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Sex differences in Alzheimer's disease and common neuropathologies of aging

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Abstract

Alzheimer's dementia is significantly more common in women than in men. However, few pathological studies have addressed sex difference in Alzheimer's disease (AD) and other brain pathologies. We leveraged post mortem data from 1453 persons who participated in one of two longitudinal community-based studies of older adults, the Religious Orders Study and the Rush Memory and Aging Project. Postmortem examination identified AD pathologies, neocortical Lewy bodies, DNA-binding protein 43 (TDP-43), hippocampal sclerosis, gross and micro infarcts, atherosclerosis, arteriolosclerosis, and cerebral amyloid angiopathy. Linear and logistic regressions examined the association of sex with each of the pathologic measures. Two thirds of subjects were women (n=971; 67%), with a mean age-at-death of 89.8 (SD=6.6) years in women and 87.3 (SD=6.6) in men. Adjusted for age and education, women had higher levels on a global measure of AD pathology (estimate=0.102, SE=0.022, p<0.001), and tau tangle density in particular (estimate=0.334, SE=0.074, p<0.001), and there was a borderline difference between women and men in amyloid- β load (estimate=0.124, SE=0.065, p=0.056). In addition, compared to men, women were more likely to have more severe arteriolosclerosis (OR=1.28, 95% CI:1.04–1.58, p=0.018), and less likely to have gross infarcts (OR=0.78, 95% CI:0.61–0.98, p=0.037), although the association with gross infarct was attenuated after controlling for vascular risk factors. These data help elucidate the neuropathologic footprint of sex difference in AD and other common brain pathologies of ageing.

Keywords

Alzheimer disease; Sex; Pathology; Tau proteins; Amyloid; Arteriolosclerosis

INTRODUCTION

Alzheimer's dementia is significantly more common in women than in men. Of the estimated 5.2 million people with Alzheimer's dementia in the United States, 3.3 million are women and 1.9 million are men [2]. While some studies have reported higher incidence of Alzheimer's dementia among women [48,60], others have found no sex difference after adjusting for age [20,32]. Further, sex difference in incidence of Alzheimer's dementia may be age dependent [23,39].

Common age-related neuropathologies, including Alzheimer's disease (AD) and other neurodegenerative and cerebrovascular pathologies, are the main causes of dementia, including Alzheimer's dementia [31,34]. Yet, to date, only a few studies have directly investigated the association between sex and AD and other common neuropathologies. Further, these included only a small sample size (less than 200 brain autopsies) [41,53], or did not examine pathologies other than AD [41,43,53].

Well-recognized risk factors for AD include age and apolipoprotein E (*APOE*) $\epsilon 4$. Little is known of the possible effects of these factors on the relation of sex with AD pathology. One clinical-pathologic study reported that the odds of AD pathology was not different between women and men if they were younger than 80 years at death, but women older than 80 years had a higher odds of AD pathology compared to men [43]. Further, another study reported that an AD pathologic diagnosis was more common among women in patients older than age 75 years [34]. Separately, clinical studies have reported that women *APOE* $\epsilon 4$ carriers had a higher risk of Alzheimer's dementia than men carriers [21]. One autopsy study found that women had more senile plaques, and the association was stronger among $\epsilon 4$ carriers [17], and another study reported that the association of *APOE* $\epsilon 4$ with neurofibrillary tangles was weaker in men than in women, while the association with senile plaques was significant in both sexes [24].

In prior work, we found that women, a subset of those in the current study, who had surgical menopause at an early age had a faster decline in cognitive function and more AD pathology at autopsy, and neuritic plaques in particular, compared to women with surgical menopause at older ages [14]. Also, in earlier work, we found that women had more AD pathology and neurofibrillary tangles in particular, but not neuritic or diffuse plaques, among 141 subjects who came to autopsy [8]. In the current study here, we build upon our prior work in several important ways. First, we included both women and men, and in a larger sample of nearly 1500 persons, and examines not only AD but also several other common dementia-related neuropathologies. Second, we used semi-quantitative outcome measure of (A β) load and tau tangles densities assessed by immunohistochemical methods rather than using neurofibrillary tangles, neuritic and diffuse plaques assessed by a Bielschowsky silver stain. Third, we considered potential confounders of age and *APOE* $\epsilon 4$. And fourth, we examine if age at surgical menopause can explain any observed difference between women and men in neuropathology.

We used post mortem data from 1453 women and men participants in one of two longitudinal community-based studies of ageing, the Religious Orders Study (ROS) and the

Memory and Aging Project (MAP), who were without dementia at study entry and agreed to annual cognitive evaluations and organ donation at the time of death. We compared AD pathological indices, including tau tangles density and A β load, and other age related neurodegenerative and cerebrovascular pathologies, between women and men. We also examined if any association of sex with pathological indices was modified by subjects' age at death or *APOE* $\epsilon 4$ status. Finally, we examined the effect of menopause-related factors, including surgical vs. natural menopause, age at surgical menopause, and use of hormone replacement therapy, on the association of sex with AD pathologies.

METHODS:

This cross-sectional study included deceased participants of one of two ongoing, longitudinal clinical pathological studies of ageing and dementia, the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP). Studies' methods have been described in detail elsewhere [10]. ROS began enrolling participants in 1994, and recruits older Catholic nuns, priests, and brothers from sites across the United States. MAP began in 1997, and enrolls participants from across the greater Chicago metropolitan area. Eligible participants in both cohorts were older women or men without known dementia at baseline. Both studies followed similar methods, including participants' agreement for annual cognitive evaluations and consenting to sign an anatomical gift act for brain donation at the time of death. With uniform data collection, it is feasible to combine data across the two cohorts to examine associations of clinical data with brain pathologies, as done in many of our prior studies [4,45]. The overall follow-up rate of both cohorts exceeds 90%, and the autopsy rate exceeds 85%.

From 1994 until November, 2017 when analyses for this study began, 1351 participants enrolled in the ROS, of whom 791 had died. Of 732 (93%) who had an autopsy, complete neuropathological data were available for inclusion in this study in 718 persons. In the MAP, out of 1766 enrolled from 1997 until November 2017, 908 died, of whom 756 (83%) had an autopsy, and 735 had complete neuropathological data and were included in this study.

Both studies were approved by the Institutional Review Board of Rush University Medical Center. Participants signed an informed consent and Anatomical Gift Act.

Clinical evaluation

Participants underwent a uniform structured annual clinical evaluation which included detailed cognitive testing. Scores from 17 cognitive tests were summarized in a global cognition score, as described previously [10,62]. Participants were evaluated by an experienced clinician who used all cognitive and clinical data to diagnose Alzheimer's and other causes of dementia.

Pathologic evaluation

The mean post-mortem interval was 9.1 (SD=7.9) hours. Details of the autopsy procedure are described elsewhere [10]. In brief, the brain is removed, one hemisphere is frozen, and the other hemisphere is fixed in 4% paraformaldehyde for at least three days. The fixed

hemisphere is cut into 1 cm slabs, and tissue blocks are obtained from pre-specified brain regions for neuropathological evaluation.

AD neuropathology.—Quantitative AD pathologic indices were determined using image analyses (A β burden) and stereology (tau tangles density) of immunohistochemically-stained brain sections [7,11]. Tissue from 8 cortical regions was dissected from the 1 cm slabs and embedded in paraffin: anterior cingulate cortex, superior frontal cortex, mid frontal cortex, inferior temporal cortex, hippocampus, entorhinal cortex, angular gyrus/supramarginal cortex, and calcarine cortex. Two or more blocks were obtained from adjacent 1 cm slabs in each region, cut in 20 μ m sections, and placed on glass slides. Paired helical filament tau was labeled with an antibody specific for phosphorylated tau, AT8 (Innogenetics, Alpharetta GA, 1:1000) in 4% horse serum, and plaque deposition of A β was labeled with three monoclonal antibodies specific for A β : 4G8 (1:9000; Covance Labs, Madison, WI), 6F/3D (1:50; Dako North America Inc., Carpinteria, CA), and 10D5 (1:600; Elan Pharmaceuticals, San Francisco, CA). Immunohistochemical analysis was performed, as previously described, with diaminobenzidine as the reporter and with 2.5% nickel sulfate to enhance immunostain contrast. All sections were run using identical incubation times on an automated immunohistochemical stainer in precisely timed runs. Control sections processed without the antibody, that displayed no specific staining, and positive controls were included in all runs.

Quantification of tau-labeled tangle density per square millimeter was performed with a stereological mapping station, which included a Leica DMRBE microscope and a computer (millennia Mme; Micron Electronic Inc, Boise, Idaho) equipped with StereoInvestigator software (Microbrightfield Inc, Colchester, Vt) [11]. After a region was delimited at low power, the software program randomly placed a grid of predetermined size over the region. Total magnification was raised to $\times 400$, and the program was engaged to direct the motorized stage on the microscope to stop at each intersection point of the grid for sampling. The operator focused through the section depth as the fields were visualized on the video monitor within the superimposed counting frame. All objects within the $150 \times 150 \mu\text{m}^2$ counting frame that did not touch the exclusion lines of the box (bottom and left sides) were counted. Approximately, 50% of the area of a delimited region of interest was quantified. Neurofibrillary tangles labeled with AT8 have a characteristic appearance and location in the neuronal cell body or as ghost tangles.

As previously reported, image analysis was used for the quantification of A β deposition [11]. A systematic random sampling scheme was used to capture video images of A β -stained sections. The following instruments were used to capture color images: an Olympus BX60 microscope, a computer-controlled motorized stage, a high sensitivity HV-C20 CCD video camera (Hitachi, Japan), and StereoInvestigator software (MicrobrightField, Inc, Colchester, VT). Then, captured 24-bit color images were converted to 8-bit gray scale images by using conversion algorithms associated with Adobe Photoshop (Adobe Systems; Tucson, AZ). Calculation of the percent area occupied by A β immunoreactive pixels was performed by algorithms using the public domain Object-Image 1.62p15 (developed by Norbert Vischer; <http://simon.bio.uva.nl/object-image.html>). The algorithm segmented each gray scale image, by use of ISODATA (Iterative Self-Organizing Data Analysis) and

triangulation thresholding approaches, along the intensity domain into two fractions, the labeled and the background compartment. The percent area occupied by the labeled compartment was calculated at each section, and the average of the percent areas was used for each brain region and across the brain regions to yield an overall A β load.

By averaging assessed regions' tangles density and A β load, separate summary measures were made for the brain's density of tau-labeled tangles and the brain's load of A β , respectively. In our previous paper, we showed these summary measures to be reliable and internally consistent [11]. The Chronbach α coefficient was 0.84 for the tangles densities and 0.88 for the A β loads, showing high item (regions' measures) total (summary measures) correlations.

A modified Bielschowsky silver stain was used to visualize neuritic plaques, diffuse plaques, and neurofibrillary tangles in five cortical areas, as in our prior publications [13]. Neuritic plaques were identified by a discrete brownish discoloration with thickened argyrophilic neurites, with and without central cores of amyloid. Diffuse plaques were identified by a diffuse blush with or without a filamentous nature but without thickened argyrophilic neurites. Although upper and lower limit size cutoffs for plaques were not used, contiguous clusters and confluent plaques were split into individual components and counted separately if possible. To determine the density of each pathologic index, we first examined the entire slide under low power (total magnification of 20 to 40 $\times$) and identified the area with the greatest density of the pathologic index. Then, using a graticule to mark a 1-mm² area we counted the total number of the pathologic index at a magnification of 100 $\times$. The procedure was followed for the three AD pathologic indices in each of the 5 brain regions. In our previous study, we found that the inter-rater reliability of the counts of AD pathologic indices were from 0.89 to 0.93, supporting reproducibility of these measures [13].

A global AD pathology score was made as an indicator variable of AD pathology burden from the three AD pathology counts (neuritic plaques, diffuse plaques, and neurofibrillary tangles). Because the regional counts had wide means, standard deviations (SD)s, and ranges, we first converted the raw counts to a standard distribution by dividing each person's count by the SD for that particular count (e.g. the number of neuritic plaques in the frontal cortex of a person by the SD for frontal cortex neuritic plaques from all participants). Next, we formed summary measures of neuritic plaques, diffuse plaques, and neurofibrillary tangles by averaging the relevant standardized scores across all brain regions. Finally, we constructed the global AD pathology score by averaging the summary measures of the three AD pathologic indices. In our previous study, we showed that the regional standardized scores were highly correlated with the global AD pathology score (r range: 0.47–0.73, median $r = 0.67$) and the Chronbach α coefficient was 0.90, indicating high internal consistency and supporting use of the global AD pathology score [13]. In addition, a board-certified neuropathologist, blinded to clinical data, determined the pathologic diagnosis of AD based on published criteria [26].

Other common degenerative pathologies.—Immunohistochemistry with α -synuclein immunostain (Zymed; 1:50) was used for assessment of Lewy bodies on sections from the following brain structures: midfrontal, midtemporal, inferior parietal, anterior cingulate,

entorhinal, hippocampus, basal ganglia, and midbrain. The immunohistochemistry was performed using the VECTASTAIN[®] ABC method with alkaline phosphatase as the color developer. Nigral Lewy bodies were identified as round, intracytoplasmic structures with a darker halo. In the cortex, Lewy bodies were identified as round intracytoplasmic structures, often lacking any halo and with eccentric nucleus. Only intracytoplasmic Lewy bodies were used as an indicator of positive staining. Simplified McKeith criteria[37] were used to summarize the brain Lewy body pathology, and can be found in more detail in the Supplemental Methods. In this study, a dichotomous summary variable was used indicative of presence or absence of neocortical-type Lewy body pathology which we previously showed to be associated with dementia [57].

Transactive response DNA-binding protein 43 (TDP-43) was assessed using immunostaining with monoclonal antibody to phosphorylated TDP-43 (pS409/410; 1:100), as previously described [63]. TDP-43 was investigated in 6 grain regions: the amygdala, hippocampus, dentate gyrus, entorhinal cortex, midfrontal cortex, and middle temporal cortex. The immunostaining procedure detects the pathologically phosphorylated TDP-43 seen in the inclusions, not the normal TDP-43. Each of the 6 regions was reviewed for the presence, severity, and location of TDP-43 cytoplasmic inclusions (both neuronal and glial), and was rated on a 6-point scale described in the Supplemental Methods. In this study, we used a 4-stages classification of TDP-43 distribution based on previous studies [30,42]: stage 0, no presence of TDP-43; stage 1, presence of TDP-43 localized to the amygdala; stage 2, extension to the hippocampus and/or entorhinal cortex; stage 3, extension to the neocortex.

Hippocampal sclerosis was evaluated unilaterally in a coronal section of the midhippocampus at the level of lateral geniculate body, as described elsewhere [42]. It was graded as absent or present based on severe neuronal loss and gliosis in CA1 and/or subiculum.

Common cerebrovascular pathologies—Uniformed examination for cerebral infarcts was conducted, as previously published [58], on the fixed slabs from one hemisphere and pictures of fresh slabs from the other hemisphere. Because acute and subacute infarctions are unlikely to be related to dementia at the last evaluation, we included only chronic infarctions. We identified age, volume, and location of all brain infarcts visible to naked eyes, including on 1cm thick coronal slabs, and dissected them for histological confirmation. Microscopic examination also provided us identification of microinfarcts, which are, by definition, not visible to the naked eyes and can only be identified under microscopy [6]. A minimum of 9 regions in 1 hemisphere were examined for microinfarcts on 6-μm paraffin-embedded sections stained with H&E. The regions were 6 cortical (mid frontal, middle temporal, entorhinal, hippocampal, inferior parietal, and anterior cingulate), 2 subcortical (anterior basal ganglia and thalamus), and mid brain. Examination of chronic microinfarcts included incomplete or cavitated infarcts, with few remaining macrophages (unless large infarct) and fibrillary gliosis. We created dichotomous summaries (presence vs. absence) for each of the infarct variables.

Atherosclerosis severity grade was assessed by evaluation of circle of Willis vessels at the base of the brain [6]. Vertebral, basilar, posterior cerebral, middle cerebral, and anterior

cerebral arteries were evaluated along with their proximal branches. Arteries were bisected for evaluation of narrowing when there was concern by visual inspection or palpation. Severity of atherosclerosis was scaled semiquantitatively (none or possible, mild to severe) on the basis of involvement of each artery and number of arteries (provided in the Supplementary Table e-1).

Severity of arteriolosclerosis was graded based on concentric hyaline thickening of vessel walls, specifically small arterioles less than approximately 50 μm in diameter [5]. The scale ranged from: 0 if there was no arteriolosclerosis; 1 for mild arteriolosclerosis, if the arterial wall was minimally thickened; 3 for moderate, if the arteriolar wall thickness was up to twice the normal thickness; 5 for severe, if the arteriolar wall thickness was more than twice the normal thickness; and 6 used when complete occlusion was seen due to a thickened arterial wall. In this analysis, like many of our previous publications [4,5], arteriolosclerosis was scaled in four levels (0=not present, 1 or 2 = mild, 3 or 4= moderate, and 5 or 6 = severe) by combining near scores in the original grading system.

Cerebral amyloid angiopathy (CAA) was examined using A β immunostaining of meningeal and parenchymal vessels in four regions (midfrontal, midtemporal, angular, and calcarine cortices) [15]. Three monoclonal antibodies against A β were used in immunostaining of sections: 4G8 (1:9000; Covance Labs, Madison, WI), 6F/3D (1:50; Dako North America Inc., Carpinteria, CA), and 10D5 (1:600; Elan Pharmaceuticals, San Francisco, CA). In this analysis, a semi-quantitatively scoring system as none, mild, moderate, and severe was used for the assessment of CAA, and can be found in more detail at the Supplementary Methods.

Other clinical variables

Presence of diabetes mellitus, hypertension, and smoking was used to construct a vascular risk factor burden score with a range from 0 to 3. Similarly, we created another construct for the burden of vascular diseases (i.e stroke, heart attack, and claudication) [10]. As vascular risk factors durations, independent of their presence, can affect vascular diseases we created a ratio (number of visits with positive history of the vascular risk factor/total years of follow up) to be used in the adjustment of statistical models of the association of sex with the neuropathologies. We did not calculate a duration variable for smoking because only 2% of the cohorts were current smokers.

Women's age at menopause was determined based on the history gathered at baseline, and data on type of menopause (surgical vs. natural) was also gathered. We assessed hormone replacement therapy (HRT) by questions about its type (pill, shot, creams, patches) and its duration, and by inspection of the prescribed bottles during the interviews.

Apolipoprotein E determination

DNA genotyping was performed at Polymorphic DNA Technologies (Alameda, CA) by investigators blinded to all clinical and pathological data. The *APOE* genotypes were determined by sequencing rs429358 (codon 112) and rs7412 (codon 158) at exon 4 of the *APOE* gene [65].

Statistical analyses

First, we used chi square test to compare women and men on categorical pathologic measures. Then, to assess for sex differences in continuous measures of AD pathology, i.e. global AD pathologic score and tau and A β , we used liner regression models adjusted for age and education. We used a square root transformation of the AD measures in the regressions, due to the positively skewed distributions of these data. Next, we added a term for the interaction between age and sex to uncover any possible change in the association of sex with AD pathological indices in subjects with different ages. Likewise, we added two terms for *APOE* $\epsilon 4$ and its interaction with sex, to examine if the association of sex with AD was different in subjects with or without *APOE* $\epsilon 4$. Also, because *APOE* $\epsilon 4$ effects differ among ethnicities [21], we repeated the above analyses by restricting to non-Latino White persons.

To uncover if the association of sex with AD pathologies were due to the type of menopause (surgical vs. natural) or the age at menopause, we first repeated the core regressions by replacing sex with a 3-levels categorical variable, women with surgical menopause, women with natural menopause, and men (reference). Then, we replaced the 3-levels variable with a 4-levels categorical variable by separating women with surgical menopause into two groups: women whose menopausal ages were equal or older than the median of age at menopause in the group, and women who were younger at their menopause. We limited the use of age for categorization to women with surgical menopause because our prior publication suggested that a younger age at menopause was associated with more AD pathologies only among women with surgical menopause [14]. Finally, we examined if age at time of HRT initiation and duration of HRT can affect the association of sex with AD pathologies by adding these variables to the regression models.

We used logistic regressions, controlled for age and education, to study association of sex with AD pathologic diagnosis, and other neurodegenerative and cerebrovascular neuropathologies. Core models were evaluated for violations of assumptions, by examining data both analytically and graphically. Analyses were done using SAS version 9.4.

RESULTS:

The demographic and clinical characteristics of the participants are shown in Table 1. On average, participants were nearly 90 years old at the time of death. Women were about 3 years older than men ($p<0.001$), had one year less of education ($p<0.001$), and had fewer vascular risk factors ($p<0.001$). We did not observe any sex difference in the *APOE* $\epsilon 4$ status ($p=0.844$), Alzheimer's dementia ($p=0.099$), or vascular diseases burden ($p=0.633$).

As our analytic sample came from two cohorts, yet uniformly designed and implemented, we compared demographic and clinical characteristics of their participants (Supplemental Table e-2). The ROS and MAP participants were different in age at death (2 years difference on average), sex (%5 more women in MAP), education (3.5 years more in ROS), vascular risk factors (more common in MAP), vascular diseases (more common in ROS), and AD dementia (7% more in ROS). However, they were not different in the frequency of diabetes, hypertension, *APOE* $\epsilon 4$ status, and the last average Mini Mental State Examination score.

Further comparison of the two cohorts in the frequency of the neuropathologies is provided at the Supplemental Table e-3.

AD pathology

We first compared the semi-quantitative global AD pathologic score, between women and men. The mean AD pathologic score was 0.7 (SD = 0.6; range 0 to 3.2) in the total group, 0.8 (SD = 0.6) for women, and 0.6 (SD = 0.6) for men. Controlling for age and education, women had a higher AD pathologic score compared to men (estimate=0.102, SE=0.022, $p<0.001$) (Figure 1). To contextualize the sex difference in the global AD score, we compared its regression coefficients with that for age of death (estimate=0.009, SE=0.002, $p<0.001$): global AD score among women were equivalent to that among men who were 12 years older. In further analyses, we added a term representing the two cohorts to the models' predictors, and the association of sex with the global AD score did not change (Supplemental Table e-4).

We next examined for specific AD pathologic markers individually. Compared to men, women had a higher tau tangle density which remained after controlling for age and education (estimate=0.334, SE=0.074, $p<0.001$) (Figure 1). This was equivalent to men who were 13 years older. Although women had a higher A β load than men ($p<0.001$), the difference was borderline significant after controlling for age and education (estimate=0.124, SE=0.065, $p=0.056$) (Figure 1). Controlling for the participants' cohort in our model did not change the association of tangles with sex, but changed the amyloid's association: women had more amyloid when we controlled for age, education, and the cohorts (estimate=0.139, SE=0.064, $p=0.031$) (Supplemental Table e-4). Then we looked at the association of sex with the tangle density and A β load at specific brain regions (Supplementary Table e-5). Women had higher levels of tangles in both mesial temporal and neocortical regions, but had higher levels of amyloid only in the mesial temporal.

In further analyses, we examined if the association of sex with AD pathologic indices were independent of other neuropathologies. We added terms for other common brain pathologies to the predictors of the core models, and the results did not change: women had more AD pathology, specifically more tau tangles density (Supplemental Table e-6). Then, we examined addition of vascular risk factors and their duration or vascular diseases to the core models and the results did not change either (Supplemental Table e-7).

More than 60% of all autopsied participants met criteria for a pathologic diagnosis of AD, and the percentage was higher among women (68%) than men (58%) ($p<0.001$). After controlling for age and education, women were 35% more likely to have a pathologic diagnosis of AD (OR=1.35, 95% CI:1.06–1.72).

Effects of age and APOE $\epsilon 4$ on the relation of sex to AD

Age may affect the association of sex with AD pathology [34,43]. After adding a term for the interaction between age and sex to the linear regression models, we observed an interaction in the association with the global AD pathologic score outcome (estimate=−0.008, SE=0.003, $p=0.023$) (Table 2). But, we did not find a significant interaction between sex and age in their association with tau tangles or A β (Table 2). As the association of age

with AD pathology may be non-linear [29,54], we replaced the continuous age with a categorical one in the models examining the interaction of sex and age in their association with the global AD pathologic score. Without the interaction term, women and older age were related to higher global AD scores. However, participants older than 90 were not different from participants aged 80–90 years ($p = 0.238$) although both groups had higher global AD pathologic scores than participants younger than 80 (both $p < 0.001$), indicating a non-linear relationship between age and AD pathology. When we added to the predictors a term for an interaction between sex and the categorical age we found the interaction to be non-significant (Table 3) implicating women had higher load of AD pathology compared to men irrespective of their age at death.

Published clinical studies found that women had higher odds for Alzheimer's dementia and this association was stronger among *APOE ε4* carriers compared to *ε3ε3* carriers [21]. Therefore, we conducted linear regression models similar to above, but with the addition of two terms, for *APOE ε4* and the interaction of *APOE ε4* with sex (Table 2). There was no interaction, suggesting that our finding of higher AD pathology in women compared to men did not vary by the *APOE ε4* status. We repeated the above analyses by restricting to non-Latino whites ($n=1377$) and results were essentially unchanged (data not shown).

Effects of menopause and HRT on the relation of sex to AD

Our previous study showed that younger menopausal age was associated with a higher burden of AD pathology among women who underwent surgical menopause, and not among women with natural menopause.[14] Therefore, we examined if age at surgical menopause could explain any difference between women and men in AD neuropathology.

About a third of women ($n=306$, 32%) reported having been exposed to surgical menopause, and menopause was induced by hysterectomy in 95% of cases. Compared to women with natural menopause, women with surgical menopause were younger at menopause (43.4 ± 7.7 vs. 48.8 ± 5.5 , $p < 0.001$), as expected, but were not different at age of death (89.6 ± 6.3 vs. 90.0 ± 6.7 , $p = 0.350$) or years of education (15.7 ± 3.4 vs. 15.9 ± 3.3 , $p = 0.458$). HRT was used by nearly 40% ($n=397$) of women in their life, was more frequently used by women with surgical than natural menopause (59% vs. 33%, $p < 0.001$), and was in the oral dosing formulation in 93% of women.

In regressions controlling for age and education, both women with natural and surgical menopause had a higher AD pathologic score compared to men (Table 3). Because women with surgical menopause were younger at menopause and more susceptible to cognitive decline [14], we repeated the regression by separating women with surgical menopause into two groups according to the median of age at menopause in the group. All three women groups, women with natural menopause and women with surgical menopause at younger or older menopausal age, had a higher AD pathologic score compared to men (Table 3). Next, we examined individual AD markers. For tau tangles density, we found a similar result to the overall AD score: both women with the natural or surgical menopause had more tau tangles density compared to men (Table 3). For A β load, although women had only a borderline higher A β after controlling for age and education, we found that women with surgical menopause had a higher A β load compared to men, but there was no difference between

women with the natural menopause and men (Table 3). Further separating women with surgical menopause into younger and older menopausal age groups, we found that only women with surgical menopause who were younger at the age of menopause had more A β compared to men (Table 3).

Finally, we examined if HRT use in the 5-year perimenopausal “window of opportunity” [51] could affect the level of AD pathology among women. In a regression model including age, education, the 4-levels sex variable, and HRT use as the predictors, HRT use in the window of opportunity was not associated with any of the AD pathological indices (Table 3). Like wise, duration of HRT use (10 or more years vs. less) was not related to AD pathology (Table 3).

Sex differences in other neurodegenerative and cerebrovascular pathologies

We examined the associations of sex with other common neuropathologies of dementia (Table 4). Controlling for age and education, women did not differ from men in their odds for other neurodegenerative pathologies including the presence of neocortical Lewy bodies, TDP-43, and hippocampal sclerosis. For cerebrovascular pathologies, women were less likely to have gross infarcts (OR=0.78, 95% CI:0.61–0.98, $p=0.037$), and there was no difference in microinfarcts. Compared to men, women had more severe arteriolosclerosis (OR=1.28, 95% CI:1.04–1.58, $p=0.018$), but we did not find sex difference in atherosclerosis or CAA. In additional analysis, the association of sex with gross infarcts became attenuated and was no longer significant after controlling for vascular risk factors (OR=0.80, 95% CI: 0.63–1.02, $p=0.072$). In contrast, women had more severe arteriolosclerosis compared to men even after controlling for vascular risk factors, vascular diseases (Table 6), and the two cohorts (Supplemental Table e-4). Finally, we examined if the association of sex with arteriolosclerosis was independent of other neuropathologies. We added terms for AD and other common dementia-related brain pathologies to our core models’ predictors, and the results did not change: women had more severe arteriolosclerosis compared to men (Supplemental Table e-8).

DISCUSSION:

Using post mortem data on nearly 1500 women and men participating in a longitudinal, clinical-pathologic community-based study of ageing, we found that women had higher levels of AD pathology and tau tangle density in particular. Moreover, we found no interaction between age and sex indicating women had higher AD pathology and tau density irrespective of age at death. Carriers of *APOE* $\epsilon 4$ had higher levels of AD pathological indices, but no interaction was seen between sex and *APOE* $\epsilon 4$ suggesting that higher AD pathologies in women compared to men did not vary by the *APOE* $\epsilon 4$ status. Furthermore, taking type of menopause (surgical vs. natural) into account did not change our results, and both women with natural and surgical menopause had higher AD pathologies compared to men. In analysis of common brain pathologies in aging, women were more likely to have severe arteriolosclerosis compared to men. Collectively, these results help to elucidate some of the neuropathological footprint of the observed sex difference in Alzheimer’s dementia as reported in the literature. In this study, we did not address interaction of sex with AD and

other pathologies in their association with cognitive impairment and dementia; this topic had been addressed in a previous study from our group [8].

Women comprise two thirds of persons with AD dementia [2]. One reason for a higher prevalence of AD dementia among women is their higher average life expectancy compared to men [61]. By contrast, higher AD dementia incidence in women may also explain higher prevalence. But, epidemiological studies showed inconsistent results with some showing no difference [9,20,32] and some others reported higher AD dementia incidence in women compared to men [48,60]. Meanwhile, few pathological studies have assessed sex differences in the frequency of AD pathologies [17,22,24,25,41,43,53]. Although two small studies found no difference between women and men in AD pathologies [41,53], others have reported more AD pathology among women [17,22,24,25,43]. We extend prior studies in three aspects. First, our study has incorporated the largest number of autopsies from two uniformly designed longitudinal clinical pathologies studies; other studies either have included less than 200 autopsies [41,53], have recruited many autopsies from clinic referrals where samples are more representative of cognitively impaired than community-dwelling subjects [17,22,24], or were derived from differentially designed cohort studies with variable target population and recruitment criteria [25]. Second, we examined the interaction of age and *APOE* ϵ 4 with sex in their associations with AD pathology, and found no interaction that is compatible with independent association of sex with AD pathology. Third, we investigated the association of other common dementia related pathologies with sex, and found that arteriolosclerosis, which is associated with increased odds of AD dementia [4], was more severe in women than in men. Together, these findings suggest that the higher AD dementia prevalence in women may be at least partially related to more AD pathologies and more severe arteriolosclerosis in women brains.

Age is thought to be the greatest risk factor for AD dementia. Indeed, one in nine American people aged 65 years and older had AD dementia compared to one in three people aged 85+ years [2]. Because women live longer than men on average, and in our study women lived 2.5 years more, the competing risk of mortality can be a factor in the observed increased frequency of AD pathologies in women. However, we considered this risk and addressed it by controlling for age of death in all of our analytic models. Also, the interaction model showed that more AD pathology in women was seen in both younger and older elderly participants. Thus, while mortality effect is likely important, it is less likely that the higher AD pathologies in women is due to this effect.

Our finding that women have more tau tangles but borderline more A β compared to men suggests a sex difference may occur in more advanced stages of the AD pathogenic disease process. We previously reported that the effect of A β on cognition was mediated by tau tangles, such that when they are both included in the same model, A β was no longer significant [11,64]. Only when restricting analyses to participants without cognitive impairment did we find associations of A β with cognition with tau tangles in the model [12]. Thus, finding a stronger association between sex and tau tangles suggests that the effect of sex may be later in the pathogenic process. Indeed, some studies have observed higher prevalence of mild cognitive impairment (MCI) in men in contrast to more dementia in women [46]. More work is needed to clarify the effect of sex on AD pathology. In an animal

model of AD, amyloid precursor protein-transgenic mouse, injury to locus ceruleus caused amyloid-related accumulation of phosphorylated tau in female, not male, rats [44]. Although pathogenetic basis of this sex difference was not investigated, difference in testosterone level was assumed to be responsible by affecting molecular pathways of stress induced tauopathy [44]. Other pathways have also been reported to bring female AD animal models vulnerability to have more AD pathology, including brain metal levels [35,36], increased cyclooxygenase-2 activity [38], myeloperoxidase polymorphism [49], level of active gamma-secretase complexes [47] or beta-secretase [55], vulnerability of female hippocampus to stressful events [19], and autophagy regulation [16].

The literature may shed some light on plausible mechanisms by which women have more AD pathology compared to men. Gonadal hormones act as neurotrophic factors in the perinatal period and throughout the life span [40]. Estradiol has been shown to induce dephosphorylation of tau and protects against tau hyperphosphorylation in human neuroblastoma cell lines and rat cortical neurons [1]. Women experience a relatively rapid loss of sex hormones at menopause, and notably after surgical menopause, compared to men who experience gradual decrease in testosterone with advancing age and do not experience severe estrogen loss because of metabolic conversion of testosterone to estrogen. In fact, we and others have shown a faster rate of cognitive decline and a higher dementia risk after bilateral oophorectomy compared to natural menopause [14,50]. So, although higher AD pathologies in women compared to men is in agreement with the neurotrophic properties of gonadal hormones, finding no association between HRT and AD pathologies is not. Additional mechanisms are likely to play a role in sex differences in AD. Apart from hormones, genes are likely to explain more AD pathology in women. Indeed, a recent study showed that ubiquitin-specific peptidase 9 is a candidate gene with highly significant different expressions in women compared to men and is associated with expression and phosphorylation of tau [33]. Also, three genes were identified in a sex-stratified genome-wide association study that were associated with tau (Osteocrin and Claudin-16) and A β (Serpine Family B Member 1) only in women's brain [18].

Like ours, a recent study, composed of 8 data sets of clinical-pathologic studies, including our studies reported here, reported no interaction between sex and *APOE* $\epsilon 4$ on AD pathology [25]. Although two other studies reported differential associations between *APOE* $\epsilon 4$ and AD pathological indices in women and men [17,24], they do not contain any formal interaction test between sex and *APOE* $\epsilon 4$. Furthermore, a recent meta-analysis of 58000 participants showed that *APOE* $\epsilon 3/\epsilon 4$ women have the same increased odds of AD dementia as *APOE* $\epsilon 3\epsilon 4$ men have, in contrast to a previous meta-analysis which reported *APOE* $\epsilon 4$ women to carry a higher risk of Alzheimer's dementia compared to *APOE* $\epsilon 4$ men [21]. More advanced statistical methods and larger samples may explain the discrepant results among recent and prior studies. Likewise, two studies that reported different sex-AD pathology association in different ages did not utilize statistical interaction modeling [34,43]. We did not find an interaction between sex and age in their association with the tau tangles and the A β load, but found an interaction with the global AD pathologic score. However, this interaction was attenuated and no longer significant when we took account a finding that the association of age with AD pathology was not linear. Therefore, women had more AD pathologies compared to men independent of their age and *APOE* $\epsilon 4$ status.

Mixed dementia, not “pure” AD, is the most common cause of dementia in the community [31,56]. We, therefore, extended our study to examine the associations of sex with 8 other common neurodegenerative and vascular causes of dementia. We found significant associations with gross infarcts and arteriolosclerosis. We found women’s brain to have more severe arteriolosclerosis, which may at least partially explain the higher prevalence of AD dementia in women given the association of arteriolosclerosis with AD dementia [4]. However, while vascular risk factors like higher blood pressure levels have been found associated with cerebral vessel disease including arteriolosclerosis [28,3], when we controlled for vascular risk factors and diseases the association of sex with arteriolosclerosis did not change. Further work is needed to elucidate the association of sex with brain small vessel diseases. One avenue of work will be in the field of genetic factors. For instance, the *ABCC9* gene, ATP-binding cassette sub-family C member 9, which is associated with cerebral arteriolosclerosis [28] is also among the gene regulatory networks of heart rhythm that are sex-dependent [27], and can be a candidate for explaining the sex-arteriolosclerosis association. In contrast to arteriolosclerosis, women had less gross infarcts compared to men. This may possibly be due to the lower frequency of vascular risk factors among women given that the association was attenuated and no longer significant after controlling for vascular risk factors.

We found that both women with natural and those with surgical menopause have higher levels of AD pathologies compared to men. This suggests that type of menopause can not account for the sex difference in AD pathology. In our previous study, we found that younger age at onset of menopause among women with surgical, not natural, menopause was associated with more AD pathologies [14]. In this study, we found that surgically menopausal women have higher AD pathologies compared to men irrespective of age of menopause. In separate analysis, we found that HRT use was not associated with AD pathology. Taken together, menopausal factors did not account for the higher AD pathology, and tau specifically, among women. While the difference between women and men in A β load was borderline significant, women with surgical menopause at younger ages had more A β compared to men. Few if any of the previous studies which investigated the association of sex with AD pathologies have examined menopausal factors [25,41,43,53]. Yet, clinical longitudinal studies have shown susceptibility of women with surgical menopause at young ages to cognitive decline [14] and dementia [52]. In animal experiments, bilaterally ovariectomized rats developed A β 1–42 in the hippocampus after exposure to stress, a finding which corroborate with our finding of more A β in women with surgical menopause at young ages [59,66]. Further work is needed to replicate our finding in different settings and using other methods (such as biomarker studies) and unravel the underlying mechanisms.

Our study has several strengths. It included about 1500 brain autopsies of participants in one of two community-based longitudinal studies of ageing, and with a large proportion of women allowing for comparison of women and men. Further, our findings are more generalizable than studies from memory or other clinics or brain banks of confirmed AD dementia subjects. Also, the cohort studies have more than 90% follow up and about 85% autopsy rates, decreasing several sources of bias, including selection bias due to attrition.

Finally, the two studies have uniform and structured procedures, and neuropathological evaluations are conducted blinded to clinical data.

Nonetheless, the study has several notable limitations. Participants had higher levels of education and more than 95% were Caucasians, reducing generalizability to other populations. Our tau tangle density measure describes only neuronal, not astrocytic, tau tangle burden, and currently we do not have data on aging-related tau astriogliopathy (ARTAG). Examining association of sex with ARTAG would be an interesting topic for future studies. Also, we do not know how much of our tangle finding was driven by the primary age-related tauopathy (PART), or what subtypes of AD, i.e. tangle-predominant form of AD, account for most of the variance in the association of sex with the AD pathologic indices. Furthermore, we only quantified the helical filament tau-positive tangles and the fibrillary form of A β by utilizing antibodies against single epitopes. It is possible that other epitopes or soluble forms of tau or A β , potentially associated with cognitive decline, were different between women and men. Lastly, the autopsy procedures were carried out on only one hemisphere, and pathologies restricted to the other hemisphere may be missed.

In conclusion, we found women to have more AD pathology, tau tangles in particular, and arteriolosclerosis compared to men that helps elucidate the neuropathologic footprint of sex difference in AD dementia. Investigating pathophysiological mechanisms of these differences may help in finding modifiable targets for prevention or treatment of AD dementia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Levels of AD pathological indices among women and men, controlled for age at death and education.

Levels of the amyloid and tangles pathologies were measured by using immunohistochemistry with the monoclonal antibodies against phosphorylated tau (AT8, Innogenetics, Alpharetta GA, 1:1000) and amyloid- β (MO0972 DAKO, 1:100). The global AD pathology is a composite measure derived from the 3 AD pathologic counts in the Bielschowsky silver stain sections: neuritic plaques, diffuse plaques, and neurofibrillary tangles.

Table 1:

Demographic, clinical, and neuropathological characteristics of participants.

Characteristic	Women (n=971)	Men (n=482)	P-value, Difference of women - men (95% CI)	All (n=1453)
Demographic				
Age at death, mean years (range)	89.8 (65.9 – 108.3)	87.3 (66.0 – 108.3)	<0.001, 2.5 (1.8 – 3.3)	88.6 (65.9 – 108.3)
Education, mean years (range)	15.8 (3.0 – 30.0)	17.2 (3.0 – 30.0)	<0.001, –1.4 (–1.0 – –1.7)	16.3 (3.0 – 30.0)
Clinical				
MMSE score, mean (range)	20.3 (0.0 – 30.0)	21.7 (0.0 – 30.0)	0.008, –1.4 (–0.4 – –2.4)	20.8 (0.0 – 30.0)
AD dementia, n (%)	424 (45%)	189 (40%)	0.099, 5% (–1% – 10%)	613 (43%)
<i>APOE ε4</i> , n(%) ^a	246 (26%)	125 (27%)	0.844, –1% (–5% – 4%)	371 (26%)
Number of vascular risk factors, mean (range) ^b	1.0 (0.0 – 3.0)	1.2 (0.0 – 3.0)	< 0.001	1.1 (0.0 – 3.0)
Number of vascular diseases, mean (range) ^b	0.7 (0.0 – 3.0)	0.7 (0.0 – 3.0)	0.873	0.7 (0.0 – 3.0)
Hypertension, n (%)	683 (70%)	267 (56%)	< 0.001, 15% (10% – 20%)	950 (65%)
Hypertension duration, average proportion of positive visits (range) ^b	0.6(0.1 – 1.0)	0.5(0.0 – 1.0)	< 0.001	0.6(0.0 – 1.0)
Diabetes, n (%)	164 (17%)	124 (26%)	< 0.001, –9% (–4% – –13%)	288 (20%)
Diabetes duration, average proportion of positive visits (range) ^b	0.1 (0.0 – 1.0)	0.2 (0.0 – 1.0)	< 0.001	0.2 (0.0 – 1.0)
Smoking, n (%)			< 0.001	
Never	752 (78%)	255 (53%)		1007 (70%)
Former	202 (21%)	201 (42%)		403 (28%)
Current	12 (1%)	22 (5%)		34 (2%)

^aWith 1 or more allele.^bWilcoxon two-samples test is used for the comparison.

Table 2:

APOE ε4 and age at death in the association of sex with Alzheimer's disease pathologies.

	Global AD Pathology		Tau Tangles		Amyloid-β	
	Estimate (S.E.)	p-Value	Estimate (S.E.)	p-Value	Estimate (S.E.)	p-Value
<i>APOE ε4</i> effect ^a						
Women	0.112 (0.024)	<0.001	0.304 (0.083)	<0.001	0.122 (0.072)	0.092
<i>APOE ε4</i>	0.294 (0.027)	<0.001	0.839 (0.092)	<0.001	0.786 (0.080)	<0.001
<i>APOE ε4</i> * Women	−0.051 (0.046)	0.275	0.059 (0.157)	0.707	−0.009 (0.137)	0.949
Age effect ^b						
Women	0.096 (0.022)	<0.001	0.326 (0.074)	<0.001	0.111 (0.065)	0.089
Age	0.006 (0.002)	<0.001	0.022 (0.006)	<0.001	0.027 (0.006)	<0.001
Age * Women	−0.008 (0.003)	0.023	−0.009 (0.011)	0.393	−0.016 (0.010)	0.090

^a Estimates are derived from regressions controlling for age and education.

^b Estimates are derived from linear regressions controlling for education.

Table 3.

Categorical age at death in the association of sex with the global Alzheimer's disease pathologic score.

	Global AD Pathology	
	Estimate (S.E.) ^a	p-Value ^a
Age ≥ 90	0.190 (0.047)	< 0.001
80 ≤ Age < 90	0.150 (0.048)	0.002
Education	0.000 (0.003)	0.968
Women	0.225 (0.066)	< 0.001
(Age ≥ 90) × Women	−0.124 (0.074)	0.094
(80 ≤ Age < 90) × Women	0.135 (0.073)	0.065

^aEstimates are derived from linear regressions.

Table 4.

Effect of type and age of menopause and hormone replacement therapy on the association of sex with AD pathological indices.

	Global AD Pathology		Tau Tangles		Amyloid- β	
	Estimate (S.E.)	p-Value	Estimate (S.E.)	p-Value	Estimate (S.E.)	p-Value
Women groups (vs. men)						
Two groups^a						
Natural menopause	0.088 (0.024)	<0.001	0.296 (0.079)	<0.001	0.069 (0.069)	0.32
Surgical menopause	0.121 (0.029)	<0.001	0.371 (0.095)	<0.001	0.205 (0.083)	0.014
Three groups^a						
Natural Menopause	0.088 (0.024)	<0.001	0.295 (0.078)	<0.001	0.069 (0.069)	0.32
Younger at surgical menopause	0.158 (0.036)	<0.001	0.511 (0.118)	<0.001	0.247 (0.105)	0.019
Older at surgical menopause	0.082 (0.036)	0.025	0.222 (0.121)	0.066	0.161 (0.106)	0.130
Hormone Replacement therapy (HRT)^b						
HRT use within 5 years postmenopausal	-0.007 (0.064)	0.909	0.016 (0.211)	0.941	-0.094 (0.187)	0.614
HRT use duration for 10 years or more	0.007 (0.034)	0.843	-0.126 (0.112)	0.260	0.010 (0.099)	0.917

Women with surgical menopause are categorized to younger or older at surgical menopause if their ages at menopause were less or more than the median of the menopausal ages, respectively.

^aEstimates are derived from linear regressions controlling for age at death and education.

^bEstimates are derived from linear regressions controlling for age at death, education, and a term for a 4-levels categorical variable: women with natural menopause, women with surgical menopause at younger, and at older age of menopause, and men (reference).

Tables 5.

Association of non-AD dementia-related common brain pathologies with sex.

Pathology	Women, n (%)	Men, n (%)	OR (95% CI), P-value
Neocortical Lewy bodies, n (%)	116 (12%)	66 (14%)	0.84(0.60 – 1.18), 0.309
TDP-43, n (%)			1.13(0.89 – 1.43), 0.313
Stage 0	391 (46%)	200 (52%)	
Stage 1	164 (19%)	72 (19%)	
Stage 2	177 (21%)	65 (17%)	
Stage 3	120 (14%)	44 (12%)	
Hippocampal sclerosis, n (%)	96 (10%)	33 (7%)	1.21 (0.79 – 1.85), 0.374
Gross infarct(s)	338 (35%)	177 (37%)	0.78 (0.61 – 0.98), 0.037
Microinfarct(s)	290 (30%)	146 (30%)	0.88 (0.69 – 1.13), 0.312
Atherosclerosis			0.88 (0.71 – 1.08), 0.218
None	178 (18%)	83 (17%)	
Mild	455 (47%)	229 (48%)	
Moderate	265 (27%)	138 (29%)	
Severe	66 (7%)	31 (6%)	
Arteriolosclerosis			1.28 (1.04 – 1.58), 0.018
None	275 (29%)	186 (39%)	
Mild	339 (35%)	150 (31%)	
Moderate	271 (28%)	99 (21%)	
Severe	79 (8%)	42 (9%)	
Cerebral Amyloid Angiopathy			1.03 (0.84 – 1.27), 0.755
None	199 (21%)	107 (23%)	
Mild	402 (42%)	198 (42%)	
Moderate	211 (22%)	109 (23%)	
Severe	134 (14%)	52 (11%)	

Estimates [Odds ratio (95% confidence interval)] and the p values are derived from logistic regression models controlled for age at death and education.

Tables 6.

Association of sex with arteriolosclerosis and gross infarcts in the presence of vascular risk factors and their duration, and vascular diseases.

Adjustment by	Arteriolosclerosis		Gross infarcts	
	Odds Ratio (95% CI) ^a	p-Value ^a	Odds Ratio (95% CI) ^a	p-Value ^a
Women (no adjustment)	1.28 (1.04 – 1.58)	0.018	0.78 (0.61 – 0.98)	0.037
Women (by hypertension)	1.31 (1.06 – 1.62)	0.011	0.75 (0.59 – 0.95)	0.018
Women (by diabetes)	1.29 (1.05 – 1.58)	0.017	0.79 (0.62 – 1.01)	0.056
Women (by sum of vascular risk factors)	1.28 (1.04 – 1.57)	0.021	0.80 (0.63 – 1.02)	0.072
Women (by sum of vascular diseases)	1.28 (1.04 – 1.58)	0.018	0.76 (0.60 – 0.97)	0.027
Women (by hypertension duration)	1.30 (1.06 – 1.60)	0.013	0.75 (0.59 – 0.95)	0.017
Women (by diabetes duration)	1.29 (1.05 – 1.59)	0.015	0.80 (0.63 – 1.01)	0.064

^a Estimates are derived from logistic regressions controlling for age at death and education.