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Identification of genetic heterogeneity of Alzheimer's disease across age

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ABSTRACT

The risk of *APOE* for Alzheimer's disease (AD) is modified by age. Beyond *APOE*, the polygenic architecture may also be heterogeneous across age. We aim to investigate age-related genetic heterogeneity of AD and identify genomic loci with differential effects across age. Stratified gene-based genome-wide association studies and polygenic variation analyses were performed in the younger (60–79 years, N = 14,895) and older (≥80 years, N = 6559) age-at-onset groups using Alzheimer's Disease Genetics Consortium data. We showed a moderate genetic correlation ($r_g = 0.64$) between the two age groups, supporting genetic heterogeneity. Heritability explained by variants on chromosome 19 (harboring *APOE*) was significantly larger in younger than in older onset group ($p < 0.05$). *APOE* region, *BIN1*, *OR2S2*, *MS4A4E*, and *PICALM* were identified at the gene-based genome-wide significance ($p < 2.73 \times 10^{-6}$) with larger effects at younger age (except *MS4A4E*). For the novel gene *OR2S2*, we further performed leave-one-out analyses, which showed consistent effects across subsamples. Our results suggest using genetically more homogeneous individuals may help detect additional susceptible loci.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is the most common form of dementia and is characterized by progressive memory loss and cognitive impairment (Winblad et al., 2016). The population with dementia worldwide, estimated to be 46.8 million in 2015, is expected to double every 20 years (Prince et al., 2015). AD is becoming one of the leading causes of death in the United States. In 2013, it was the sixth leading cause of death and deaths attributed to AD increased 71%, whereas other major causes decreased between 2000 and 2013 (Alzheimer's Association, 2016).

Age is the most important risk factor of AD. Although this may result from a considerable accumulation of environmental exposures, genetic components also play a substantial role in AD, with heritability estimates of approximately 60% based on the twin study design (Gatz et al., 2006) and 24%–53% based on genome-wide association studies (GWAS) (Lee et al., 2013; Ridge et al., 2013, 2016). For late-onset sporadic AD, *APOE* is the most hazardous susceptibility gene with moderate-risk allele (ε4 allele) frequency (~15% in the United States [Raber et al., 2004]). People with 1 or 2 copies of the ε4 allele were found to have, respectively, 3 or 12 times higher risk (Bertram et al., 2007) and earlier ages of AD onset (Raber et al., 2004) compared with noncarriers of the ε4 allele (Bertram et al., 2007). The lifetime risk of AD by age 85 years was estimated to be 18%–35% for one-copy-ε4 carriers and 51%–68% for two-copy-ε4 carriers, relative to the estimated 4%–12% risk for non-ε4 carriers in the European ancestry population (Genin et al., 2011). However, *APOE* alone only accounts for 6% of the phenotypic variations (Ridge et al., 2013). Several international collaborative consortia have conducted GWAS and identified at least 20 susceptibility loci with common allele frequencies but smaller effects (odds ratio <2) on AD than *APOE* ε4 (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009,

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2013; Naj et al., 2011; Seshadri et al., 2010). These genetic loci associated with AD reaching GWAS significance accounted for only 2% of the phenotypic variations (Ridge et al., 2013). In addition, these susceptibility genes are largely involved in cholesterol and lipid metabolism, immune response, and endosomal vesicle cycling pathways. Some are also associated with putative related etiology of AD including clearance of amyloid β and tau toxicity (Van Cauwenberghe et al., 2016). Beyond *APOE* and known susceptibility loci of AD, 25% of the phenotypic variations remain to be attributable to genetic variations (Ridge et al., 2013), suggesting that many risk loci are still to be discovered.

One potential reason for the relatively few identified risk genes is that the population of AD is genetically heterogeneous. *APOE* effects on AD have been shown to be heterogeneous across age in both retrospective (Farrer et al., 1997; Genin et al., 2011) and prospective (Bonham et al., 2016) studies, and in particular, the ε4 allele was associated with greater increase in risk among those aged 60–75 years than in older adults (Bonham et al., 2016; Farrer et al., 1997; Genin et al., 2011). The association between age at onset of AD cases and *APOE* has been observed in genome-wide linkage studies (Choi et al., 2011; Li et al., 2002) and recently confirmed by two GWAS (Kamboh et al., 2012; Naj et al., 2014) which reported that 1 additional copy of the ε4 allele decreased age at onset in patients by 2.45 years (Naj et al., 2014). In addition, genome-wide studies also found that multiple chromosomal regions and loci other than *APOE* were involved in age at onset (Choi et al., 2011; Dickson et al., 2008; Holmans et al., 2005; Kamboh et al., 2012; Li et al., 2002; Naj et al., 2014), suggesting that the effect of these loci on AD may interact with age in a similar way as *APOE*. Such genetic heterogeneity across age has not been investigated in genome-wide studies.

Here, we investigated the genetic heterogeneity of AD by performing stratified analyses for two age-at-onset groups (60–79 years and ≥80 years, details in Materials and methods) in terms of single-nucleotide polymorphism (SNP) heritability estimates and their genetic correlation using a genome-wide complex trait analysis (GCTA) tool based on a multicohort sample from Alzheimer's Disease Genetics Consortium (ADGC). We hypothesized that stratified analyses would reveal genetic heterogeneity between younger and older age at onset of AD, and enable identification of loci with differential effects on the two age groups.

2. Materials and methods

2.1. ADGC sample

Briefly, phase 1 of ADGC enrolled 15 cohorts from 1989 to 2011 based on case-control data, including 18,844 individuals with European ancestry aged ≥60 years and known covariates such as age, sex, and top 10 principal components for correcting population stratification. Phase 2 enrolled 15 cohorts, including 5342 European ancestry individuals with covariates as phase 1 aforementioned, in which all participants were aged ≥60 years (except 1 AD case with age at onset of 58 years). The details of each cohort in phase 1 (Jun et al., 2010; Naj et al., 2011, 2014) and phase 2 (Jun et al., 2016) have been described elsewhere. The sample quality control included genotyping call rate, X-chromosome analysis for sex, identity by descent for relatedness, and sample duplication (Naj et al., 2011, 2014). Genotyped SNPs were excluded because of low minor allele frequencies (<0.02 for Affymetrix chips or <0.01 for Illumina chips) or violation of Hardy-Weinberg equilibrium (p value $<10^{-6}$). Genome-wide SNP imputation was performed in each cohort using 1000 Genomes reference panel and imputed SNPs were removed if imputation quality (R^2) < 0.5 (Naj et al., 2014).

In our study, age of participants was defined as age at onset for AD cases and age at last visit for unaffected individuals (Desikan et al., 2017). We stratified participants into two age-at-onset groups in the stratification analyses of heritability and GWAS: 60–79 years (including 1 case with age 58 years) and ≥80 years. We chose an age cutoff of ≥80 years based on previous findings that ε4 effects are reduced in this age group relative to younger ages (Bonham et al., 2016). In addition, our preliminary analyses revealed a smaller genetic correlation (r_g), indicating greater genetic heterogeneity, between the older and younger groups based on the age ≥80 years ($r_g = 0.64$) as cutoff than when age ≥75 years ($r_g = 0.75$) was selected as the cutoff.

2.2. Statistical analysis

2.2.1. Whole-genome heritability and genetic correlation estimation

The heritability of AD was estimated by calculating the proportion of phenotypic variance explained by SNPs from the whole genome, which is implemented by GCTA (Yang et al., 2011a). GCTA fits effects of all SNPs simultaneously as random effects and effects of other covariates as fixed effects in a mixed linear model. In the regression model, the variance explained by SNPs can be estimated by the restricted maximum likelihood approach using the genetic relationship matrix (GRM), which reflects the genetic correlations across all SNPs between individuals (Yang et al., 2010). In our analysis, SNPs with minor allele frequencies >0.01 were retained to estimate GRM and we excluded related individuals using an individual-pairwise GRM threshold of <0.025. The overall and stratified heritability estimates in the two age groups (60–79 years and ≥80 years) were calculated based on GRMs (random effects) in the mixed-model regression analyses with covariates (fixed effects) such as age, sex, cohort indicators, and 10 principal components. The heritability estimates were also partitioned into chromosome 19 and other chromosomes by using 2 GRMs, 1 generated from chromosome 19 and the other generated from the other 21 autosomal chromosomes, in the mixed model (Yang et al., 2011b). After removing individuals with GRM >0.025, we combined ADGC phase 1 and 2 samples, including 12,698 and 5198 individuals in age groups of 60–79 years and ≥80 years, respectively, for estimations of heritability and genetic correlation (Lee et al., 2012). The genetic correlation between the two age groups was estimated using the bivariate restricted maximum likelihood method (Lee et al., 2012), and we determined whether the resulting correlation significantly differed from 1, which implies genetic heterogeneity between the two groups. In the case-control study design, the prevalence of AD in the population should be used to correct ascertainment due to oversampled cases in the case-control ADGC sample (Lee et al., 2011). In our study, we assigned the prevalence of AD for the population aged ≥60 years at 0.0613, aged 60–79 years at 0.0259, and aged ≥80 years at 0.2217. Detailed information of the prevalence of AD source and calculation is shown in the section of "Prevalence of AD."

2.2.2. Estimation of genetic effects of APOE ε4 alleles on AD

In whole-genome heritability estimation, heritability can be partitioned by chromosome. We estimated heritability for chromosome 19 and the other 21 chromosomes simultaneously in a mixed linear model including 2 GRMs and covariates (such as age, sex, cohort indicators, and 10 principal components). To estimate heritability of the *APOE* ε4 alleles, in the mixed linear model, we only included 1 GRM generated from the other 21 autosomal chromosomes and covariates and then calculated the best linear unbiased prediction (BLUP), which is the total genetic effect, and residual effect, which is the difference between BLUP and the phenotypic value for each individual (Yang et al., 2011a). We regressed residuals generated from BLUP estimation on the number of *APOE* ε4 alleles and obtained R^2, which is the proportion of the

variance of residuals that can be explained by *APOE* ε4 alleles. Therefore, the heritability of *APOE* ε4 alleles was denoted as R^2 in combined ADGC phase 1 and 2 samples (N = 17,046) and across the two age groups (60–79 years, N = 12,064, and ≥80 years, N = 4982). We also estimated effects of ε4 alleles in terms of odds ratios using logistic regression in the younger and older groups. We simultaneously calculated effects of 1 ε4 allele and 2 ε4 alleles for AD in the same model. The sample sizes were slightly smaller than those for whole-genome estimation due to missing *APOE* ε4 status for some individuals.

2.2.3. Prevalence of AD

As AD accounts for most dementia cases, we used age-specific dementia prevalence (Supplementary Table 1) in the United States from a systemic meta-analysis, which included 5-year prevalence for those over 60 years of age, to estimate the prevalence of AD (Prince et al., 2013). We recalculated average prevalence for those aged 60–79 years and ≥80 years (Supplementary Table 1) weighted by annual estimates of the resident population by single year of age of the United States in 2015 from United States Census Bureau (Supplementary Table 1, https://www2.census.gov/programs-surveys/popest/datasets/2010-2015/national/asrh/nc-est2015-agesex-res.csv).

2.2.4. Stratified SNP-based GWAS in the age groups 60–79 and ≥80 years

Genome-wide association analyses for 38,043,082 SNPs were performed in the two age-at-onset groups using logistic regressions implemented in PLINK 1.9 (Chang et al., 2015). Age, sex, cohort indicators, and the top 10 principal components for population structure correction were included as covariates. Individual-pairwise GRM >0.1 were excluded from analyses to ensure sample independence. In the age group of 60–79 years, 11,358 individuals (5703 cases and 5655 controls) in phase 1 and 3537 individuals (1613 cases and 1924 controls) in phase 2 were included; in the age group of ≥80 years, 4801 individuals (1942 cases and 2859 controls) in phase 1 and 1758 individuals (457 cases and 1301 controls) in phase 2 were included (see details in Supplementary Table 2). We combined phase 1 and 2 samples for association analyses in the two age groups (N = 14,895 and 6559 for the age 60–79 and ≥80 years groups), respectively, and obtained significant SNPs at the genome-wide significance level of p value = 5×10^{-8}. Linkage disequilibrium (LD)-independent SNPs were identified within the significant loci after removing correlated SNPs at LD $r^2 > 0.1$ that are within 250 kb of the top SNP base on empirical LD from European reference panel of 1000 Genomes Project phase 3 (released in May 2013) (Genomes Project et al., 2015) using PLINK 1.9. These remaining SNPs were LD-independent and the most significant in the LD block.

2.2.5. Stratified gene-based analyses in the age groups 60–79 and ≥80 years

To reduce the number of tests that were conducted in SNP-based GWAS and aggregate the weak effect of each SNP within a gene, we then performed gene-based analyses using MAGMA (de Leeuw et al., 2015) implemented in FUMA (Watanabe et al., 2017). We used stratified whole GWAS results in the two age groups. The gene-based p value was calculated based on the mean of the summary statistic (χ^2 statistic) of GWAS for the SNPs in a gene (de Leeuw et al., 2015; Watanabe et al., 2017). SNPs with minor allele frequencies ≥0.01 in the European reference panel of 1000 Genomes Project were included. The distance between two LD blocks <250 kb were merged into a locus. In our analyses, SNPs within the genes were mapped to 18,334 loci (genes). The p value significance threshold was corrected by Bonferroni method, which is 2.73×10^{-6}, that is, 0.05 divided by the number of genes (18,334) and, in addition, the suggestive threshold was set to be 10^{-5}. The stratified gene-based analyses in the age groups 60–79 and ≥80 years using summary statistics generated by stratified GWAS were performed to obtain significant genes for the two age groups, respectively. In addition, we showed the most significant SNP within each gene for the two age groups and performed Cochran's Q-test implemented in METAL (Willer et al., 2010) for heterogeneity of the SNP effects between the younger and older groups.

3. Results

3.1. Heritability estimates and genetic correlations

The heritability estimate of AD in combined phase 1 and 2 samples (N = 17,896) was 18.8% (95% CI 15.0%–22.6%) for the full sample using GCTA. The heritability estimates in the two age groups and chromosomal partitioned estimates are shown in Fig. 1. The contributions of chromosome 19 were considerably different between the two age groups, with chromosome 19 having a larger impact on the younger population of AD. The genetic correlation (r_g) between the two age groups was 0.64 (95% CI 0.30–0.97, p value for H_0: $r_g = 1$ was 0.043), suggesting divergent genetic components in the two age groups.

3.2. Heritability of APOE and APOE effects stratified by age

The heritability of *APOE* ε4 was estimated to be 9.56% in the combined phase 1 and 2 samples (N = 17,046) and, 12.49% and 4.30% in the younger (N = 12,064) and older (N = 4982) age groups, respectively. In terms of *APOE* effects (odds ratio) on AD, 1 *APOE* ε4

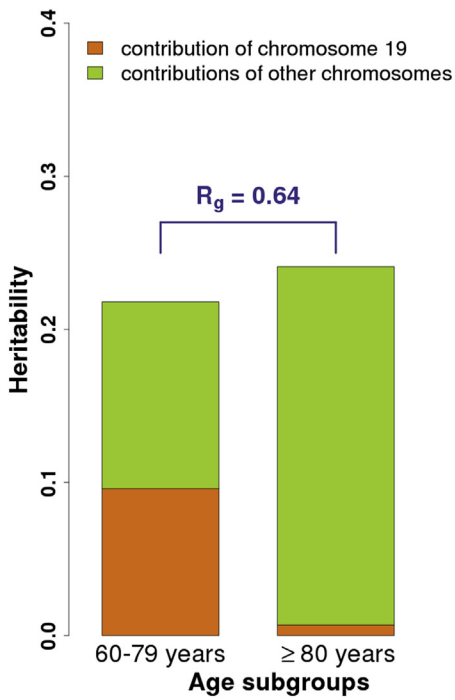

Fig. 1. The overall and partitioned heritability estimates in combined phase 1 and 2 samples across two age groups (age 60–79 and ≥80 years). The heritability estimates are 16.9% (95% CI 12.9%–20.9%) and 24.1% (95% CI 5.6%–42.6%) for the younger (N = 12,698) and older (N = 5198) age-at-onset groups, respectively. In the younger group, heritability estimates of chromosome 19 and others are 9.6% (95% CI 8.1%–11.1%) and 12.2% (8.5%–15.9%), whereas in the older group, they are 0.7% (0%–3.8%) and 23.4% (5.1%–41.8%), respectively. In addition, the genetic correlation (r_g) between the two age groups is 0.64 (95% CI 0.30–0.97) which significantly differs from 1 (p value for H_0: $r_g = 1$ is 0.043).

Table 1
Significant (p value $<2.73 \times 10^{-6}$) and suggestive (p value $<10^{-5}$) genes identified by stratified gene-based genome-wide analyses in ages 60–79 and ≥80 y using ADGC combined phase 1 and 2 samples

		p value	Top SNP in gene	A1/A2	Age 60–79 y (younger)				Age ≥80 y (older)				Heterogeneity between the younger & older age groups
Gene	Chr				Frq	N	OR (95% CI)	p value	Frq	N	OR (95% CI)	p value	p value
Top genes in age 60–79 y													
CR1	1	4.70×10^{-6}	rs3818361	A/G	0.204	14,867	1.21 (1.14, 1.29)	5.41×10^{-9}	0.195	6554	1.12 (1.01, 1.23)	0.027	0.159
BIN1	**2**	$\mathbf{3.67 \times 10^{-8}}$	**rs6431219**	**T/C**	**0.419**	**14,225**	**1.23 (1.17, 1.30)**	$\mathbf{6.06 \times 10^{-14}}$	**0.408**	**6341**	**1.04 (0.96, 1.13)**	**0.340**	$\mathbf{6.86 \times 10^{-4}}$
OR2S2	**9**	$\mathbf{8.45 \times 10^{-7}}$	**rs12378268**	**T/C**	**0.320**	**14,185**	**1.14 (1.08, 1.21)**	$\mathbf{5.43 \times 10^{-6}}$	**0.320**	**6339**	**1.04 (0.96, 1.13)**	**0.363**	**0.0695**
MS4A6A	11	8.68×10^{-6}	rs1834550	C/T	0.389	14,677	0.89 (0.84, 0.94)	1.08×10^{-5}	0.402	6473	0.86 (0.80, 0.94)	3.38×10^{-4}	0.587
MS4A4E	11	2.10×10^{-6}	rs184909761	C/T	0.353	12,169	0.86 (0.81, 0.92)	1.45×10^{-6}	0.360	5340	0.85 (0.78, 0.93)	5.53×10^{-4}	0.863
PICALM	**11**	$\mathbf{8.90 \times 10^{-8}}$	**rs639012**	**A/G**	**0.309**	**14,880**	**0.86 (0.82, 0.91)**	$\mathbf{1.86 \times 10^{-7}}$	**0.322**	**6552**	**0.98 (0.90, 1.06)**	**0.601**	**0.0132**
GNPNAT1	**14**	$\mathbf{4.16 \times 10^{-6}}$	**rs73298734**	**G/A**	**0.108**	**14,297**	**1.23 (1.13, 1.35)**	$\mathbf{1.69 \times 10^{-6}}$	**0.096**	**6311**	**0.95 (0.83, 1.09)**	**0.455**	$\mathbf{1.29 \times 10^{-3}}$
APOE[a]	19	1.49×10^{-154}	rs429358	C/T	0.248	13,641	3.93 (3.65, 4.23)	4.90×10^{-286}	0.126	6212	2.39 (2.11, 2.70)	3.90×10^{-43}	1.41×10^{-11}
Top genes in age ≥ 80 y													
MYOZ2	**4**	$\mathbf{4.13 \times 10^{-6}}$	**rs4277762**	**T/C**	**0.299**	**14,704**	**0.98 (0.93, 1.04)**	**0.476**	**0.306**	**6466**	**1.20 (1.10, 1.30)**	$\mathbf{2.05 \times 10^{-5}}$	$\mathbf{8.32 \times 10^{-5}}$
APOE[a]	19	4.57×10^{-30}	rs429358	C/T	0.248	13,641	3.93 (3.65, 4.23)	4.90×10^{-286}	0.126	6212	2.39 (2.11, 2.70)	3.90×10^{-43}	1.41×10^{-11}
ST13	**22**	$\mathbf{2.82 \times 10^{-6}}$	**rs6002167**	**T/A**	**0.052**	**14,844**	**1.04 (0.93, 1.17)**	**0.502**	**0.051**	**6543**	**0.67 (0.55, 0.80)**	$\mathbf{2.27 \times 10^{-5}}$	$\mathbf{8.01 \times 10^{-5}}$

The top SNPs with smallest p values within genes are shown.
The genes in bold face are shown distinct genetic effects in the younger and older groups for AD in addition to genes on chromosome 19. Their effects are significant in the younger or older group but not (p value >0.05) in the other group. The confidence intervals of those effects in the two age groups are largely nonoverlapping and significantly different based on Cochran's Q-test for heterogeneity.
Key: Chr, chromosome; A1, effect allele; A2, noneffect allele; Frq, allele frequency of A1; N, sample size; OR, odds ratio; 95% CI, 95% confidence interval.
[a] APOE is shown as representatives of significant genes on chromosome 19.

allele was estimated to increase risk of AD by 4.59-fold (95% CI 4.17–5.04) and 2 alleles 14.98-fold (95% CI 12.22–18.52) in the younger group. Corresponding values in the older group were 2.83-fold (95% CI 2.46–3.27) and 3.62-fold (95% CI 2.16–6.20). The results suggest differential contribution of *APOE* ε4 alleles to risk of AD in the two age groups, with ε4 being a more important risk factor in the younger population of AD.

3.3. Stratified SNP-based GWAS: younger (age 60–79 years) and older (age ≥80) group

To characterize diverse genetic impacts between the younger and older age groups, we performed SNP-based and gene-based genome-wide association analyses in each group.

In the younger group (N = 11,358), we identified 28 significant LD-independent SNPs on 4 different chromosomes (Supplementary Table 3) at p value $<5 \times 10^{-8}$. Among those SNPs, 24 SNPs were located on chromosome 19 and 4 SNPs on chromosomes 1, 2, and 11. In addition to chromosome 19, 3 SNPs on chromosomes 2 and 11 were significant in the younger group but not (p value >0.05) in the older group and their genetic effects for AD were stronger in the younger than the older group (Supplementary Table 3). The loci on chromosomes 1, 2, and 11, where significant SNPs were located, have been reported in previous GWAS (Harold et al., 2009; Lambert et al., 2009, 2013). In the older group (N = 4801), we identified 2 significant LD-independent SNPs on chromosomes 19 (Supplementary Table 3), which were also identified in the younger group within *APOE* (Supplementary Table 3).

3.4. Stratified gene-based GWAS: younger (age 60–79 years) and older (age ≥80) group

To detect novel associated loci for AD, stratified gene-based analyses were then performed in the two age groups. In the younger group (N = 14,895), in addition to genes on chromosome 19 surrounding *APOE* region, we identified 4 significant genes (*BIN1*, *OR2S2*, *MS4A4E*, and *PICALM*) on chromosomes 2, 9, and 11 at p value $<2.73 \times 10^{-6}$, and 3 genes (*CR1*, *MS4A6A*, and *GNPNAT1*) on chromosomes 1, 11, and 14 are suggestive at p value $<10^{-5}$ (Table 1 and Fig. 2A). Two novel genes, *OR2S2* (at the significance level) and *GNPNAT1* (at the suggestive level), were not reported in previous GWAS of AD. It is notable that effects of *BIN1*, *OR2S2*, *PICALM*, and *GNPNAT1* were suggestively significant (p value $<10^{-5}$) in the younger group but not (p value >0.05) in older group in terms of the SNPs with smallest p values within genes (Table 1). The confidence intervals of odds ratios of 4 SNPs (rs6431219, rs1237868, rs639012, and rs73298734) in the younger and older groups were largely nonoverlapping and their p values for testing heterogeneity (Cochran's Q-test) between the two age groups were significant, except rs1237868, which was borderline significant. The results indicated their genetic effects for AD were distinct in the younger and older groups and, furthermore, they were stronger in the younger than the older group (Table 1). In the older group (N = 6559), other than chromosome 19, we identified 2 suggestive genes, *MYOZ2* and *ST13*, on chromosomes 4 and 22 (Table 1 and Fig. 2B), which are novel for AD. Similarly, their effects had lower p values (p value $\approx 10^{-5}$) in the older group but not (p value >0.05) in the younger with nonoverlapping confidence intervals (significant for heterogeneity from the Cochran's Q-test) and their genetic effects for AD were stronger in the older group than in the younger group (Table 1).

For the significant locus, *OR2S2*, that we identified by gene-based GWAS in the younger group, we verified that the differential effects across age were observed in the phase 1 and phase 2 data sets (phase 1: Z value: 4.07, p value: 2.37×10^{-5} for the younger group, and Z value: −1.24, p value >0.05 for the older group; phase 2: Z value:

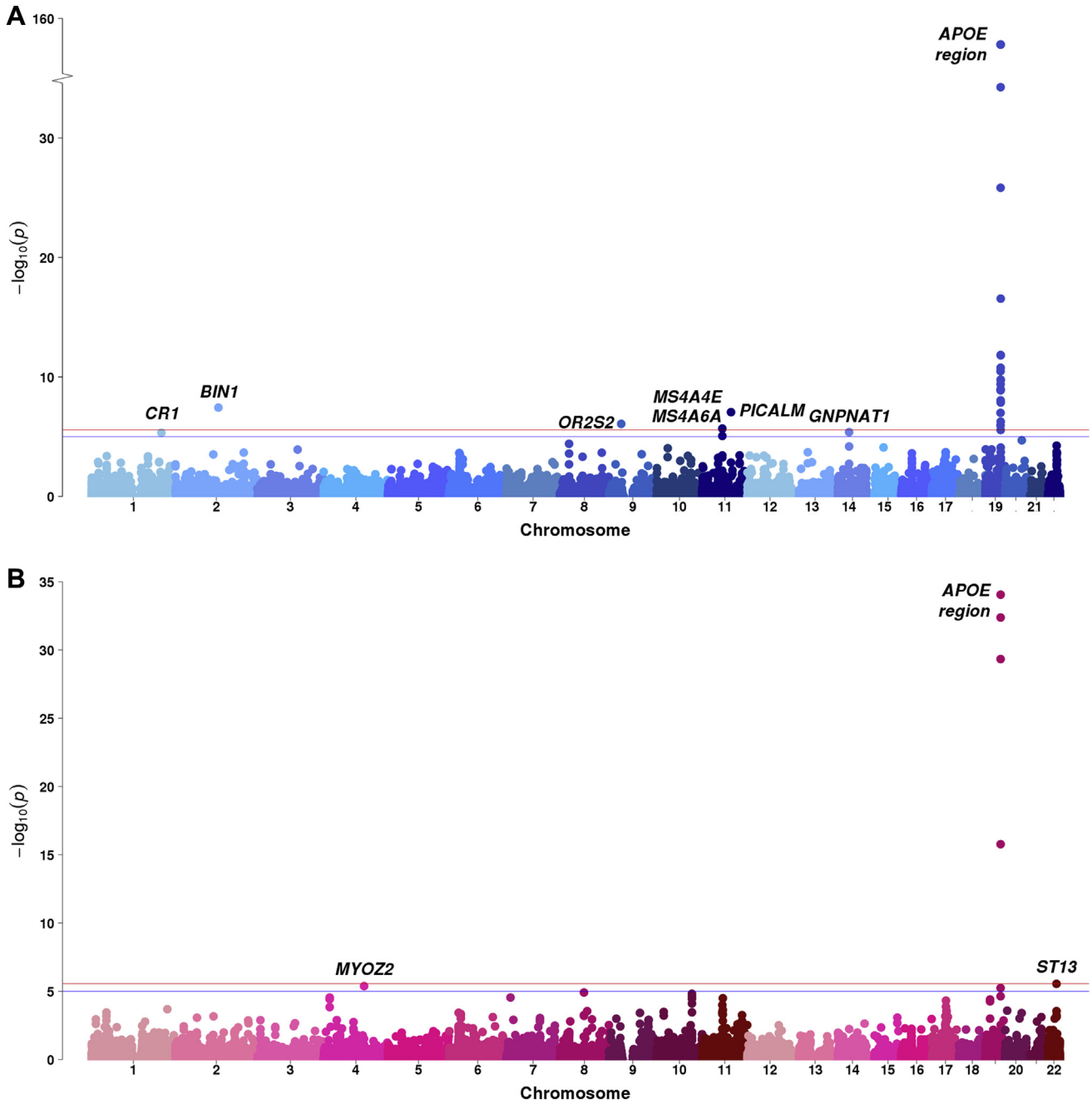

Fig. 2. Manhattan plots of gene-based genome-wide association analyses in ADGC combined phase 1 and phase 2 samples of (A) the younger age (age 60–79 years, N = 11,358) and (B) the older age (age ≥80 years, N = 4801). The red line denotes the gene-based genome-wide significance level of p value = 2.73×10^{-6} and blue line denotes the suggestive level of p value = 2.73×10^{-6}. The gene symbols are shown here if their p values calculated by gene-based analyses are less than the suggestive level. *APOE* region is shown as representatives of significant genes on chromosome 19. Abbreviations: ADGC, alzheimer's disease genetics consortium. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

1.59, p value = 0.056 for the younger group, and p value >0.05 for the older group). Because the phase 2 data are under power, we were only able to observe the trend. Furthermore, to evaluate the consistency of associations within the individual cohorts in phase 1 and phase 2, we performed stratified gene-based analyses in the subsets of the whole sample (ADGC combined phase 1 and 2 samples) using a leave-one-out cohort approach. All analytic procedures and covariates for adjustment followed the stratified gene-based GWAS. This leave-one-out method verified that p values of *OR2S2* in the all subsets (Supplementary Table 4) were consistent with the p value calculated from the whole ADGC sample (Table 1).

4. Discussion

The partitioned heritability results showed that chromosome 19 explained approximately a half of heritability in the younger age-at-onset group compared with a very small proportion in the older age-at-onset group, suggesting different genetic architectures between these two age groups (Fig. 1). Using age-stratified gene-based GWAS, in addition to *APOE* region on chromosome 19, we identified 1 significant novel locus, *OR2S2*, which were not reported in previous GWAS using AD cases across all age groups, in the younger group (age 60–79 years). The *APOE* region, *BIN1*, *PICALM*,

and *OR2S2* that we identified had stronger effects in the younger than the older group. We further performed leave-one-out analyses, which showed consistent effects of *OR2S2* across subsamples. Our findings suggested that analysis in more restricted age groups with genetically homogeneous AD cases may help detect potential susceptibility loci.

An interaction of *APOE* with age in risk of developing AD has been shown in candidate-gene approach (Bonham et al., 2016; Farrer et al., 1997; Genin et al., 2011; Sando et al., 2008), genome-wide linkage analysis (Choi et al., 2011; Li et al., 2002), and GWAS, using age at onset as the outcome variable (Kamboh et al., 2012; Naj et al., 2014). In particular, a recent longitudinal study observed that the risk of AD for *APOE* ε4 carriers showed an inverted U-shaped function with peak risk between ages 70 and 75 years (Bonham et al., 2016). Our results based on genome-wide analyses from the large ADGC sample are consistent with this finding. We found that *APOE* ε4 was more deleterious for the younger cases of AD (age 60–79 vs. ≥ 80 years). In addition to AD, *APOE* ε4 has been reported to increase the risk of other age-related phenotypes (Ang et al., 2008), such as cognitive decline (Ihle et al., 2012; Izaks et al., 2011; Schiepers et al., 2012), cardiovascular disease (Eichner et al., 2002; Wilson et al., 1994), and mortality (Rosvall et al., 2009). Collectively, these findings support the idea that younger carriers of *APOE* ε4 are more vulnerable to these adverse outcomes than older carriers. Once carriers age successfully, the risks associated with *APOE* ε4 appear to be reduced, suggesting the potential presence of counteracting effects (Bonham et al., 2016), such as other protective genes and/or behaviors, for example, physical activity, which may reduce age-related neuroinflammation (Soto et al., 2015).

In addition to several significant hits on chromosome 19, 4 significant genes (*BIN1*, *OR2S2*, *MS4A4E,* and *PICALM*) and 3 suggestive genes (*CR1*, *MS4A6A,* and *GNPNAT1*) on chromosomes 2, 9, and 11 were identified in age 60–79 years (Table 1). We also found that *APOE*, *BIN1*, *OR2S2,* and *PICALM* have larger effects for AD in younger age group. Our findings supported associations between age at onset and know susceptibility loci of AD (*BIN1*, *PICALM,* and *APOE*) which has been reported in a GWAS using age at onset as the outcome variable (Naj et al., 2014). The novel gene, *OR2S2* (olfactory receptor family 2 subfamily S member 2), is a member of olfactory receptors involved in a neuronal response that triggers the perception of a smell and has been reported to be associated with urate levels (Huffman et al., 2015) in previous GWAS.

Chromosome 19 contributes the partition of heritability by 9.6% and 0.7% for the younger and older age groups, respectively (Fig. 1). Similarly, *APOE* ε4 explained 12.79% and 4.45% of the phenotypic variation in the younger and older groups, respectively. The chromosomal partition heritability was estimated using GCTA based on the restricted maximum likelihood, which fits all SNPs jointly in a random-effect model so that each SNP effect is fitted conditioning on the joint effects of all the other SNPs (i.e., it accounts for LD between the SNPs) (Yang et al., 2016), and therefore, in this case, *APOE* ε4 explains higher variations in the regression model (Yang et al., 2016). Our result highly supported that AD is polygenic, particularly in older AD cases with higher heritability, which may be contributed from other genes in addition to *APOE*.

In our study, heritability of AD was estimated to be 18.8% (N = 17,896, including ADGC phase 1 and 2) compared with 53.24% (N = 9,699, ADGC phase 1) in a recent study (Ridge et al., 2016), that also used subsets of the ADGC sample. The discrepancy of heritability estimates between the two studies might be due to differences in covariate adjustments, prevalence of AD, quality control criteria for including subjects, and methods to generate principal components. First, we included covariates, such as age, sex, source cohorts of individuals recruited (cohort indicators), and 10 principal components, but cohort indicators were not included in the study of Ridge et al. To compare with the previous estimations, we then excluded cohort indicators from the model of the present study, and thereafter heritability was estimated to be 31.9% (95% CI 27.7%–36.2%), which is comparable with 33% from a previous study of Ridge et al. in 2013 using a subset of ADGC phase 1 sample (Ridge et al., 2013). As a result, adjustment for cohort indicators showed high impact on heritability reduction in our study. Second, for case-control study design with ascertainment bias of proportion of cases, heritability estimate is required to adjust for the prevalence of AD in the population (Lee et al., 2011). Based on 2015 age-specific population from United States Census Bureau and age-specific dementia prevalence from a systemic meta-analysis (Prince et al., 2013), we estimated the prevalence of AD to be 0.0613 among population over age 60 years (Supplementary Table 1), in contrast with 0.13 among population over age 65 years (Alzheimer's Association, 2012) applied in the study of (Ridge et al. 2016). Third, all individuals in our heritability analysis were nonmissing for AD status, age, sex, and principal components as well as their GRM <0.025, whereas Ridge et al. removed individuals with more closely related rather than third cousins, and those with missing data for AD status, age, sex, principal components, *APOE* genotype, or genotypes of the 21 known susceptibility loci of AD (Ridge et al., 2016). Finally, 10 principal components were calculated within each cohort in our study, whereas Ridge et al. estimated principal components using whole ADGC sample (Ridge et al., 2016).

In our stratified analyses to investigate heterogeneity of AD based on a continuous variable, age, we were confronted with 3 challenges: constant diagnostic accuracy across age, dichotomization of age, and reduction of sample size in each age group. First, the previous study showed that the positive predictive value of AD based on the clinical diagnosis was 83% (Beach et al., 2012), which indicates that 17% of AD cases might be misdiagnosed. If the positive predictive value decreases with age at onset, we will include more noncases in the older group than in the younger, and such misclassification will dilute effect sizes of susceptibility genes of AD and lead to being undetected. Second, the detrimental effect of *APOE* ε4 allele is higher among those aged 60–75 years and gradually diminishes in older adults (Bonham et al., 2016; Farrer et al., 1997; Genin et al., 2011). Moreover, our results from polygenic analyses showed contributions from other genetic risks become more evident to support genetic heterogeneity between AD cases younger and older than 80 years. Third, in general for stratified analysis that splits the sample into two subgroups, sample size is halved at most in one group and GWAS are vulnerable to insufficient sample size, although GWAS power may increase in genetically more homogeneous groups. Future studies that include more older cases with AD (age ≥80 years), for which *APOE* have a moderate impact may aid in identifying potential genes that may function to neutralize or postpone the effect of *APOE*.

Our analytical strategy for stratified analyses first used polygenic modeling to detect genetic heterogeneity of AD in terms of age at onset (60–79 vs. ≥ 80 years) and second used GWAS to uncover susceptibility genes (i.e., *APOE*, *BIN1, OR2S2,* and *PICALM*) with different effects in younger and older cases with AD. This strategy may help identify divergent biological mechanisms for AD cases with distinct features and/or subtypes of AD cases and provide insights for pharmaceutical development in personalized medicine.

Disclosure

M-TL is employed by and receives salaries from Ambry Genetics after the first submission of this article. Other authors report no actual or potential conflict of interest.

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The authors confirmed that all authors have reviewed the contents of the article being submitted, approve of its contents, and validate the accuracy of the data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.02.022.

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