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Sex-dependent autosomal effects on clinical progression of Alzheimer's disease

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#Deceased.

Sex differences in the manifestations of Alzheimer's disease are under intense investigation. Despite the emerging importance of polygenic predictions for Alzheimer's disease, sex-dependent polygenic effects have not been demonstrated. Here, using a sex crossover analysis, we show that sex-dependent autosomal genetic effects on Alzheimer's disease can be revealed by characterizing disease progress via the hazard function. We first performed sex-stratified genome-wide associations, and then applied derived sex-dependent weights to two independent cohorts. Relative to sex-mismatched scores, sex-matched polygenic hazard scores showed significantly stronger associations with age-at-disease-onset, clinical progression, amyloid deposition, neurofibrillary tangles, and composite neuropathological scores, independent of apolipoprotein E. Models without using hazard weights, i.e. polygenic risk scores, showed lower predictive power than polygenic hazard scores with no evidence for sex differences. Our results indicate that revealing sex-dependent genetic architecture requires the consideration of temporal processes of Alzheimer's disease. This has strong implications not only for the genetic underpinning of Alzheimer's disease but also for how we estimate sex-dependent polygenic effects for clinical use.

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Abbreviations: ADGC = Alzheimer's Disease Genetic Consortium; GWAS = genome-wide association studies; NACC = National Alzheimer's Coordinate Center; f/mPHS = female/male polygenic hazard score; PRS = polygenic risk score; ROSMAP = Religious Orders Study and Rush Memory and Aging Project; SNP = single nucleotide polymorphism

Introduction

Sex, as both an endogenous and an exogenous factor modulating human biology, has a ubiquitous impact on the pathogenesis of complex diseases (Khramtsova *et al.*, 2019). Evidence on sex-dependent clinicopathological progressions of Alzheimer's disease is just beginning to emerge (Ferretti *et al.*, 2018). Compared to males, females show later manifestation of verbal memory deficits, faster decline after disease onset (Caldwell *et al.*, 2017), and some differences in neuropathological characteristics, such as tau tangle density (Damoiseaux *et al.*, 2012; Oveisgharan *et al.*, 2018). Results from studies on incidence rate and prevalence are less consistent (Winblad *et al.*, 2016; Nebel *et al.*, 2018), yet females are often reported to have increased incidence of Alzheimer's disease in older ages (Ruitenberg *et al.*, 2001) and higher prevalence (Mazure and Swendsen, 2016). Although some studies have suggested sex-dependent autosomal effects on Alzheimer's disease pathologies (Cellini *et al.*, 2009; Li *et al.*, 2017; Deming *et al.*, 2018), sex-dependent differences in polygenic effects remain unresolved. So far only apolipoprotein E (*APOE* ε4) has been found to have a differential impact on age-at-onset between males and females (Farrer, 1997; Altmann *et al.*, 2014), despite evidence suggesting that Alzheimer's disease is highly polygenic, with a heritability as high as 79% (Gatz *et al.*, 2006). Given this unmet need for better understanding of sex differences in Alzheimer's disease, we wanted to investigate whether there is sex-dependent genetic risk on disease processes of Alzheimer's disease.

This sex-agnostic *status quo* is particularly problematic for disease prediction based on polygenic effects. By aggregating the estimated regression weights of autosomal single nucleotide polymorphisms (SNPs) from genome-wide association studies (GWAS), polygenic scores have been used to assist in several important clinical functions, including disease prediction (Khera *et al.*, 2018), risk stratification (Torkamani *et al.*, 2018), enriching clinical trials (Tan *et al.*, 2018, 2019), and facilitating disease screening (Seibert *et al.*, 2018). However, because the standard practice in GWAS is to treat sex as a confounding factor for autosomal effects, the basis of polygenic scores, the estimated odds ratios are devoid of sex-dependent effects. Given the complexity of the moderating effects of sex on disease aetiology (Khramtsova *et al.*, 2019), applying sex-agnostic polygenic scores may produce substantially biased risk quantifications. Such scores could underestimate the genetic risk of Alzheimer's disease for females, since *APOE* ε4, one of the most well-established risk factors for Alzheimer's disease, has stronger effects on Alzheimer's disease onset among females than among males (Altmann *et al.*, 2014). Given the heightened awareness of utilizing polygenic effects beyond *APOE* as biomarkers for Alzheimer's disease (Sabuncu *et al.*, 2012; Escott-Price *et al.*, 2015; Mormino *et al.*, 2016; Desikan *et al.*, 2017; Ge *et al.*, 2018; Tan *et al.*, 2019), understanding the sex-dependent polygenic effects for Alzheimer's disease is imperative for their application to clinical settings.

To investigate whether there are sex-dependent polygenic effects in addition to *APOE*, we performed a sex crossover study (see the 'Materials and methods' section and Fig. 1) whereby we derived polygenic scores from separate GWAS on males and females in the training cohorts (Alzheimer's Disease Genetic Consortium, ADGC, $n = 17\,855$; see the 'Materials and methods' section and Table 1), and then applied each of the sex-dependent regression weights to both males and females in independent cohorts (National Alzheimer's Coordinate Center cohort, NACC, $n = 6076$; Religious Orders Study and Rush Memory and Aging Project, ROSMAP, $n = 599$) to determine if there was differential performance in predicting Alzheimer's disease. Importantly, as the sex differences in Alzheimer's disease onset can be the end results of complex interplays between pathological process and cognitive resilience (Cellini *et al.*, 2009; Caldwell *et al.*, 2017; Li *et al.*, 2017; Deming *et al.*, 2018; Ferretti *et al.*, 2018), we focused our validation on predictive performance in different aspects of Alzheimer's disease manifestations, i.e. the age of Alzheimer's disease onset, cognitive decline, and neuropathological findings.

Materials and methods

Study design

The crossover analysis is illustrated in Fig. 1. The training samples and validating cohorts are described in the following section and in Table 1. In all of our analyses, we restricted our sample to participants with European ancestry only, as specified in previous main GWAS results of the ADGC (Naj *et al.*, 2011). We further used the allele frequency spectrums to calculate the proportion of global ancestry and excluded any individuals with less than 80% of European genetic ancestry (Chen *et al.*, 2013). We also excluded individuals with more than 20% of missing genotypes and individuals with discordant self-report and genetically inferred sex. For SNP quality control, we filtered SNPs based on minor allele frequencies $>1\%$, in Hardy-Weinberg equilibrium (HWE P-value $> 1 \times 10^{-7}$) and missing rate $<10\%$.

First, we performed sex-stratified genome-wide analyses on Alzheimer's disease, using imputed genotypes and phenotypic data from the ADGC (Naj *et al.*, 2011; Lambert *et al.*, 2013; Kunkle *et al.*, 2019). The ADGC datasets consist of case-control, prospective, and family based sub-studies of participants with Alzheimer's disease occurrence after age 60 years old derived from Alzheimer's Disease Centres across the USA and healthy controls from the general community, enrolled from 1984 to 2012. Participants with *APP*, *PSEN1*, and *PSEN2* were

excluded. The training samples contain ADGC Phase 1 and Phase 2 data, excluding individuals from the National Institute of Aging Alzheimer's Disease Center (NIA ADC) and ROSMAP. To ensure independence between the training and validation cohorts, we performed an extensive check on potential sample overlap and removed any overlapping individuals from the training data. The final training data included 7158 males and 10 697 females (Table 1). Genome-wide Cox regression analyses were performed on males and females separately to obtain sex-dependent weights. Detailed descriptions of the analytical methods can be found in the following section and in the Supplementary material.

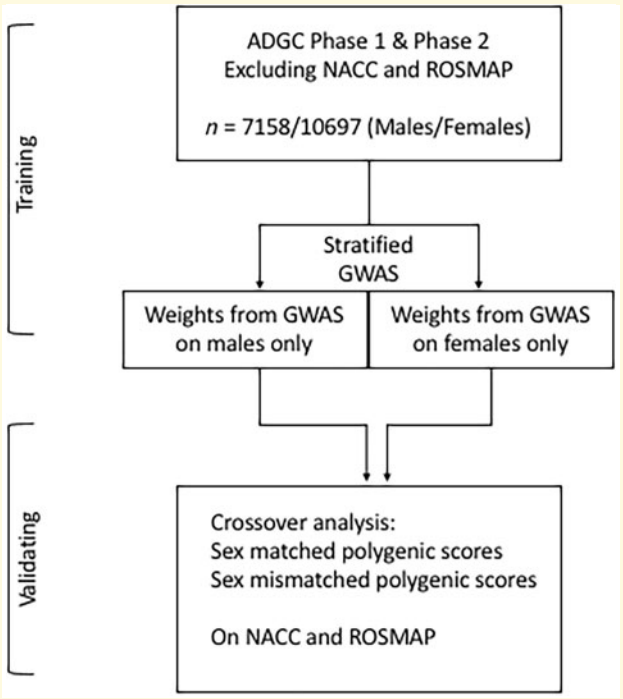

Figure 1 Flow chart of the sex crossover analysis. We stratified the ADGC cohort by sex and performed a GWAS on males and females separately to obtain sex-specific weights for PHS and PRS. We then generated the sex matched and sex mismatched PHS and PRS on the validation datasets, i.e. NACC and ROSMAP. We assessed the predictive performances of sex-matched and sex-mismatched PHS and PRS on Alzheimer's disease processes in using NACC and ROSMAP.

After obtaining the sex-dependent Cox regression weights for each autosomal SNP from the ADGC data, we applied these weights to two independent cohorts (Table 1), generating male-dependent polygenic hazard score (mPHS) and female-dependent PHS (fPHS) for every participant. Thus, we can compare whether sex-matched models (mPHS on males and fPHS on females) have better predictive power than sex-mismatched models (fPHS on males and mPHS on females), as a cross-over comparison (Fig. 1).

The first independent cohort was obtained from the NACC. The NACC recruits case series as a nationwide recruiting effort funded by the NIA, involving clinical centres across the USA. Given the longitudinal design of the NACC, we examined whether sex-matched PHS predicted dementia onset better than sex-mismatched PHS. The cohort characteristics of the NACC can be found in Table 1.

The second independent cohort was the ROSMAP. ROS and MAP are two community-based cohort studies that enrolled individuals without dementia, all of whom agreed to longitudinal follow-up and organ donation, enabling us to examine the distribution of neuropathology among participants as a function of sex-specific PHS. All participants signed an informed consent, Anatomic Gift Act, and repository consent allowing their data to be shared. Both studies were approved by an Institutional Review Board of Rush University. Details of the studies, generation of genomic data, and neuropathological data collection have been previously reported (Bennett *et al.*, 2018; De Jager *et al.*, 2018). We investigated whether sex-matched PHS has stronger associations with neuropathology in the brain than sex-mismatched PHS. Those who have both genotyping data and autopsy results were included in this analysis ($n = 599$). Detailed characteristics of ROSMAP can be found in Table 1.

For comparison purposes, we also examined the performance of polygenic risk scores (PRS) in the same manner as described above, except using weights from logistic regressions while controlling for age-at-ascertainment. This is intended to investigate the benefit of using Cox regressions in contrast to the standard GWAS approach.

Estimating sex-dependent hazards for autosomal SNPs

To obtain sex-dependent weights for each SNP, we fitted genome-wide Cox regression models on males and females separately. This stratified approach was intended to capture sex-specific effects from autosomal SNPs without explicitly modelling interaction terms. This stratified approach also allows for differences in the shape of the baseline hazard function

Table 1 Characteristics of training samples and independent validating cohorts

	Training samples		Independent validation cohorts			
	ADGC[a]		NACC		ROSMAP	
	Males	Females	Males	Females	Males	Females
Total *n*	7158	10697	2628	3448	220	379
Age, years (SD)	75.4 (7.7)	75.9 (8.2)	78.6 (9.4)	79.1 (9.8)	86.4 (6.3)	89.4 (6.2)
Alzheimer's disease cases/events	42.7%	47.6%	52.3%	41.5%	37.7%	43.8%
APOE ε4 carriers	40.9%	43.3%	40.9%	37.9%	29.5%	28.4%

[a]Excluded any overlapping samples with NIA ADCs and ROSMAP.

between males and females. As noted in prior studies on sex-dependent genetic effects (Khramtsova *et al.*, 2019), although the total sample size for GWAS is thus reduced by half, stratified models are computationally simple and avoid the need for additional assumptions on the nature of sex interactions. Furthermore, hazard ratio estimation is facilitated by utilizing Martingale residuals under null (Therneau *et al.*, 1990):

$$\hat{\beta} = (x^T x)^{-1} x^T M_0 \quad (1)$$

where x is the mean centred genotype dosage and M_0 is the Martingale residuals of the null model. More detailed discussion about the hazard estimates from case-control studies can be found in the Supplementary material.

For ADGC data, we used the age-at-onset as the time-to-event and the age-at-last-visit as the censoring time for Cox regression while controlling for dosages of *APOE* ε2 and ε4, the first five genetic principal components, and indicators of recruiting sites. In addition to filtering SNPs that failed quality controls, we also filtered SNPs located outside of *APOE* (19q13.32) and major histocompatibility complex regions, resulting in 6 784, 887 imputed SNPs in our analyses. The resulting male- and female-derived hazard ratios were used to generate the corresponding sex-dependent PHS. For comparison purposes, we also performed standard GWAS with logistic regressions for the same 6 784, 887 SNPs. All covariates are the same in the models except age-at-last-visit is now treated as one of the covariates. The estimated sex-dependent odds ratios were then used to generate the corresponding PRS. Because our focus was on polygenic effects over and above the effects of *APOE*, we excluded any SNPs located within *APOE* region when we calculated all polygenic scores.

Deriving polygenic hazard scores and polygenic risk scores

The polygenic scores are the product sum of GWAS obtained weights and genotypes of individuals in the two test cohorts:

$$S_i = \sum_{j=1}^{M} G_{ij}\beta_j \quad (2)$$

for individual i, the score S_i is the product sum of genotypes G_{ij} and weights β_j for M SNPs. To make PHS and PRS comparable, we used the identical pruning and clumping process to select independent SNPs for generating the scores. The parameters include clumping within 250 kb and linkage disequilibrium >0.1, resulting in 251 040 independent SNPs for generating the scores. Although our initial validation analyses indicated that the predictive performance can benefit slightly from imposing a liberal *P*-value threshold for SNP selection (*P*-value of 0.5; Supplementary Fig. 1), the potential for over-fitting by choosing optimal thresholding for each of the stratified models overshadowed the slight benefit of using *P*-value thresholding. Therefore, in the main analyses, we imposed no *P*-value thresholds to avoid using different numbers of SNPs between the PHS and the PRS, ensuring the comparisons were based on the signals from the same set of SNPs. Male-derived scores used weights for SNPs based on the GWAS of males in ADGC, and similarly, female-derived scores only used weights from GWAS of females in ADGC. Both male- and female-derived scores were then computed for each participant in the validation cohorts using the same autosomal SNPs. Crossover analyses can thus be used to compare the predictive performance of sex-matched vs. sex-mismatched scores in the validation cohorts.

Statistical analysis

We implemented genome-wide Cox regression for efficiently estimating hazard ratios across millions of SNPs. *P*-values of the Cox regressions were obtained using score tests (Chen *et al.*, 2014). The logistic regression GWAS were performed using PLINK. All genome-wide analyses were done using ADGC data, separately for males and females. To provide an intuitive interpretation on the obtained weights, we also calculated gene-based effect sizes using Pascal (Lamparter *et al.*, 2016). Pascal obtained gene-based *P*-values are based on a linkage-disequilibrium weighted average of effect sizes of SNPs located within 50 kb regions of the gene body.

Because sex is the matching factor, we included sex as covariates in all validation analyses to ensure the association signals are driven by the polygenic effects *per se*. In NACC, we used: (i) Cox regression to examine the predictive power of polygenic scores on Alzheimer's disease age-at-onset; and (ii) linear mixed effects model to examine the associations between polygenic scores and rate of clinical progression, defined as changes in Cognitive Dementia Rating – Sum of Boxes (CDR-SB). All models controlled for *APOE* status (dosages of ε2 and ε4) and education levels. The main analysis of NACC included 2628 males and 3448 females. We also examined whether the patterns of association remained constant if we restricted analyses to neuropathologically-confirmed cases; 817 males and 706 females from the NACC had post-mortem neuropathological examinations. To ensure the consistency of the units, all results are based on standardized polygenic scores, comparing changes in 1 standard deviation (SD) of scores.

In ROSMAP, we analysed the relationship between the neuropathological burden at autopsy and sex-dependent polygenic scores. Four quantifications of neuropathology were included, i.e. the percentage area occupied by amyloid-β, and the density of tau-positive neurofibrillary tangles. Because those neuropathological measures were skewed, we performed a square root transformation to normalize the neuropathology data. We also determined Braak stage, and Consortium to Establish a Registry for Alzheimer's disease (CERAD) score. All regression models controlled for *APOE* status (dosages of ε2 and ε4), age-at-death, and education level. To ensure the consistency of the units, all results are based on standardized polygenic scores and neuropathological data, comparing neuropathological variations in 1 SD of scores.

For all comparisons between sex-matched and sex-mismatched models, we used bootstrapping to calculate the 95% confidence intervals (CIs) and determine significance accordingly. We chose this approach because we wanted to specifically examine the differences in the polygenic estimation while controlling for all other potential confounds.

Data availability

The summary statistics for genome-wide hazard estimates and gene-based analyses can be found in the Supplementary material.

Results

Distribution of hazard weights

First, we performed genome-wide Cox regressions for Alzheimer's disease outcomes on ADGC individuals (males/females = 7158/10 697). The models controlled for first five genetic principal components, *APOE* status, and recruiting sites ('Materials and methods' section). The results showed different top hits between males and females (Fig. 2A and B). Males had a GWAS-significant locus on 1q32.2, encompassing *CR1*, and females had a GWAS-significant locus on 2q14.3, encompassing *BIN1*. In addition to GWAS-significant loci, polygenic signals below the GWAS-significant threshold are important for deriving polygenic scores. To provide an intuitive summary on the sex-dependent polygenic effects, we performed gene-based analyses using Pascal (Lamparter *et al.*, 2016). Figure 2C illustrates the sex-dependent distributions from gene-based analyses. Gene clusters on 19q13.32 continue to show consistent effects between males and females, with trends for sex-specific genetic effects. For example, the effect sizes of *BIN1*, *MS4A6A*, *DNAJA2*, and *FERMT2* are larger among females while *FAM193B*, *C2orf47*, *TYW5* have larger effect sizes among males. Additionally, the tau-related gene, *MAPT*, shows stronger effects on males than on females.

Predicting clinical manifestations in the NACC

By aggregating the hazard weights obtained from genome-wide Cox regressions of ADGC, we derived fPHS and mPHS using standard pruning and clumping process for every individual in the NACC cohort (males/females = 2628/3448), resulting in sex-matched model (males with mPHS and females with fPHS) and sex-mismatched model (males with fPHS and females with mPHS). To avoid the confounding of *APOE* due to imputations, we excluded any genetic variants located in the *APOE* region ('Materials and methods' section). For clinically determined Alzheimer's disease onset, the sex-matched model consistently performed more accurately than the sex-mismatched model (Fig. 3A). After controlling for *APOE* status, sex-matched PHS has a hazard ratio of 1.26 (95% CI: 1.26–1.32, $P < 1 \times 10^{-16}$) and sex-mismatched PHS has a hazard ratio of 1.14 (95% CI: 1.09–1.19, $P = 1 \times 10^{-10}$). Sex-matched PHS performed significantly better than sex-mismatched PHS ($P = 0.001$). Subgroup analyses indicate that stronger predictive power in sex-matched models than sex-mismatched models is evident for both males and females (Supplementary Fig. 1A). When we limited our analysis to those with neuropathological disease confirmation ($n = 1523$), the crossover effects were consistent (hazard ratio: 1.21, $P = 2 \times 10^{-9}$; Fig. 3B and Supplementary Fig. 1B), and retaining significant difference

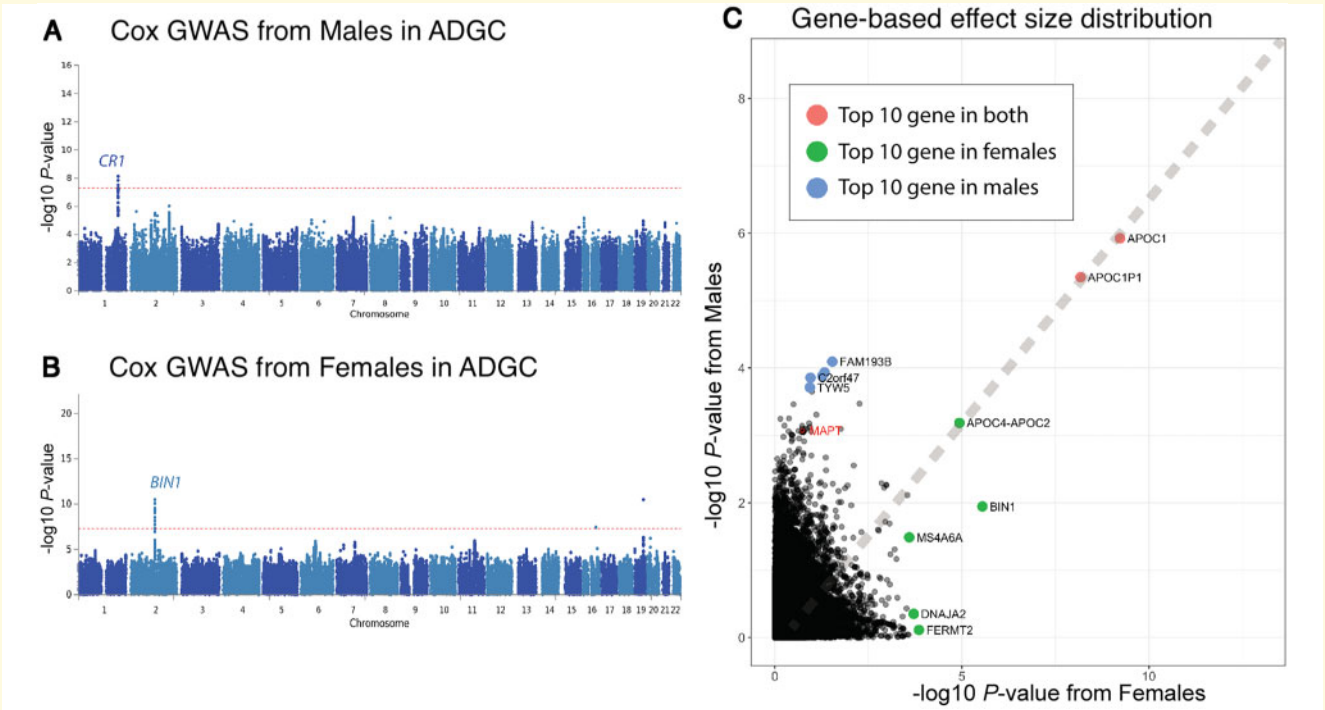

Figure 2 Effect size distributions of obtained hazard weights from sex stratified genome-wide Cox regressions. (**A**) Manhattan plot from genome-wide Cox regression from males in ADGC. (**B**) Manhattan plot from genome-wide Cox regression from females in ADGC. (**C**) Results from gene-based analysis. The diagonal dashed line represents the equivalent effect sizes given the sample size differences. We listed top 10 rank genes in terms of –log10(*P*) from the Pascal. Genes in both top 10 rank list of males and females are coloured in red. Genes in only top 10 rank list of females are coloured in green and of males are coloured in blue.

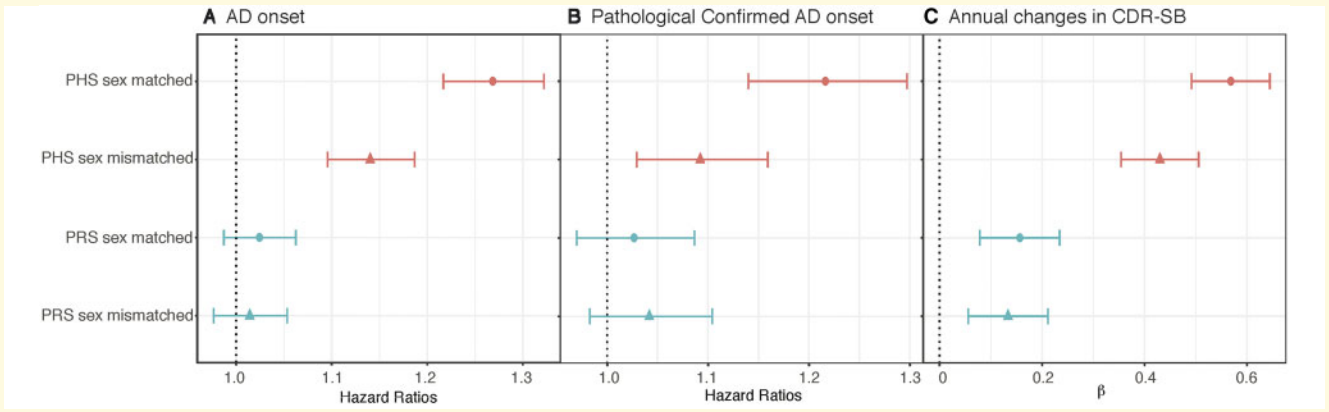

Figure 3 Predictive performance of polygenic components in NACC. Weights from Cox regressions of training data were applied to all participants in NACC, yielding both mPHS and fPHS for all participants. The hazard ratios of comparing 1 SD differences in PHS, after controlling *APOE* and education levels, are shown. (**A**) Prediction of clinically defined Alzheimer's disease (AD). (**B**) Prediction in neuropathologically confirmed Alzheimer's disease cases. (**C**) Prediction of Cognitive Dementia Rating – Sum of Boxes (CDR-SB) changes.

between sex-matched and mismatched models ($P = 0.008$). Figure 3C shows the performance of polygenic scores in predicting clinical progressions as CDR-SB changes during longitudinal follow-up in the NACC. Sex-matched PHS was predictive of annual changes of CDR-SB (β: 0.057, 95% CI: 0.049–0.064, $P < 1 \times 10^{-16}$) and performed better than sex-mismatched PHS (β: 0.043, 95% CI: 0.035–0.050, $P < 1 \times 10^{-16}$). The difference between sex-matched PHS and sex-mismatched PHS was statistically significant ($P = 0.006$). In contrast, PRS from logistic regressions showed lower effect sizes than PHS and showed no evidence for sex-dependent effects (Fig. 3A–C).

Predicting neuropathology in ROSMAP

Figure 4 demonstrates the association strengths across four types of neuropathology. After controlling for age at death, education levels, and *APOE* status, sex-matched models showed significantly stronger associations than sex-mismatched models for all neuropathological measures (*P*-values for differences in effect sizes between sex-matched and sex-mismatched PHS as 5×10^{-5}, 4×10^{-7}, 0.007, and 5×10^{-4} for amyloid deposition, CERAD score, tau-associated neurofibrillary tangles, and Braak score, respectively). None of the sex-mismatched models reached statistical significance in predicting neuropathology based on polygenic components. Table 2 summarizes the variance explained for subgroup analyses on each neuropathology. Compared to sex-mismatched models, fPHS applied to females increased the variance explained by 6%, 5%, 3%, and 6% for amyloid deposition, CERAD score, neurofibrillary tangles, and Braak score, respectively; applying mPHS to males increased the variance explained for these same measures by 1%, 3%, 3%, and 4%, respectively. In general, variance explained attributable to the polygenic components for sex matched models can reach up to 89% of variance explained by *APOE* only. In contrast, sex-matched PRS had no significant association with any neuropathology except CERAD score, with no evidence of sex differences after controlling for *APOE* (Fig. 4 and Supplementary Fig. 2).

Discussion

By modelling the disease courses as time-to-clinical-onset, the polygenic hazard approach revealed sex-dependent autosomal effects on Alzheimer's disease after controlling for *APOE*. Sex-matched PHS showed better prediction of both clinical age-at-onset and neuropathological manifestations than sex-mismatched PHS, implying that genetic risk factors differ between males and females. These finding have implications not only for the aetiology of Alzheimer's disease, but also offer a new approach to examine sex differences in genetic risks.

Many of the genes highlighted by our analyses have been implicated in Alzheimer's disease in prior reports (Hollingworth *et al.*, 2011; Naj *et al.*, 2011; Lambert *et al.*, 2013; Kunkle *et al.*, 2019). Yet, our survival analyses revealed a complex landscape of sex-dependency across the genome. Loci such as *BIN1*, *MS4A6A*, *DNAJA2*, and *FERMT2* contribute higher risk to females than to males. Previous GWAS have identified *BIN1* and *MS4A6A* as risk loci for Alzheimer's disease (Hollingworth *et al.*, 2011), but our results indicate that their effects may be sex dependent, especially for pathological ageing processes. Experimental studies have found that *FERMT2* is associated with amyloid deposition (Chapuis *et al.*, 2017) whereas *DNAJA2* interacts with protein tau aggregation (Mok *et al.*, 2018). When aggregating those differences as PHS, the sex-dependency of the genetic effects emerged, indicating there are divergent pathological pathways between males and females.

In addition to the pathogenesis of Alzheimer's disease, these crossover analyses also highlight an important aspect for modeling genetic risks: time. Alzheimer's disease is an

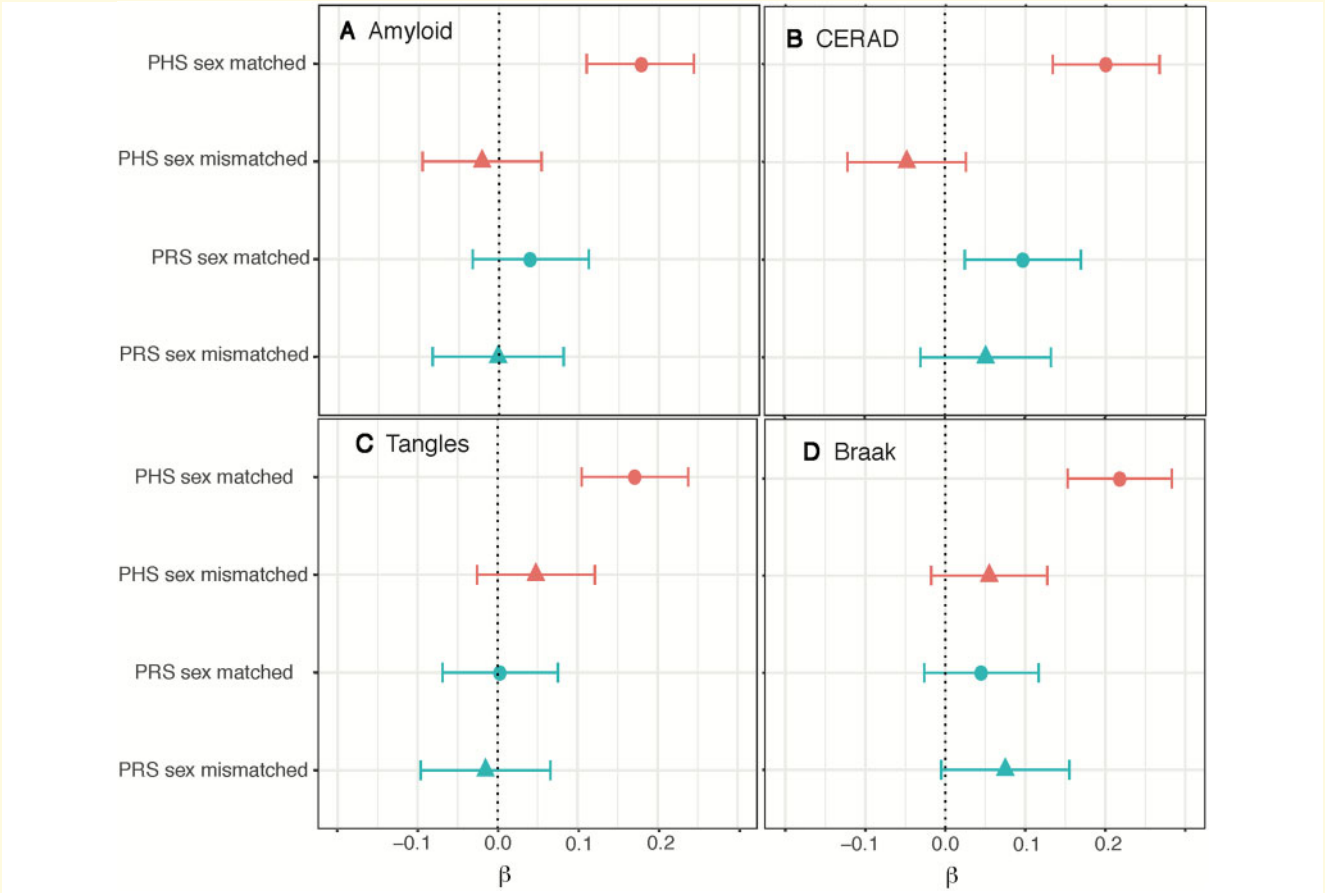

Figure 4 Associations with neuropathology in ROSMAP. Sex-dependent polygenic scores were obtained for all participants in ROSMAP. The colouring schemes are consistent with Fig. 3. All models controlled for age at death, education levels, and *APOE* status. (**A**) Associations with amyloid deposition. (**B**) Associations with CERAD score. (**C**) Associations with neurofibrillary tangles. (**D**) Associations with Braak score.

Table 2 Variance explained of neuropathological indices for crossover models in ROSMAP

Pathology		Validation subjects	Covariates only	*APOE* + ε2 + ε4	+Sex-matched PHS
Amyloid-related pathology	Amyloid	Females	2%	12%	17%
	Amyloid	Males	5%	12%	13%
	CERAD	Females	1%	11%	16%
	CERAD	Males	3%	9%	12%
Tau-related pathology	Tangles	Females	2%	15%	19%
	Tangles	Males	4%	10%	13%
	Braak	Females	5%	11%	17%
	Braak	Males	8%	15%	19%

Sex-mismatched PHS not shown because of no improvement on the variance explained over covariates *APOE* + ε2 + ε4 model.

insidious, progressive disease. When the genetic effects on disease risks are differentially expressed across time, the mean liability model cannot readily capture differences in the underlying genetic risks (Falconer, 1965). In our analyses, PRS had limited predictive accuracy on both Alzheimer's disease onset and neuropathology, regardless of sex dependencies. This does not discredit the utility of PRS, as many have shown the unstratified PRS models with *APOE* for Alzheimer's disease can have 70% of accuracy in classifying the prevalent cases and controls of Alzheimer's disease (Sabuncu *et al.*, 2012; Escott-Price *et al.*, 2015; Mormino *et al.*, 2016; Ge *et al.*, 2018). However, our results suggest that explicit modeling of time of clinical disease onset using survival analyses is needed to reveal sex-dependent effects in polygenic signals. Considering that one of the key differences between males and females with respect to Alzheimer's disease is the temporal disease course, and hence the underlying hazard function, sex-dependent polygenic

effects may largely modulate the temporal disease course for Alzheimer's disease. Interestingly, prior reports on sex-dependent autosomal effects on Alzheimer's disease have been discovered through analysing the endophenotype or molecular phenotype of Alzheimer's disease instead of the binary diagnostic Alzheimer's disease status (Cellini *et al.*, 2009; Li *et al.*, 2017; Deming *et al.*, 2018). Taken together with our results, this indicates that sex-dependent effects for Alzheimer's disease are particularly important for predicting disease progression.

Meanwhile, because PHS explicitly take the age-at-Alzheimer's disease-onset into consideration, factors that impact the determination of the age-at-onset would also impact on the predictive performance of PHS. Given the insidious nature of Alzheimer's disease onset, the exact onset of Alzheimer's disease is oftentimes difficult to establish. The sex differences can also be the end results of complex interactions between cumulations of neuropathologies and the cognitive reserves. This limitation further highlights the need for validations not only in the prediction for age-at-onset, but also in other metrics related to Alzheimer's disease process. We have validated the sex-dependent PHS for the age of Alzheimer's disease onset, cognitive decline, and neuropathological findings. Our results show the sex-dependent autosomal effects exist in multiple domains of Alzheimer's disease progressions.

Sex differences are ubiquitous in human biology and disease manifestations, yet are rarely reported in terms of genetic risks (Khramtsova *et al.*, 2019). Our results indicate that by explicitly modeling age-dependent hazards in sex-stratified analyses, we can reveal these sex-dependent effects. In addition to providing insight about sex-differences in Alzheimer's disease pathophysiology, we also hope this study will encourage improvements in GWAS study design to consider sex differences regarding time of disease onset.

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Competing interests

C.C.F. is a consultant of Multimodal Imaging Service, dba Healthlytix, in addition to his research appointment at the University of California, San Diego. A.M.D. is a founder of and holds equity interest in CorTechs Labs and serves on its scientific advisory board. He is also a member of the Scientific Advisory Board of Healthlytix and receives research funding from General Electric Healthcare (GEHC). The terms of these arrangements have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies.

Supplementary material

Supplementary material is available at *Brain* online.

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