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REPORT

Sex differences in the genetic predictors of Alzheimer's pathology

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Autopsy measures of Alzheimer's disease neuropathology have been leveraged as endophenotypes in previous genome-wide association studies (GWAS). However, despite evidence of sex differences in Alzheimer's disease risk, sex-stratified models have not been incorporated into previous GWAS analyses. We looked for sex-specific genetic associations with Alzheimer's disease endophenotypes from six brain bank data repositories. The pooled dataset included 2701 males and 3275 females, the majority of whom were diagnosed with Alzheimer's disease at autopsy (70%). Sex-stratified GWAS were performed within each dataset and then meta-analysed. Loci that reached genome-wide significance ($P < 5 \times 10^{-8}$) in stratified models were further assessed for sex interactions. Additional analyses were performed in independent datasets leveraging cognitive, neuroimaging and CSF 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 ($P = 2.5 \times 10^{-8}$) but not females ($P = 0.85$, sex-interaction $P = 2.9 \times 10^{-4}$). In follow-up analyses, rs34331204 was also associated with hippocampal volume, executive function, and age-at-onset only among males. 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.

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Abbreviations: eQTL = expression quantitative trait locus; GWAS = genome-wide association studies; NFT = neurofibrillary tangles

Introduction

Two-thirds of Alzheimer's disease cases are female (Mielke *et al.*, 2014; Mazure and Swendsen, 2016) and emerging evidence has highlighted notable sex differences in Alzheimer's disease 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 Alzheimer's disease shows a stronger association among females compared to males, particularly between ages 65 and 75 years (Neu *et al.*, 2017). Despite growing evidence of sex differences in the genetic drivers of Alzheimer's disease (Deming *et al.*, 2018), limited work has systematically explored sex-specific genetic associations with Alzheimer's disease 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 genome-wide association studies (GWAS) to identify novel genetic loci for Alzheimer's disease (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 sex-specific analyses because identified associations will highlight points along the disease cascade where sex differences emerge.

This study leverages six autopsy cohorts to assess sex-specific genetic associations with Alzheimer's disease neuropathology. First, we perform a sex-stratified GWAS in 5976 participants with autopsy measures of plaques and 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 Alzheimer's disease.

The identification of sex-specific effects will advance our understanding of the genetic architecture of Alzheimer's disease.

Materials and methods

Participants

Data were drawn from a previous GWAS (Beecham *et al.*, 2014), which provided detailed descriptions of the following six well-characterized autopsy cohorts: 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). All participants agreed to brain donation and were evaluated at each site. Alzheimer's disease diagnoses were made through consensus criteria excluding participants with a documented history of stroke, substantial cerebrovascular disease, if they met criteria for another dementia/aphasia, or with an active neurological disease or medication/medical co-morbidity that may impact cognition (McKhann *et al.*, 1984, 2011). All neuropathological data were reviewed and harmonized by a single neuropathologist.

Quantification of neuropathology outcomes

Autopsy measures of neurofibrillary tangles (Braak staging) and neuritic plaques (CERAD score) were collected and harmonized previously (Beecham *et al.*, 2014). Thal stage was not collected or included in our staging definitions. Both measures were analysed as binary outcomes. Binary neuritic plaque status was defined based on established neuropathological criteria for Alzheimer's disease (Hyman *et al.*, 2012) whereby a CERAD score of 'none' or 'sparse' was considered 'neuritic plaque negative', and 'moderate' or 'frequent' was considered 'neuritic plaque positive'. Similarly, the binary neurofibrillary tangles (NFT) status was defined whereby Braak stages 0/I/II were considered 'NFT negative' and stages III/IV/V/VI were considered 'NFT positive' (Hyman *et al.*, 2012).

Genotyping and quality control

Genome-wide genotyping was carried out by each study on a variety of platforms. All participants were of European descent, and all genotype data were processed using the same imputation and standard quality control protocols (Supplementary material).

Statistical analyses

Descriptive statistics were quantified in R v3.3.1 (https://www.r-project.org/). Sex-stratified analyses of NFT and neuritic plaques were performed using logistic regression in PLINK (https://www.cog-genomics.org/plink/1.9). Analyses were carried out within each cohort, 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). Meta-analysis results were limited to SNPs (single nucleotide polymorphisms) that were genotyped or imputed, passed quality control, and were polymorphic in at least three of the six cohorts. Statistical significance was set at the standard GWAS level ($\alpha = 5 \times 10^{-8}$). All significant sex-stratified effects were assessed for sex × SNP interactions. Miami plots were generated using EasyStrata v16.0 (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 (v2018Apr16). Forest plots were generated using the R package Metafor.

Six haplotype tagging SNPs were used to test for associations between the *MAPT* locus and NFT positivity. Tags included the H1 haplotype (rs8070723) and H1 sub-haplotypes (rs1467967, rs242557, rs3785883, rs2471738, and rs7521) (Pittman *et al.*, 2005; Höglinger *et al.*, 2011).

Analysis of Alzheimer's disease endophenotypes

SNPs with sex-specific associations were further assessed for correlation with relevant Alzheimer's disease endophenotypes using linear regression in R, co-varying 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 Alzheimer's disease (Huang *et al.*, 2017). See Supplementary material for additional details.

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.

Significant eQTL genes were further assessed for sex-specific associations with Alzheimer's disease neuropathology leveraging prefrontal cortex gene expression data from ROS/MAP participants at autopsy (Supplementary material).

Results

A total of 2701 males and 3275 females across six independent autopsy datasets were analysed. In general, females were older than males (males: 79 ± 9 years, females: 81 ± 9 years, $P < 0.001$) and were more frequently Alzheimer's disease cases (males: 68%, females: 71%, $P = 0.02$), *APOE* $\varepsilon4$ carriers (males: 49%, females: 46%, $P = 0.03$), and neuritic plaque- 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 intergenic SNP on chr7p21.1 (rs34331204; Fig. 1 and Supplementary Fig. 2) outside of the *APOE* locus reached

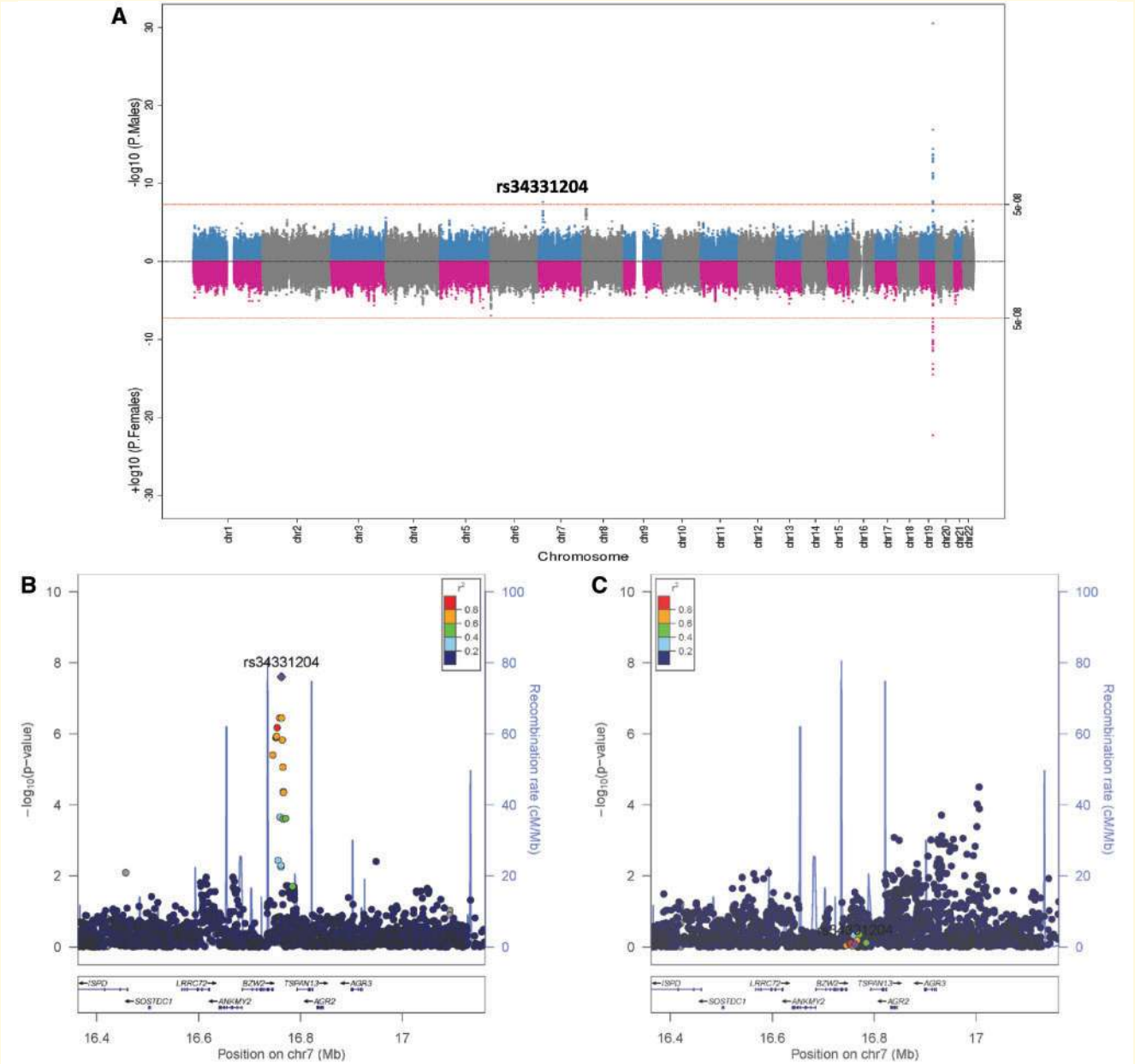

Figure 1 Sex-stratified genome-wide association results for tangle positivity. (**A**) Miami plot illustrating neurofibrillary tangle positivity genome-wide association results stratified by males and females. Male findings are plotted in blue and grey on the *top* and female results are plotted in pink and grey at the *bottom*. The red lines represent the genome-wide threshold for statistical significance ($P < 5 \times 10^{-8}$). Regional association plots for the rs34331204 association with neurofibrillary tangle positivity within (**B**) males and (**C**) females.

genome-wide significance in males ($\beta = -0.720$; $P = 2.48 \times 10^{-8}$) but not in females ($\beta = -0.027$; $P = 0.85$). Furthermore, rs34331204 showed an interaction with sex on NFT ($\beta = 0.71$; $P = 2.93 \times 10^{-4}$), whereby the minor allele C (MAF = 0.07) 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 previously published GWAS that did not incorporate sex-stratified models ($\beta = -0.39$; $P = 2.63 \times 10^{-5}$). Additional models using different Braak staging cut-off points are presented in Supplementary Figs 3–6.

No associations reached genome-wide significance in the sex-stratified GWAS analysis of neuritic plaque (Supplementary Fig. 7). The top meta-analysis results from sex-stratified GWAS of neuritic plaque and NFT are presented in Supplementary Tables 2–5.

As expected, a strong signal was seen at the *APOE* locus in all four sex-stratified GWAS (*P*-values $< 4.96 \times 10^{-20}$) (Fig. 1 and Supplementary Fig. 7). As the sex-specific effect of *APOE* on Alzheimer's disease biomarkers has been previously reported in detail (Hohman *et al.*, 2018), our focus was on associations outside of the *APOE* locus. However,

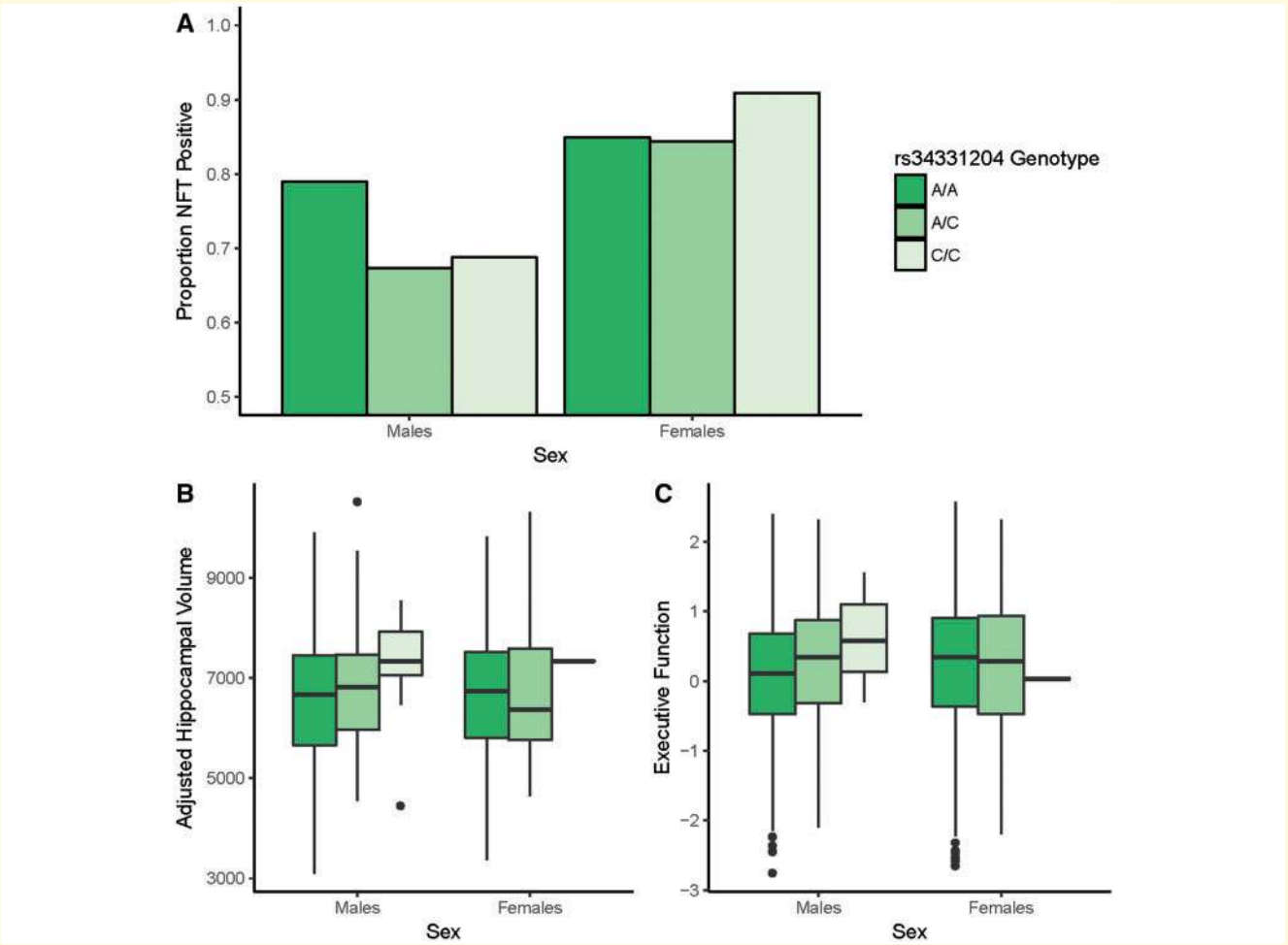

Figure 2 Male-specific SNP (rs34331204) associated with protection from neurofibrillary tangles also relates to hippocampal volume and executive function. Sex-specific association of rs34331204 with (**A**) NFT, (**B**) hippocampal volume, and (**C**) executive function. Within each panel, males are presented on the *left* and females on the *right*. Outcomes are presented on the *y*-axes. Bar colours 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.

as it is possible that some significant signal(s) in the *APOE* locus could be independent of *APOE* haplotype, we co-varied for both *APOE* ε4 and ε2 allele carrier status. All signals in the *APOE* locus were strongly attenuated after co-varying for *APOE* haplotype (*P*-values $> 1.46 \times 10^{-3}$) (Supplementary Figs 8 and 9).

Another candidate locus we evaluated was *MAPT*, which encodes the tau protein. Of the six *MAPT* locus haplotype tagging SNPs tested, rs242557 was nominally associated with NFT positivity in males ($P = 0.0043$) but not in females ($P = 0.30$; sex-interaction $P = 0.015$) (Supplementary Table 6).

The putative sex-specific GWAS locus (rs34331204) was then assessed for associations with cognition, amyloidosis, neurodegeneration, and age-at-onset using publicly available data sources. Results are presented in Table 1. SNP 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 analysed 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 7). 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.

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**	**0.001**	−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	17 603	−0.091	0.052	0.043	0.27	**0.136**	**0.022**
Age of onset in CERAD	3552	0.076	0.47	0.077	0.35	0.015	0.91

Bold 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 were collected from prefrontal cortex tissue of participants from the Religious Orders Study/Memory and Aging Project (males: $n = 213$, females: $n = 380$).

Discussion

The present study evaluated sex-specific genetic associations with Alzheimer's disease 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 follow-up 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 × 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 Alzheimer's disease ($P = 6 \times 10^{-11}$) (Sherva *et al.*, 2014). The protein product of *ANKMY2* has been shown to interact with FKBP38 in the mouse brain, regulating the Sonic hedgehog signalling pathway (Saita *et al.*, 2014), but FKBP38 also acts as a BCL2 chaperone that has been implicated in an apoptotic pathway downstream of amyloidosis (Kudo *et al.*, 2012). However, *ANKMY2* has not been directly implicated in Alzheimer's disease previously. The present results suggest future functional and fine-mapping work in the rs34331204 region should focus on potential sex-specific effects.

In addition to the GWAS associations, we also observed weak evidence of a male-specific association between an SNP (rs242557) that partially tags the H1c sub-haplotype and tangle burden. This sub-haplotype has been associated with increased risk of Alzheimer's disease (Myers *et al.*, 2005) and increased expression of tau (Myers *et al.*, 2007) in previous work, and our findings suggest a possible sex difference may contribute to this effect.

It is important to note that our results provide evidence of a sex-specific effect of rs34331204 on tangle load at

autopsy, but not on CSF tau or CSF p-tau (Table 1). Furthermore, our results do not appear to overlap with those of a recent large sex-specific GWAS of CSF Alzheimer's disease biomarkers (Deming *et al.*, 2018). Deming and colleagues identified three female-specific associations with CSF biomarker levels, but none of these loci showed evidence of sex-specific associations with the relevant neuropathology in this study (Supplementary Table 8). Interestingly, in a previous study, we observed a similar lack of consistency across autopsy and CSF datasets for sex differences in the *APOE* association, whereby sex-specific effects of *APOE* ε4 on CSF tau, but not on autopsy measures of neurofibrillary tangles, were seen (Hohman *et al.*, 2018). It is also notable that the loci identified in GWAS for CSF biomarkers and autopsy measures of neuropathology (across sexes) similarly did not show overlap with one another, highlighting that the discrepant results are characteristic of the endophenotypes rather than the sex-specific analytical pipelines. While the differing results between CSF tau and autopsy measures of tangles seem counterintuitive, there are multiple contributing factors. First, autopsy staging of tangles reflects a process that is distinct from CSF biomarkers of tau. Autopsy measures (and PET tau measures) are best characterized as 'stage' markers that signify how far the disease process has progressed, while CSF biomarkers are 'state' biomarkers that appear to measure the intensity of the disease process (Blennow and Hampel, 2003; Mattsson *et al.*, 2017). In addition to the different biological processes that are tagged by autopsy and CSF metrics, there are also notable cohort differences between the CSF datasets and autopsy datasets that could contribute to the discrepancy. The autopsy datasets included here were older on average and include a higher proportion of individuals with clinical Alzheimer's disease compared to the younger cohorts evaluated in the previous CSF studies. It is certainly possible that the genetic architecture of Alzheimer's disease neuropathology is different at older ages than at younger ages, and different during the preclinical stages of disease compared to end stage disease. Ultimately, larger CSF and autopsy datasets will be needed to disentangle the complex contributors to discrepant signals in CSF and autopsy-based endophenotype analyses.

This study had multiple strengths including the large sample size gathered across multiple cohort studies, the comprehensive follow-up 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 Alzheimer's disease 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 ageing and dementia. Nevertheless, our results highlight a novel sex-specific candidate locus for Alzheimer's disease and demonstrate the utility of incorporating sex considerations into genetic models of disease.

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Competing interests

E.B.L. reports royalties from UpToDate. J.A.S. reports personal fees from Avid Radiopharmaceuticals, personal fees from Navidea Biopharmaceuticals, outside the submitted work. H.Z. 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. K.B. 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.

Supplementary material

Supplementary material is available at *Brain* online.

References

Altmann A, Tian L, Henderson VW, Greicius MD. Sex modifies the APOE-related risk of developing Alzheimer disease. Ann Neurol 2014; 75: 563–73.

Apostolova LG, Dinov ID, Dutton RA, Hayashi KM, Toga AW, Cummings JL, et al. 3D comparison of hippocampal atrophy in amnestic mild cognitive impairment and Alzheimer's disease. Brain 2006; 129: 2867–73.

Barnes LL, Wilson RS, Bienias JL, Schneider JA, Evans DA, Bennett DA. Sex differences in the clinical manifestations of Alzheimer disease pathology. Arch Gen Psychiatry 2005; 62: 685–91.

Beecham GW, Hamilton K, Naj AC, Martin ER, Huentelman M, Myers AJ, et al. Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. PLoS Genet 2014; 10: e1004606.

Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol 2003; 2: 605–13.

Buckley RF, Mormino EC, Amariglio RE, Properzi MJ, Rabin JS, Lim YY, et al. Sex, amyloid, and APOE ε4 and risk of cognitive decline in preclinical Alzheimer's disease: findings from three well-characterized cohorts. Alzheimers Dement 2018; 14: 1193–203.

Cheng DD, Li SJ, Zhu B, Yuan T, Yang QC, Fan CY. Downregulation of BZW2 inhibits osteosarcoma cell growth by inactivating the Akt/mTOR signaling pathway. Oncol Rep 2017; 38: 2116–22.

Deming Y, Dumitrescu L, Barnes LL, Thambisetty M, Kunkle B, Gifford KA, et al. Sex-specific genetic predictors of Alzheimer's disease biomarkers. Acta Neuropathol 2018; 136: 857–72.

Höglinger GU, Melhem NM, Dickson DW, Sleiman PMA, Wang L-S, Klei L, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. Nat Genet 2011; 43: 699.

Hohman TJ, Dumitrescu L, Barnes LL, Thambisetty M, Beecham GW, Kunkle B, et al. Sex-specific effects of Apolipoprotein E with cerebrospinal fluid levels of tau. JAMA Neurol 2018; 75: 989–98.

Hua X, Hibar DP, Lee S, Toga AW, Jack CR, Weiner MW, et al. Sex and age differences in atrophic rates: an ADNI study with n = 1368 MRI scans. Neurobiol Aging 2010; 31: 1463–80.

Huang K-L, Marcora E, Pimenova A, Di Narzo A, Kapoor M, Jin SC, et al. A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheime's disease. Nat Neurosci 2017; 20: 1052.

Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 2012; 8: 1–13.

Koran MI, Wagener MA, Hohman TJ. Sex differences in the association between AD biomarkers and cognitive decline. Brain Imaging Behav 2017; 11: 205–13.

Kudo W, Lee HP, Smith MA, Zhu X, Matsuyama S, Lee Hg. Inhibition of Bax protects neuronal cells from oligomeric Aβ neurotoxicity. Cell Death Dis 2012; 3: e309.

Le Pichon CE, Meilandt WJ, Dominguez S, Solanoy H, Lin H, Ngu H, et al. Loss of dual leucine zipper kinase signaling is protective in animal models of neurodegenerative disease. Sci Transl Med 2017; 9: eaag0394.

Mägi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. BMC Bioinform 2010; 11: 288.

Mattsson N, Schöll M, Strandberg O, Smith R, Palmqvist S, Insel PS, et al. 18F-AV-1451 and CSF T-tau and P-tau as biomarkers in Alzheimer's disease. EMBO Mol Med 2017; 9: 1212–23.

Mazure CM, Swendsen J. Sex differences in Alzheimer's disease and other dementias. Lancet Neurol 2016; 15: 451–2.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34: 939.

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheime's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer Dement 2011; 7: 263–9.

Mielke MM, Vemuri P, Rocca WA. Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. Clin Epidemiol 2014; 6: 37–48.

Myers AJ, Kaleem M, Marlowe L, Pittman AM, Lees AJ, Fung HC, et al. The H1c haplotype at the MAPT locus is associated with Alzheimer's disease. Hum Mol Genet 2005; 14: 2399–404.

Myers AJ, Pittman AM, Zhao AS, Rohrer K, Kaleem M, Marlowe L, et al. The MAPT H1c risk haplotype is associated with increased expression of tau and especially of 4 repeat containing transcripts. Neurobiol Dis 2007; 25: 561–70.

Neu SC, Pa J, Kukull W, Beekly D, Kuzma A, Gangadharan P, et al. Apolipoprotein E genotype and sex risk factors for Alzheimer disease: a meta-analysis. JAMA Neurol 2017; 74: 1178–89.

Pittman AM, Myers AJ, Abou-Sleiman P, Fung HC, Kaleem M, Marlowe L, et al. Linkage disequilibrium fine mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration. J Med Genet 2005; 42: 837–46.

Saita S, Shirane M, Ishitani T, Shimizu N, Nakayama KI. Role of the ANKMY2-FKBP38 axis in regulation of the Sonic hedgehog (Shh) signaling pathway. J Biol Chem 2014; 289: 25639–54.

Sherva R, Tripodis Y, Bennett DA, Chibnik LB, Crane PK, de Jager PL, et al. Genome-wide association study of the rate of cognitive decline in Alzheimer's disease. Alzheimers Dement 2014; 10: 45–52.

Voevodskaya O, Simmons A, Nordenskjold R, Kullberg J, Ahlstrom H, Lind L, et al. The effects of intracranial volume adjustment approaches on multiple regional MRI volumes in healthy aging and Alzheimer's disease. Front Aging Neurosci 2014; 6: 264.

Winkler TW, Kutalik Z, Gorski M, Lottaz C, Kronenberg F, Heid IM. EasyStrata: evaluation and visualization of stratified genome-wide association meta-analysis data. Bioinformatics 2014; 31: 259–61.