Genetics

Sex differences in the association between apolipoprotein E ε4 allele and Alzheimer's disease markers

Erin E. Sundermann[a,*], My Tran[b], Pauline M. Maki[c], Mark W. Bondi[a,d],
for the Alzheimer's Disease Neuroimaging Initiative[1]

[a]Department of Psychiatry, University of California, San Diego, San Diego, CA, USA
[b]Department of Psychology, San Diego State University, San Diego, San Diego, CA, USA
[c]Departments of Psychiatry and Psychology, University of Illinois at Chicago, Chicago, IL, USA
[d]Veterans Affairs San Diego Healthcare System, San Diego, CA, USA

Abstract

Introduction: We determined whether the effect of apolipoprotein E (*APOE*)-ε4 genotype on Alzheimer's disease (AD) markers differs in men and women across AD stages.

Methods: Among normal control (NC) participants (N = 702) and participants with mild cognitive impairment (N = 576) and AD (N = 305), we examined the associations of sex and *APOE*-ε4 carrier status with cortical amyloid-β (Aβ) burden, hippocampal volume ratio (HpVR; hippocampal volume/intracranial volume $\times 10^3$), brain glucose metabolism, and verbal memory.

Results: In NC, *APOE*-ε4 related to greater Aβ burden and poorer verbal memory across sex but to smaller HpVR and hypometabolism in men only. In mild cognitive impairment, *APOE*-ε4 related to smaller HpVR, hypometabolism, greater Aβ burden, and poorer verbal memory across sex. In AD, *APOE*-ε4 related to greater Aβ burden in men only and smaller HpVR across sex and showed no association with hypometabolism or verbal memory.

Discussion: Sex differences in the association between *APOE*-ε4 and AD markers vary by disease stage.
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Keywords: *APOE*; Sex differences; Amyloid-β plaque deposition; Hippocampal volume; Brain glucose metabolism; Verbal memory

1. Background

The ε4 allele of the apolipoprotein E gene (*APOE*-ε4) is the most common genetic risk factor for Alzheimer's disease (AD) [1,2]. *APOE*-ε4 is associated with AD-related biological and clinical markers including cortical amyloid-β (Aβ) plaque burden, hippocampal atrophy [3–5], and accelerated cognitive decline in healthy aging [6–8]. However, there have been conflicting/mixed findings with some reporting that *APOE*-ε4 is not associated with hippocampal volume [9,10] or cognitive decline in healthy aging [9,10] or with risk of converting from mild cognitive impairment (MCI) to AD [11,12]. These inconsistencies may be due to the potentially critical modulating role of sex in the association between *APOE*-ε4 and AD.

Early cross-sectional studies indicated that *APOE*-ε4 confers a greater risk for AD in women than in men [13–15]. These early findings are supported by a longitudinal study [16] and a meta-analysis that reported an increased risk of incident AD in female *APOE*-ε4 heterozygotes versus male heterozygotes among adults aged 65 to 75 years [17]. Among older normal controls, the adverse effect of *APOE*-ε4 on AD biomarkers including cerebrospinal fluid total tau levels [18], brain metabolism [19], cortical thinning [19], and functional brain connectivity in the default mode network [18] was stronger in women versus men, although not consistently [19,20]. In MCI, the effect of *APOE*-ε4 on brain atrophy [21], total tau levels [16], and the tau/Aβ ratio [16] was stronger in women versus men. To our knowledge, only one study examined sex differences in the effects of *APOE*-ε4 in AD patients and reported a stronger association between *APOE*-ε4 and Aβ burden in parietal, cingulate, and frontal regions in men versus women [22], suggesting a possible reversal in the moderating role of sex on *APOE*-ε4 in the MCI-to-AD transition that warrants further exploration.

Surprisingly, few studies have examined the interactive effects of sex and *APOE*-ε4 on cognitive performance [21,23,24], particularly verbal memory, even though verbal memory is the cognitive domain that shows the earliest and most severe deficits in AD [25]. Among community-dwelling older adults, the association between *APOE*-ε4 and accelerated cognitive decline, as measured by a global cognitive measure, was stronger in women versus men [23,24]. In a MCI sample, female heterozygous or homozygous *APOE*-ε4 carriers showed worse performance on a delayed (5 minute) word recall task from the Alzheimer's Disease Assessment Scale Cognitive Subscale compared with female noncarriers, whereas the *APOE*-ε4 effect was only evident in homozygous men [21]. In addition, few studies have examined the sex by *APOE* interaction across the AD continuum [22] despite evidence of a temporal ordering of AD markers, whereby Aβ deposition occurs first, followed by neurodegenerative biomarkers and cognitive impairment [26,27]. We systematically examined the separate and interactive effects of sex and *APOE* on multiple AD-related markers including Aβ deposition, hippocampal volume, brain glucose metabolism, and verbal memory performance in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Effects were examined in each disease stage (NC, MCI, AD), in which MCI versus NC was defined using the Jak/Bondi diagnostic method, an actuarial, neuropsychological diagnostic approach that has produced more discernible cognitive phenotypes, more stable diagnoses, stronger associations with AD biomarkers, and better predication of progression to dementia than conventional diagnostic criteria [28,29]. Consistent with the broader literature, we predicted that the adverse effects of *APOE*-ε4 would be stronger in women versus men, and in-line with the temporal sequence of AD-related markers

[26,27], this sex difference in the *APOE*-ε4 effect will manifest at the NC stage for earlier AD events (Aβ deposition) and in MCI for later events (hippocampal atrophy, brain hypometabolism, and memory deficits).

2. Methods

2.1. Participants and data source

Data were extracted from the ADNI database (adni.loni.usc.edu). ADNI is a longitudinal, multisite cohort study that began in 2003 as a public-private partnership. Information about ADNI can be found at www.adni-info.org. ADNI study visits involve neuroimaging, neuropsychological, and clinical assessments. We included participants who had *APOE* genotype and baseline data on one of the AD-related markers examined herein. We limited our sample to ADNI's largest race/ethnic group, Caucasians, to minimize potential population stratification bias that could complicate interpretation of genetic data. Analyses were repeated while excluding *APOE*-ε2 allele carriers because the protective effect of *APOE*-ε2 could mask the adverse effect of *APOE*-ε4.

2.2. Verbal memory assessment

Our verbal memory measure was the Rey Auditory Verbal Learning Test (AVLT) [30]. The AVLT is a multitrial list learning and memory test with immediate and delayed recall and recognition outcomes. Immediate recall scores (range: 0–75) were the primary outcome because they were not used in diagnostic criteria, and learning deficits may better discriminate preclinical AD from normal controls than retention deficits [31,32].

2.3. Biomarkers

Biomarkers included neuroimaging measures of hippocampal volume, brain glucose metabolism, and cortical Aβ deposition. Structural MRI scans were collected on a 1.5T scanner according to a standardized protocol [33]. Hippocampal volume data were analyzed using FreeSurfer version 4.3 (https://surfer.nmr.mgh.harvard.edu) at the University of California–San Francisco (http://adni.loni.ucla.edu/wp-content/uploads/2010/12/UCSF-FreeSurfer-Overview-and-QC_-Template_Format.pdf) [34]. To control for sex differences in head size, we calculated a hippocampal volume ratio (HpVR) using the formula, hippocampal/intracranial volume $\times 10^3$.

Brain glucose metabolism was measured by $[^{18}F]$ fludeoxyglucose (FDG) positron emission tomography (PET). Images were preprocessed following a standard procedure described in http://adni.loni.usc.edu/methods/pet-analysis/pre-processing/. ADNI investigators at the University of California, Berkeley, established a "MetaROI" of brain regions that commonly demonstrate metabolic changes in MCI/AD which correlate with cognitive

performance in a meta-analysis [11,35]. The "MetaROI" was comprised of bilateral posterior cingulate gyrus, bilateral angular gyri, and middle/inferior temporal gyrus. Standardized uptake value ratios (SUVRs) were calculated by averaging FDG uptake across the MetaROI and dividing by a reference region of pons and cerebellum [11,35]. FDG PET image analysis is described in http://www.adni-info.org/Scientists/ADNIStudy Procedures.aspx.

Cortical Aβ burden was measured by [^{18}F]florbetapir positron emission tomography (AV45 PET) as described in http://www.adni-info.org. Mean AV45 uptake was measured within frontal, anterior/posterior cingulate, lateral parietal, and lateral temporal regions. SUVRs were calculated by averaging across regions and dividing by whole cerebellum.

2.4. Diagnostic classification

Diagnosis of NC versus MCI was based on the Jak/Bondi diagnostic method [28]. This method included six neuropsychological tests representing three cognitive domains: (1) Trail-Making Tests A and B (psychomotor speed/executive function); (2) Category Fluency and Boston Naming Test (language); and (3) AVLT Delayed Recall and Recognition Tests (episodic memory). An impaired score was defined as >1 SD below the age-corrected normative mean. MCI diagnosis required one of three criteria: (1) impaired score on two tests within a cognitive domain; (2) one impaired score in each of the three cognitive domains; and/or (3) a score of 9 on the Functional Assessment Questionnaire indicating dependence in at least three daily activities. If no criterion was met, a NC diagnosis was provided. Diagnostic criteria for AD were based on the standard National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria [36].

2.5. Statistical analysis

The APOE genotype was categorized into APOE-ε4 carriers and noncarriers because of the low prevalence of APOE-ε4 homozygotes when stratifying by sex and diagnostic group (e.g., 8 women and 22 men in NC group). Analyses were conducted within diagnostic group. Differences in sample characteristics and AD-related markers between sex and APOE-ε4 status were examined using independent t-tests for continuous variables and Chi-square tests for categorical variables. We used multivariable linear regression analyses modeling the separate and interactive associations of sex and APOE-ε4 status with each AD-associated marker (HpVR, FDG SUVR, AV45 SUVR, and AVLT scores) while adjusting for age and education. The interaction was removed from the model if $P \geq .10$. Analyses were performed using SPSS 24 (SPSS Inc., Chicago, IL). Significance was defined as $P \leq .05$ (two-sided).

Table 1
Sample size by sex, APOE-ε4 status, and diagnostic group for each AD-related marker

Diagnostic group/outcome	Total sample	APOE-ε4+ Women	APOE-ε4+ Men	APOE-ε4− Women	APOE-ε4− Men
NC					
AVLT Immediate Recall	702	107	126	228	241
HpVR	630	101	109	205	215
FDG SUVR	562	88	101	180	193
AV45 SUVR	430	78	71	143	138
MCI					
AVLT Immediate Recall	576	127	183	101	165
HpVR	490	112	152	84	142
FDG SUVR	414	87	135	73	119
AV45 SUVR	260	58	78	52	72
AD					
AVLT Immediate Recall	305	90	120	45	58
HpVR	250	73	97	36	44
FDG SUVR	220	63	83	25	49
AV45 SUVR	130	39	48	15	28

Abbreviations: AD, Alzheimer's disease; APOE-ε4, apolipoprotein ε4 allele; AV45, florbetapir positron emission tomography; AVLT, Rey Auditory Verbal Learning Test; FDG, fluodeoxyglucose; HpVR, hippocampal volume ratio (hippocampal/intracranial volume × 10^3); MCI, mild cognitive impairment; NC, normal control; SUVR, standardized uptake value ratio.

3. Results

Our sample included 1583 participants with AVLT scores, 1370 with HpVR, 998 with FDG SUVR, and 820 with AV45 SUVR. Table 1 displays sample sizes by sex and APOE-ε4 status for each diagnostic group and AD-related marker. In the largest sample (participants with AVLT scores), there were no sex differences in the distribution of APOE-ε4 carriers in any diagnostic group. In all diagnostic groups, women were younger and had less years of education than men (P's < .05; Table 2). Mean Mini-Mental State Examination score was higher in NC women versus NC men ($P = .002$), although the difference was only half a point. APOE-ε4 carriers were younger than noncarriers in all diagnostic groups ($P < .01$). APOE-ε4 carriers had a lower mean Mini-Mental State Examination and a higher mean Clinical Dementia Rating–Sum of Boxes scores than noncarriers in MCI ($P < .001$). Differences between sex and APOE-ε4 status groups on AD-related markers are examined in the linear regressions that are described in the following.

3.1. Normal control

Inconsistent with hypotheses, there was a significant sex by APOE interaction on HpVR (Table 3) and FDG SUVR in NC (Fig. 1). The APOE-ε4 allele was associated with smaller HpVR and lower FDG SUVR in men (HpVR: B = −0.26, SE = 0.07, P < .001; FDG SUVR: B = −0.06, SE = 0.02, P < .001) but not in women (HpVR: B = 0.01, SE = 0.07, P = .86; FDG SUVR: B = −0.01, SE = 0.01, P = .33). The interaction

Table 2
Characteristics of each diagnostic group as a function of sex and *APOE*-ε4 status

Parameters	Sex		Women versus men *P* value	*APOE*-ε4 status		*APOE*-ε4+ versus *APOE*-ε4− *P* value
	Women	Men		*APOE*-ε4+	*APOE*-ε4−	
NC						
Age	73.0 (6.6)	74.1 (6.9)	.01	72.4 (6.9)	74.1 (6.7)	.001
Education, years	15.8 (2.6)	17.0 (2.5)	<.001	16.2 (2.5)	16.5 (2.6)	ns
MMSE	28.9 (1.3)	28.6 (1.5)	.002	28.6 (1.6)	28.8 (1.3)	ns
AVLT Immediate Recall	47.7 (8.9)	44.1 (10.1)	<.001	43.4 (10.6)	44.5 (9.8)	ns
HpVR	5.0 (0.6)	4.7 (0.7)	<.001	4.8 (0.7)	4.9 (0.7)	ns
FDG SUVR	1.32 (0.11)	1.29 (0.12)	<.001	1.28 (0.12)	1.31 (0.11)	.002
AV45 SUVR	1.2 (0.2)	1.1 (0.2)	.04	1.2 (0.2)	1.1 (0.2)	<.001
MCI						
Age	72.5 (7.4)	74.5 (6.8)	.003	72.9 (6.7)	74.6 (7.4)	<.001
Education, years	15.4 (2.8)	16.2 (2.8)	.003	15.8 (2.9)	15.9 (2.7)	ns
MMSE	27.4 (1.9)	27.4 (1.8)	ns	27.2 (1.8)	27.7 (1.8)	<.001
CDR-SOB	1.5 (1.0)	1.6 (1.0)	ns	1.7 (1.0)	1.4 (1.0)	<.001
AVLT Immediate Recall	32.6 (8.7)	29.3 (7.5)	<.001	29.5 (7.7)	31.9 (8.5)	<.001
HpVR	4.4 (0.8)	4.2 (0.7)	.005	4.2 (0.8)	4.4 (0.7)	.02
FDG SUVR	1.22 (0.14)	1.22 (0.13)	ns	1.20 (0.13)	1.24 (0.13)	.002
AV45 SUVR	1.2 (0.2)	1.3 (0.2)	ns	1.3 (0.2)	1.1 (0.2)	<.001
AD						
Age	73.8 (7.9)	76.0 (7.6)	.03	74.1 (7.2)	76.8 (8.6)	.005
Education, years	14.4 (2.6)	16.0 (2.8)	<.001	15.2 (2.8)	15.5 (2.8)	ns
MMSE	23.3 (2.1)	23.2 (2.0)	ns	23.4 (2.0)	23.1 (2.1)	ns
CDR-SOB	4.5 (1.7)	4.4 (1.6)	ns	4.3 (1.6)	4.6 (1.8)	ns
AVLT Immediate Recall	23.8 (7.9)	21.5 (6.8)	.03	22.8 (7.0)	21.9 (8.0)	ns
HpVR	3.9 (0.7)	3.7 (0.6)	.02	3.7 (0.5)	3.9 (0.8)	.05
FDG SUVR	1.06 (0.15)	1.07 (0.14)	ns	1.07 (0.14)	1.07 (0.15)	ns
AV45 SUVR	1.5 (0.2)	1.4 (0.2)	.001	1.5 (0.2)	1.3 (0.3)	<.001

Abbreviations: AD, Alzheimer's disease; *APOE*-ε4, apolipoprotein ε4 allele; AV45, florbetapir positron emission tomography; AVLT, Rey Auditory Verbal Learning Test; CDR-SOB, Clinical Dementia Rating–Sum of Boxes; FDG, fluodeoxyglucose; HpVR, hippocampal volume ratio (hippocampal/intracranial volume × 10³); MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NC, normal control; SUVR, standardized uptake value ratio.

NOTE. Values are expressed as mean (standard deviation). Sample characteristics are based on the largest sample (participants with AVLT Immediate Recall data). The pattern of sex and *APOE*-ε4 status differences in sample characteristics was similar in all AD marker subsamples.

Table 3
Results of multivariable linear regression analyses modeling the separate and interactive associations of sex and *APOE*-ε4 status with AD-related markers

Sample/outcome	Multivariable linear regression models					
	Sex (male versus female)		*APOE*-ε4 (ε4 carriers versus noncarriers) status		Sex × *APOE*-ε4 status	
	B, β (SE)	*P* value	B, β (SE)	*P* value	B, β (SE)	*P* value
NC						
HpVR	−0.16, −0.12 (0.06)	.007	0.02, 0.01 (0.07)	.81	−0.28, −0.16 (0.1)	**.004**
FDG SUVR	−0.02, −0.10 (0.01)	.04	−0.02, −0.06 (0.01)	.29	−0.04, −0.15 (0.02)	**.02**
AV45 SUVR	−0.04, −0.18 (0.01)	<.001	−0.03, −0.18 (0.01)	.002	0.01, 0.02 (0.03)	.78
AVLT Immediate Recall	−7.33, −0.36 (0.69)	<.001	−1.46, −0.07 (0.71)	.04	−1.55, −0.06 (1.42)	.27
MCI						
HpVR	0.01, 0.03 (0.01)	.60	−0.25, −0.18 (0.01)	<.001	0.05, 0.03 (0.13)	.69
FDG SUVR	−0.01, −0.09 (0.02)	.22	−0.08, −0.30 (0.02)	<.001	0.04, 0.15 (0.03)	.11
AV45 SUVR	0.00, 0.00 (0.03)	.99	0.21, 0.45 (0.03)	<.001	−0.09, −0.18 (0.05)	.09
AVLT Immediate Recall	−3.29, −0.20 (0.67)	<.001	−2.79, −0.17 (0.65)	<.001	0.39, 0.02 (1.33)	.77
AD						
HpVR	−0.20, −0.16 (0.08)	.008	−0.23, −0.17 (0.08)	.004	0.05, 0.04 (0.16)	.76
FDG SUVR	−0.01, −0.04 (0.02)	.55	0.02, 0.05 (0.02)	.40	−0.01, −0.05 (0.04)	.74
AV45 SUVR	−0.21, −0.49 (0.06)	.001	0.03, 0.07 (0.06)	.61	0.20, 0.45 (0.07)	**.007**
AVLT Immediate Recall	−2.70, −0.18 (0.89)	.003	1.04, 0.07 (0.90)	.25	−2.29, −0.15 (1.78)	.20

Abbreviations: AD, Alzheimer's disease; *APOE*-ε4, apolipoprotein ε4 allele; AV45 PET, florbetapir positron emission tomography; AVLT, Rey Auditory Verbal Learning Test; B, unstandardized regression coefficient; FDG, fluodeoxyglucose; HpVR, hippocampal volume ratio (hippocampal/intracranial volume × 10³); MCI, mild cognitive impairment; NC, normal controls; SUVR, standardized uptake value ratio; β, standardized regression coefficient.

NOTE. All analyses were adjusted for age and education. Bolded text indicates statistical significance at *P* < .05.
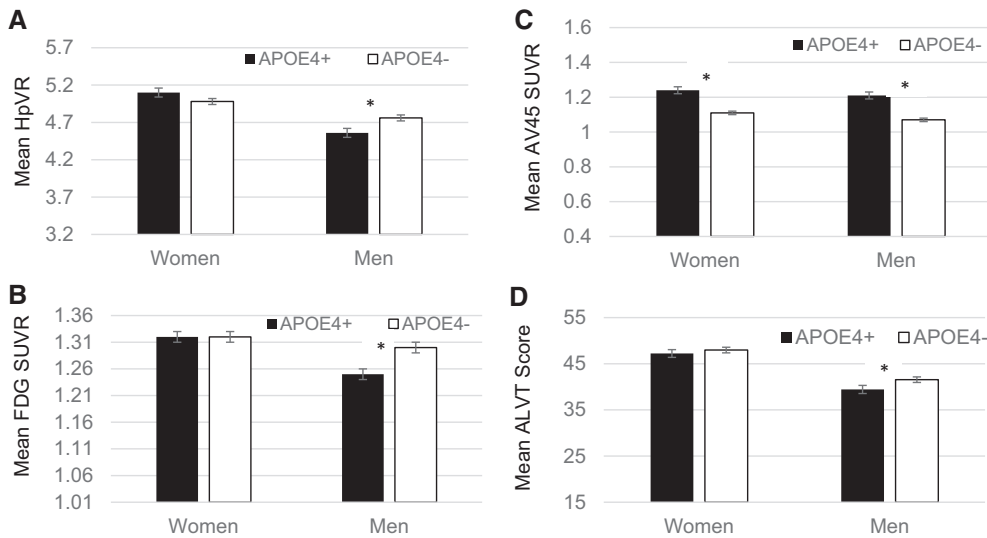
Fig. 1. Mean AD biomarkers (A–C) and AVLT Immediate Recall scores (D) as a function of sex and *APOE*-ε4 status in NC. *Significant mean difference at *P* < .05 while adjusting for age and education. Abbreviations: *APOE*4+, apolipoprotein ε4 allele carrier; *APOE*4−, apolipoprotein ε4 allele noncarrier; AVLT score, Immediate Recall outcome of the Rey Auditory Verbal Learning Test (range, 0–75); FDG, fludeoxyglucose; HpVR, hippocampal volume ratio; SUVR, standardized uptake volume ratio.

was not significant for AVLT scores or AV45 SUVR. Rather, there was a main effect of sex and *APOE*-ε4 for both markers in NC; however, as seen in Fig. 1D, the adverse effect of *APOE*-ε4 on AVLT scores was only significant among NC men in sex-stratified analyses. Women demonstrated higher AVLT scores and AV45 SUVR than men (*P* ≤ .001), and *APOE*-ε4 carriers demonstrated higher AV45 SUVR and lower AVLT scores than noncarriers (*P* < .05).

3.2. Mild cognitive impairment

In the MCI sample, there were no significant sex by *APOE* interactions on any AD-related marker (Fig. 2). There was a main effect of sex on AVLT scores in MCI whereby scores were higher in women than in men (*P* < .001). There was a main effect of *APOE*-ε4 for all markers in MCI. *APOE*-ε4 was associated with smaller HpVR, lower FDG SUVR, higher AV45 SUVR, and poorer AVLT scores (*P*'s < .001).
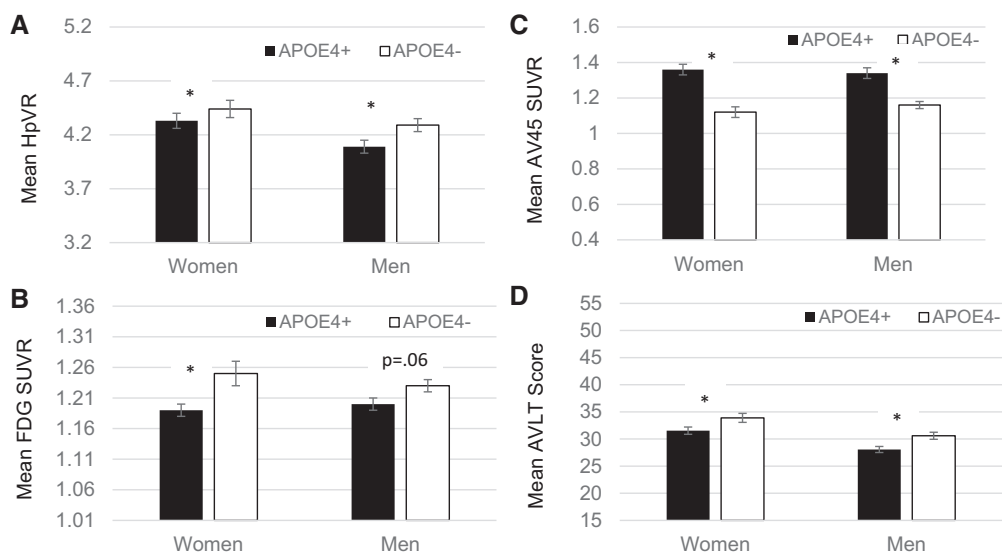

Fig. 2. Mean AD biomarkers (A–C) and AVLT Immediate Recall scores (D) as a function of sex and *APOE*-ε4 status in MCI. *Significant mean difference at *P* < .05 while adjusting for age and education. Abbreviations: *APOE*4+, apolipoprotein ε4 allele carrier; *APOE*4−, apolipoprotein ε4 allele noncarrier; AVLT score, Immediate Recall outcome of the Rey Auditory Verbal Learning Test (range, 0–75); FDG, fludeoxyglucose; HpVR, hippocampal volume ratio; SUVR, standardized uptake volume ratio.
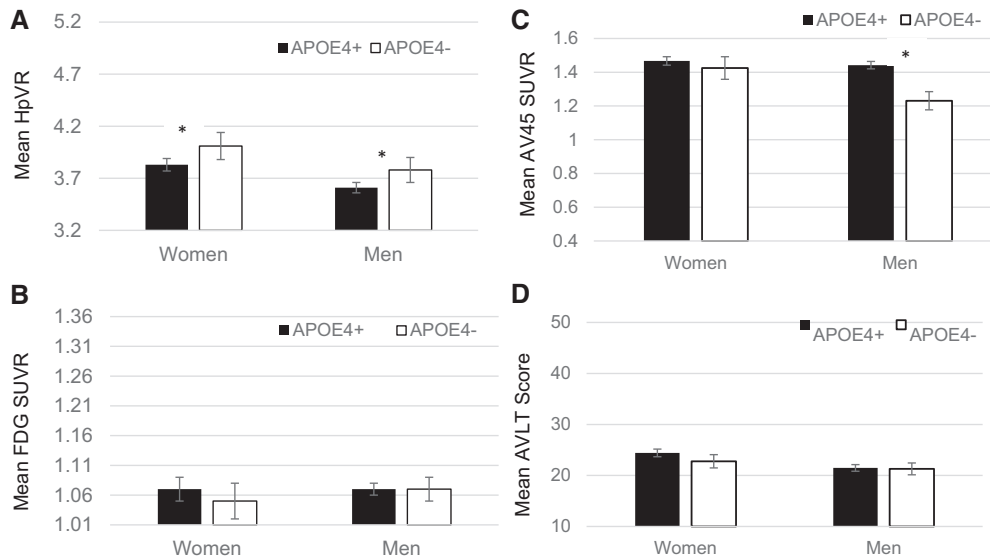
Fig. 3. Mean AD biomarkers (A–C) and AVLT Immediate Recall scores (D) as a function of sex and *APOE*-ε4 status in AD. *Significant mean difference at *P* < .05 while adjusting for age and education. Abbreviations: *APOE*4+, apolipoprotein ε4 allele carrier; *APOE*4–, apolipoprotein ε4 allele noncarrier; AVLT score, Immediate Recall outcome of the Rey Auditory Verbal Learning Test (range, 0–75); FDG, fludeoxyglucose; HpVR, hippocampal volume ratio; SUVR, standardized uptake volume ratio.

3.3. Alzheimer's disease

Among AD patients, there was a significant sex by *APOE* interaction on AV45 SUVR (*P* = .007; Fig. 3). *APOE*-ε4 was associated with a higher AV45 SUVR in men (B = 0.23, SE = 0.05, *P* < .001) but not in women (B = 0.02, SE = 0.05, *P* = .67). The interaction was not significant for the other markers. As in the other diagnostic groups, there was a main effect of sex on AVLT scores in AD. AVLT scores were higher in women versus men (*P* = .003). There was also a main effect of sex on HpVR and AV45 SUVR in AD. HpVR and AV45 SUVR were higher in women versus men (*P* < .01). There was no main effect of sex on FDG SUVR. There was a main effect of *APOE*-ε4 on HpVR in AD. HpVR was lower in *APOE*-ε4 carriers versus noncarriers (*P* = .004). There were no main effects of *APOE*-ε4 on other markers.

When excluding *APOE*-ε2 carriers, results were unchanged in the NC and AD groups. In contrast to the primary results, the sex by *APOE* interaction was significant for FDG SUVR (B = 0.05, SE = 0.03, *P* = .05) in MCI. *APOE*-ε4 was associated with lower FDG SUVR in MCI women (B = −0.09, SE = 0.02, *P* < .001) but not in MCI men (B = −0.03, SE = 0.02, *P* = .13). In addition, there was a significant sex by *APOE* interaction for AV45 SUVR in MCI (B = −0.11, 0.06, *P* = .04). The *APOE*-ε4 and AV45 SUVR association was stronger in MCI women (B = 0.26, SE = 0.04, *P* < .001) versus MCI men (B = 0.16, SE = 0.04, *P* < .001).

4. Discussion

Broadly, our findings support evidence of sex differences in the adverse effect of *APOE*-ε4 on AD-related markers. We extend previous findings by performing the following: (1) comparing these sex differences across biomarker and clinical outcomes; (2) identifying changes in sex differences that occur across disease stages; and (3) by defining MCI using an algorithmic, neuropsychological diagnostic criteria that have shown better prognostic value in terms of progression to dementia than more conventional criteria. Our novel findings are that sex differences in *APOE*-ε4 effects on AD-related markers varied by marker and disease stage and that the effect of *APOE*-ε4 on brain structure/function manifested at a later disease stage in women versus men.

Consistent with previous findings, we found that AV45 SUVR was higher in NC women versus NC men regardless of *APOE* genotype. We also replicated the well-evidenced finding that women outperform men in verbal memory and indicated that this difference is regardless of *APOE* genotype. The dichotomy of better verbal memory performance and greater Aβ pathology in NC women versus NC men suggests that women may be better equipped to compensate for disease pathology and maintain normal verbal memory performance. Consistent with some past reports [37–40], we also found that NC women had a larger HpVR and a higher FDG SUVR than NC men possibly because of hormonal factors associated with female sex, such as the organization and activation effects of estrogen on the brain. A larger and a more efficient neural network in the brain region that regulates verbal memory may serve as a "brain reserve" [41,42] that allows for compensation of the greater Aβ deposition in women and supports the female advantage in verbal memory. Inconsistent with hypotheses, sex by *APOE* interactions on HpVR and FDG PET in the NC group indicated an

association between *APOE*-ε4 and lower HpVR and FDG SUVR in men only. The association between *APOE* and brain structure/function in healthy aging has been controversial with some, but not all, studies reporting an association [19,43–47]. Neglecting *APOE* by sex interactive effects on brain structure/function could be a contributing factor to discordant results.

The sex differences in FDG SUVR, Aβ deposition, and HpVR (after adjusting for covariates) observed in NC were absent in MCI; however, women were able to sustain their verbal memory advantage over men in MCI despite poorer AVLT scores in MCI versus NC. In contrast to the NC group, the adverse effects of *APOE*-ε4 on HpVR and FDG SUVR were observed in MCI women in addition to MCI men. Findings suggest that whereas *APOE*-ε4 effects on brain structure/function manifest in men during normal cognitive aging or preclinical AD, effects do not manifest in women until MCI. Perhaps, in the MCI stage, the pathophysiological brain changes have reached a threshold of severity past which compensatory mechanisms are overwhelmed in women and the adverse effect of *APOE*-ε4 on verbal memory is exposed. Conversely, the adverse effect of *APOE*-ε4 on higher amyloid deposition was observed across sex in both NC and MCI possibly because of the hypothesized temporal evolution of AD biomarkers whereby Aβ deposition occurs before changes in brain structure and functional changes and up to a decade before clinical deficits [48].

In AD, overall sex differences included a larger HpVR and the continued female advantage in verbal memory advantage in women versus men although the sex difference in AVLT scores was much smaller than that observed in the NC and MCI groups suggesting that the advantage is attenuated with increasing disease severity. *APOE*-ε4 related to smaller HpVR and greater Aβ deposition but not FDG SUVR or verbal memory in AD suggesting that the effects of the disease at this advanced stage overwhelm *APOE*-ε4 effects on brain function and verbal memory. There was a sex by *APOE* interaction on AV45 SUVR in AD whereby *APOE*-ε4 related to AV45 SUVR in men only. As shown in Figs. 2C and 3C, the manifestation of this interaction in AD but not in MCI resulted from greater Aβ deposition in female *APOE*-ε4 noncarriers in AD versus MCI so that Aβ deposition was similarly high in female carriers and noncarriers with AD. Conversely, male noncarriers had similar Aβ deposition levels in MCI and AD. Similarly, Tosun et al. [22] reported a sex by *APOE* interaction on Aβ deposition that was driven by lower Aβ deposition in male *APOE*-ε4 noncarriers versus carriers and similarly high levels in female carriers and noncarriers. An increase in Aβ deposition in female noncarriers in the MCI-to-AD transition requires investigation in longitudinal studies and, if found, would challenge theories that Aβ deposition plateaus before AD diagnosis in women [48].

Our results are in contrast to some previous reports of a stronger *APOE* effect in women versus men on AD-related

outcomes in healthy aging and on cognitive function in MCI. Discrepant findings may result from multiple contributing factors. Mixed race/ethnicity groups in prior studies may have introduced bias from population stratification that can complicate interpretation of genetic data. Race differences have been reported in the effects of *APOE*-ε4 on cognitive decline [49]. Studies may also have differed in the age of study samples and the specific biomarkers (e.g., cortical thickness vs. ROI-based volumetry) or cognitive outcomes examined. Many studies examined a global cognitive measure, whereas we specifically examined verbal memory, a clinical marker more reflective of AD risk. The female advantage on verbal memory may influence the effect of *APOE* on verbal memory. Differences in diagnostic criteria might also contribute to discrepant findings. We used the Jak/Bondi criteria, an actuarial neuropsychological diagnostic approach that eliminates use of rating scales, global cognitive screens, and subjective cognitive complaints that are commonly used in conventional diagnostic methods. When comparing Jak/Bondi and conventional criteria, conventional MCI diagnostic methods resulted in about 33% false-positive and 7% false-negative error rates [28,29,50]. In support of the Jak/Bondi criteria, the "false-positive" subgroup performed within normal limits across the cognitive battery and showed cerebrospinal fluid biomarker levels, cortical thickness maps, and rates of progression to dementia that were comparable to a robust NC group [28,29,51]. The "false-negative" group showed cerebrospinal fluid biomarker levels that were comparable to the MCI group and progressed to dementia at double the rate of the overall NC sample [50]. These diagnostic errors resulting from conventional diagnoses may have skewed results of prior studies of sex by *APOE* interactive effects within NC or MCI.

Our study has limitations. Our cross-sectional design precluded us from comparing rates of biomarker progression and memory decline by sex and *APOE*-ε4 status. Our differing and, at times, small sample sizes for each marker and diagnostic group may have led to varying and limited degrees of statistical power; however, we were able to detect a significant sex by *APOE* interaction in the smallest sample (AD patients with AV45 SUVR data). Our statistical power was too limited for significant findings to survive a multiple comparison correction; however, we sought to minimize comparisons by conducting sex-stratified analyses only in the presence of a significant sex by *APOE*-ε4 interaction. The risk for type 1 error should be considered when interpreting results. Our low number of *APOE*-ε4 homozygotes prohibited us from examining the additive effects of *APOE*-ε4 (noncarriers vs. heterozygotes vs. homozygotes). Finally, ADNI participants represent a convenience sample of volunteers who are predominantly Caucasian and well educated, which limits generalizability of results.

A study strength was our examination of the sex by *APOE* interaction across disease stages and across clinical and biological AD-related markers. Comparing results among diagnostic groups enabled us to discern a pattern of effects whereby the adverse effect of *APOE*-ε4 on brain structure/function manifests in NC men but not until clinical disease stages in women. We also revealed important sex differences in AD-related markers regardless of *APOE*-ε4 status. The female advantage in verbal memory in conjunction with higher greater Aβ pathology in NC women versus NC men suggests that women may be better equipped to compensate for disease pathology and maintain their verbal memory advantage.

APOE-ε4 status is a common selection criterion for intervention trials in preclinical AD. Considering that men and women may differ in the strength and trajectory of *APOE*-ε4 effects, failure to account for sex differences in *APOE*-ε4 effects on study outcomes could complicate interpretation of trials. Our findings may help to explain inconsistencies in the literature regarding *APOE*-ε4 effects on AD-related markers, particularly during the preclinical phase, and offer insight into biological mechanisms underlying sex differences in *APOE*-ε4–associated AD risk.

Acknowledgments

RESEARCH IN CONTEXT

1. Systemic review: Apolipoprotein E (*APOE*)-ε4 genotype may confer a greater risk for Alzheimer's disease (AD) in women than in men. We are the first to examine the moderating role of sex on the effects of *APOE*-ε4 on multiple AD-related markers at all disease stages.

2. Interpretation: Sex differences in the adverse effect of *APOE*-ε4 varied by AD-related marker and disease stage. Effects of *APOE*-ε4 on hippocampal volume and brain metabolism manifested in normal control men, whereas these effects did not manifest in women until mild cognitive impairment (MCI). In AD dementia, *APOE*-ε4 related to greater amyloid-β burden in men only.

3. Future directions: *APOE*-ε4 status is a common selection criterion for intervention trials in preclinical AD. Considering that the strength and trajectory of *APOE*-ε4 effects differ in men and women, failure to account for sex differences in *APOE*-ε4 effects could complicate interpretation of trial findings.

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