

ORIGINAL ARTICLE

Sex differences in the neuropathological hallmarks of Alzheimer's disease: focus on cognitively intact elderly individuals

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Abstract

Aims: Women are more vulnerable to Alzheimer's disease (AD) than men. We investigated (i) whether and at what age the AD hallmarks, that is, β -amyloid ($A\beta$) and hyperphosphorylated Tau (p-Tau) show sex differences; and (ii) whether such sex differences may occur in cognitively intact elderly individuals.

Methods: We first analysed the entire post-mortem brain collection of all non-demented 'controls' and AD donors from our Brain Bank (245 men and 403 women), for the presence of sex differences in AD hallmarks. Second, we quantitatively studied possible sex differences in $A\beta$, $A\beta_{42}$ and p-Tau in the entorhinal cortex of well-matched female ($n = 31$) and male ($n = 21$) clinically cognitively intact elderly individuals.

Results: Women had significantly higher Braak stages for tangles and amyloid scores than men, after 80 years. In the cognitively intact elderly, women showed higher levels of p-Tau, but not $A\beta$ or $A\beta_{42}$, in the entorhinal cortex than men, and a significant interaction of sex with age was found only for p-Tau but not $A\beta$ or $A\beta_{42}$.

Conclusions: Enhanced p-Tau in the entorhinal cortex may play a major role in the vulnerability to AD in women.

KEYWORDS

β -amyloid, Alzheimer's disease, hyperphosphorylated Tau, sex difference

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INTRODUCTION

Alzheimer's disease (AD) is two to three times more prevalent in women than in men, even after correcting for the longer lifespan of women.^{1,2} In addition, women have more severe cognitive decline during the course of AD,³ and a higher rate of atrophy both of the hippocampus⁴ and of the nucleus basalis of Meynert.⁵ The underlying reasons for this sex difference are largely unexplored. It is unknown whether and at what age AD hallmarks, that is, β -amyloid (A β) in plaques and hyperphosphorylated Tau (p-Tau) that forms the neurofibrillary tangles (NFTs), show sex differences. In addition, it is unknown whether such sex differences may occur in the cognitively intact elderly, which is where the likely therapeutic window for AD treatment lies.⁶

A β is formed by proteolytic cleavage of amyloid precursor protein (APP) by γ - and β -secretases. Cleavage can result in both A β ₄₂ and A β ₄₀, with the former being more amyloidogenic and cytotoxic.^{7,8} Although A β is a key pathological hallmark of AD, there is little correlation between A β and clinical AD symptoms were reported.⁹ On the other hand, p-Tau accumulation, which is presumed to be accelerated in AD and driven by amyloid pathology,¹⁰ is found to correlate significantly with cognitive decline in AD.^{11,12} NFTs have been used to stage the development of AD according to their appearance in specific brain regions by Braak and Braak.¹³ Braak stage 0 represents no NFTs in any brain area, Braak I-II is characterised by NFTs appearing in the entorhinal cortex and its surrounding regions, in Braak III-IV NFTs are found in the limbic system including the hippocampus, temporal cortex and amygdala, and Braak V-VI NFTs are present in almost all isocortical areas. Braak I-II individuals rarely show clinical symptoms, even though the entorhinal cortex and hippocampus which process short-term memory¹⁴ are already starting to develop NFTs.

Extracellular A β and intracellular p-Tau can both appear decades prior to clinical AD.¹⁵ It remains a crucial question whether a putative sex difference occurs in the earliest AD stages and whether it is reflected in A β and/or p-Tau at the neuropathological level. In this study, we first analysed the entire collection of post-mortem brains of all non-demented 'controls' and AD donors, based on neuropathological scoring in the Netherlands Brain Bank (NBB), for the possible presence of sex differences in AD hallmarks. Second, we quantitatively studied the possible sex differences in the accumulation of A β , A β ₄₂ and p-Tau in the entorhinal cortex of stringently selected, well-matched male and female cognitively intact elderly individuals, according to clinical diagnoses, selected as with Reisberg scale 1-2.¹⁶

MATERIAL AND METHODS

Experiment 1: Assessment of neuropathological scoring

Post-mortem human brain material was obtained from the NBB. Clinical files and neuropathological data ranging from non-demented 'controls' to demented AD patients from NBB were studied. Subjects with other neurological or psychiatric principal diagnoses were

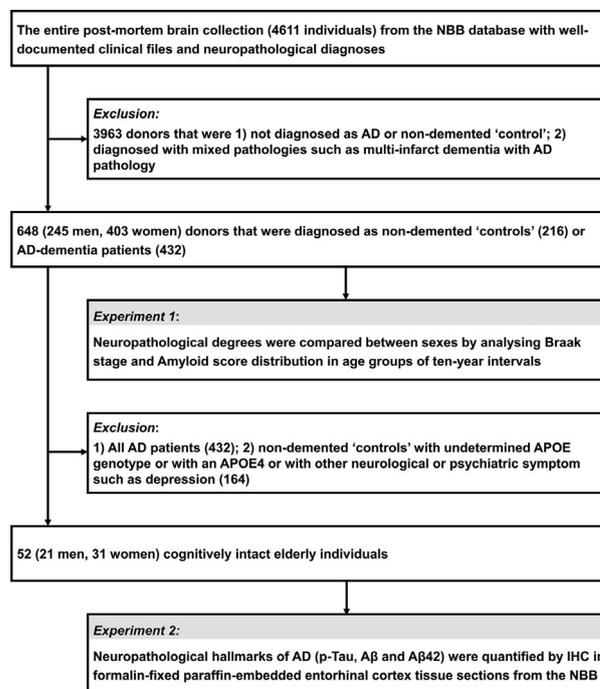


FIGURE 1 Flow chart of the studies. AD = Alzheimer's disease, APOE4 = apolipoprotein E ϵ 4 genotype, IHC = immunohistochemistry, NBB = Netherlands Brain Bank

excluded (Figure 1). In total, 648 subjects were recruited, including 403 women (age range 41-111 years) and 245 men (age range 40-103 years). Braak stages (0-VI) and amyloid scores (O, A, B, C) were made by board-certified neuropathologists for each subject, based upon the Braak scoring system.¹³

Experiment 2: Immunohistochemical quantification of A β and p-Tau deposition in the entorhinal cortex

Immunohistochemical quantification of A β , A β ₄₂ and p-Tau deposition was performed in the entorhinal cortex in cognitively intact elderly (Reisberg scale 1-2), that is, 31 women (age range 46-93 years) and 21 well-matched men (age range 49-92 years), selected from the above-mentioned 648 subjects. The age distribution between men and women showed no significant difference ($p = 0.76$, Kolmogorov-Smirnov test). See subjects' characteristics in Table 1. The exclusion criteria included apolipoprotein E (ApoE) ϵ 4 genotype (APOE4) or unknown ApoE genotype; and any other neurological or psychiatric disorder such as depression accompanying AD (Figure 1). The putative confounding factors, including age, cerebrospinal fluid (CSF) pH, post-mortem delay (PMD), fixation time (FT), clock time of death (CTD) and month of death (MOD), were carefully matched between sexes and checked by the following statistical analysis (see below).

Formalin-fixed paraffin-embedded tissue blocks containing the entorhinal cortex were stored at room temperature (RT) until use. They were serially sectioned at 6 μ m thickness along the rostro-caudal axis using a microtome (Leitz 1512, Germany). Most subjects

TABLE 1 Pathological characteristics of subjects in Experiment 2

Sex	Age	Braak stages	Amyloid scores	Sex	Age	Braak stages	Amyloid scores
Women (n = 31)	46	0	O	Men (n = 21)	49	0	O
	52	0	O		51	0	O
	53	0	O		55	0	O
	61	0	O		56	0	O
	69	I	B		62	0	O
	70	II	A		62	I	O
	70	II	A		71	I	O
	71	I	A		73	0	O
	72	I	A		76	III	B
	74	II	O		77	1	O
	76	II	O		79	II	A
	77	I	A		80	0	A
	77	I	B		80	0	O
	77	II	O		81	II	O
	78	I	A		84	I	A
	78	II	A		87	I	A
	81	I	B		87	III	A
	82	II	B		89	I	B
	82	II	O		91	I	O
	82	II	B		92	II	C
83	I	B	92	IV	C		
83	I	B	Mean (SD)	75.0 (13.9)			
83	I	B					
84	II	A					
84	II	B					
85	I	B					
85	II	B					
85	III	A					
89	II	B					
90	III	O					
93	II	O					
Mean (SD)	76.5 (11.1)						

Note: Braak stages (I-IV) and amyloid scores (O, A, B, C) were made by board-certified neuropathologists for each subject, based upon the Braak scoring system (Braak and Braak, 1991).

had only a single block available; some had two blocks of different levels of entorhinal cortex, which were used to verify the uniformity of A β and p-Tau expression along the rostro-caudal axis. Since no significant difference was found (A β : $p \geq 0.20$, p-Tau: $p \geq 0.10$), these blocks were thus used interchangeably. Because of the very low background of immunohistochemical staining in brain sections, thionine staining was first performed as previously described¹⁷ to visualise the anatomical borders of the entorhinal cortex in an adjacent section for each subject. The specificity of antibodies 4G8,¹⁸ H31L21¹⁹ and AT8²⁰ was confirmed in previous studies. Except for A β 42, which was stained on one section per tissue block, A β and p-Tau were each stained in three adjacent sections respectively.

All sections were first deparaffinised in xylene (2x10 min) and hydrated in graded ethanols (100%-50%, 5 min each). For A β and A β 42 staining, sections were then briefly washed in antigen retrieval buffer (0.01 M citrate buffer +0.05% tween-20, pH 6.0), preheated for approximately 5 min until boiling, then 2x5 min at 800 W in a microwave. After cooling to RT, sections were washed in aqua dest, after which they were subjected to 10 min 70% fresh formic acid (FA) treatment. Sections were then washed 3x10 min in Tris-buffered saline (TBS) and pre-incubated in 5% milk powder (ELK, the Netherlands) TBS (w/v) for 30 min at RT to reduce the background. Primary antibody incubation was performed with monoclonal mouse anti-A β 4G8 (Signet, MA, USA; 1:20000) or monoclonal rabbit anti-A β 42 H31L21 (Invitrogen,

CA, USA; 1:1000) in supermix (0.05 M Tris-HCl, 0.15 M NaCl, 0.25% gelatin, 0.05% Triton X-100 (v/v), pH 7.6) with 5% milk powder overnight at RT in a moist chamber. For p-Tau staining, after deparaffinisation and hydration, sections were directly washed 3x10 min in TBS and pre-incubated in TBS with 5% milk powder for 30 min at RT. Primary antibody, the mouse monoclonal anti-p-Tau AT8 (Thermo, USA; 1:300), was incubated in supermix with 5% milk powder for 1 h at RT and overnight at 4°C in a moist chamber.

On the second day, all sections were washed 3x10 min in TBS then incubated with horse anti-mouse-HRP (DAKO, Denmark; 1:400) or biotinylated horse anti-rabbit (Vector Labs, USA; 1:400) secondary antibody in supermix for 1 h at RT. Next, the sections were washed for 3x10 min in TBS. Sections were incubated with ABC (Vector Labs, USA) at 1:800 in supermix for 1 h at RT and washed again for 3x10 min in TBS. Sections were then developed with diaminobenzidine (DAB) substrate solution (0.5 mg/ml DAB (Sigma, USA), 0.01% H₂O₂ in TBS), and reactions were followed under the microscope (development time 12 min). The reaction was stopped in aqua dest. Sections were dehydrated in graded ethanols (50%–100%, 5 min each), cleared in xylene for 2x10 min, coverslipped with Entellan and dried overnight.

Image analysis for quantitative immunohistochemistry

Images from brain sections were taken by a black and white camera (SONY XC-77E) mounted on a microscope (Zeiss Axioskop with Plan-NEOFLUAR Zeiss objectives, Carl Zeiss GmbH, Jena, Germany) at 10x magnification. The grey matter thickness of the entorhinal cortex was outlined based on the adjacent thionine-stained section. As for the anatomical boards of the entorhinal cortex, we took the region from (i) the end of the subiculum until (ii) prior to the sulcus of the transentorhinal cortex, and (iii) to the white matter²¹ (Figure 4A). Images were analysed using Image Pro 6.3 software, and signal quantification was based on optical density (OD) measurements and thresholding, as described in detail in Zhu et al¹⁷. In brief, the threshold was set at OD = 0.1, which is approximately three times the value of the background. Within the outlined area, the computer then determined the OD values (density mask) and the surface area covered by immunocytochemical signal (area mask). The integrated optical density (IOD) was calculated by multiplying density mask with area mask. Since the size of the outlined entorhinal cortex varied across subjects, the final value was corrected by dividing the IOD value by the total outlined area to obtain the corrected IOD (cIOD) value. Researchers were blind to the identity of all the sections.

Statistical analysis

In Experiment 1, the non-normally distributed neuropathological data from post-mortem brain samples of the 648 subjects were analysed by the Mann-Whitney test for differences in age, Braak stage

and amyloid score distribution between women and men. In addition, after grouping the subjects within ten-year age intervals, neuropathological changes between sexes were analysed by the Kruskal-Wallis test for contingency tables. In Experiment 2, for the immunohistochemical study on AD pathological markers in the entorhinal cortex of cognitively intact elderly individuals, the Mann-Whitney test was used to verify the uniformity of A β and p-Tau levels in respective individuals who have two entorhinal cortex tissue blocks. Restricted maximum likelihood estimation fitted generalised least squares (GLS) models were employed to compare the A β (4G8), A β 42 (H31L21) and p-Tau (AT8) cIOD values between sexes, with data centred on the mean age (78 years). Effects of the putative confounding factors including age, CSF pH, PMD and FT were also checked by GLS models. Data are represented as mean \pm 95% confidence interval. Correlation analyses were performed with the Spearman test. The Kruskal-Wallis test and GLS were performed with TIBCO Spotfire S+ (version 8.2.0), the other statistics were performed with SPSS (version 17.0). $p < 0.05$ was considered to be significant.

RESULTS

Women exhibit more severe AD neuropathological hallmarks than men from age 80

Among the NBB cohort of 648 subjects, Braak stages and amyloid scores were found to be significantly positively correlated (men: rho = 0.80, $p < 0.001$; women: rho = 0.75, $p < 0.001$). In addition, women showed significantly older ages at death than men (Mann-Whitney test, $p < 0.001$, Figure 2A), neuropathological changes were, therefore, analysed by grouping the subjects in ten-year age intervals. Due to the small number of subjects under 50 (3 men, 5 women) and over 100 (4 men, 6 women), subjects under the age of 50 were combined with 50–59 (i.e., ~59) and those over 100 years old were combined with 90–99 (i.e., 90–), Figure 2B. It should be noted that there were no sex differences found in the ApoE genotypes proportion in each age group ($p \geq 0.14$, Mann-Whitney test, Figure 2C–D). Kruskal-Wallis test for contingency tables was performed with ten-year age intervals. We found that the interaction of sex with age was significant for both Braak stages and amyloid scores ($p < 0.001$ and $p = 0.003$ respectively), and post-hoc multiple comparisons showed that neuropathological sex differences were present only after 80 years of age ($p \leq 0.003$, Figure 3A and $p \leq 0.008$, Figure 3B, respectively). Of note, in men, the Braak stage was significantly lower in 80–89 and 90–99 age groups compared with 60–69 group ($p < 0.001$ and $p < 0.001$, respectively). Similarly, amyloid score showed a trend of decrease in 80–89 group compared with 60–69 group ($p = 0.06$). While in women no differences were found among these age groups for Braak stages or amyloid scores ($p = 0.13$ and $p = 0.46$ respectively). Although this does not affect the direct comparisons of men and women in the over 80 groups, it provides an interesting premise for a possible survival bias in men, which is worth future consideration.

FIGURE 2 Age characteristics of the 648 subjects in Experiment 1. (A) Women had significantly older ages at death than men ($***p < 0.001$). Data are presented as median (range). (B) The number of subjects in each age group is mentioned on the top of each bar, and the mean (standard deviation) of age is shown inside the bar. (C-D) No sex differences in the proportion of ApoE genotypes were found in each age group ($p \geq 0.14$)

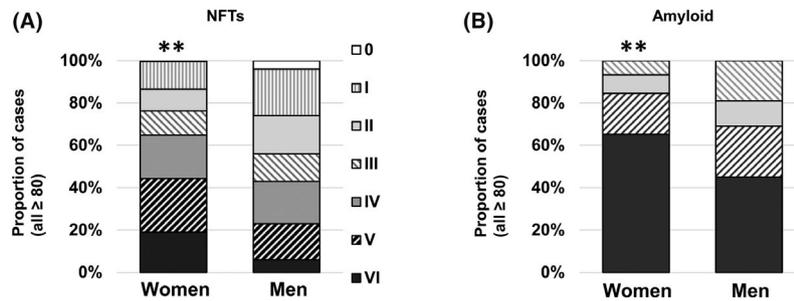
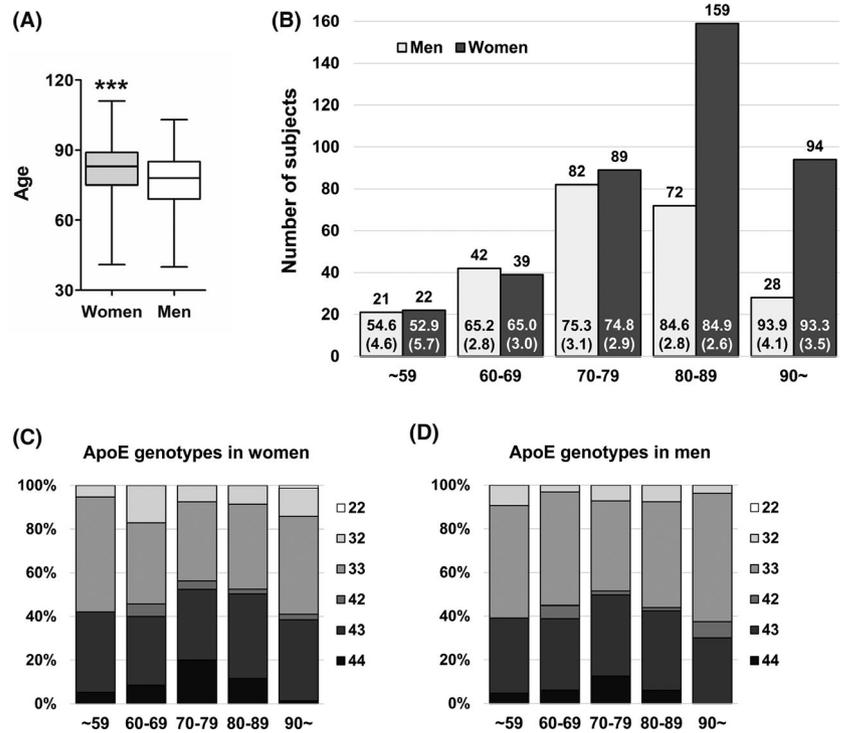


FIGURE 3 Sex differences in Alzheimer's disease (AD) neuropathological hallmarks. Among the 648 subjects from Netherlands Brain Bank donors (245 men and 403 women), after performing a Kruskal-Wallis test for contingency tables, the significant sex differences (Braak stage: $p \leq 0.003$, A; amyloid scores: $p \leq 0.008$, B) were present only over 80 years of age (100 men and 253 women). (*) compared with corresponding men. $**p < 0.01$

Cognitively intact elderly women have higher p-Tau load in the entorhinal cortex

To quantify AD pathology, we stained with AT8 antibody for p-Tau the NFTs, neuropil threads and neuritic plaques. The sites forming the AT8 epitopes (S202/T205) are frequently phosphorylated from the early stages of AD and in control brains,²² since we focused on the cognitively intact elderly, we chose to use this p-Tau antibody. We stained with 4G8 antibody for A β and we mainly observed diffuse amyloid plaques with some had cores. In addition, we stained with H31L21 for A β 42 and observed mainly diffuse amyloid plaques and rarely dense core plaques. Significantly higher levels of p-Tau were observed in the entorhinal cortex of cognitively intact elderly women than in well-matched men (GLS, $p = 0.008$, Figure 4B-D). However, no significant sex difference for A β (GLS, $p = 0.43$, Figure 4E-G) or A β 42 (GLS, $p = 0.10$, Figure 4H-J) accumulation was observed. See details in Table 2. The interaction

of sex with age was significant in p-Tau model (GLS, $p = 0.02$), but not for A β nor for A β 42 models (GLS, $p = 0.89$ and $p = 0.26$ respectively). In addition, p-Tau significantly positively correlated with age in both sexes (men: $\rho = 0.78$, $p < 0.001$, and women: $\rho = 0.55$, $p < 0.01$), while no correlation was found between age and A β (men: $\rho = 0.31$, $p = 0.18$; women: $\rho = 0.24$, $p = 0.19$) or age and A β 42 (men: $\rho = 0.35$, $p = 0.11$; women: $\rho = 0.35$, $p = 0.06$). Moreover, a significant positive correlation was present between A β 42 and A β levels (men: $\rho = 0.60$, $p = 0.004$; women: $\rho = 0.80$, $p < 0.001$). In men, a significant positive correlation was present between A β 42 and p-Tau levels ($\rho = 0.49$, $p = 0.03$), while in women a trend towards a positive correlation ($\rho = 0.31$, $p = 0.09$) was observed. Since it is not certain whether all Braak 0 subjects will finally develop AD, we also did analysis after removal of Braak 0 subjects, and we observed that the sex differences did not change, that is, p-Tau levels in the cognitively intact elderly female entorhinal cortex were significantly higher than in well-matched males (GLS, $p = 0.009$), while

TABLE 2 Generalized least squares (GLS) models for hyperphosphorylated Tau (p-Tau), β -amyloid (A β) and A β 42

		Intercept	Regression coefficient	Mean	Lower 95% confidence interval	Upper 95% confidence interval
P-Tau	Men	0.0004	0.00001	0.0007	0.0005	0.001
	Women	0.003	0.0001	0.003	0.002	0.004
A β	Men	0.0004	0.00001	0.0003	0.0002	0.0005
	Women	0.0006	0.00001	0.0009	0.0006	0.001
A β 42	Men	0.002	0.00007	0.002	0.0003	0.004
	Women	0.005	0.0002	0.005	0.002	0.007

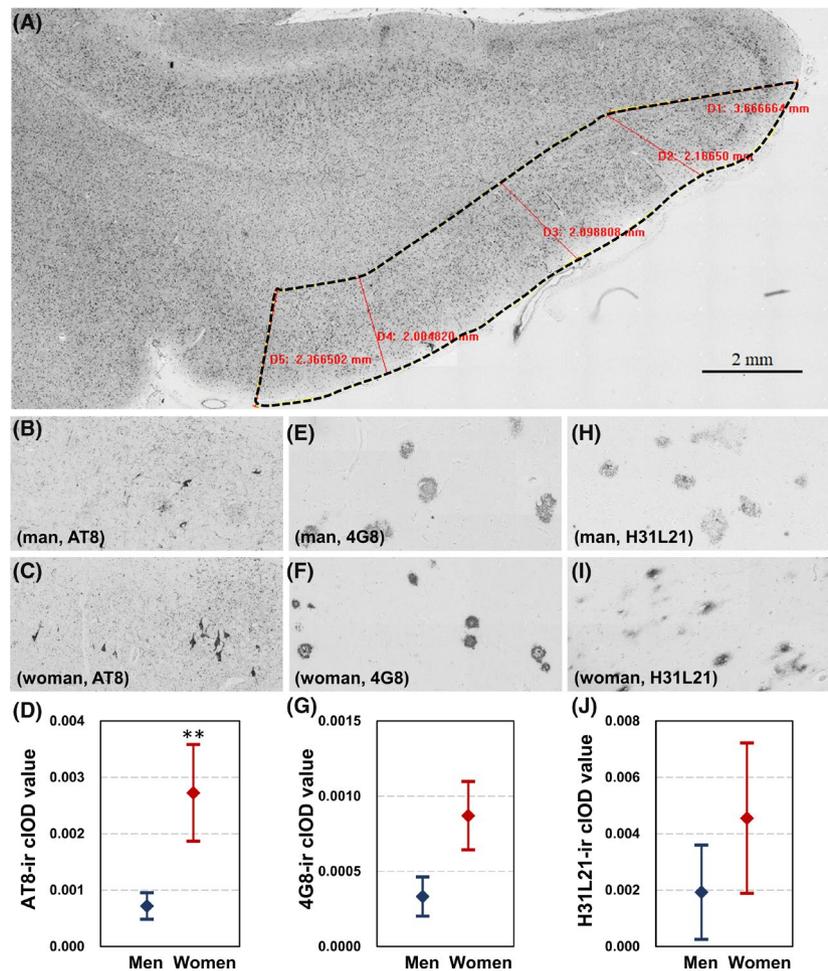


FIGURE 4 Thionine staining and representative immunohistochemical staining of hyperphosphorylated Tau (p-Tau), β -amyloid (A β) and A β 42 in human entorhinal cortex. (A) The thickness of grey matter within one section was measured at multiple points in order to delineate the entorhinal cortex in an adjacent immunohistochemical section for A β or p-Tau, as grey matter thickness was undetectable in these staining. The entorhinal cortex is outlined with a dashed line, starting from the end of the subiculum and ending prior to the transentorhinal cortex. The presented exemplar section was from a 77-year-old Braak stage I woman with amyloid score A (NBB number 2004-049). Anatomical definition was according to Insauti and Amaral.²² Typical images of staining (40x) by antibody AT8 (recognises Tau phosphorylated at S202/T205, B-C), 4G8 (against residues 17-24 of A β , E-F) and H31L21 (specific to A β 42, H-I) in the entorhinal cortex of men and women. Presented typical sections were from a 92-year-old Braak stage II man with amyloid score C (NBB number 1999-092, B,E,H), an 83-year-old Braak stage I woman with amyloid score B (NBB number 2011-049, C,F) and an 83-year-old Braak stage I woman with amyloid score B (NBB number 2006-014, I). Note that there are more positive signals of all antibodies in women. Data for (D,G,J) is represented as mean \pm 95% confidence interval (21 men and 31 women). (*) compared with corresponding men. ** $p < 0.01$

no significant sex difference in A β accumulation was observed (GLS, $p = 0.27$) or A β 42 (GLS, $p = 0.37$).

DISCUSSION

In the entire collection of post-mortem brains of all non-demented 'controls' and AD donors in the NBB, we observed a clear sex difference, that is, women had higher Braak stages and amyloid scores than men, only after 80 years of age. Another novel finding was that, in the cognitively intact elderly, more p-Tau, rather than A β or A β 42, accumulated in the entorhinal cortex of women, as compared to men. This suggests that p-Tau rather than A β may crucially be involved in the sex differences in AD pathogenesis in the entorhinal cortex.

In Experiment 1, we included all the non-demented 'controls' and AD patients in the NBB without matching, in order to see whether there are sex differences in the neuropathological scores for AD changes in the population. Indeed, we observed in Experiment 1 survival bias, that is, women had significantly older ages at death than men ($p < 0.001$), which is in accordance with the longer lifespan of women in the population.²³ In order to make clear when the sex difference starts to show significance, we subsequently performed stringent statistics of Kruskal-Wallis test for contingency tables and observed that the more severe neuropathological stages in women, as compared to men, appeared only after 80 years of age. Previous post-mortem human brain studies performed with samples of men and women with an average age of death over 83 also showed that women tend to have more AD pathology than men.^{24,25} What is new in our data is that it shows that such a sex difference does not exist in those who died at younger ages. It is also of interest to note that in the oldest men, but not in women (i.e., ≥ 80 years of age), neuropathology is less severe than in younger age-groups. These findings suggest another possible survival bias, whereby individuals predisposed to AD are less likely to become very old.²⁶ However, another post-mortem study²⁷ did not observe this levelling-off effect. It should be noted that in this paper, a longitudinal study was performed in which all participants had entered without dementia while only around 60% of all autopsied participants met criteria for a pathological AD diagnosis. In addition, the authors first measured the burden of both A β and p-Tau immunohistochemically, then semi-quantitatively determined a composite indicator called 'global AD pathologic score' and they observed higher scores in participants older than 80 than those younger than 80. Our Exp-1, on the other hand, was a retrospective study that excluded those not being diagnosed as non-demented 'controls' or AD, and those who had mixed pathologies (Figure 1). In addition, our results compared the AD hallmarks spreading in the brain,¹³ rather than how much they accumulated in the brain. These different characteristics may account for the different findings between studies.

With the purpose of investigating whether there are sex differences in AD neuropathological hallmarks in the cognitively intact early stages (clinical Reisberg scale 1–2), in Experiment 2, we quantified neuropathological AD changes in the entorhinal cortex, one of the early affected regions in AD.¹³ The confounding factors mentioned above were very well matched between sexes (Mann-Whitney or Mardia-Watson-Wheeler test, $p \geq 0.22$) except for PMD

(Mann-Whitney, $p < 0.001$), while by checking them within the GLS models, the possible significant effect on the pathological hallmarks in our present study was excluded ($p \geq 0.06$). In addition, we excluded subjects that had an APOE4 genotype or those whose ApoE genotype was not yet determined by the NBB. APOE4 may increase A β deposition and decrease A β clearance²⁸ and is accompanied by diminished neuronal metabolism.²⁹ In addition, APOE4 may increase Tau hyperphosphorylation more in female than in male AD patients.³⁰ We also excluded subjects with any neuropsychiatric disorder diagnoses other than AD, such as depression, as it is one of the most common co-morbid psychiatric disorders in dementia.³¹ Another reason to exclude such patients is that they showed a higher number of neuritic plaques and NFT deposits in the cerebral cortex³² and hippocampus³³ compared with those without depression. We observed in Experiment 2 that women showed significantly more p-Tau loads, but not more A β nor A β 42 loads, in the entorhinal cortex than men did. These findings are in agreement with those of Mufson et al³⁴ who showed that women without cognitive impairment were more likely to have higher Braak stages than corresponding men, although their study did not quantify p-Tau, A β or A β 42 load in any brain area that is affected in early AD. Indeed, p-Tau correlates better than A β with cognitive decline during the course of AD as exemplified in previous studies.^{11,12} A putative alternative interpretation of our findings in the cognitively intact elderly may, theoretically, be that the sex difference in p-Tau observed in our study may reflect 'resilience' to AD pathology in women, which may agree with the hypothesis of Wang et al. that neurons acutely overexpressing p-Tau are more resistant to apoptosis stimulated by oxidative stress.³⁵ However, Kopeikina et al. showed that although the formation of NFTs may protect neurons acutely from the effect of toxic soluble forms of p-Tau, the long-term inhibition of cellular transport by NFTs may finally lead to cell death.³⁶ Since these experiments were based upon *in vitro* or mouse models,^{35,36} while AD in the human brain is an extremely slow process over decades,^{15,37} the possible acute protective effect of p-Tau is a less probable explanation of our findings. It should be noted, however, that we, as well as others,³⁴ observed a positive correlation between p-Tau and A β loads in the entorhinal cortex, which indicates that the contribution of A β to the early pathogenesis of AD cannot be ruled out. Our finding that the sex difference in AD pathology during the cognitively intact elderly stage is mainly related to p-Tau is an important starting point for future studies into the mechanism behind this feature.

The sex differences in AD neuropathology may be due to the changes in sex hormones and/or gonadotropins in response to the drastic reduction of oestrogen level during menopause in women.³⁸ The neuroprotective effects of oestrogens have been well documented.³⁹ Studies have shown that the age-related drop in oestrogen levels in post-menopausal women increases the susceptibility to AD pathology,⁴⁰ and beneficial effects on cognition were shown in some studies when treatment was started in early post-menopause (most commonly at ages 50–60 years),⁴¹ while effective therapeutic oestrogen intervention has yet to be realized.⁴² Brain luteinizing hormone (LH) was recently considered as another important candidate

involved in women's vulnerability for AD. For instance, reduced LH-mRNA levels were found in the hippocampus and cortex of AD women.⁴³ In addition, in the triple-transgenic AD mouse harbouring human PS1^{M146V}, APP^{Swe} and Tau^{P301L} transgenes,⁴⁴ brain LH levels were reduced in the superior colliculus by ovariectomy, while a gonadotropin-releasing hormone receptor agonist improves cognitive performance and increases spine density of pyramidal neurons residing in the retrosplenial cortex and cingulate cortex layer II/III.⁴⁵

In summary, our study provides further evidence suggesting women are more vulnerable to AD. In addition, this vulnerability may be present early in the disease process. Our data amplify the importance of early intervention in AD, and possible sex-specific intervention, with potential future research focusing on the contribution of hormonal changes as a contributor to the sex differences in the formation and aggregation especially of p-Tau in the brain of early AD.

ETHICAL APPROVALS AND PATIENT CONSENTS

The post-mortem human brain study was approved by the Medical Ethics Committee of the VU Medical Centre (for Ethical and legal declaration of the Netherlands Brain Bank (NBB) see: <https://www.brainbank.nl/about-us/the-nbb/>). All the donors or their next of kin had provided the NBB written informed consent for a brain autopsy and for the use of the material and clinical information for research purposes.

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AUTHOR CONTRIBUTIONS

The authors Y.H., D.S. and A.B. contributed to the conception and design of the study. Y.H., J.B., H.M., J.S., A.S., R.B. and R.V. contributed to the acquisition and analysis of data. Y.H., J.B., H.M., J.S., D.S. and A.B. contributed to drafting the text and preparing the figures.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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