Menopause Status Moderates Sex Differences in Tau Burden: A Framingham PET Study

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Objective: Women have a higher lifetime risk of Alzheimer's disease (AD) than men. Among cognitively normal (CN) older adults, women exhibit elevated tau positron emission tomography (PET) signal compared with men. We explored whether menopause exacerbates sex differences in tau deposition in middle-aged adults.
Methods: 328 CN participants from the Framingham Study (mean age = 57 years (±10 years), 161 women, of whom, 104 were post-menopausal) underwent tau and β-amyloid (Aβ)-PET neuroimaging. We examined global Aβ-PET, and tau-PET signal in 5 regions identified *a priori* as demonstrating significant sex differences in older adults (in temporal, inferior parietal, middle frontal, and lateral occipital regions). We examined sex and menopause status-related differences in each region-of-interest, using linear regressions, as well as interactions with Aβ and APOEε4 genotype.
Results: Women exhibited higher tau-PET signal ($p < 0.002$), and global Aβ-PET ($p = 0.010$), than men in inferior parietal, rostral middle frontal, and lateral occipital regions. Compared with age-matched men, post-menopausal women showed significantly higher tau-PET signal in parieto-occipital regions ($p < 0.0001$). By contrast, no differences in tau-PET signal existed between pre-menopausal women and men. Aβ-PET was not associated with menopausal status or age. Neither Aβ-PET nor APOEε4 status moderated sex or menopause associations with tau-PET.
Interpretation: Clear divergence in tauopathy between the sexes are apparent approximately 20 years earlier than previously reported. Menopause status moderated sex differences in Aβ and tau-PET burden, with tau first appearing post-menopause. Sex and menopause differences consistently appeared in middle frontal and parieto-occipital regions but were not moderated by Aβ burden or APOEε4, suggesting that menopause-related tau vulnerability may be independent of AD-related pathways.

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Women demonstrate an elevated risk for progression to dementia compared to men,[1,2] particularly among those at greater genetic risk of sporadic Alzheimer's disease (AD) dementia.[3–5] Women also have a higher risk of elevated AD-related pathology,[6,7] specifically tau,[1,8–12] and faster rates of brain atrophy compared to men.[13–15] *In vivo* studies of clinically normal older women have consistently shown higher tau positron emission tomography (PET) signal relative to men,[8,11,12,16] and elevated levels of cerebrospinal fluid (CSF) total tau in apolipoprotein ε4 (*APOE*ε4) carriers relative to men.[1,9,10,17] Specifically, regions of the rostral middle frontal, inferior parietal, temporal, and lateral occipital cortices have been implicated as sites of sex-differentiated tau vulnerability.[11,12,16] In those with abnormal levels of Aβ, however, women also exhibit higher tau-PET signal in regions of the medial temporal lobe.[8,16] As such, it is clear that both Aβ-relevant and Aβ-independent sex differences exist in tau deposition in older adults.

Hormonal changes during menopause have been proposed as a rationale for the appearance of sex differences in AD pathology and clinical progression.[18,19] A reduction in circulating endogenous 17β-estradiol is associated with a proliferation of AD pathology in animal models.[20] In humans, the later stages of menopause have been associated with increased levels of Aβ, loss of white matter integrity and glucose hypometabolism.[12,21,22] Further illustrating the potential importance of this hormonal transitional period, surgically induced menopause at an earlier age has been associated with cognitive decline and increased AD neuropathology at post-mortem.[23,24] A recent observational study also reported that prior history of hormone therapy was protective against abnormal levels of tau-PET in women.[12] Taken together, these findings support a nexus of AD-related pathological risk associated with the menopausal transition. Given the controversial history of the protective effects of hormone therapy in relation to risk of progression to dementia in clinical trials[25,26] the association between menopause and *in vivo* AD biomarkers has been understudied to date, particularly in relation to tau-PET. Elucidating these associations will be critical for precision medicine approaches aimed at identifying sex dimorphic responses to treatment,[19,20] particularly with regard to tau-targeting interventions.

Our objective was to examine sex and menopause associations with PET markers of global Aβ and regional tau in a group of participants spanning 32 to 88 years (mean of 57 years) from the Framingham Study. We investigated whether sex differences in Aβ and tau-PET were evident in a largely middle-aged cohort, and the extent to which menopause status moderated these sex differences. We hypothesized that middle-aged women would exhibit higher tau deposition than men in *a priori* regions of interest. Furthermore, we hypothesized that menopausal status and age-at-menopause would serve as moderators of this association. Finally, we explored the influence of Aβ and *APOE*ε4 status to moderate associations between tau-PET signal and either sex or menopause status.

Methods

Participants. A total of 328 cognitively normal adults (age = 57(SD 10), range = 32 to 88, women = 161(49%), post-menopausal women = 104 (65% of all women)) from the Framingham Study 2nd (Gen 2) and 3rd (Gen 3) generation cohorts underwent a ^{18}F-Flortaucipir (FTP)-PET and/or ^{11}C-Pittsburgh Compound-B (PiB)-PET scan (see https://github.com/rfbuckley/FHS_menopause for participant flow chart). Sex was categorized on the basis of self-report. In this cross-sectional study, 94% of Aβ-PET and tau-PET scans occurred on the same day, and all occurred within 6 months of each other. We conducted the PET neuroimaging procedures for this study under the ethical guidelines stipulated by the Massachusetts General Brigham Human Research Committee, and written consent was obtained in each cohort.

Menopause information. Menopause included those who underwent natural or surgically induced menopause. Women who answered the following question "What is the best way to describe your periods?" with 'Not stopped', 'Periods stopped due to pregnancy, breast feeding, or hormonal contraceptive', 'Periods stopped due to low body weight, heavy exercise, or due to medication or health condition such as thyroid disease, pituitary tumor, hormone imbalance, stress', 'Periods stopped for less than 1 year', or 'Periods stopped, but now have periods induced by hormones' were categorized as pre-menopausal. Those who answered the question with "Periods stopped for 1 year or more" (in Gen 2 and Gen 3 cohorts) or "definitely menopausal" (Gen 2 cohort) were categorized as post-menopausal. Age-at-menopause was collected with the item, "Age when periods stopped"; for the purposes of the current study, this variable was dichotomized at 50 years of age and treated as younger age vs. older age at menopause. Data on menopausal status and age-at-menopause were collected a median of 1 year (interquartile range: 0.5–2.6) from the most recent PET scan.

Magnetic Resonance Imaging. Structural T1-weighted anatomical images were acquired using a Philips 3T Achieva [repetition time (TR), 6,800 ms; echo time (TE), 3.1 ms; angle, 9°; voxels, 0.98 × 0.98 × 1.2 mm]. Images were processed with FreeSurfer version 6.0 to identify gray-white as well as pial surfaces and produce automatic Desikan-Killany cortical and subcortical region of interest (ROI)

parcellations, with quality control measures published previously.[27]

PiB Positron Emission Tomography. The PiB-PET acquisition parameters have been published previously.[28,29] In brief, distribution volume ratios (DVRs) were computed using Logan plotting techniques 40–60 minutes post injection. PET images were co-registered to the corresponding T1 image (SPM12), and FreeSurfer-derived ROIs were sampled. A global Aβ-PET composite was computed from a weighted average within a large aggregate cortical ROI consisting of precuneus, rostral anterior cingulate, medial orbitofrontal, superior frontal, rostral middle frontal, inferior parietal, inferior temporal, and middle temporal (termed FLR) regions. This FLR composite was referenced to cerebellar gray, and log-transformed for normality. When Aβ-PET was examined as a predictor, we divided the distribution into quintiles to address any non-linear associations with tau-PET (see Model 5). PiB and FTP scans were acquired from 2 cameras: the 5-ring GE Discovery MI[30] (n = 109) and the Siemens ECAT HR+ (n = 219). To harmonize data across these cameras, GE Discovery images were smoothed with a 6 mm Gaussian filter. Data were not partial volume corrected.

FTP Positron Emission Tomography. FTP-PET, formerly AV1451 or T807, acquisition parameters have been published.[28] Standard uptake volume ratios (SUVrs) were calculated from images acquired 80–100 mins post-injection and referenced to cerebellar gray.[29] Five tau ROIs were examined as regions implicated in preclinical AD (entorhinal and inferior temporal),[29] and those that demonstrate large sex differences in CN older adults (entorhinal, rostral middle frontal, inferior parietal, and lateral occipital cortices).[8,11,12,16] Primary models involved data that were not partial volume corrected; however, partial volume corrected results are also reported. Partial volume correction was conducted using Geometric Transfer Matrix (GTM) method.[31]

Statistical analyses. Analyses were run in SAS version 9.4. We first examined demographic differences between the sexes and by menopausal status using t-tests and chi-squared tests of independence (Fisher's exact *p*-value reported if cell $n < 5$) for continuous and categorical variables, respectively. Our primary aims were investigated using a series of linear regression models that adjusted for age at PET and type of PET camera. First, the main effect of sex was examined in association with Aβ and tau-PET

TABLE 1. Demographic Comparisons Between the Sexes

	Sex		
	Women	Men	*p*
	N = 161	N = 167	
Age at PET, mean (SD)	57 (10)	58 (10)	0.45
Education, n (%)			0.30
≤HS degree	12 (7%)	21 (12%)	
Some college	45 (28%)	43 (26%)	
≥College degree	104 (65%)	103 (62%)	
APOEε4 positive, n (%)	36 (23%)	38 (24%)	0.86
Discovery GE camera n (%)	58 (36%)	51 (31%)	
White n (%)	161 (100%)	167 (100%)	

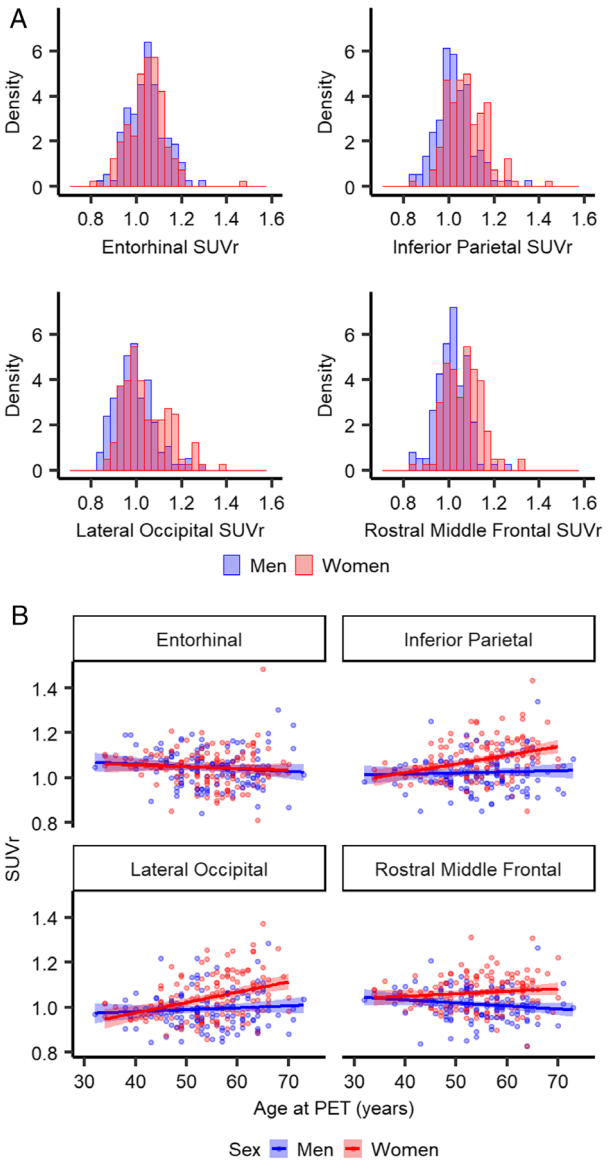

FIGURE 1: Multi-panel histogram/scatterplots of entorhinal, rostral middle frontal, inferior parietal and lateral occipital (unadjusted, raw scores) by (A) sex and (B) sex across the age span.

TABLE 2. Association Between Sex and Global Aβ-PET and Regional Tau-PET Signal (SDUs)

	Model 1		Model 2		Model 3	
	Sex (reference = men)		Menopause Status Among Women (reference = pre-menopause)		Menopause Age (reference = <50 years)	
	β (SE)	p	β (SE)	p	β (SE)	p
Aβ-PET DVR	**N = 323**		**N = 160**		**N = 100**	
Global FLR[a]	**0.274 (0.101)**	**0.010**[a]	−0.176 (0.235)	0.454	−0.082 (0.234)	0.728
Tau-PET SUVr	N = 259		N = 134		N = 84	
Entorhinal	−0.001 (0.127)	0.995	−0.244 (0.268)	0.365	−0.106 (0.244)	0.665
Inf temporal	−0.024 (0.120)	0.839	−0.174 (0.242)	0.473	−0.139 (0.210)	0.511
Inf parietal	**0.452 (0.103)**	**<0.0001**[b]	0.256 (0.218)	0.242	−0.271 (0.198)	0.175
Rostral mid frontal	**0.644 (0.120)**	**<0.0001**[b]	0.193 (0.261)	0.261	**−0.486 (0.238)**	**0.045**
Lat occipital	**0.292 (0.093)**	**0.002**[b]	0.327 (0.200)	0.103	**−0.407 (0.184)**	**0.030**

Note: Each row denotes a different linear regression model adjusted for age at PET scan and camera.
[a]Log transformed for normality; $p < 0.05$ is bolded.
[b]Indicates FDR-corrected $p < 0.05$.
FLR = frontal, lateral temporoparietal and retrosplenial regions; Inf = inferior; Lat = lateral; Mid = middle.

TABLE 3. Demographic Comparisons Between Menopause Groups Among Women

	Menopause group		
	Pre	Post	p
	N = 57	N = 104	
Age at PET, mean (SD)	48 (6)	62 (7)	<0.0001
[min, max]	[34–63]	[42–81]	
Age at menopause	–	49 (5)	–
Education, n (%)			0.08
≤HS degree	4 (7%)	8 (8%)	
Some college	10 (18%)	35 (33%)	
≥College degree	43 (75%)	61 (59%)	
APOEε4 positive, n (%)	12 (22%)	24 (24%)	0.81
Discovery GE camera n (%)	16 (28%)	42 (40%)	

signal. In women only, we then tested the association of menopausal status (pre-menopause versus post-menopause) with both Aβ and tau-PET signal. Furthermore, in post-menopausal women only, we explored the association of age-at-menopause with PET signal. Chronological age is an inherent confound of menopause status as well as one of the strongest risk factors for abnormal AD biomarkers. As such, it is an important, and yet non-trivial, task to try to extricate the menopause status effect from the age effect. Unfortunately, due to the almost universal age at menopause (50 years of age[32]), we found only minimal overlap in the current age of women considered to be pre-menopausal and post-menopausal. Due to this issue, we decided to instead use age-matched males as a control comparison against the pre-menopausal and post-menopausal women. We paired pre- and post-menopausal women with age- and camera-matched men, using a 1-year caliper matching scheme for age. This pairing, which we refer to as 'menopausal status-matched' groups, resulted in 32 men matched to pre-menopausal women and 69 men matched to post-menopausal women. This analysis was intended to provide a different control to account for pre-menopausal women being significantly younger than post-menopausal women. As an additional analysis to explore the potential age confound, we replaced the menopausal status-matched group with a group that split by age above and below 50 years (the average age-at-menopause in the population). Finally, in exploratory analyses, we investigated the moderating impact of Aβ and *APOEε4* status on the association between sex or menopause status on PET signal. In all analyses, global Aβ-PET and the 5 tau-PET regions were examined as dependent variables in separate models. Model

TABLE 4. Demographic Comparisons Between Matched Groups

	Matched group					
	Pre-menopausal Cases (women) N = 32	Pre-menopausal Controls (men) N = 32	*p*	Post-menopausal Cases (women) N = 69	Post-menopausal Controls (men) N = 69	*p*
Age at PET, mean (SD)	47 (4)	47 (5)	1.00	58 (5)	58 (5)	0.98
Education, n (%)			0.27			0.01
≤HS degree	4 (13%)	1 (3%)		4 (6%)	13 (19%)	
Some college	8 (25%)	6 (19%)		29 (42%)	16 (23%)	
≥College degree	20 (62%)	25 (78%)		36 (52%)	40 (58%)	
APOEε4 positive, n (%)	4 (13%)	11 (37%)	0.03	19 (28%)	15 (22%)	0.46

TABLE 5. Interaction Between Sex and Matched-Menopause Status on Tau-PET Signal Followed by Stratification by Matched Menopause Status (SDUs)

	Model 4		
Outcome Variable Strata	Interaction Between Sex and Matched-Menopause	Association Between Sex (women are referent) and tau-PET in Strata[a]	
	p	*β* (SE)	*p*
Tau-PET SUVr	N = 202		
Entorhinal	0.945		
Pre-meno (N = 61)		N/A	
Post-meno (N = 134)		N/A	
Inferior temporal	0.600		
Pre-meno (N = 61)		N/A	
Post-meno (N = 135)		N/A	
Inferior parietal	0.050		
Pre-meno (N = 61)		−0.047 (0.198)	0.814
Post-meno (N = 135)		**−0.588 (0.162)**	**0.0004**[b]
Rostral middle frontal	0.058		
Pre-meno (N = 61)		−0.330 (0.234)	0.163
Post-meno (N = 135)		**−0.829 (0.170)**	**<0.0001**[b]
Lateral occipital	**0.038**[b]		
Pre-meno (N = 61)		0.078 (0.201)	0.699
Post-meno (N = 135)		**−0.499 (0.165)**	**0.003**[b]

Note: Each section contains a different interaction model, and subsequent stratification models if the interaction was significant; N/A = Not applicable, stratification only applicable when interaction *p*-value <0.10.

[a]Strata: PET ~ sex + education + *APOE*ε4 status, with men matched to pre-menopause ($n = 32$ men matched to $n = 32$ women)/post-menopause ($n = 69$ men matched to 69 women, but missing *APOE*ε4 status reduced the group size); $p < 0.05$ is bolded.

[b]Indicates FDR-corrected $p < 0.05$.

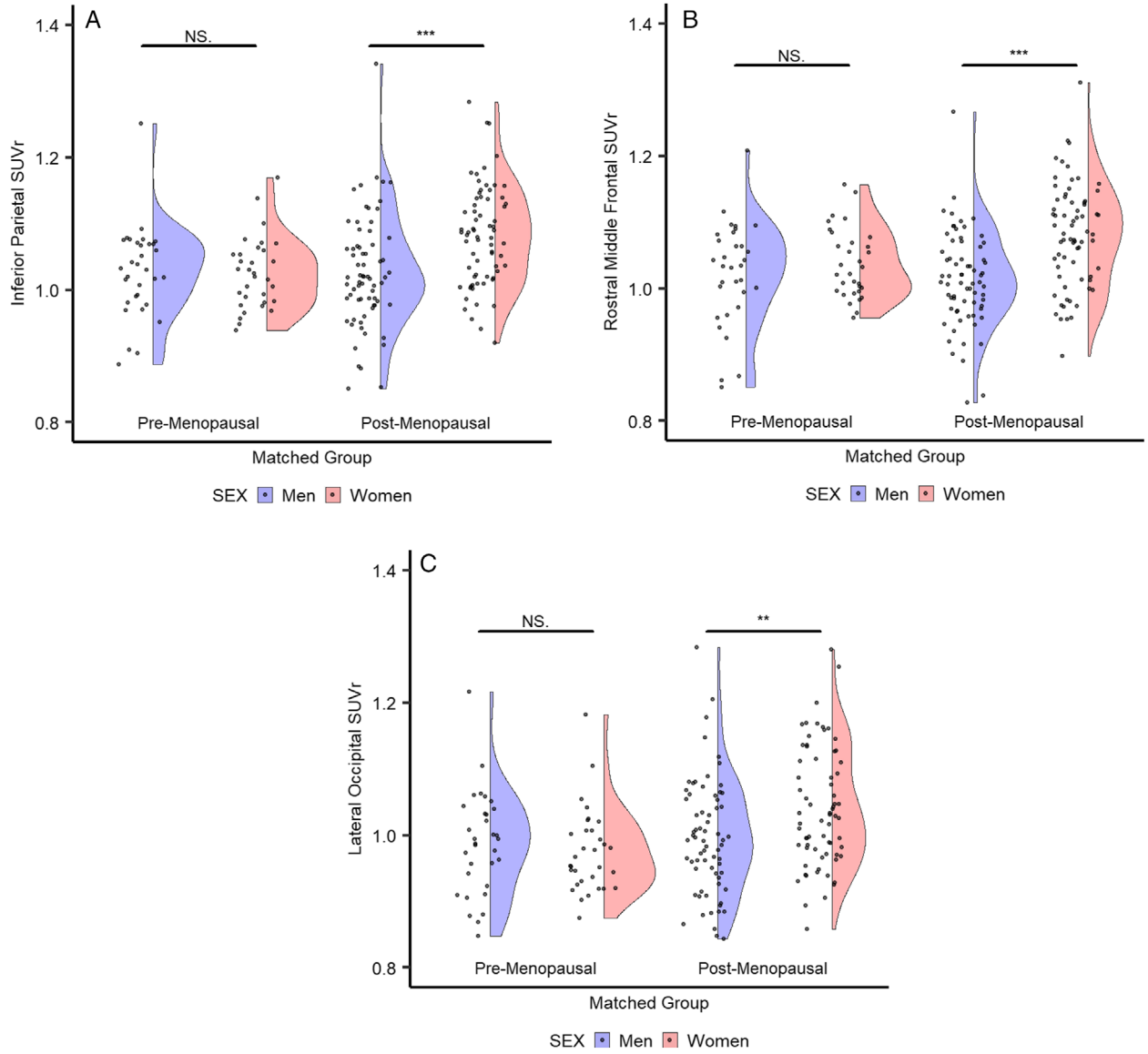

FIGURE 2: Interaction plot showing the moderating effect of menopause on the relationship between sex and FTP-PET signal in (A) the inferior parietal, (B) rostral middle frontal and (C) lateral occipital regions (* indicates p<0.001).**

estimates in tables are reported as standard deviation units (SDUs), although sex differences are reported in-text using unstandardized PET DVR or SUVr units. We set overall $\alpha = 0.05$ and adjusted for multiple comparisons using false discovery rate (FDR) correction (results $p < 0.05$ reported for interest). In each table, we report raw p-values and indicate FDR-corrected p-values below 0.05. We report raw p-values in text. We conducted the FDR correction separately for all analyses in each table, such that p-values are corrected for the number of analyses presented together. Analyses are listed below for clarity:

Model 1: PET ~ Sex (women vs. men) + current age + camera.

Model 2: PET ~ Menopause status (post-menopausal women vs. pre-menopausal women) +- current age + camera.

Model 3: PET ~ (Age-at-Menopause<50 vs. Age-at-Menopause≥50) + current age + camera (post-menopausal women only).

Model 4: PET ~ Sex * menopausal status-matched group + current age + camera.

Model 4A: PET ~ Sex * </> 50 years-matched group + current age + camera.

Model 5: PET ~ Risk modifier (Aβ OR *APOE*ε4)* Group (sex OR menopause status) + current age + camera.

Results

Sex differences in PET signal. No demographic differences were found by sex (see Table 1). Women exhibited higher tau-PET signal in the inferior parietal, rostral middle frontal

TABLE 6. Interaction Between Sex and Matched-Age Group on PET Signal Followed by Stratification by Matched-Age Group Status (SDUs)

	Model 4A		
Outcome variable / **Strata**	**Interaction Between Sex and Matched Age Group**	**Association Between Sex (women are referent) and FTP in Strata***	
	p	***β* (SE)**	***p***
FTP-PET SUVr		N = 202	
Entorhinal	0.961		
Age < 50 Group (N = 54)		N/A	
Age > 50 Group (N = 148)		N/A	
Inferior temporal	0.454		
Age < 50 Group (N = 54)		N/A	
Age > 50 Group (N = 148)		N/A	
Inferior parietal	**0.029**		
Age < 50 Group (N = 54)		0.012 (0.186)	0.950
Age > 50 Group (N = 148)		**−0.567 (0.143)**	**0.0001**
Rostral middle frontal	**0.001**		
Age < 50 Group (N = 54)		0.046 (0.211)	0.829
Age > 50 Group (N = 148)		**−0.889 (0.154)**	**<0.0001**
Lateral occipital	**0.032**		
Age < 50 Group (N = 54)		0.154 (0.198)	0.441
Age > 50 Group (N = 148)		**−0.430 (0.147)**	**0.004**

Note: Each section contains a different interaction model, and subsequent stratification models if the interaction was significant; N/A = Not applicable, stratification only applicable when interaction *p*-value < 0.10. Strata: PET ~ age <50 in matched pre-menopause/post-menopause; $p < 0.05$ is bolded.
*Indicates an interactive term (mathematical symbol).

and lateral occipital regions than men (β = ~0.3–0.6, $p < 0.002$; see Fig 1 and Table 2). Women also demonstrated higher global Aβ-PET signal (β = 0.3, $p = 0.010$). Using PVC data, we found the pattern of effects to remain largely the same, except in the lateral occipital region where the difference became attenuated ($p = 0.90$).

Menopausal status, age-at-menopause and PET signal. Post-menopausal women were older than pre-menopausal women (see Table 3). No relationship was found between current age at PET scan and age-at-menopause (rho = 0.10, $p = 0.34$). We found no main effect of menopause status on global Aβ-PET signal or regional tau-PET signal in women (see Table 2). Constricting the age range to those aged between 50–60 years of age in both groups resulted in a similar pattern of effects. In those who reported age-at-menopause below 50 years (n = 40), we found numerically higher tau-PET signal in the rostral middle frontal ($p = 0.05$) and lateral occipital regions ($p = 0.03$) relative to those with later age-at-menopause ($n = 61$), although these results did not survive FDR correction. Examining PVC data did not change the direction of these findings.

Sex-by-menopause-matched group interaction on PET signal. Pre-menopausal women showed slightly lower *APOEε4* carriership to age-matched men, and post-menopausal women showed slightly higher levels of education than age-matched males (see Table 4). As such, we adjusted these models with *APOEε4* status and years of education. Menopause status moderated the association between sex and tau-PET signal in the inferior parietal and lateral occipital regions ($p = 0.01$), as well as the rostral middle frontal region ($p = 0.02$; see Table 5). Using stratification models, post-menopausal women exhibited higher signal than matched men (β = ~ −0.5-0.9,

TABLE 7. Effect Modification of Aβ-PET Quintiles and APOEε4 Status on the Relationship Between Sex or Menopause on Aβ and Tau-PET

Outcome Variable	Model 5 Interactions			
	Sex*Aβ-PET^	Sex* APOEε4	Menopause*PiB[a]	Menopause* APOEε4
	p	*p*	*p*	*p*
Aβ-PET DVR		N = 312		N = 156
Global FLR[b]	–	0.47	–	0.99
Tau-PET SUVr	N = 254	N = 250	N = 133	N = 130
Entorhinal	0.88	0.70	0.37	0.34
Inferior temporal	0.54	0.77	0.57	0.28
Rostral middle frontal	0.21	0.49	0.12	0.44
Inferior parietal	0.17	0.35	0.73	**0.03**
Lateral occipital	0.26	0.74	0.82	0.06

Note: Each row denotes a different model; $p < 0.05$ is bolded.
[a]Aβ-PET is PIB-PET DVR treated as quintiles (with top quintile (Q5) the referent).
[b]Global FLR log-transformed for normality.

$p < 0.001$; see Fig 2AB). No differences in tau-PET signal were found between pre-menopausal women and either matched-group of men. Examining PVC data resulted in an attenuated effect in the lateral occipital region; however, the pattern of findings in the other regions were unaffected. To explore the confound of age, we also found women over 50 years of age at the time of PET scan exhibited higher levels of tau-PET signal in both regions than matched men. Women younger than 50 years at PET scan did not exhibit differences in tau-PET signal relative to either matched group of men (Table 6).

Moderating influences of Aβ-PET and APOEε4. Aβ-PET did not moderate the influence of sex or menopause status on regional tau-PET signal (see Table 7). When examining the interaction between menopause status and *APOEε4* on regional tau-PET signal, we found sub-threshold interactive effects on the inferior parietal ($p = 0.03$) and lateral occipital ($p = 0.06$), which was not significant after FDR correction. No interaction between sex and *APOEε4* was found on Aβ or tau-PET.

Discussion

Our study examined the influence of sex and menopause status on global Aβ and regional tau-PET signal. Relative to age-matched men, post-menopausal women exhibited higher levels of Aβ and regional tau, with differences in tau-PET signal appearing in inferior parietal and lateral occipital regions. By contrast, no difference was found between pre-menopausal women and either age-matched group of men, supporting the notion that the menopause transition is a critical time in which tau deposition appears to diverge between the sexes. Post-menopausal women with early onset (below 50 years) showed numerically elevated levels of tau-PET burden in these regions. Chronological age is strongly associated with PET markers of both Aβ[33] and tau[34,35] in clinically unimpaired older adults, and we also found that women aged over 50 showed higher tau-PET signal in the same regions relative to women aged under 50 and both groups of matched men. These findings signal a critical watershed period of tau vulnerability in women that occurs around the time of menopause. Examining the menopause transition with tau is confounded by age. As such, these findings must also be interpreted within the milieu of other midlife risk profiles that come to the fore during the menopausal transition, such as increased risk for metabolic syndrome[36] and midlife vascular risk exposure.[37] For instance, women are at greater risk of mid-life diabetes, obesity and hypertension, which has considerable impact on vascular-related cognitive dysfunction.[38]

We found sex differences in regional tau deposition in individuals approximately 20 years younger than those included in prior studies reporting sex differences.[11,12,16] Our findings replicate recent work in a diverse sample of later middle-aged individuals who also reported sex differences in cortical tau deposition independent of Aβ burden.[39] We

extend these findings by reporting Aβ-independent sex differences in regional tau-PET deposition in areas not typically found to have early AD-related tau deposition.[7,21,29] We found no sex by *APOE*ε4 or sex by Aβ-PET interactions associated with tau-PET signal in any of our *a priori* regions, supporting the hypothesis that sex differences in these regions may not necessarily be influenced by AD-related processes. An important caveat to note is the smaller samples sizes when considering these interactions. In our previous findings, sex by Aβ-PET effects on tau-PET signal were confined to the temporal lobe,[8] suggesting that perhaps 2 levels of sex dimorphic tau pathways exist: 1 exacerbated by Aβ processes, and the other driven by other pathological pathways. Unlike previous findings, we did not see any sex by Aβ-PET associations with inferior temporal tau.[8,16] A recent cohort of late middle-aged adults found higher levels of middle and inferior temporal tau in women,[39] but also no interaction between sex and Aβ on regional tau, suggesting that sex by Aβ interactions on tau deposition may appear later in life. We found elevated levels of global Aβ-PET signal in women, supporting previous findings amongst middle aged adults.[22] We did not find any menopause status associations with Aβ-PET, sitting in contrast to prior studies[21,22] that found post-menopausal women exhibited higher global Aβ load, lower white matter integrity, regional hypometabolism, and reduced gray matter volume relative to pre-menopausal women. The age range in our study was greater, which may have obscured associations with Aβ-PET, as sex differences in Aβ-PET do not traditionally appear in older adults.

A question remains as to the rationale behind sex differences in neocortical tau-PET signal in middle-aged adults. Braak staging of tau deposition[40] places the appearance of neurofibrillary tangles in these regions at latter stages (Braak stages V-VI), when cognitive impairment is apparent.[41] Tau-PET signal in the lateral occipital region in preclinical AD has become of particular interest to the field. Most commonly, posterior cortical atrophy patients display a characteristic pattern of lateral occipital tau-PET signal.[42] Some studies report an over-representation of women by up to 50% in the PCA patient group,[43,44] although this is not necessarily reflected by estimated prevalence rates.[45] It is possible, however, that there may be elevated sex-specific risk for a "preclinical" PCA pathological profile. Early onset AD dementia cases characterized by prominent visuospatial dysfunction also show significant burden in occipital regions relative to age-matched controls.[46] It is unlikely, however, that sex differences in these regions are highlighting preclinical stages of these rarer forms of AD. In a recent data-reduction of voxel-wise tau-PET data across a range of clinical unimpaired and impaired individuals, a distinctive cluster of lateralized lateral occipital signal was reported in those with high Aβ burden.[47] Other studies of preclinical AD have also shown elevated tau-PET signal in inferior occipital and inferior parietal regions.[35] As such, there is mounting evidence of lateral occipital and inferior parietal tau-PET signal appearing in cases of preclinical AD. The biological relevance of sex differences in tau deposition in these regions, however, remains unclear. It is possible, however, that sex-specific regional tau vulnerability outside the medial temporal lobe is driven by sex hormonal or sex chromosomal factors, or that rapid tau proliferation beyond the medial temporal region is a byproduct of a post-menopausal environment (hormonal, vascular, metabolic, etc).

These findings introduce a wider implication of what it means to be "tau abnormal" for women relative to men. Given the mounting evidence across multiple independent studies of higher tau in women across cortical regions,[6,7,11,12,16,39] even in middle age,[39] this raises the question of how to understand and interpret tau deposition in women relative to men in observational cohorts as well as clinical trials. At a broad level, this has implications for how we understand tauopathies and the extent to which certain tau species propagate in a sex-specific manner. From a pragmatic standpoint, defining individuals with 'high tau' in the A/T/N model[48] may require sex adjustment. Furthermore, tau therapeutic clinical trials may need to consider sex stratifications for their primary endpoints to better estimate treatment response in men and women. From a methodological standpoint, there are implications for how tau-PET composites are created across both sexes; for instance, we would recommend avoiding the rostral middle frontal, parietal, and lateral occipital regions when creating cortical tau composites as these may introduce bias. Future studies will need to directly explore the impact of these implications particularly within the context of longitudinal study designs.

When examining the effect of menopause status or age-at-menopause in women only, we found no significant differences in tau-PET signal although the pattern of effects remained the same. This was particularly true for age-at-menopause in post-menopausal women, where age-at-menopause below 50 years was associated with numerically higher tau-PET signal in rostral middle frontal and lateral occipital regions. The window of opportunity hypothesis suggests that a shorter duration of exposure to endogenous estrogen increases risk for AD pathology.[49] Earlier age at menopause, whether natural or surgically induced, increases the risk of a range of conditions, such as cardiovascular disease, psychiatric illness, osteoporosis, as well as early mortality.[50] Age at premature menopause is typically considered to be between 40 and 45 years,[51] much lower than the median-age cutoff defined in the current study. Thus, it is possible we underestimated the effects of age-at-menopause on tau-PET.

Given the older age-range of our sample, our analyses would have been underpowered to adopt a younger age cutoff.

The strength of this study is the large sample of middle-aged adults with both Aβ and tau-PET imaging and carefully collected menopause status information close to the time to neuroimaging. Some limitations should be acknowledged. An important consideration is our use of the age-matched male comparison group against pre- and post-menopausal groups. While this reference is not an ideal benchmark, age-matched men provide a useful tool for extricating the impact of age so as to provide an understanding of age-related tau increase that is unrelated to the menopause milieu. One interesting investigation would be of post-menopausal women on hormone therapy versus those who were not. In the current study, there were only 18 women who had any prior or current use of hormone therapy, which limited our capacity to address this question. In addition, we did not examine the impact of surgery (unilateral or bilateral oophorectomy) or circulating sex hormones (estrogen, progesterone, testosterone) in our analyses. As such, it remains unclear which component of the menopause transition may be driving our findings. Two PET scanners were used in this study necessitating smoothing harmonization procedures[52] when combining data. We have, however, internally demonstrated that analyses with only those scanned on the GE Discovery (the camera in which the bulk of participants were scanned) provide the same pattern of results. Furthermore, examining *a priori* regions of the brain, while reducing the risk of a Type I error, precluded exploration of sex differences across the entire cortex in this middle-aged sample. An additional methodological consideration is the impact of sex differences in off-target binding.[53] Smith and colleagues[53] recently reported that off-target meningeal binding is greater in women than men for the Flortaucipir tracer. While this may be evident, our own preliminary work has suggested that it does not significantly impact sex differences in target signal in regions of interest.[54] Finally, this sample is well-educated and predominantly Caucasian, and as such, may not be generalizable.

In summary, we show a moderating effect of menopause status on the association between sex and Aβ and tau deposition in middle-aged clinically normal adults. Post-menopausal women demonstrate higher tau-PET signal than age-matched men, as well as the pre-menopausal age-matched groups. Our study externally validates a pattern of sex divergence in tau-PET signal in the rostral middle frontal, lateral occipital, and inferior parietal regions in a cohort approximately 20 years younger compared to prior studies. Taken together, these findings suggest a role for menopause to play to increase risk for tau-PET signal in women relative to men of the same age. There remains a critical question, however, about the impact of age vs the menopause hormonal transition, specifically, on vulnerability to AD pathology in women. Future studies exploring the association between sex hormones (biomarkers of the menopausal transition), as well as age at menarche and parity and tau-PET signal are required to examine the influence of other critical transitional hormonal periods on female vulnerability to tauopathy. It would also be important to examine the impact of education and other socioeconomic status indicators to tease apart how the impact of gender may influence tau-PET signal. Finally, it will be critical to follow pre- and post-menopausal women longitudinally to more robustly assess the point at which tau accumulation diverges across the reproductive timespan.

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Author Contributions

Study concept and design was carried out by R.F.B., A.O., E.M., H.I.L.J., C.L., C.S., S.G., Z.R., J.M., R.E.A., K.A.J., S.S., A.S.B. Data acquisition and analysis was carried out by A.O., S.S., A.S.B. Drafting the manuscript and figures was conducted R.F.B., A.O., E.M., S.S., A.S.B.

Potential Conflict of Interest

Nothing to report.

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