Sex Differences in the Genetic Predictors of Alzheimer's Pathology

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http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Abstract

Autopsy measures of Alzheimer's disease (AD) neuropathology have been leveraged as endophenotypes in previous genome-wide association studies (GWAS). However, despite evidence of sex differences in AD risk, sex-stratified models have not been incorporated into previous GWAS analyses. We looked for sex-specific genetic associations with AD endophenotypes from 6 brain bank data repositories. The pooled dataset included 2701 males and 3275 females, the majority of whom were diagnosed with AD at autopsy. Sex-stratified GWAS were performed within each dataset and then meta-analyzed. Loci that reached genome-wide significance ($p<5x10^{-8}$) in stratified models were further assessed for sex interactions. Additional validation was performed in independent datasets leveraging cognitive, neuroimaging, and cerebrospinal fluid endophenotypes, along with age-at-onset data. Outside of the *APOE* region, one locus on chromosome 7 (rs34331204) showed a sex-specific association with neurofibrillary tangles among males ($p=2.5x10^{-8}$) but not females ($p=0.85$, sex-interaction $p=2.9x10^{-4}$). In validation analyses, rs34331204 was also associated with hippocampal volume, executive function, and age-at-onset only among males. Expression quantitative trait loci results from brain highlight *BZW2* and *ANKMY2* as candidate genes at this locus. These results implicate a novel locus that confers male-specific protection from tau pathology and highlight the value of assessing genetic associations in a sex-specific manner.

Keywords: Alzheimer's disease; neuropathology; sex difference; amyloid; tau; GWAS

1. Introduction

Two-thirds of Alzheimer's disease (AD) cases are female (Mielke *et al.*, 2014; Mazure and Swendsen, 2016) and emerging evidence has highlighted notable sex differences in AD risk (Altmann *et al.*, 2014; Neu *et al.*, 2017; Buckley *et al.*, 2018), presentation (Barnes *et al.*, 2005; Apostolova *et al.*, 2006; Hua *et al.*, 2010; Hohman *et al.*, 2018), and progression (Barnes *et al.*, 2005; Koran *et al.*, 2017). Notably, the Apolipoprotein E (*APOE*) gene which is the strongest genetic risk factor for AD shows a stronger association among females compared to males, particularly between 65 and 75 years of age (Neu *et al.*, 2017). Despite the growing evidence of sex differences in AD neuropathology and the genetic drivers of AD neuropathology (Deming *et al.*, 2018), limited work has systematically explored sex-specific genetic associations with AD neuropathology across the genome.

Autopsy measures of neuropathology, including the Consortium to Establish a Registry for Alzheimer's disease (CERAD) neuritic plaque staging and Braak neurofibrillary tangle staging, have been leveraged in previous GWAS to identify novel genetic loci for AD that were not identified in case/control analyses (Beecham *et al.*, 2014). These endophenotypes provide an invaluable opportunity to better understand the underlying disease process by providing biological measures that are more proximal to gene function. Moreover, these metrics provide ideal outcomes for the investigation of sex-specific analyses because identified associations will highlight points along the disease cascade where sex differences begin to emerge.

This study leverages 6 autopsy cohorts to assess sex-specific genetic associations with AD neuropathology. First, we perform a sex-stratified GWAS in 5,976

participants with autopsy measures of neuritic plaques and neurofibrillary tangles. Second, we validate observed sex-specific associations leveraging complementary biomarker data from independent datasets. Our central hypothesis is that certain genetic factors act in a sex-specific manner to drive the neuropathological presentation of AD. The identification of sex-specific effects will advance our understanding of the genetic architecture of AD and clarify the underlying pathways that contribute to AD neuropathology.

2. Materials and methods

2.1 Participants

Participants were drawn from a previously published genome-wide neuropathology analysis (Beecham *et al.*, 2014) which includes detailed descriptions of the six well-characterized cohorts used in this study. Studies included the National Institute on Aging Late–Onset Alzheimer's Disease Family Study (LOAD), Mayo Clinic (Mayo), the Adult Changes in Thought (ACT) study, the National Alzheimer's Coordinating Center (NACC), the Religious Orders Study and Rush Memory and Aging Project (ROS/MAP), and the Translational Genomics Research Institute (TGEN). Additionally, we excluded individuals younger than 60 or missing a clinical dementia diagnosis. All participants agreed to brain donation and were evaluated at each site.

2.2 Quantification of Neuropathology Outcomes

Autopsy measures of neurofibrillary tangles (Braak staging) and neuritic plaques (CERAD score) were collected in each cohort and harmonized for a previously published GWAS (Beecham *et al.*, 2014). Thal stage was not collected in all studies so

was not included in our staging definitions. Both measures were analyzed as binary outcomes. Binary neuritic plaque (NP) status was defined based on established neuropathological criteria for AD (Hyman *et al.*, 2012) whereby a CERAD score of "none" or "sparse" was considered "NP negative", and "moderate" or "frequent" was considered "NP positive". Similarly, the binary neurofibrillary tangles (NFT) status was defined whereby Braak stages none, I, or II were considered "NFT negative" and stages III, IV, V, or VI were considered "NFT positive" (Hyman *et al.*, 2012).

2.3 Quantification of Clinical, Structural, and Laboratory Biomarkers of AD

Validation analyses were performed using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and using cerebrospinal fluid (CSF) data from a previously published GWAS (Deming *et al.*, 2018). We leveraged preprocessed ADNI amyloid positron emission tomography (PET) data, specifically baseline mean cortical florbetapir uptake calculated from six established regions of interest, normalized to whole cerebellum (Landau *et al.*, 2012). Episodic memory and executive function composite scores were calculated in ADNI previously (Crane *et al.*, 2012; Gibbons *et al.*, 2012). Hippocampal and intracranial volumes were captured using MRI and quantified using FreeSurfer as previously described (Mormino *et al.*, 2009). Quantification of CSF levels of Aβ42, total tau (t-tau), and phosphorylated tau (p-tau) were performed independently in seven cohorts (ADNI, Mayo, University of Pennsylvania, University of Washington, BIOCARD, Knight ADRC, and a multi-center AD study from Sweden) as described in a previous GWAS publication (Deming *et al.*, 2017). Finally, age-at-onset data from a previous GWAS analysis (Huang *et al.*, 2017) was used to evaluate sex-specific associations with AD progression.

2.4 Measures of Gene Expression and Semi-Quantitative Neuropathology in ROS/MAP Brain Tissue

Gene expression data from ROS/MAP were obtained through the Accelerating Medicines Partnership AD project. RNA expression was processed from frozen, manually dissected sections of dorsolateral prefrontal cortex (PFC) tissue as previously described (Lim *et al.*, 2014). For the present analyses, low abundance genes (expressed in <10% of the cohort) were removed. Semi-quantitative measures of AD neuropathology were measured using immunohistochemistry and quantified by image analysis and stereology (Bennett *et al.*, 2012a; Bennett *et al.*, 2012b). Tangle density was quantified with AT8 antibody across 8 regions of the brain (Bennett *et al.*, 2012a; Bennett *et al.*, 2012b).

2.5 Genotyping and Quality Control

Genome-wide genotyping was performed by each study on a variety of genotyping platforms. Imputation was performed on the University of Michigan Imputation Server in all datasets using the HRC r1.1.2016 reference panel, SHAPEIT phasing, and the EUR (European) population. Prior to imputation, all genotype data were processed through the same quality control protocol, including removing single nucleotide polymorphisms (SNPs) that had a poor call rate (<98% calls on Illumina platforms, <95% calls on Affymetrix platforms) or outside of Hardy-Weinberg equilibrium ($p<1x10^{-6}$). Samples were limited to those of European-descent and were excluded for sex inconsistency or cryptic relatedness (Pihat>0.4). Following imputation, SNPs with MAF>0.01 and imputation $R^2>0.3$ were retained for subsequent analysis.

2.6 Statistical Analyses

Sex differences in clinical and demographic characteristics were evaluated using an independent samples t-test for continuous variables and a $\chi 2$ test for categorical variables in R (https://www.r-project.org/). Sex-stratified analyses of NFT and NP were performed using logistic regression in PLINK (Version 1.9, https://www.cog-genomics.org/plink/1.9). Analyses were performed within each cohort separately, used additive coding, and covaried for age at death. Fixed-effects meta-analysis was performed using GWAMA (http://www.geenivaramu.ee/en/tools/gwama) (Mägi and Morris, 2010). Results were limited to SNPs that were present in at least three of the six cohorts. The threshold for statistical significance was set at the standard GWAS level of $p=5x10^{-8}$. All significant sex-stratified effects were assessed for sex x SNP interactions. Sex-stratified Miami plots were generated using EasyStrata (version 16.0) (Winkler *et al.*, 2014). Genomic inflation factors for the GWAS analyses ranged from $\lambda=0.99-1.04$ (Supplementary Fig. 1). SNP annotation was performed using ANNOVAR (version 2018Apr16). Forest plots were generated using the R package Metafor.

SNPs with putative sex-specific associations with neuropathology were further assessed for correlation with baseline episodic memory, executive function, amyloid PET, hippocampal volume, and CSF levels of Aβ42, t-tau, and p-tau using linear regression in R, covarying for baseline age. Hippocampal volumes were normalized by intracranial volume using established procedures (Voevodskaya *et al.*, 2014). Finally, putative SNPs were also evaluated in sex-specific associations with age-at-onset using data from a previously published survival analysis of AD (Huang *et al.*, 2017).

2.7 Expression Quantitative Trait Analysis

SNPs that showed a sex-specific association were further assessed for expression quantitative trait locus (eQTL) associations using data from Braineac (http://caprica.genetics.kcl.ac.uk/BRAINEAC/). Correction for multiple comparisons was completed using the false discovery rate (FDR) procedure, correcting for the total number of gene-tissue combinations.

Significant eQTL genes were further assessed for sex-specific associations with AD neuropathology leveraging gene expression data from ROS/MAP. Sex-stratified and sex-interaction analyses were completed using linear regression covarying for age at death. Tangle load was square-root transformed to better approximate a normal distribution.

3. Results

A total of 2701 males and 3275 females across six independent autopsy datasets were analyzed. In general, females were older than males (males: 79±9 years, females: 81±9 years, $p<0.001$) and were more frequently AD cases (males: 68% , females: 71% , $p=0.02$), *APOE*-ε4 carriers (males: 49% , females: 46%, $p=0.03$), and NP- and NFT-positive individuals (males: 72% and 77% , females: 76% and 85%, p-values<0.01) than males. Participant characteristics by cohort are presented in Supplementary Table 1.

In the sex-stratified GWAS analysis of NFT, one SNP (rs34331204; **Fig. 1**; Supplementary Fig. 2) outside of the *APOE* locus reached genome-wide significance in males ($\beta=-0.720$; $p=2.48\times10^{-8}$) but not in females ($\beta=-0.027$; $p=0.85$). Furthermore, rs34331204 showed an interaction with sex on NFT ($\beta=0.71$; $p=2.93\times10^{-4}$), with the

minor allele was associated with a lower risk of NFT positivity in males (**Fig. 2**). It is notable that this association did not meet genome-wide significance in the larger sex-combined sample (β=-0.39; $p=2.63x10^{-5}$) and, thus, may have been overlooked if we did not explicitly model sex. No associations reached genome-wide significance in the sex-stratified GWAS analysis of NP (Supplementary Fig. 3). The top meta-analysis results from sex-stratified GWAS of NP and NFT are presented in Supplementary Tables 2-5.

The putative sex-specific locus was then assessed for associations with cognition, amyloidosis, neurodegeneration, and age-at-onset using publicly available data sources. Results are presented in **Table 1.** The sex-specific association with NFT (rs34331204) showed a comparable male-specific association with executive function performance and hippocampal volume (**Fig. 2**), with mixed evidence of a sex difference in the age-at-onset analysis. No sex-specific associations with CSF tau or p-tau were observed.

Lastly, we used eQTL mapping in Braineac to identify candidate genes within the rs34331204 locus and analyzed expression of these genes in brain tissue. Significant eQTL associations (FDR-corrected aveALL p-value<0.05 in Braineac) were seen for eight genes (*BZW2*, *TSPAN13*, *AGR3*, *ANKMY2*, *LRRC72*, *AGR2*, *ISPD*, and *AHR;* Supplementary Table 6). Two of these genes were not highly expressed in ROS/MAP PFC (*AGR3* and *LRRC72*), so we assessed six genes for sex-specific associations with tau load (**Table 2**). Surprisingly, *BZW2* and *ANKMY2* showed evidence of female-specific associations with tau load (p-values<0.002), but no male-specific associations or sex-interactions were observed.

4. Discussion

The present manuscript evaluated sex-specific genetic associations with AD neuropathology measured at autopsy. Results implicate one novel genetic locus, rs34331204 on chromosome 7 proximal to *BZW2*, that is associated with neurofibrillary tangles only among males. Additional evidence of a male-specific neuroprotective effect was observed in validation analyses in which the minor allele of rs34331204 was also associated with larger hippocampal volume, better executive function, and a later age-at-onset among males. It is important to note that the association between rs34331204 and NFT fell below the threshold of genome-wide significance when males and females were combined, and sex was simply included as a covariate in a post-hoc analysis, highlighting the utility of sex-stratified analyses in uncovering novel potential disease loci.

There are a number of potential candidate genes within the associated locus, and rs34331204 was a strong eQTL for eight of them, complicating the picture. Among the implicated genes, *ANKMY2* and *BZW2* showed some weak evidence of association with tangle burden. It should be noted that the gene expression effects of these two genes were observed among females rather than males, counter to the male-specific SNP effects. The female-specific gene expression association may suggest that there is a male-specific eQTL effect (which we could not test from available data as Braineac does not offer results stratified by sex), or that these two genes are not the functional genes driving the male-specific association. However, given the sex x gene expression interaction was not significant, and there are more females than males in the ROS/MAP

expression sample, it is probably safest to assume the gene expression effect is not sex-specific while the SNP effect is male-specific.

Both implicated genes, *ANKMY2* and *BZW2*, are interesting candidates. *BZW2* is a basic leucine zipper protein with a known role in cell proliferation through the Akt/mTOR pathway, particularly in cancer (Cheng *et al.*, 2017). Associations between dual leucine zipper proteins and neurodegenerative disease have been reported in the literature recently (Le Pichon *et al.*, 2017). While no functional association between *BZW2* and neurodegeneration has been reported to date, it is notable that a SNP within *BZW2* (rs58370486) previously showed an association with cognitive decline in AD ($p=6x10^{-11}$) (Sherva *et al.*, 2014). The protein product of *ANKMY2* has been shown to interact with FKBP38 in the mouse brain, regulating the Sonic hedgehog signaling pathway (Saita *et al.*, 2014), but FKBP38 also acts as a BCL2 chaperone which has been implicated in an apoptotic pathway downstream of amyloidosis (Kudo *et al.*, 2012). However, *ANKMY2* has not been directly implicated in AD previously. The present results suggest future functional and fine-mapping work in the rs34331204 region should focus on potential sex-specific effects.

This study had multiple strengths including the large sample size gathered across multiple cohort studies, the comprehensive validation analyses in independent studies using complementary endophenotypes of amyloidosis, tau, neurodegeneration, and cognition, and the functional assessment of gene expression in prefrontal cortex tissue providing evidence of sex-specific associations at the gene level. However, there were also important limitations, including the noted age difference between males and females, the high percentage of AD cases, and the high percentage of *APOE* ε4 carriers

within the leveraged datasets. These limitations leave open the strong possibility that additional sex-specific genetic loci for amyloid and tau pathology, particularly in the preclinical stages of disease, were probably undetected in our analyses. Further, the present analyses were restricted to individuals of European ancestry, leaving open the possibility that findings may not extend to other racial or ancestral backgrounds. Future work extending to datasets with more cognitively normal individuals and a more representative sample will be important to better understand sex-specific associations across the spectrum of normal aging and dementia. Nevertheless, our results highlight a novel sex-specific candidate locus for AD and demonstrate the utility of incorporating sex considerations into genetic models of disease.

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6. Potential Conflicts of Interest

Dr. Larson reports royalties from UpToDate. Dr. Schneider reports personal fees from Avid Radiopharmaceuticals, personal fees from Navidea Biopharmaceuticals, outside the submitted work. Dr. Zetterberg has served at advisory boards of Eli Lilly, Roche Diagnostics and Pharmasum Therapeutics and is one of the founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. Dr. Blennow has served at advisory boards of Alzheon, BioArctic, Eli Lilly, IBL International, Fujirebio, Merck, and Roche Diagnostics and is one of the founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

7. Tables

Table 1. Associations between rs34331204 and Relevant Alzheimer's Disease Endophenotypes

Outcome	N	Males		Females		Sex-Interaction	
		Beta	P	Beta	P	Beta	P
CSF Tau	2926	0.006	0.69	-0.009	0.58	-0.013	0.55
CSF P-tau	2759	-0.002	0.89	-0.011	0.47	-0.010	0.63
Episodic Memory	1182	0.104	0.14	0.038	0.73	-0.063	0.62
Executive Function	1182	**0.266**	**0.0010**	-0.016	0.88	**-0.283**	**0.039**
Hippocampal Volume	1086	**252.17**	**0.014**	-33.18	0.80	-284.70	0.09
Age of Onset in ADGC	17603	-0.091	0.052	0.043	0.27	**0.136**	**0.022**
Age of Onset in GERAD	3552	0.076	0.47	0.077	0.35	0.015	0.91

Boldface font signifies p<0.05.

Table 2. Associations between Tau Load and rs34331204 cis Gene Expression in Brain Tissue

Gene	Males		Females		Sex-Interaction	
	Beta	P	Beta	P	Beta	P
AGR2	-2.069	0.59	-0.824	0.60	1.263	0.76
AHR	-0.233	0.42	0.189	0.29	0.420	0.21
ANKMY2	-0.037	0.22	-0.078	9.93×10^{-4}	-0.041	0.28
BZW2	-0.027	0.48	-0.093	2.02×10^{-3}	-0.064	0.18
ISPD	-0.395	0.18	-0.537	0.018	-0.145	0.70
TSPAN13	-0.001	0.94	-0.010	0.049	-0.010	0.30

Gene expression data was collected from prefrontal cortex tissue of participants from the Religious Orders Study/Memory and Aging Project (Males: n=213, Females: n=380).

8. Figure Legends

Fig. 1. Sex-Stratified Genome-Wide Association Results for Tangle Positivity. (a) Miami plot illustrating neurofibrillary tangle positivity genome-wide association results stratified by males and females. Male findings are plotted in blue and grey on the top and female results are plotted in pink and grey at the bottom. The red lines at the top and bottom represent the genome-wide threshold for statistical significance ($p<5x10-8$). Regional association plots for the rs34331204 association with neurofibrillary tangle positivity within **(b)** males and **(c)** females.

Fig. 2. Male-Specific SNP (rs34331204) Associated with Protection from Neurofibrillary Tangles Also Relates to Hippocampal Volume and Executive Function. Sex-specific association of rs34331204 with neurofibrillary tangles (Panel A), hippocampal volume (Panel B), and executive function (Panel C). Within each panel, males are presented on the left and females are presented on the right. Outcomes are presented on the y-axes. Bar colors represent rs34331204 genotype. Homozygous carriers of the A allele are presented in dark green on the left, heterozygotes in light green in the middle, and homozygous carriers of the C allele in the lightest green on the right. A neuroprotective effect of the rs34331204 C allele is observed among males, but not females.