex, and entorhinal cortex. This olfactory cortex has a projection extending to other brain areas, including the thalamus, hypothalamus, orbitofrontal cortex, and hippocampus.

Older rats were less reactive than younger rats in a test of cat odor avoidance, but they expressed similar amounts of cat odor-induced Fos (a neuronal activity marker) in the posterior accessory OB, a critical region for processing predator odor stimulus. Thus, the loss of reactivity may be due to changes in the more central olfactory processing (Hunt et al., 2011).

PREVALENCE OF AGE-RELATED OLFACTORY DYSFUNCTION IN HUMANS

Several epidemiological surveys have examined the prevalence of olfactory dysfunction in the general population. The methods included questionnaire-based self-assessment of olfactory function (Hoffman et al., 1998; Lee et al., 2013; Rawal et al., 2016) and use of psychophysical olfactory tests (Larsson et al., 2000; Murphy et al., 2002; Bramerson et al., 2004; Landis et al., 2004; Ross et al., 2008; Vennemann et al., 2008; Shu et al., 2009; Wehling et al., 2011; Gopinath et al., 2012b; Mullol et al., 2012). In general, the prevalence of olfactory impairment based on self-assessment is lower than that based on olfactory tests. For example, Hoffman et al. (1998) estimated based on a questionnaire survey from 42,000 households in the United States that 1.4% of American adults experience olfactory problems. Lee et al. (2013) reported based on a national survey in the Korean population (4,000 households) that the prevalence of subjective olfactory dysfunction in adults was 4.5%. In contrast, in a population-based study in Sweden, the olfactory function of 1,387 adults was tested using a smell identification test and 19.1% of respondents showed olfactory dysfunction (Bramerson et al., 2004). Likewise, in the population-based survey in Germany (n = 1,312, 25–75 years) using a smell identification test, the prevalence of olfactory dysfunction was estimated as 21.6% (Vennemann et al., 2008). This discrepancy is probably due to common unawareness of olfactory deterioration, especially in the elderly population (Murphy et al., 2002; Shu et al., 2009; Wehling et al., 2011). Furthermore, the prevalence of olfactory dysfunction based on olfactory tests varies among studies, possibly because of differences in the population examined, type of olfactory test, and definition of olfactory impairment. Despite such discrepancies in their methods, these studies have consistently demonstrated that the prevalence of olfactory dysfunction increases with age. For example, the National survey by Hoffman et al. (1998) in the United States demonstrated that the prevalence of subjective olfactory dysfunction was 2.0% in the 55–64 age range but was 4.6% in the 75 years and older population. Furthermore, in a population-based survey using an odor identification test in the United States, the prevalence of olfactory impairment was 6.1% in individuals aged 53–59 years but was 29.2% in individuals aged 70–79 years, and as much as 62.5% in the group aged 80–97 years (Murphy et al., 2002).

Some studies have estimated the risk of developing olfactory dysfunction by testing human subjects after specific time intervals and the results of these studies have demonstrated that risk increases with age. For example, the risk of

developing olfactory dysfunction during the following 5 years was 4.1% in the 53–59 age category but 21% in the 70–79 age category and 47.1% in the 80–97 age category (Schubert et al., 2011). In another study that evaluated 57–85-year-old American adults twice at 5-year intervals, the olfactory identification ability deteriorated more rapidly in older individuals and men than in their respective counterparts (Pinto et al., 2015).

RISK FACTORS FOR AGE-RELATED OLFACTORY DYSFUNCTION

Several risk factors have been identified for olfactory dysfunction. While most of the studies assessed olfactory dysfunction in general, a few have focused on identifying risk factors specific to olfactory dysfunction in the aging process. The results, however, vary depending on the studies.

Most of the epidemiological studies have demonstrated that males have a higher risk of being hyposmic, that is, have a reduced ability to smell and detect odors (Doty et al., 1984; Vennemann et al., 2008; Schubert et al., 2012, 2015; Dong et al., 2017). Beyond gender, ethnicity may influence olfactory decline. Dong et al. (2017) reported that the incidence of anosmia (loss of the sense of smell) in the older population is higher in black people compared to white people. Pinto et al. (2015) also reported that olfaction in African Americans deteriorates more rapidly than in Whites. Moreover, adults with Down's Syndrome (DS) show significantly poorer odor thresholds and odor identification abilities than age- and cognitively-matched control subjects (Murphy and Jinich, 1996; Nijjar and Murphy, 2002). Also, adults with DS show poorer odor identification ability than children and younger adults with DS, suggesting that age-related olfactory dysfunction progresses more rapidly in DS than in normal subjects (Nijjar and Murphy, 2002).

Another possible risk factor for olfactory dysfunction is smoking. In cross-sectional population-based surveys, ongoing chronic smoking increased the risk for impairment of olfactory function (Vennemann et al., 2008; Schubert et al., 2012; Glennon et al., 2019). A systematic review and meta-analysis also demonstrated that current smoking, but not former smoking, was associated with a significantly increased risk of olfactory dysfunction, suggesting that the effects of smoking on olfaction may be reversible (Ajmani et al., 2017). However, olfactory impairment in smokers has been reported to persist 15 years after quitting (Siegel et al., 2019). In animal studies, intranasal administration of tobacco solution induced degeneration of the olfactory neuroepithelium (Ueha et al., 2016a,b, 2018c).

Other suggested risk factors include heavy alcohol use (Schubert et al., 2011; Rawal et al., 2016; Glennon et al., 2019), sinonasal diseases (Schubert et al., 2011, 2012; Rawal et al., 2016), history of head injury (Rawal et al., 2016), income <110% poverty threshold (Rawal et al., 2016), and body weight loss (Gopinath et al., 2012b; Aiello et al., 2019). On the other hand, regular exercise was reported to be associated with a lower 10-year cumulative incidence of olfactory impairment (Schubert et al., 2013).

To determine the prognostic factors of olfactory dysfunction, London et al. (2008) assessed olfactory function in 542 patients using olfactory test scores on two occasions, which were separated from one another by a varying duration ranging from 3 months to 24 years. Patient age, the severity of initial olfactory loss, and the patient-reported duration of dysfunction at the first testing were significant predictors of improvement in olfactory function. Etiology, sex, the time between the two tests, and initial smoking behavior were not significant predictors. Moreover, Doty et al. (2011) measured odor identification ability in a population-based cohort of 1,222 twins and singletons of very old age. Sex, age, cognitive function, and smoking were significant predictors of olfactory test scores. The study also demonstrated that the effects of heritability on odor identification decline with age, suggesting that adverse environmental factors contribute more than genetic factors to such olfactory deterioration, especially at older ages (Doty et al., 2011).

PATHOLOGY OF THE AGE-RELATED OLFACTORY MUCOSA IN HUMANS AND LABORATORY ANIMALS

Because of the difficulty to obtain samples, information regarding age-related histological changes in the human olfactory mucosa is limited. Biopsy and cadaver studies have demonstrated that the surface of the olfactory mucosa decreases with age. Moreover, within the olfactory mucosa area, disruption of the zonal distribution of supporting, ORNs, and basal cells and patchy replacement of olfactory neuroepithelium by respiratory epithelium could be observed with increasing age (Nakashima et al., 1984; Paik et al., 1992; Holbrook et al., 2011).

In rodents, it has been shown that the olfactory mucosa surface decreases with age, especially in the anterior portion of the nasal cavity, and that the olfactory mucosa undergoes degenerative changes such as the irregular boundary of olfactory and respiratory regions, reduced number of ORNs, and inclusion bodies, as also seen in humans (Loo et al., 1996; Breckenridge et al., 1997; Rosli et al., 1999; Kondo et al., 2009). The lesions in the neuroepithelium and underlying Bowman's glands tend to be spatially co-localized, suggesting a close association between their pathogenesis (Kondo et al., 2009).

Age-related changes in organs may be due to both physiological degenerations associated with increasing lifetime and age-related changes associated with pathologies. It is difficult to discriminate these two causes for the change in the clinical setting. In laboratory animals, even animals kept under very clean air conditions show degenerative changes in the olfactory neuroepithelium (Loo et al., 1996). Therefore, age-related mucosal degenerative changes could be, at least in part, a result of mere physiological aging. Conversely, in the clinical setting, the incidence of postviral olfactory disorders is more frequent among middle-aged and elderly patients, and recovery becomes increasingly incomplete with increasing age (Reden et al., 2006), suggesting that aging may affect the susceptibility of the olfactory neural system against damaging

factors and its regenerative capacity after injury. In laboratory animals, rats can still detect food odor with more than 90% of the olfactory mucosa being degenerated (Youngentob et al., 1997), suggesting taht the peripheral olfactory system has a large spare ability. Should this also be the case in humans, patients who have just noticed olfactory deterioration may be in the final stages of olfactory neuroepithelial degeneration after the long-term latent progress of degeneration.

AGE-RELATED DECREASE IN BASAL CELL PROLIFERATION

Because the olfactory neuroepithelium has self-renewal capacity, the balance between olfactory neurogenesis and cell death is responsible for the maintenance of an adequate number of ORNs. Basal cell proliferation both in undamaged tissues and after injury decreases with age (Fung et al., 1997; Weiler and Farbman, 1997; Ducray et al., 2002; Kondo et al., 2010; Jia and Hegg, 2015; Ueha et al., 2018b; **Figure 1B**). Suzukawa et al. (2011) evaluated age-related changes in neuroepithelial regeneration after chemical injury in mice and demonstrated that: (1) the chronological pattern in neuronal cell proliferation and differentiation was similar among the different age groups; (2) the extent of neuroepithelial cell proliferation after injury decreased with age; and (3) the final histological recovery of the olfactory neuroepithelium and the innervation of the OB was significantly lower in the aged group than in younger age groups. These results suggest that the age-related decline in olfactory neuroepithelial regeneration capacity is associated with a decreased proliferative activity, rather than with changes in the neuronal differentiation process.

While apoptosis, the other cellular event that may be involved in the maintenance of the olfactory neuroepithelium cell population, has been investigated in several studies, it remains controversial to date whether or not apoptosis increases with age (Fung et al., 1997; Robinson et al., 2002; Conley et al., 2003; Kondo et al., 2010; Ueha et al., 2018b).

THE MOLECULAR BASIS UNDERLYING AGE-RELATED NEUROEPITHELIAL CHANGES

The molecular basis underlying age-related changes in the olfactory neuroepithelium remains largely unclear. While there have been an increasing number of studies addressing the molecular mechanisms underlying olfactory neural regeneration, most of these did not specifically investigate the aging process.

During basal cell proliferation, several transcription factors operate concomitantly to further or stop the cell division process (Beites et al., 2005; Schwob et al., 2017). The expression of cyclin D, a transcription factor promoting cell proliferation, decreases with age (Legrier et al., 2001). The expression of various growth factors such as epidermal growth factor, insulin-like growth factor-1 (IGF-1), and neuropeptide Y signaling decreases with age (Enwere et al., 2004; Chaker et al., 2015; Jia and Hegg, 2015; Ueha et al., 2018b), which may also be associated with the decrease in basal cell proliferation.

The aging of stem cells, in addition to extrinsic factors, may also be associated with the age-related decrease in basal cell proliferation. For example, the number of basal cells which express the neural stem cell marker Musashi-1decreases with age (Watanabe et al., 2007). Telomerase-deficient mice, in which there is a shortening of telomere lengths, show more partial neuroepithelial recovery compared with wild type mice after olfactory mucosal injury (Watabe-Rudolph et al., 2011). Child et al. (2018) have demonstrated that in transgenic mice in which the diphtheria toxin is expressed in ORNs under the control of the olfactory marker protein (OMP) promoter, ORNs apoptosis increases and cell turnover in the neuroepithelium is accelerated. In this model, the neurogenetic capacity of basal cells is "exhausted" and neuroepithelial degeneration persists even after toxin expression has ceased. This suggests that when excess basal cell division occurs due to the repeated damage of ORNs, the capacity of the basal cell to proliferate is eventually lost and neuroepithelial degeneration occurs (Child et al., 2018).

In the aging process, cells are exposed to many sources of oxidative stress and can stop mitotic activity irreversibly. Some affected cells undergo apoptosis, but others survive and begin to secret inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). This phenomenon is designated as the senescence-associated secretory phenotype (Zhu et al., 2014). A similar process may occur in the olfactory mucosa, as supported by the higher concentration of IL-6 in the olfactory mucosa of aged mice compared to young mice (Ueha et al., 2018b). In a human study, hyposmia was correlated with increased IL-6 concentrations in serum and nasal mucus (Henkin et al., 2013). It has also been shown that in a transgenic mouse model of olfactory inflammation, in which TNF-α expression is induced specifically within the olfactory epithelium, inflammation induces the suppression of basal cell proliferation with the resulting degeneration of the olfactory neuroepithelium (Lane et al., 2010). These observations suggest that the elevation of inflammatory cytokines in the aged olfactory neuroepithelium may be associated with the loss of ORNs and suppression of basal cell proliferation.

DNA microarray analysis of olfactory mucosa in senescence-accelerated mouse (SAM) has demonstrated that changes in the expression of genes associated with chemosensory detection, immune barrier function, xenobiotic metabolism, cell cycle progression, and cell death were particularly prominent in old SAM strains (Getchell et al., 2003, 2004). Conversely, Rimbault et al. (2009) have demonstrated using microarray and quantitative polymerase chain reaction (PCR) that, when gene expression in the olfactory mucosa was compared between newborn, 9-week, and 22-month-old Brown Norway rats, overall gene expression did not change considerably across ages, with only 0.25% of the transcripts showing differential expression. This suggests that age-related changes in gene expression in the olfactory mucosa may be strain-dependent.

AGE-RELATED CHANGES IN THE EXPRESSION OF ODORANT RECEPTORS

Several studies have explored whether the expression pattern of odorant receptors (OR) changes with age. Khan et al. (2013) have reported using Nanostring assay that the gene expression profile of odorant receptors in the C57B6 mice strain is almost stable within the 2–31 month age range. Rimbault et al. (2009) have also demonstrated using microarray and quantitative PCR that the OR gene expression is not different between 9-week and 22-month-old Brown Norway rats. Conversely, in another mouse study conducted by Ueha et al. (2018b) using microarray analysis, the expression of many OR genes changed with age. Also, in mice, although the overall number of ORNs decreases with age, the extent of the loss of ORNs differs according to the type of odorant receptor the neurons express (Lee et al., 2009). In contrast, the number of ORs expressed on individual ORNs is stable (Lee et al., 2009). In humans, the response of individual ORNs to odors is not affected by aging, while the specificity of the response of each ORN to the odor decreases with age (Rawson et al., 2012). If the expression level of specific ORs on the olfactory mucosa decreases with age, elderly people may show decreased sensitivity for these specific odors. In other words, it is possible that the quality of the odorant perception when smelling changes with age, because the combination of ORs activated by the odor could change. Parosmia is a dysfunction in the field of smell detection characterized by the inability to properly identifying an odor's natural smell. In humans, parosmia is often observed in cases of postviral or traumatic olfactory disorders, whereas it is rarely observed in age-related slowly progressing olfactory decline (Nordin et al., 2007). This finding suggests that in humans, although the overall number of ORNs decreases with age, the proportion of each OR gene expression in the olfactory mucosa may not be different between young and older individuals.

AGE-RELATED CHANGES IN THE STRUCTURE AND CELL DYNAMICS OF THE OLFACTORY BULB

Human studies have reported that the volume of the OB, the glomerular layer thickness, the number of glomeruli, and the concentration of mitral cells per unit area all decrease with increasing age (Bhatnagar et al., 1987; Meisami et al., 1998; Yousem et al., 1998). Studies in rodents have demonstrated more mixed results. In mice, the bulb volume did not change (Richard et al., 2010), or rather increased with age (Mirich et al., 2002). The number of mitral cells is not altered considerably during physiological aging (Richard et al., 2010). In rats, from 24 to 30 months of age, a significant decrease occurs in the volume of the bulb layers, and the number of mitral cells decreases (Hinds and McNelly, 1977). The number of olfactory axodendritic synapses in the glomeruli and the total volume of glomerular dendrites, especially in the glomerulus layer, both decrease with age (Richard et al., 2010). Such decreases appear to reflect the decrease in synapse formation between the dendrites of the neurons, as well as the decrease in the number of ORNs (Buschhüter et al., 2008). It is thus suggested that the atrophic changes in the OB may be in part secondary to changes in the ORNs. The number of synapses in the glomeruli appears to decline less markedly with age than the number of ORNs, and a significant increase in the number of synapses per ORN occurs in the oldest group studied (33 months), suggesting a compensatory increase in the relative number of synapses per ORN (Hinds and McNelly, 1979, 1981).

The number of stem cells and their proliferation in the SVZ, and the migration of new neurons as well as their integration into the neural system in the OB, are all reduced with age, and the elimination of adult-born neurons in the OB is promoted with age (**Figure 1B**; Maslov et al., 2004; Honda et al., 2009; Choi et al., 2010; Bouab et al., 2011). Because the survival of granule cells is influenced by odorant stimulation and food-intake activity (Yokoyama et al., 2011), the decrease in the migration of new neurons may be a secondary change due to the reduced sensory input caused by the decrease in the number of ORNs (Yoshihara et al., 2012).

Interestingly, the granule cell density in the OB does not significantly decrease with age (Richard et al., 2010), but rather may increase (Enwere et al., 2004). This suggests that the turnover in the granule cell population decreases, with granule cells living longer in aged animals than in young animals (Sui et al., 2012). The relevance of these findings to olfactory function remains unclear, but the decrease in the turnover of granule cells may lead to a less flexible reorganization of neural connections in response to a new odorant. It has also been suggested that neurogenesis, rather than the total number of interneurons, is important for fine olfactory discrimination (Enwere et al., 2004).

If the change in the cell turnover of the OB represents the primary change, changes in cell turnover may reflect changes in the availability of growth factors. It has been reported that the expression of IGF-1 decreases with age (Ferrari et al., 2003; Chaker et al., 2015) and that insulin binding decreases in the OB with age, probably because of the decrease in the insulin receptor number in the OB (Tchilian et al., 1990). The accumulation of oxidative stress and the resulting cell aging may also cause age-related changes in the OB (Vaishnav et al., 2007; Romero-Grimaldi et al., 2008).

AGE-RELATED CHANGES IN THE CENTRAL OLFACTORY PATHWAY AND OLFACTORY CORTEX

To date, there is limited evidence regarding age-related changes in the central olfactory pathway and olfactory cortex regions such as the anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala cortical nucleus, and entorhinal cortex. However, changes in the central olfactory pathway also appear to be involved in age-related functional deficits.

Human morphometrical studies using magnetic resonance imaging (MRI) have yielded mixed results. One study suggests that among the olfactory-related brain structures, the volume of the piriform cortex and that of the amygdala cortex do not reduce with age, while the volume of the orbitofrontal cortex significantly decreases (Shen et al., 2013). Another study examining normal subjects across ages showed that the volume of the OB and tract showed an initial increase up to the 4th decade of life, followed by a decrease with increasing age (Yousem et al., 1998). Conversely, functional MRI studies suggest that activation of the central olfactory region, including the piriform cortex, entorhinal cortex, and amygdala, and of the orbitofrontal cortex decreases in older age (Cerf-Ducastel and Murphy, 2003; Wang et al., 2005). During early aging, the activity of the primary olfactory cortex under olfactory stimulation is not correlated with age, while the activity of the secondary and higher central olfactory structures (prefrontal cortex, insular cortex, and orbitofrontal cortex) is negatively correlated. This suggests that in the early aging process, an age-related functional decline in the human brain is more prominent in the secondary and higher-order central olfactory structures than in other regions (Wang et al., 2017). Furthermore, a positron emission tomography (PET) study has demonstrated that age-related reduction in the binding potential for the striatal dopamine transporter in the putamen is associated with the age-related olfactory deficit (Larsson et al., 2009). Consistent with these findings, the olfactory event-related potential shows an age-related decrease in its amplitude and processing speed with increasing age (Yousem et al., 1999; Murphy et al., 2000; Suzuki et al., 2001).

In animal studies, the histological analysis of rat brains has also demonstrated that the volume of the piriform cortex does not decline with age (Curcio et al., 1985). Conversely, electrophysiological recordings in the anterior piriform cortex have demonstrated that the synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is decreased during aging, suggesting that glutamatergic synaptic function changes with age (Gocel and Larson, 2013).

PUTATIVE GENE POLYMORPHISM ASSOCIATED WITH AGE-RELATED OLFACTORY DYSFUNCTION IN HUMANS

Recent analyses have demonstrated the association of gene variants with olfactory dysfunction, but few of these analyses have addressed age-related olfactory dysfunction. Dong et al. (2015) performed the first genome-wide meta-analysis on the sense of smell among 6,252 U.S. older adults of European descent. The results suggest that the microtubule-associated protein tau locus may play a role in regulating the sense of smell in older adults. Furthermore, the effect of the brain-derived neurotrophic factor (BDNF) val66met polymorphism on olfactory function changes was examined in a large-scale, longitudinal population-based sample (Hedner et al., 2010). The magnitude of the olfactory decline in the older age cohort was larger for the VAL homozygote carriers than for the MET carriers.

ASSOCIATION OF AGE-RELATED CHANGES IN TRIGEMINAL AND AUTONOMIC NERVOUS SYSTEMS WITH OLFACTORY DYSFUNCTION

Another sensory system in the nasal cavity is the trigeminal sensory system. It has been suggested that the trigeminal system contributes to olfaction. Compared to younger subjects, older people have a reduced sensitivity to the intranasal trigeminal system (Frasnelli and Hummel, 2003). Furthermore, patients with olfactory dysfunction have lower scores in the lateralization task than controls, indicating decreased trigeminal sensitivity compared to healthy controls (Hummel et al., 2003). Since it has been reported that the loss of trigeminal sensitivity reduces olfactory sensitivity (Husner et al., 2006), the function of the trigeminal and olfactory nervous system may be linked. There is a neural connection between these two nervous systems in the OB (Finger and Böttger, 1993; Schaefer et al., 2002). Both the trigeminal and autonomic nervous system innervations in the olfactory mucosa show age-related changes (Chen et al., 1993). The distribution of adrenergic innervation in the human olfactory mucosa reveals a decrease in the innervation density of blood vessels over 60 years of age. These age-related changes may be involved in age-related olfactory sensitivity.

OLFACTORY DYSFUNCTION ASSOCIATED WITH NEURODEGENERATIVE DISEASES

More and more attention has been paid to the association between olfactory dysfunction and neurodegenerative diseases whose incidence increases with aging (Kovács, 2004; Barresi et al., 2012). The olfactory central pathway has recently gained more attention clinically, since the early pathological changes of neurodegenerative diseases, including AD and PD, occur in the central olfactory pathways.

In AD, the most frequent neurodegenerative disease globally, characteristic pathological changes such as senile plaque and neurofibrile changes appear in the OB, anterior olfactory nucleus, and entorhinal cortex (Braak and Braak, 1991; Hyman et al., 1991; Kovács et al., 1999; Daulatzai, 2015). The OB as well as the olfactory neuroepithelium show degenerative changes in patients with AD (Trojanowski et al., 1991; Yamagishi et al., 1998; Kovács et al., 2001; Attems et al., 2005; Thomann et al., 2009). Patients with AD show olfactory dysfunction at the early phase of the disease (Doty et al., 1987; Serby et al., 1991; Peters et al., 2003; Hori et al., 2015; Silva et al., 2018; Jung et al., 2019; **Figure 3**). Odor identification deteriorates first followed by odor detection (Murphy et al., 1990; Serby et al., 1991). When elderly individuals with normal cognitive function were prospectively followed up, subjects with olfactory dysfunction showed a faster cognitive decline (Dintica et al., 2019) and developed mild cognitive impairment (MCI) more

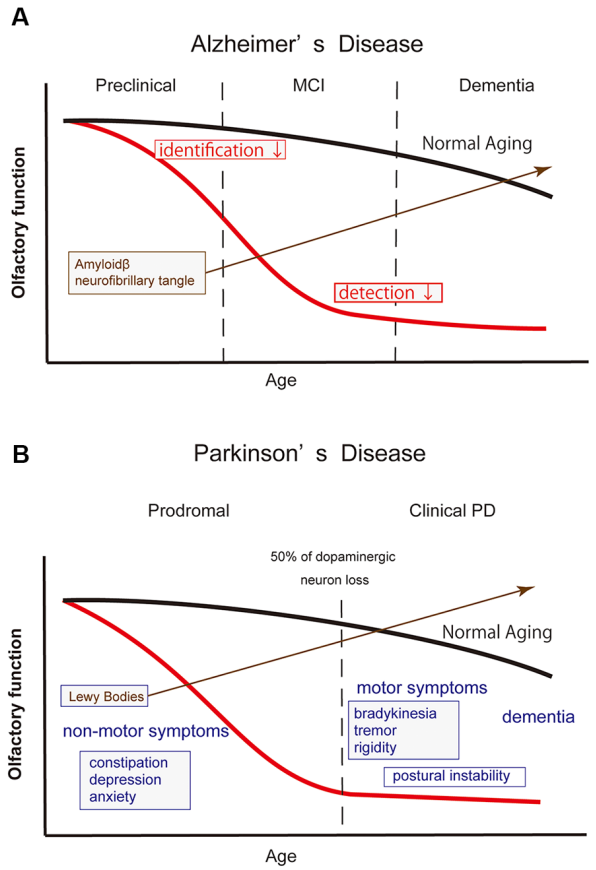

FIGURE 3 | Schematic drawing illustrating the olfactory dysfunction and time course of Alzheimer's disease (AD) and Parkinson's disease (PD). In both neurodegenerative diseases, the emergence of olfactory dysfunction precedes their definite diagnosis. The red and black lines in the drawing indicate the time course of olfactory function in patients and normal elderly individuals, respectively. **(A)** In AD, olfactory dysfunction emerges in its preclinical phase when cognitive function is still preserved. At the time, however, the accumulation of amyloid β already begins in the brain structures, including in the olfactory pathway. Odor identification ability deteriorates first, followed by a worsening in odor detection. **(B)** Definite diagnosis of PD is made by the emergence of motor symptoms when approximately 50% of dopaminergic neurons are lost in the substantia nigra. Olfactory dysfunction emerges in the prodromal phase, along with other non-motor symptoms. Lewy bodies are already observed in the olfactory system, including the OB. In clinical PD, the severity of olfactory dysfunction does not appear to be correlated with the disease duration or disease stages, suggesting that the degenerative changes in the olfactory neural structures have progressed at the time of diagnosis.

frequently than the subjects with normal olfaction (Wilson et al., 2007; MacDonald et al., 2018). Furthermore, MCI patients with olfactory dysfunction transited to AD more frequently than patients with normal olfaction (Devanand et al., 2000; Roberts et al., 2016). Therefore, olfactory dysfunction is expected to be one of the biomarkers to predict progression from normal cognitive status or MCI to AD (Adams et al., 2018; Windon et al., 2020). One study reported that a specific pattern of olfactory identification deficit may differentiate AD from age-related olfactory loss (Woodward et al., 2018).

PD represents the second-largest neurodegenerative population after AD. Patients with PD show olfactory dysfunction (Doty et al., 1988; Iijima et al., 2008; Watanabe et al., 2017), and the olfactory test score is not correlated with motor function, disease duration, or disease stages (Doty et al., 1988; Iijima et al., 2008; **Figure 3**). The Movement Disorders Society has officially adopted olfactory dysfunction as a supporting diagnostic criterion for clinical PD (Postuma et al., 2015), as well as a supporting research criterion for prodromal PD (Berg et al., 2015). Hyposmia has a high discriminatory power to differentiate PD from differentials such as multiple system atrophy, progressive supranuclear palsy, drug-induced parkinsonism, and essential tremor, with >80% sensitivity and specificity (Mahlknecht et al., 2016). Older people with olfactory dysfunction are at higher risk of developing PD (Ross et al., 2008; Berg et al., 2012; Chen et al., 2017; Fullard et al., 2017). Two recent studies have reported that the relative risk of developing incident PD in hyposimc subjects over non-hyposmic subjects up to a 10-year follow-up period was 3–4 (Chen et al., 2017; Mahlknecht et al., 2020). These findings suggest that there is a long prodromal phase of the illness and that the patients in this phase may be underdiagnosed as merely having "age-related olfactory dysfunction."

Characteristic pathological changes of PD such as Lewy bodies and the accumulation of alpha-synuclein are observed at the early phase of the disease (Braak et al., 2004; Funabe et al., 2013). Histological studies have suggested that the deposition of Lewy bodies appears preferentially in the olfactory tract, including the OB and anterior olfactory nucleus (Hubbard et al., 2007; Funabe et al., 2013). Interestingly, the presence of Lewy bodies in the brain is associated with olfactory dysfunction in otherwise asymptomatic elderly individuals (Ross et al., 2006; Wilson et al., 2011) and this is thought to represent a presymptomatic stage of PD. Taken together, olfactory dysfunction could be a useful screening biomarker to identify those at high risk for developing PD. It has also been reported that olfactory impairment predicts cognitive decline in early PD patients (Baba et al., 2012; Fullard et al., 2016; Domellöf et al., 2017).

Early diagnosis and intervention of patients at risk and earlier stages of the disease appear to be essential for any successful neuroprotection. In PD, the degenerative change in the dopaminergic neurons in the substantia nigra has already progressed severely (approximately 50% of loss) when neurologists can make a diagnosis according to the accepted clinical diagnostic criteria (Becker et al., 2002). Neuroprotective therapy starting at such an advanced stage of the disease may not be effective enough to stop the degenerative process. Therefore, the identification of non-motor symptoms, especially the olfactory function, may be useful for the early diagnosis and treatment of PD. However, as a single marker, hyposmia is not specific to predict PD, and therefore additional markers are needed for an accurate diagnosis. Berg et al. (2015) have proposed a formula to estimate the probability of prodromal PD using several parameters. Jennings et al. (2017) also have proposed a two-step approach (olfactory test followed by dopamine transporter imaging) to identify individuals from the general population at risk for conversion to a clinical diagnosis of PD.

MANAGEMENT STRATEGIES FOR AGE-RELATED OLFACTORY DYSFUNCTION

The prevention of olfaction dysfunction may lead to happier and more successful aging. In the case of olfactory impairment, clinical management may help patients to overcome the difficulties associated with their impairment. Although, several drugs have been tested for the treatment of age-related sensorineural olfactory dysfunction including zinc, vitamins, and herbal medicines, no evidence-based medicine has been established to improve age-related olfactory dysfunction (Miwa et al., 2019).

Recently, olfactory training has been reported to be useful for the treatment of sensorineural olfactory disorders (Hummel et al., 2009; Damm et al., 2014). The original method reported by Hummel et al. (2009) required patients to expose themselves twice daily to four odors [phenyl ethyl alcohol (PEA): rose, eucalyptol: eucalyptus, citronellal: lemon, and eugenol: cloves]. Olfactory training has been reported to improve age-related olfactory loss (Birte-Antina et al., 2018), although further studies are warranted to confirm the efficacy.

The mechanism underlying odor stimulation-dependent improvement is unclear, but as described above, the number of interneurons in the OB is regulated depending on odor stimulation (Yokoyama et al., 2011). Also, in the olfactory neuroepithelium, odor stimulation is important to maintain the survival of newly-generated ORNs, especially in the critical periods of making synapses (Kikuta et al., 2015).

Although still at the experimental stage, the intranasal application of growth factors, gene therapy, and stem cell transplantation have been tested as a treatment of sensorineural olfactory degeneration (Choi and Goldstein, 2018; Kurtenbach et al., 2019). Intranasal application of drugs/genes/cells may also be useful for age-related olfactory dysfunction because the most prominent pathological feature of the age-related changes in olfactory neuroepithelium is the reduced basal cell proliferation (Weiler and Farbman, 1997; Kondo et al., 2010; Suzukawa et al., 2011). The olfactory mucosa is exposed to an airway and offers the advantage of easy accessibility. Animal studies have demonstrated that intranasal application of fibroblast growth factor-2 and IGF-1 promote neuroepithelial regeneration after chemical injury in aged mice (Fukuda et al., 2018). Another study in which IGF-1 was administered subcutaneously to aged mice demonstrated that while low-dose IGF-1 administration increases the numbers of olfactory progenitors, immature ORNs, and mature ORNs in the olfactory neuroepithelium (OE), high-dose IGF-1 administration increases only the number of immature ORNs, with a concurrent increase in apoptotic cells (Ueha et al., 2018a). This finding suggests that in designing drug therapies, the use of an appropriate dose is important.

Intranasal administration of drugs has also been extensively studied as a treatment of central nervous system diseases, because the olfactory mucosa may be used as a route to deliver drugs to the intracranial space bypassing the blood-brain barrier (Chapman et al., 2013). The provision of daily-life advice, especially to guarantee patient safety and the appreciation of food is also important to manage age-related olfactory impairment. With olfactory deterioration, patients tend to fail the detection of hazardous odors, such as gas leakage and fire smoke odors. In a family of an elderly couple, possibly none can detect such hazardous odors. For such patients, the use of odor detection machines is recommended (Miwa et al., 2001). Patients may also fail to notice the smell of spoiled food. In such a situation, it is recommended to pay attention to food conditions by checking the expiration date label, especially in the summertime.

Another problem to be addressed is malnutrition due to olfactory impairment. It is reported that the addition of flavor to the food may increase appetite and improve the nutritional condition (Schiffman and Warwick, 1993). Conversely, patients with a neural disorder such as postviral and traumatic olfactory dysfunction, frequently experience parosmia, which causes food such as fish, oily food, some vegetables and fruits, and fermented goods to have unpleasant odors during the recovery period. Therefore, adequate food choices while cooking are important to maintain the joy of the meals.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. SK and KK designed the figures. All authors reviewed and approved the final version of the manuscript.

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REFERENCES

Adams, D. R., Kern, D. W., Wroblewski, K. E., McClintock, M. K., Dale, W., and Pinto, J. M. (2018). Olfactory dysfunction predicts subsequent dementia in older U.S. adults. *J. Am. Geriatr. Soc.* 66, 140–144. doi: 10.1111/jgs.15048

Aiello, M., Parma, V., De Carlo, S., Hummel, T., and Rumiati, R. I. (2019). Cognitive, olfactory, and affective determinants of body weight in aging individuals. *Arch. Clin. Neuropsychol.* 34, 637–647. doi: 10.1093/arclin/acy072

Ajmani, G. S., Suh, H. H., Wroblewski, K. E., and Pinto, J. M. (2017). Smoking and olfactory dysfunction: a systematic literature review and meta-analysis. *Laryngoscope* 127, 1753–1761. doi: 10.1002/lary.26558

Attems, J., Lintner, F., and Jellinger, K. A. (2005). Olfactory involvement in aging and Alzheimer's disease: an autopsy study. *J. Alzheimers Dis.* 7, 149–157; discussion 173–180. doi: 10.3233/jad-2005-7208

Attems, J., Walker, L., and Jellinger, K. A. (2015). Olfaction and aging: a mini-review. *Gerontology* 61, 485–490. doi: 10.1159/000381619

Baba, T., Kikuchi, A., Hirayama, K., Nishio, Y., Hosokai, Y., Kanno, S., et al. (2012). Severe olfactory dysfunction is a prodromal symptom of dementia associated with Parkinson's disease: a 3 year longitudinal study. *Brain* 135, 161–169. doi: 10.1093/brain/awr321

Barresi, M., Ciurleo, R., Giacoppo, S., Foti Cuzzola, V., Celi, D., Bramanti, P., et al. (2012). Evaluation of olfactory dysfunction in neurodegenerative diseases. *J. Neurol. Sci.* 323, 16–24. doi: 10.1016/j.jns.2012.08.028

Becker, G., Muller, A., Braune, S., Buttner, T., Benecke, R., Greulich, W., et al. (2002). Early diagnosis of Parkinson's disease. *J. Neurol.* 249, 40–48. doi: 10.1007/s00415-002-1309-9

Beites, C. L., Kawauchi, S., Crocker, C. E., and Calof, A. L. (2005). Identification and molecular regulation of neural stem cells in the olfactory epithelium. *Exp. Cell Res.* 306, 309–316. doi: 10.1016/j.yexcr.2005.03.027

Berg, D., Marek, K., Ross, G. W., and Poewe, W. (2012). Defining at-risk populations for Parkinson's disease: lessons from ongoing studies. *Mov. Disord.* 27, 656–665. doi: 10.1002/mds.24985

Berg, D., Postuma, R. B., Adler, C. H., Bloem, B. R., Chan, P., Dubois, B., et al. (2015). MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* 30, 1600–1611. doi: 10.1002/mds.26431

Bergman, U., Ostergren, A., Gustafson, A. L., and Brittebo, B. (2002). Differential effects of olfactory toxicants on olfactory regeneration. *Arch. Toxicol.* 76, 104–112. doi: 10.1007/s00204-002-0321-2

Bhatnagar, K. P., Kennedy, R. C., Baron, G., and Greenberg, R. A. (1987). Number of mitral cells and the bulb volume in the aging human olfactory bulb: a quantitative morphological study. *Anat. Rec.* 218, 73–87. doi: 10.1002/ar.1092180112

Birte-Antina, W., Ilona, C., Antje, H., and Thomas, H. (2018). Olfactory training with older people. *Int. J. Geriatr. Psychiatry* 33, 212–220. doi: 10.1002/gps.4725

Bouab, M., Paliouras, G. N., Aumont, A., Forest-Bérard, K., and Fernandes, K. J. (2011). Aging of the subventricular zone neural stem cell niche: evidence for quiescence-associated changes between early and mid-adulthood. *Neuroscience* 173, 135–149. doi: 10.1016/j.neuroscience.2010.11.032

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 82, 239–259. doi: 10.1007/BF00308809

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., and Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res.* 318, 121–134. doi: 10.1007/s00441-004-0956-9

Bramerson, A., Johansson, L., Ek, L., Nordin, S., and Bende, M. (2004). Prevalence of olfactory dysfunction: the skovde population-based study. *Laryngoscope* 114, 733–737. doi: 10.1097/00005537-200404000-00026

Brämerson, A., Nordin, S., and Bende, M. (2007). Clinical experience with patients with olfactory complaints and their quality of life. *Acta Otolaryngol.* 127, 167–174. doi: 10.1080/00016480600801357

Brann, J. H., and Firestein, S. J. (2014). A lifetime of neurogenesis in the olfactory system. *Front. Neurosci.* 8:182. doi: 10.3389/fnins.2014.00182

Breckenridge, L. J., Cameron, J., Puri, N., Reid, O., McGadey, J., and Smith, R. A. (1997). Localised degeneration occurs in aged mouse olfactory epithelium. *J. Anat.* 191, 151–154. doi: 10.1046/j.1469-7580.1997.19110151.x

Buschhüter, D., Smitka, M., Puschmann, S., Gerber, J. C., Witt, M., Abolmaali, N. D., et al. (2008). Correlation between olfactory bulb volume and olfactory function. *NeuroImage* 42, 498–502. doi: 10.1016/j.neuroimage.2008.05.004

Cerf-Ducastel, B., and Murphy, C. (2003). FMRI brain activation in response to odors is reduced in primary olfactory areas of elderly subjects. *Brain Res.* 986, 39–53. doi: 10.1016/s0006-8993(03)03168-8

Chaker, Z., Aid, S., Berry, H., and Holzenberger, M. (2015). Suppression of IGF-I signals in neural stem cells enhances neurogenesis and olfactory function during aging. *Aging Cell* 14, 847–856. doi: 10.1111/acel.12365

Chapman, C. D., Frey, W. H. II., Craft, S., Danielyan, L., Hallschmid, M., Schioth, H. B., et al. (2013). Intranasal treatment of central nervous system dysfunction in humans. *Pharm. Res.* 30, 2475–2484. doi: 10.1007/s11095-012-0915-1

Chen, Y., Getchell, T. V., Sparks, D. L., and Getchell, M. L. (1993). Patterns of adrenergic and peptidergic innervation in human olfactory mucosa: age-related trends. *J. Comp. Neurol.* 334, 104–116. doi: 10.1002/cne.903340109

Chen, H., Shrestha, S., Huang, X., Jain, S., Guo, X., Tranah, G. J., et al. (2017). Olfaction and incident Parkinson disease in US white and black older adults. *Neurology* 89, 1441–1447. doi: 10.1212/WNL.0000000000004382

Child, K. M., Herrick, D. B., Schwob, J. E., Holbrook, E. H., and Jang, W. (2018). The neuroregenerative capacity of olfactory stem cells is not limitless: implications for aging. *J. Neurosci.* 38, 6806–6824. doi: 10.1523/jneurosci.3261-17.2018

Choi, J. H., Yoo, K. Y., Lee, C. H., Park, O. K., Yan, B. C., Li, H., et al. (2010). Comparison of newly generated doublecortin-immunoreactive neuronal progenitors in the main olfactory bulb among variously aged gerbils. *Neurochem. Res.* 35, 1599–1608. doi: 10.1007/s11064-010-0220-5

Choi, R., and Goldstein, B. J. (2018). Olfactory epithelium: cells, clinical disorders and insights from an adult stem cell niche. *Laryngoscope Investig. Otolaryngol.* 3, 35–42. doi: 10.1002/lio2.135

Conley, D. B., Robinson, A. M., Shinners, M. J., and Kern, R. C. (2003). Age-related olfactory dysfunction: cellular and molecular characterization in the rat. *Am. J. Rhinol.* 17, 169–175. doi: 10.1177/194589240301700311

Croy, I., Nordin, S., and Hummel, T. (2014). Olfactory disorders and quality of life--an updated review. *Chem. Senses* 39, 185–194. doi: 10.1093/chemse/bjt072

Curcio, C. A., McNelly, N. A., and Hinds, J. W. (1985). Aging in the rat olfactory system: relative stability of piriform cortex contrasts with changes in olfactory bulb and olfactory epithelium. *J. Comp. Neurol.* 235, 519–528. doi: 10.1002/cne.902350409

Damm, M., Pikart, L. K., Reimann, H., Burkert, S., Goktas, O., Haxel, B., et al. (2014). Olfactory training is helpful in postinfectious olfactory loss: a randomized, controlled, multicenter study. *Laryngoscope* 124, 826–831. doi: 10.1002/lary.24340

Daulatzai, M. A. (2015). Olfactory dysfunction: its early temporal relationship and neural correlates in the pathogenesis of Alzheimer's disease. *J. Neural Transm.* 122, 1475–1497. doi: 10.1007/s00702-015-1404-6

Devanand, D. P., Lee, S., Manly, J., Andrews, H., Schupf, N., Masurkar, A., et al. (2015). Olfactory identification deficits and increased mortality in the community. *Ann. Neurol.* 78, 401–411. doi: 10.1002/ana.24447

Devanand, D. P., Michaels-Marston, K. S., Liu, X., Pelton, G. H., Padilla, M., Marder, K., et al. (2000). Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. *Am. J. Psychiatry* 157, 1399–1405. doi: 10.1176/appi.ajp.157.9.1399

Dintica, C. S., Marseglia, A., Rizzuto, D., Wang, R., Seubert, J., Arfanakis, K., et al. (2019). Impaired olfaction is associated with cognitive decline and neurodegeneration in the brain. *Neurology* 92, e700–e709. doi: 10.1212/wnl.0000000000006919

Domellöf, M. E., Lundin, K. F., Edstrom, M., and Forsgren, L. (2017). Olfactory dysfunction and dementia in newly diagnosed patients with Parkinson's disease. *Parkinsonism Relat. Disord.* 38, 41–47. doi: 10.1016/j.parkreldis.2017.02.017

Dong, J., Pinto, J. M., Guo, X., Alonso, A., Tranah, G., Cauley, J. A., et al. (2017). The prevalence of anosmia and associated factors among U.S. black and white older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 72, 1080–1086. doi: 10.1093/gerona/glx081

Dong, J., Yang, J., Tranah, G., Franceschini, N., Parimi, N., Alkorta-Aranburu, G., et al. (2015). Genome-wide meta-analysis on the sense of smell among US older adults. *Medicine* 94:e1892. doi: 10.1097/md.0000000000001892

Doty, R. L. (2017). Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? *Lancet Neurol.* 16, 478–488. doi: 10.1016/S1474-4422(17)30123-0

Doty, R. L., Deems, D. A., and Stellar, S. (1988). Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology* 38, 1237–1244. doi: 10.1212/wnl.38.8.1237

Doty, R. L., and Kamath, V. (2014). The influences of age on olfaction: a review. *Front. Psychol.* 5:20. doi: 10.3389/fpsyg.2014.00020

Doty, R. L., Petersen, I., Mensah, N., and Christensen, K. (2011). Genetic and environmental influences on odor identification ability in the very old. *Psychol. Aging* 26, 864–871. doi: 10.1037/a0023263

Doty, R. L., Reyes, P. F., and Gregor, T. (1987). Presence of both odor identification and detection deficits in Alzheimer's disease. *Brain Res. Bull.* 18, 597–600. doi: 10.1016/0361-9230(87)90129-8

Doty, R. L., Shaman, P., Applebaum, S. L., Giberson, R., Siksorski, L., and Rosenberg, L. (1984). Smell identification ability: changes with age. *Science* 226, 1441–1443. doi: 10.1126/science.6505700

Ducray, A., Bondier, J. R., Michel, G., Bon, K., Millot, J. L., Propper, A., et al. (2002). Recovery following peripheral destruction of olfactory neurons in young and adult mice. *Eur. J. Neurosci.* 15, 1907–1917. doi: 10.1046/j.1460-9568.2002.02044.x

Enwere, E., Shingo, T., Gregg, C., Fujikawa, H., Ohta, S., and Weiss, S. (2004). Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis and deficits in fine olfactory discrimination. *J. Neurosci.* 24, 8354–8365. doi: 10.1523/jneurosci.2751-04.2004

Farbman, A. I. (1990). Olfactory neurogenesis: genetic or environmental controls? *Trends Neurosci.* 13, 362–365. doi: 10.1016/0166-2236(90)90017-5

Ferrari, C. C., Johnson, B. A., Leon, M., and Pixley, S. K. (2003). Spatiotemporal distribution of the insulin-like growth factor receptor in the rat olfactory bulb. *Neurochem. Res.* 28, 29–43. doi: 10.1023/a:1021639926941

Finger, T. E., and Böttger, B. (1993). Peripheral peptidergic fibers of the trigeminal nerve in the olfactory bulb of the rat. *J. Comp. Neurol.* 334, 117–124. doi: 10.1002/cne.903340110

Frasnelli, J., and Hummel, T. (2003). Age-related decline of intranasal trigeminal sensitivity: is it a peripheral event? *Brain Res.* 987, 201–206. doi: 10.1016/s0006-8993(03)03336-5

Fukuda, Y., Katsunuma, S., Uranagase, A., Nota, J., and Nibu, K. I. (2018). Effect of intranasal administration of neurotrophic factors on regeneration of chemically degenerated olfactory epithelium in aging mice. *Neuroreport* 29, 1400–1404. doi: 10.1097/wnr.0000000000001125

Fullard, M. E., Morley, J. F., and Duda, J. E. (2017). Olfactory dysfunction as an early biomarker in Parkinson's disease. *Neurosci. Bull.* 33, 515–525. doi: 10.1007/s12264-017-0170-x

Fullard, M. E., Tran, B., Xie, S. X., Toledo, J. B., Scordia, C., Linder, C., et al. (2016). Olfactory impairment predicts cognitive decline in early Parkinson's disease. *Parkinsonism Relat. Disord.* 25, 45–51. doi: 10.1016/j.parkreldis.2016.02.013

Funabe, S., Takao, M., Saito, Y., Hatsuta, H., Sugiyama, M., Ito, S., et al. (2013). Neuropathologic analysis of Lewy-related α-synucleinopathy in olfactory mucosa. *Neuropathology* 33, 47–58. doi: 10.1111/j.1440-1789.2012.01329.x

Fung, K. M., Peringa, J., Venkatachalam, S., Lee, V. M., and Trojanowski, J. Q. (1997). Coordinate reduction in cell proliferation and cell death in mouse olfactory epithelium from birth to maturity. *Brain Res.* 761, 347–351. doi: 10.1016/s0006-8993(97)00467-8

Genter, M. B., Deamer, N. J., Blake, B. L., Wesley, D. S., and Levi, P. E. (1995). Olfactory toxicity of methimazole: dose-response and structure-activity studies and characterization of flavin-containing monooxygenase activity in the Long-Evans rat olfactory mucosa. *Toxicol. Pathol.* 23, 477–486. doi: 10.1177/019262339502300404

Getchell, M. L., and Getchell, T. V. (1991). Immunohistochemical localization of components of the immune barrier in the olfactory mucosae of salamanders and rats. *Anat. Rec.* 231, 358–374. doi: 10.1002/ar.1092310310

Getchell, T. V., Peng, X., Green, C. P., Stromberg, A. J., Chen, K. C., Mattson, M. P., et al. (2004). In silico analysis of gene expression profiles in the olfactory mucosae of aging senescence-accelerated mice. *J. Neurosci. Res.* 77, 430–452. doi: 10.1002/jnr.20157

Getchell, T. V., Peng, X., Stromberg, A. J., Chen, K. C., Paul Green, C., Subhedar, N. K., et al. (2003). Age-related trends in gene expression in the chemosensory-nasal mucosae of senescence-accelerated mice. *Ageing Res. Rev.* 2, 211–243. doi: 10.1016/s1568-1637(02)00066-1

Glennon, S. G., Huedo-Medina, T., Rawal, S., Hoffman, H. J., Litt, M. D., and Duffy, V. B. (2019). Chronic cigarette smoking associates directly and indirectly with self-reported olfactory alterations: analysis of the 2011–2014 national health and nutrition examination survey. *Nicotine Tob. Res.* 21, 818–827. doi: 10.1093/ntr/ntx242

Gocel, J., and Larson, J. (2013). Evidence for loss of synaptic AMPA receptors in anterior piriform cortex of aged mice. *Front. Aging Neurosci.* 5:39. doi: 10.3389/fnagi.2013.00039

Gopinath, B., Anstey, K. J., Kifley, A., and Mitchell, P. (2012a). Olfactory impairment is associated with functional disability and reduced independence among older adults. *Maturitas* 72, 50–55. doi: 10.1016/j.maturitas.2012.01.009

Gopinath, B., Sue, C. M., Kifley, A., and Mitchell, P. (2012b). The association between olfactory impairment and total mortality in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 67, 204–209. doi: 10.1093/gerona/glr165

Gottfried, J. A. (2010). Central mechanisms of odour object perception. *Nat. Rev. Neurosci.* 11, 628–641. doi: 10.1038/nrn2883

Graziadei, G. A., and Graziadei, P. P. (1979). Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. *J. Neurocytol.* 8, 197–213. doi: 10.1007/bf01175561

Hahn, C. G., Han, L. Y., Rawson, N. E., Mirza, N., Borgmann-Winter, K., Lenox, R. H., et al. (2005). *in vivo* and *in vitro* neurogenesis in human olfactory epithelium. *J. Comp. Neurol.* 483, 154–163. doi: 10.1002/cne.20424

Hedner, M., Nilsson, L. G., Olofsson, J. K., Bergman, O., Eriksson, E., Nyberg, L., et al. (2010). Age-related olfactory decline is associated with the BDNF Val66met polymorphism: evidence from a population-based study. *Front. Aging Neurosci.* 2:24. doi: 10.3389/fnagi.2010.00024

Henkin, R. I., Schmidt, L., and Velicu, I. (2013). Interleukin 6 in hyposmia. *JAMA Otolaryngol. Head Neck Surg.* 139, 728–734. doi: 10.1001/jamaoto.2013.3392

Hinds, J. W., and McNelly, N. A. (1977). Aging of the rat olfactory bulb: growth and atrophy of constituent layers and changes in size and number of mitral cells. *J. Comp. Neurol.* 72, 345–367. doi: 10.1002/cne.901710304

Hinds, J. W., and McNelly, N. A. (1979). Aging in the rat olfactory bulb: quantitative changes in mitral cell organelles and somato-dendritic synapses. *J. Comp. Neurol.* 184, 811–820. doi: 10.1002/cne.901840412

Hinds, J. W., and McNelly, N. A. (1981). Aging in the rat olfactory system: correlation of changes in the olfactory epithelium and olfactory bulb. *J. Comp. Neurol.* 203, 441–453. doi: 10.1002/cne.902030308

Hoffman, H. J., Ishii, E. K., and MacTurk, R. H. (1998). Age-related changes in the prevalence of smell/taste problems among the United States adult population. Results of the 1994 disability supplement to the National Health Interview Survey (NHIS). *Ann. N Y Acad. Sci.* 855, 716–722. doi: 10.1111/j.1749-6632.1998.tb10650.x

Holbrook, E. H., Wu, E., Curry, W. T., Lin, D. T., and Schwob, J. E. (2011). Immunohistochemical characterization of human olfactory tissue. *Laryngoscope* 121, 1687–1701. doi: 10.1002/lary.21856

Honda, N., Sakamoto, H., Inamura, K., and Kashiwayanagi, M. (2009). Age-dependent spatial distribution of bromodeoxyuridine-immunoreactive cells in the main olfactory bulb. *Biol. Pharm. Bull.* 32, 627–630. doi: 10.1248/bpb.32.627

Hori, Y., Matsuda, O., and Ichikawa, S. (2015). Olfactory function in elderly people and patients with Alzheimer's disease. *Psychogeriatrics* 15, 179–185. doi: 10.1111/psyg.12092

Hubbard, P. S., Esiri, M. M., Reading, M., McShane, R., and Nagy, Z. (2007). α-synuclein pathology in the olfactory pathways of dementia patients. *J. Anat.* 211, 117–124. doi: 10.1111/j.1469-7580.2007.00748.x

Hummel, T., Futschik, T., Frasnelli, J., and Huttenbrink, K. B. (2003). Effects of olfactory function, age, and gender on trigeminally mediated sensations: a study based on the lateralization of chemosensory stimuli. *Toxicol. Lett.* 140–141, 273–280. doi: 10.1016/s0378-4274(03)00078-x

Hummel, T., Kobal, G., Gudziol, H., and Mackay-Sim, A. (2007). Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur. Arch. Otorhinolaryngol.* 264, 237–243. doi: 10.1007/s00405-006-0173-0

Hummel, T., Rissom, K., Reden, J., Hahner, A., Weidenbecher, M., and Huttenbrink, K. B. (2009). Effects of olfactory training in patients with olfactory loss. *Laryngoscope* 119, 496–499. doi: 10.1002/lary.20101

Hummel, T., Whitcroft, K. L., Andrews, P., Altundag, A., Cinghi, C., Costanzo, R. M., et al. (2017). Position paper on olfactory dysfunction. *Rhinol. Suppl.* 54, 1–30. doi: 10.4193/Rhino16.248

Hunt, G. E., Van Nieuwenhuijzen, P. S., Chan-Ling, T., and McGregor, I. S. (2011). "When an old rat smells a cat": a decline in defense-related, but not accessory olfactory, Fos expression in aged rats. *Neurobiol. Aging* 32, 737–749. doi: 10.1016/j.neurobiolaging.2009.03.014

Hurtt, M. E., Thomas, D. A., Working, P. K., Monticello, T. M., and Morgan, K. T. (1988). Degeneration and regeneration of the olfactory epithelium following inhalation exposure to methyl bromide: pathology,

cell kinetics, and olfactory function. *Toxicol. Appl. Pharmacol.* 94, 311–328. doi: 10.1016/0041-008x(88)90273-6

Husner, A., Frasnelli, J., Welge-Lussen, A., Reiss, G., Zahnert, T., and Hummel, T. (2006). Loss of trigeminal sensitivity reduces olfactory function. *Laryngoscope* 116, 1520–1522. doi: 10.1097/01.mlg.0000225946.37489.4c

Hyman, B. T., Arriagada, P. V., and Van Hoesen, G. W. (1991). Pathologic changes in the olfactory system in aging and Alzheimer's disease. *Ann. N Y Acad. Sci.* 640, 14–19. doi: 10.1111/j.1749-6632.1991.tb00184.x

Ihara, S., Yoshikawa, K., and Touhara, K. (2013). Chemosensory signals and their receptors in the olfactory neural system. *Neuroscience* 254, 45–60. doi: 10.1016/j.neuroscience.2013.08.063

Iijima, M., Kobayakawa, T., Saito, S., Osawa, M., Tsutsumi, Y., Hashimoto, S., et al. (2008). Smell identification in Japanese Parkinson's disease patients: using the odor stick identification test for Japanese subjects. *Intern. Med.* 47, 1887–1892. doi: 10.2169/internalmedicine.47.1345

Jafek, B. W., Murrow, B., Michaels, R., Restrepo, D., and Linschoten, M. (2002). Biopsies of human olfactory epithelium. *Chem. Senses* 27, 623–628. doi: 10.1093/chemse/27.7.623

Jennings, D., Siderowf, A., Stern, M., Seibyl, J., Eberly, S., Oakes, D., et al. (2017). Conversion to Parkinson disease in the PARS hyposmic and dopamine transporter-deficit prodromal cohort. *JAMA Neurol.* 74, 933–940. doi: 10.1001/jamaneurol.2017.0985

Jia, C., and Hegg, C. C. (2015). Effect of IP3R3 and NPY on age-related declines in olfactory stem cell proliferation. *Neurobiol. Aging* 36, 1045–1056. doi: 10.1016/j.neurobiolaging.2014.11.007

Jung, H. J., Shin, I. S., and Lee, J. E. (2019). Olfactory function in mild cognitive impairment and Alzheimer's disease: a meta-analysis. *Laryngoscope* 129, 362–369. doi: 10.1002/lary.27399

Kavoi, B., Makanya, A., Hassanali, J., Carlsson, H. E., and Kiama, S. (2010). Comparative functional structure of the olfactory mucosa in the domestic dog and sheep. *Ann. Anat.* 192, 329–337. doi: 10.1016/j.aanat.2010.07.004

Khan, M., Vaes, E., and Mombaerts, P. (2013). Temporal patterns of odorant receptor gene expression in adult and aged mice. *Mol. Cell. Neurosci.* 57, 120–129. doi: 10.1016/j.mcn.2013.08.001

Kikuta, S., Sakamoto, T., Nagayama, S., Kanaya, K., Kinoshita, M., Kondo, K., et al. (2015). Sensory deprivation disrupts homeostatic regeneration of newly generated olfactory sensory neurons after injury in adult mice. *J. Neurosci.* 35, 2657–2673. doi: 10.1523/jneurosci.2484-14.2015

Kondo, K., Suzukawa, K., Sakamoto, T., Watanabe, K., Kanaya, K., Ushio, M., et al. (2010). Age-related changes in cell dynamics of the postnatal mouse olfactory neuroepithelium: cell proliferation, neuronal differentiation, and cell death. *J. Comp. Neurol.* 518, 1962–1975. doi: 10.1002/cne.22316

Kondo, K., Watanabe, K., Sakamoto, T., Suzukawa, K., Nibu, K., Kaga, K., et al. (2009). Distribution and severity of spontaneous lesions in the neuroepithelium and Bowman's glands in mouse olfactory mucosa: age-related progression. *Cell Tissue Res.* 335, 489–503. doi: 10.1007/s00441-008-0739-9

Konstantinidis, I., Hummel, T., and Larsson, M. (2006). Identification of unpleasant odors is independent of age. *Arch. Clin. Neuropsychol.* 21, 615–621. doi: 10.1016/j.acn.2006.05.006

Kovács, T. (2004). Mechanisms of olfactory dysfunction in aging and neurodegenerative disorders. *Ageing Res. Rev.* 3, 215–232. doi: 10.1016/j.arr.2003.10.003

Kovács, T., Cairns, N. J., and Lantos, P. L. (1999). β-amyloid deposition and neurofibrillary tangle formation in the olfactory bulb in ageing and Alzheimer's disease. *Neuropathol. Appl. Neurobiol.* 25, 481–491. doi: 10.1046/j.1365-2990.1999.00208.x

Kovács, T., Cairns, N. J., and Lantos, P. L. (2001). Olfactory centres in Alzheimer's disease: olfactory bulb is involved in early Braak's stages. *Neuroreport* 12, 285–288. doi: 10.1097/00001756-200102120-00021

Kraemer, S., and Apfelbach, R. (2004). Olfactory sensitivity, learning and cognition in young adult and aged male Wistar rats. *Physiol. Behav.* 81, 435–442. doi: 10.1016/j.physbeh.2004.01.012

Kurtenbach, S., Goss, G. M., Goncalves, S., Choi, R., Hare, J. M., Chaudhari, N., et al. (2019). Cell-based therapy restores olfactory function in an inducible model of hyposmia. *Stem Cell Rep.* 12, 1354–1365. doi: 10.1016/j.stemcr.2019.05.001

Landis, B. N., Konnerth, C. G., and Hummel, T. (2004). A study on the frequency of olfactory dysfunction. *Laryngoscope* 114, 1764–1769. doi: 10.1097/00005537-200410000-00017

Lane, A. P., Turner, J., May, L., and Reed, R. (2010). A genetic model of chronic rhinosinusitis-associated olfactory inflammation reveals reversible functional impairment and dramatic neuroepithelial reorganization. *J. Neurosci.* 30, 2324–2329. doi: 10.1523/JNEUROSCI.4507-09.2010

Larsson, M., Farde, L., Hummel, T., Witt, M., Lindroth, N. E., and Backman, L. (2009). Age-related loss of olfactory sensitivity: association to dopamine transporter binding in putamen. *Neuroscience* 161, 422–426. doi: 10.1016/j.neuroscience.2009.03.074

Larsson, M., Finkel, D., and Pedersen, N. L. (2000). Odor identification: influences of age, gender, cognition and personality. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 55, P304–P310. doi: 10.1093/geronb/55.5.p304

Lazarini, F., and Lledo, P. M. (2011). Is adult neurogenesis essential for olfaction? *Trends Neurosci.* 34, 20–30. doi: 10.1016/j.tins.2010.09.006

Lee, A. C., Tian, H., Grosmaitre, X., and Ma, M. (2009). Expression patterns of odorant receptors and response properties of olfactory sensory neurons in aged mice. *Chem. Senses* 34, 695–703. doi: 10.1093/chemse/bjp056

Lee, W. H., Wee, J. H., Kim, D. K., Rhee, C. S., Lee, C. H., Ahn, S., et al. (2013). Prevalence of subjective olfactory dysfunction and its risk factors: korean national health and nutrition examination survey. *PLoS One* 8:e62725. doi: 10.1371/journal.pone.0062725

Legrier, M. E., Ducray, A., Propper, A., Chao, M., and Kastner, A. (2001). Cell cycle regulation during mouse olfactory neurogenesis. *Cell Growth Differ.* 12, 591–601.

Ling, G., Gu, J., Genter, M. B., Zhuo, X., and Ding, X. (2004). Regulation of cytochrome P450 gene expression in the olfactory mucosa. *Chem. Biol. Interact.* 147, 247–258. doi: 10.1016/j.cbi.2004.02.003

Liu, B., Luo, Z., Pinto, J. M., Shiroma, E. J., Tranah, G. J., Wirdefeldt, K., et al. (2019). Relationship between poor olfaction and mortality among community-dwelling older adults: a cohort study. *Ann. Intern. Med.* 170, 673–681. doi: 10.7326/m18-0775

London, B., Nabet, B., Fisher, A. R., White, B., Sammel, M. D., and Doty, R. L. (2008). Predictors of prognosis in patients with olfactory disturbance. *Ann. Neurol.* 63, 159–166. doi: 10.1002/ana.21293

Loo, A. T., Youngentob, S. L., Kent, P. F., and Schwob, J. E. (1996). The aging olfactory epithelium: neurogenesis, response to damage, and odorant-induced activity. *Int. J. Dev. Neurosci.* 14, 881–900. doi: 10.1016/s0736-5748(96)00046-9

MacDonald, S. W. S., Keller, C. J. C., Brewster, P. W. H., and Dixon, R. A. (2018). Contrasting olfaction, vision, and audition as predictors of cognitive change and impairment in non-demented older adults. *Neuropsychology* 32, 450–460. doi: 10.1037/neu0000439

Mahlknecht, P., Pechlaner, R., Boesveldt, S., Volc, D., Pinter, B., Reiter, E., et al. (2016). Optimizing odor identification testing as quick and accurate diagnostic tool for Parkinson's disease. *Mov. Disord.* 31, 1408–1413. doi: 10.1002/mds.26637

Mahlknecht, P., Stockner, H., Marini, K., Gasperi, A., Djamshidian, A., Willeit, P., et al. (2020). Midbrain hyperechogenicity, hyposmia, mild parkinsonian signs and risk for incident Parkinson's disease over 10 years: a prospective population-based study. *Parkinsonism Relat. Disord.* 70, 51–54. doi: 10.1016/j.parkreldis.2019.12.008

Markovic, K., Reulbach, U., Vassiliadu, A., Lunkenheimer, J., Lunkenheimer, B., Spannenberger, R., et al. (2007). Good news for elderly persons: olfactory pleasure increases at later stages of the life span. *J. Gerontol. A Biol. Sci. Med. Sci.* 62, 1287–1293. doi: 10.1093/gerona/62.11.1287

Maslov, A. Y., Barone, T. A., Plunkett, R. J., and Pruitt, S. C. (2004). Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J. Neurosci.* 24, 1726–1733. doi: 10.1523/JNEUROSCI.4608-03.2004

Matarazzo, V., Zsürger, N., Guillemot, J. C., Clot-Faybesse, O., Botto, J. M., Dal Farra, C., et al. (2002). Porcine odorant-binding protein selectively binds to a human olfactory receptor. *Chem. Senses* 27, 691–701. doi: 10.1093/chemse/27.8.691

Matulionis, D. H. (1975). Ultrastructural study of mouse olfactory epithelium following destruction by ZnSO4 and its subsequent regeneration. *Am. J. Anat.* 142, 67–89. doi: 10.1002/aja.1001420106

Meisami, E., Mikhail, L., Baim, D., and Bhatnagar, K. P. (1998). Human olfactory bulb: aging of glomeruli and mitral cells and a search for the accessory olfactory bulb. *Ann. N Y Acad. Sci.* 855, 708–715. doi: 10.1111/j.1749-6632.1998.tb10649.x

Mellert, T. K., Getchell, M. L., Sparks, L., and Getchell, T. V. (1992). Characterization of the immune barrier in human olfactory mucosa. *Otolaryngol. Head Neck Surg.* 106, 181–188. doi: 10.1177/019459989210600221

Mery, S., Gross, E. A., Joyner, D. R., Godo, M., and Morgan, K. T. (1994). Nasal diagrams: a tool for recording the distribution of nasal lesions in rats and mice. *Toxicol. Pathol.* 22, 353–372. doi: 10.1177/019262339402200402

Mirich, J. M., Williams, N. C., Berlau, D. J., and Brunjes, P. C. (2002). Comparative study of aging in the mouse olfactory bulb. *J. Comp. Neurol.* 454, 361–372. doi: 10.1002/cne.10426

Miwa, T., Furukawa, M., Tsukatani, T., Costanzo, R. M., DiNardo, L. J., and Reiter, E. R. (2001). Impact of olfactory impairment on quality of life and disability. *Arch. Otolaryngol. Head Neck Surg.* 127, 497–503. doi: 10.1001/archotol.127.5.497

Miwa, T., Ikeda, K., Ishibashi, T., Kobayashi, M., Kondo, K., Matsuwaki, Y., et al. (2019). Clinical practice guidelines for the management of olfactory dysfunction—secondary publication. *Auris Nasus Larynx* 46, 653–662. doi: 10.1016/j.anl.2019.04.002

Mobley, A. S., Rodriguez-Gil, D. J., Imamura, F., and Greer, C. A. (2014). Aging in the olfactory system. *Trends Neurosci.* 37, 77–84. doi: 10.1016/j.tins.2013.11.004

Moran, D. T., Rowley, J. C. III., Jafek, B. W., and Lovell, M. A. (1982). The fine structure of the olfactory mucosa in man. *J. Neurocytol.* 11, 721–746. doi: 10.1007/bf01153516

Moreno, M., Richard, M., Landrein, B., Sacquet, J., Didier, A., and Mandairon, N. (2014). Alteration of olfactory perceptual learning and its cellular basis in aged mice. *Neurobiol. Aging* 35, 680–691. doi: 10.1016/j.neurobiolaging.2013.08.034

Mori, K., Nagao, H., and Yoshihara, Y. (1999). The olfactory bulb: coding and processing of odor molecule information. *Science* 286, 711–715. doi: 10.1126/science.286.5440.711

Morrison, E. E., and Costanzo, R. M. (1990). Morphology of the human olfactory epithelium. *J. Comp. Neurol.* 297, 1–13. doi: 10.1002/cne.902970102

Morrison, E. E., and Costanzo, R. M. (1995). Regeneration of olfactory sensory neurons and reconnection in the aging hamster central nervous system. *Neurosci. Lett.* 198, 213–217. doi: 10.1016/0304-3940(95)11943-q

Mullol, J., Alobid, I., Mariño-Sánchez, F., Quintó, L., de Haro, J., Bernal-Sprekelsen, M., et al. (2012). Furthering the understanding of olfaction, prevalence of loss of smell and risk factors: a population-based survey (OLFACAT study). *BMJ Open* 2:e001256. doi: 10.1136/bmjopen-2012-001256

Murphy, C., Gilmore, M. M., Seery, C. S., Salmon, D. P., and Lasker, B. R. (1990). Olfactory thresholds are associated with degree of dementia in Alzheimer's disease. *Neurobiol. Aging* 11, 465–469. doi: 10.1016/0197-4580(90)90014-q

Murphy, C., and Jinich, S. (1996). Olfactory dysfunction in Down's syndrome. *Neurobiol. Aging* 17, 631–637. doi: 10.1016/0197-4580(96)00008-5

Murphy, C., Morgan, C. D., Geisler, M. W., Wetter, S., Covington, J. W., Madowitz, M. D., et al. (2000). Olfactory event-related potentials and aging: normative data. *Int. J. Psychophysiol.* 36, 133–145. doi: 10.1016/s0167-8760(99)00107-5

Murphy, C., Schubert, C. R., Cruickshanks, K. J., Klein, B. E., Klein, R., and Nondahl, D. M. (2002). Prevalence of olfactory impairment in older adults. *JAMA* 288, 2307–2312. doi: 10.1001/jama.288.18.2307

Nakashima, T., Kimmelman, C. P., and Snow, J. B. Jr. (1984). Structure of human fetal and adult olfactory neuroepithelium. *Arch. Otolaryngol.* 110, 641–646. doi: 10.1001/archotol.1984.00800360013003

Nijjar, R. K., and Murphy, C. (2002). Olfactory impairment increases as a function of age in persons with Down syndrome. *Neurobiol. Aging* 23, 65–73. doi: 10.1016/s0197-4580(01)00263-9

Nordin, S., Brämerson, A., Millqvist, E., and Bende, M. (2007). Prevalence of parosmia: the Skovde population-based studies. *Rhinology* 45, 50–53.

Paik, S. I., Lehman, M. N., Seiden, A. M., Duncan, H. J., and Smith, D. V. (1992). Human olfactory biopsy. The influence of age and receptor distribution. *Arch. Otolaryngol. Head Neck Surg.* 118, 731–738. doi: 10.1001/archotol.1992.01880070061012

Patel, R. C., and Larson, J. (2009). Impaired olfactory discrimination learning and decreased olfactory sensitivity in aged C57Bl/6 mice. *Neurobiol. Aging* 30, 829–837. doi: 10.1016/j.neurobiolaging.2007.08.007

Peters, J. M., Hummel, T., Kratzsch, T., Lotsch, J., Skarke, C., and Frolich, L. (2003). Olfactory function in mild cognitive impairment and Alzheimer's disease: an investigation using psychophysical and electrophysiological techniques. *Am. J. Psychiatry* 160, 1995–2002. doi: 10.1176/appi.ajp.160.11.1995

Pinto, J. M., Wroblewski, K. E., Kern, D. W., Schumm, L. P., and McClintock, M. K. (2014). Olfactory dysfunction predicts 5-year mortality in older adults. *PLoS One* 9:e107541. doi: 10.1371/journal.pone.0107541

Pinto, J. M., Wroblewski, K. E., Kern, D. W., Schumm, L. P., and McClintock, M. K. (2015). The rate of age-related olfactory decline among the general population of older U.S. adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 1435–1441. doi: 10.1093/gerona/glv072

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., et al. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* 30, 1591–1601. doi: 10.1002/mds.26424

Rawal, S., Hoffman, H. J., Bainbridge, K. E., Huedo-Medina, T. B., and Duffy, V. B. (2016). Prevalence and risk factors of self-reported smell and taste alterations: results from the 2011–2012 US national health and nutrition examination survey (NHANES). *Chem. Senses* 41, 69–76. doi: 10.1093/chemse/bjv057

Rawson, N. E., Gomez, G., Cowart, B. J., Kriete, A., Pribitkin, E., and Restrepo, D. (2012). Age-associated loss of selectivity in human olfactory sensory neurons. *Neurobiol. Aging* 33, 1913–1919. doi: 10.1016/j.neurobiolaging.2011.09.036

Reden, J., Mueller, A., Mueller, C., Konstantinidis, I., Frasnelli, J., Landis, B. N., et al. (2006). Recovery of olfactory function following closed head injury or infections of the upper respiratory tract. *Arch. Otolaryngol. Head Neck Surg.* 132, 265–269. doi: 10.1001/archotol.132.3.265

Ressler, K. J., Sullivan, S. L., and Buck, L. B. (1994). Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79, 1245–1255. doi: 10.1016/0092-8674(94)90015-9

Richard, M. B., Taylor, S. R., and Greer, C. A. (2010). Age-induced disruption of selective olfactory bulb synaptic circuits. *Proc. Natl. Acad. Sci. U S A* 107, 15613–15618. doi: 10.1073/pnas.1007931107

Rimbault, M., Robin, S., Vaysse, A., and Galibert, F. (2009). RNA profiles of rat olfactory epithelia: individual and age related variations. *BMC Genomics* 10:572. doi: 10.1186/1471-2164-10-572

Roberts, R. O., Christianson, T. J., Kremers, W. K., Mielke, M. M., Machulda, M. M., Vassilaki, M., et al. (2016). Association between olfactory dysfunction and amnestic mild cognitive impairment and alzheimer disease dementia. *JAMA Neurol.* 73, 93–101. doi: 10.1001/jamaneurol.2015.2952

Robinson, A. M., Conley, D. B., Shinners, M. J., and Kern, R. C. (2002). Apoptosis in the aging olfactory epithelium. *Laryngoscope* 112, 1431–1435. doi: 10.1097/00005537-200208000-00019

Romero-Grimaldi, C., Moreno-López, B., and Estrada, C. (2008). Age-dependent effect of nitric oxide on subventricular zone and olfactory bulb neural precursor proliferation. *J. Comp. Neurol.* 506, 339–346. doi: 10.1002/cne.21556

Rosli, Y., Breckenridge, L. J., and Smith, R. A. (1999). An ultrastructural study of age-related changes in mouse olfactory epithelium. *J. Electron Microsc.* 48, 77–84. doi: 10.1093/oxfordjournals.jmicro.a023653

Ross, G. W., Abbott, R. D., Petrovitch, H., Tanner, C. M., Davis, D. G., Nelson, J., et al. (2006). Association of olfactory dysfunction with incidental Lewy bodies. *Mov. Disord.* 21, 2062–2067. doi: 10.1002/mds.21076

Ross, G. W., Petrovitch, H., Abbott, R. D., Tanner, C. M., Popper, J., Masaki, K., et al. (2008). Association of olfactory dysfunction with risk for future Parkinson's disease. *Ann. Neurol.* 63, 167–173. doi: 10.1002/ana.21291

Saito, S., Ayabe-Kanamura, S., Takashima, Y., Gotow, N., Naito, N., Nozawa, T., et al. (2006). Development of a smell identification test using a novel stick-type odor presentation kit. *Chem. Senses* 31, 379–391. doi: 10.1093/chemse/bjj042

Schaefer, M. L., Bottger, B., Silver, W. L., and Finger, T. E. (2002). Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. *J. Comp. Neurol.* 444, 221–226. doi: 10.1002/cne.10143

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. *JAMA* 278, 1357–1362. doi: 10.1001/jama.1997.03550160077042

Schiffman, S. S., and Warwick, Z. S. (1993). Effect of flavor enhancement of foods for the elderly on nutritional status: food intake, biochemical indices

and anthropometric measures. *Physiol. Behav.* 53, 395–402. doi: 10.1016/0031-9384(93)90224-4

Schubert, C. R., Cruickshanks, K. J., Fischer, M. E., Huang, G. H., Klein, B. E., Klein, R., et al. (2012). Olfactory impairment in an adult population: the beaver dam offspring study. *Chem. Senses* 37, 325–334. doi: 10.1093/chemse/bjr102

Schubert, C. R., Cruickshanks, K. J., Fischer, M. E., Huang, G. H., Klein, R., Tsai, M. Y., et al. (2015). Carotid intima media thickness, atherosclerosis, and 5-year decline in odor identification: the beaver dam offspring study. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 879–884. doi: 10.1093/gerona/glu158

Schubert, C. R., Cruickshanks, K. J., Klein, B. E., Klein, R., and Nondahl, D. M. (2011). Olfactory impairment in older adults: five-year incidence and risk factors. *Laryngoscope* 121, 873–878. doi: 10.1002/lary.21416

Schubert, C. R., Cruickshanks, K. J., Nondahl, D. M., Klein, B. E., Klein, R., and Fischer, M. E. (2013). Association of exercise with lower long-term risk of olfactory impairment in older adults. *JAMA Otolaryngol. Head Neck Surg.* 139, 1061–1066. doi: 10.1001/jamaoto.2013.4759

Schubert, C. R., Fischer, M. E., Pinto, A. A., Klein, B. E. K., Klein, R., Tweed, T. S., et al. (2017). Sensory impairments and risk of mortality in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 72, 710–715. doi: 10.1093/gerona/glw036

Schwob, J. E. (2002). Neural regeneration and the peripheral olfactory system. *Anat. Rec.* 269, 33–49. doi: 10.1002/ar.10047

Schwob, J. E., Jang, W., Holbrook, E. H., Lin, B., Herrick, D. B., Peterson, J. N., et al. (2017). Stem and progenitor cells of the mammalian olfactory epithelium: taking poietic license. *J. Comp. Neurol.* 525, 1034–1054. doi: 10.1002/cne.24105

Schwob, J. E., Szumowski, K. E., and Stasky, A. A. (1992). Olfactory sensory neurons are trophically dependent on the olfactory bulb for their prolonged survival. *J. Neurosci.* 12, 3896–3919. doi: 10.1523/JNEUROSCI.12-10-03896.1992

Schwob, J. E., Youngentob, S. L., and Mezza, R. C. (1995). Reconstitution of the rat olfactory epithelium after methyl bromide-induced lesion. *J. Comp. Neurol.* 359, 15–37. doi: 10.1002/cne.903590103

Serby, M., Larson, P., and Kalkstein, D. (1991). The nature and course of olfactory deficits in Alzheimer's disease. *Am. J. Psychiatry* 148, 357–360. doi: 10.1176/ajp.148.3.357

Shen, J., Kassir, M. A., Wu, J., Zhang, Q., Zhou, S., Xuan, S. Y., et al. (2013). MR volumetric study of piriform-cortical amygdala and orbitofrontal cortices: the aging effect. *PLoS One* 8:e74526. doi: 10.1371/journal.pone.0074526

Shu, C. H., Hummel, T., Lee, P. L., Chiu, C. H., Lin, S. H., and Yuan, B. C. (2009). The proportion of self-rated olfactory dysfunction does not change across the life span. *Am. J. Rhinol. Allergy* 23, 413–416. doi: 10.2500/ajra.2009.23.3343

Siegel, J. K., Wroblewski, K. E., McClintock, M. K., and Pinto, J. M. (2019). Olfactory dysfunction persists after smoking cessation and signals increased cardiovascular risk. *Int. Forum Allergy Rhinol.* 9, 977–985. doi: 10.1002/alr.22357

Silva, M. M. E., Mercer, P. B. S., Witt, M. C. Z., and Pessoa, R. R. (2018). Olfactory dysfunction in Alzheimer's disease Systematic review and meta-analysis. *Dement. Neuropsychol.* 12, 123–132. doi: 10.1590/1980-57642018dn12-020004

Sinding, C., Puschmann, L., and Hummel, T. (2014). Is the age-related loss in olfactory sensitivity similar for light and heavy molecules? *Chem. Senses* 39, 383–390. doi: 10.1093/chemse/bju004

Stevens, J. C., and Spencer, N. A. (1994). Olfactory detection of a complex versus a simple substance in advanced age. *Chem. Senses* 19, 365–369. doi: 10.1093/chemse/19.4.365

Sui, Y., Horne, M. K., and Stanic, D. (2012). Reduced proliferation in the adult mouse subventricular zone increases survival of olfactory bulb interneurons. *PLoS One* 7:e31549. doi: 10.1371/journal.pone.0031549

Suzukawa, K., Kondo, K., Kanaya, K., Sakamoto, T., Watanabe, K., Ushio, M., et al. (2011). Age-related changes of the regeneration mode in the mouse peripheral olfactory system following olfactotoxic drug methimazole-induced damage. *J. Comp. Neurol.* 519, 2154–2174. doi: 10.1002/cne.22611

Suzuki, Y., Critchley, H. D., Suckling, J., Fukuda, R., Williams, S. C., Andrew, C., et al. (2001). Functional magnetic resonance imaging of odor identification: the effect of aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 56, M756–M760. doi: 10.1093/gerona/56.12.m756

Takeuchi, H., and Sakano, H. (2014). Neural map formation in the mouse olfactory system. *Cell. Mol. Life Sci.* 71, 3049–3057. doi: 10.1007/s00018-014-1597-0

Tchilian, E. Z., Zhelezarov, I. E., Petkov, V. V., and Hadjiivanova, C. I. (1990). 125I-insulin binding is decreased in olfactory bulbs of aged rats. *Neuropeptides* 17, 193–196. doi: 10.1016/0143-4179(90)90035-w

Thomann, P. A., Dos Santos, V., Toro, P., Schonknecht, P., Essig, M., and Schroder, J. (2009). Reduced olfactory bulb and tract volume in early Alzheimer's disease—a MRI study. *Neurobiol. Aging* 30, 838–841. doi: 10.1016/j.neurobiolaging.2007.08.001

Trojanowski, J. Q., Newman, P. D., Hill, W. D., and Lee, V. M. (1991). Human olfactory epithelium in normal aging, Alzheimer's disease, and other neurodegenerative disorders. *J. Comp. Neurol.* 310, 365–376. doi: 10.1002/cne.903100307

Ueha, R., Kondo, K., Ueha, S., and Yamasoba, T. (2018a). Dose-dependent effects of insulin-like growth factor 1 in the aged olfactory epithelium. *Front. Aging Neurosci.* 10:385. doi: 10.3389/fnagi.2018.00385

Ueha, R., Shichino, S., Ueha, S., Kondo, K., Kikuta, S., Nishijima, H., et al. (2018b). Reduction of proliferating olfactory cells and low expression of extracellular matrix genes are hallmarks of the aged olfactory mucosa. *Front. Aging Neurosci.* 10:86. doi: 10.3389/fnagi.2018.00086

Ueha, R., Ueha, S., Kondo, K., Kikuta, S., and Yamasoba, T. (2018c). Cigarette smoke-induced cell death causes persistent olfactory dysfunction in aged mice. *Front. Aging Neurosci.* 10:183. doi: 10.3389/fnagi.2018.00183

Ueha, R., Ueha, S., Kondo, K., Sakamoto, T., Kikuta, S., Kanaya, K., et al. (2016a). Damage to olfactory progenitor cells is involved in cigarette smoke-induced olfactory dysfunction in mice. *Am. J. Pathol.* 186, 579–586. doi: 10.1016/j.ajpath.2015.11.009

Ueha, R., Ueha, S., Sakamoto, T., Kanaya, K., Suzukawa, K., Nishijima, H., et al. (2016b). Cigarette smoke delays regeneration of the olfactory epithelium in mice. *Neurotox. Res.* 30, 213–224. doi: 10.1007/s12640-016-9617-5

Vaishnav, R. A., Getchell, M. L., Poon, H. F., Barnett, K. R., Hunter, S. A., Pierce, W. M., et al. (2007). Oxidative stress in the aging murine olfactory bulb: redox proteomics and cellular localization. *J. Neurosci. Res.* 85, 373–385. doi: 10.1002/jnr.21130

Vennemann, M. M., Hummel, T., and Berger, K. (2008). The association between smoking and smell and taste impairment in the general population. *J. Neurol.* 255, 1121–1126. doi: 10.1007/s00415-008-0807-9

Wang, J., Eslinger, P. J., Smith, M. B., and Yang, Q. X. (2005). Functional magnetic resonance imaging study of human olfaction and normal aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 510–514. doi: 10.1093/gerona/60.4.510

Wang, J., Sun, X., and Yang, Q. X. (2017). Early aging effect on the function of the human central olfactory system. *J. Gerontol. A Biol. Sci. Med. Sci.* 72, 1007–1014. doi: 10.1093/gerona/glw104

Watabe-Rudolph, M., Begus-Nahrmann, Y., Lechel, A., Rolyan, H., Scheithauer, M. O., Rettinger, G., et al. (2011). Telomere shortening impairs regeneration of the olfactory epithelium in response to injury but not under homeostatic conditions. *PLoS One* 6:e27801. doi: 10.1371/journal.pone.0027801

Watanabe, K., Kondo, K., Takeuchi, N., Okano, H., and Yamasoba, T. (2007). Musashi-1 expression in postnatal mouse olfactory epithelium. *Neuroreport* 18, 641–644. doi: 10.1097/wnr.0b013e3280bef7e2

Watanabe, Y., Suzuki, K., Miyamoto, T., Miyamoto, M., Numao, A., Fujita, H., et al. (2017). A card-type odor identification test for japanese patients with Parkinson's disease and related disorders. *Intern. Med.* 56, 2871–2878. doi: 10.2169/internalmedicine.8565-16

Wehling, E., Nordin, S., Espeseth, T., Reinvang, I., and Lundervold, A. J. (2011). Unawareness of olfactory dysfunction and its association with cognitive functioning in middle aged and old adults. *Arch. Clin. Neuropsychol.* 26, 260–269. doi: 10.1093/arclin/acr019

Weiler, E., and Farbman, A. I. (1997). Proliferation in the rat olfactory epithelium: age-dependent changes. *J. Neurosci.* 17, 3610–3622. doi: 10.1523/JNEUROSCI.17-10-03610.1997

Whitman, M. C., and Greer, C. A. (2009). Adult neurogenesis and the olfactory system. *Prog. Neurobiol.* 89, 162–175. doi: 10.1016/j.pneurobio.2009.07.003

Wilson, R. S., Schneider, J. A., Arnold, S. E., Tang, Y., Boyle, P. A., and Bennett, D. A. (2007). Olfactory identification and incidence of mild cognitive impairment in older age. *Arch. Gen. Psychiatry* 64, 802–808. doi: 10.1001/archpsyc.64.7.802

Wilson, R. S., Yu, L., Schneider, J. A., Arnold, S. E., Buchman, A. S., and Bennett, D. A. (2011). Lewy bodies and olfactory dysfunction in old age. *Chem. Senses* 36, 367–373. doi: 10.1093/chemse/bjq139

Windon, M. J., Kim, S. J., Oh, E. S., and Lin, S. Y. (2020). Predictive value of olfactory impairment for cognitive decline among cognitively normal adults. *Laryngoscope* 130, 840–847. doi: 10.1002/lary.28166

Woodward, M. R., Hafeez, M. U., Qi, Q., Riaz, A., Benedict, R. H. B., Yan, L., et al. (2018). Odorant item specific olfactory identification deficit may differentiate alzheimer disease from aging. *Am. J. Geriatr. Psychiatry* 26, 835–846. doi: 10.1016/j.jagp.2018.02.008

Yamagishi, M., Getchell, M. L., Takami, S., and Getchell, T. V. (1998). Increased density of olfactory receptor neurons immunoreactive for apolipoprotein E in patients with Alzheimer's disease. *Ann. Otol. Rhinol. Laryngol.* 107, 421–426. doi: 10.1177/000348949810700511

Yokoyama, T. K., Mochimaru, D., Murata, K., Manabe, H., Kobayakawa, K., Kobayakawa, R., et al. (2011). Elimination of adult-born neurons in the olfactory bulb is promoted during the postprandial period. *Neuron* 71, 883–897. doi: 10.1016/j.neuron.2011.05.046

Yoshihara, S., Takahashi, H., Nishimura, N., Naritsuka, H., Shirao, T., Hirai, H., et al. (2012). 5T4 glycoprotein regulates the sensory input-dependent development of a specific subtype of newborn interneurons in the mouse olfactory bulb. *J. Neurosci.* 32, 2217–2226. doi: 10.1523/JNEUROSCI.5907-11.2012

Youngentob, S. L., Schwob, J. E., Sheehe, P. R., and Youngentob, L. M. (1997). Odorant threshold following methyl bromide-induced lesions of the olfactory epithelium. *Physiol. Behav.* 62, 1241–1252. doi: 10.1016/s0031-9384(97)00301-6

Yousem, D. M., Geckle, R. J., Bilker, W. B., and Doty, R. L. (1998). Olfactory bulb and tract and temporal lobe volumes. Normative data across decades. *Ann. N Y Acad. Sci.* 855, 546–555. doi: 10.1111/j.1749-6632.1998.tb10624.x

Yousem, D. M., Maldjian, J. A., Hummel, T., Alsop, D. C., Geckle, R. J., Kraut, M. A., et al. (1999). The effect of age on odor-stimulated functional MR imaging. *Am. J. Neuroradiol.* 20, 600–608.

Zhu, Y., Armstrong, J. L., Tchkonia, T., and Kirkland, J. L. (2014). Cellular senescence and the senescent secretory phenotype in age-related chronic diseases. *Curr. Opin. Clin. Nutr. Metab. Care* 17, 324–328. doi: 10.1097/mco.0000000000000065

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