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Genetics

Single-nucleotide polymorphisms are associated with cognitive decline at Alzheimer's disease conversion within mild cognitive impairment patients

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Abstract

Introduction: The growing public threat of Alzheimer's disease (AD) has raised the urgency to quantify the degree of cognitive decline during the conversion process of mild cognitive impairment (MCI) to AD and its underlying genetic pathway. The aim of this article was to test genetic common variants associated with accelerated cognitive decline after the conversion of MCI to AD.
Methods: In 583 subjects with MCI enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI; ADNI-1, ADNI-Go, and ADNI-2), 245 MCI participants converted to AD at follow-up. We tested the interaction effects between individual single-nucleotide polymorphisms and AD diagnosis trajectory on the longitudinal Alzheimer's Disease Assessment Scale-Cognition scores.
Results: Our findings reveal six genes, including *BDH1*, *ST6GAL1*, *RAB20*, *PDS5B*, *ADARB2*, and *SPSB1*, which are directly or indirectly related to MCI conversion to AD.
Discussion: This genome-wide association study sheds light on a genetic mechanism of longitudinal cognitive changes during the transition period from MCI to AD.

Keywords: Alzheimer's disease; GWAS; Mild cognitive impairment; Cognitive decline; Longitudinal study

1. Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that imposes social, psychological, and financial burden on both patients and their caregivers. Hebert et al. predict that the total number of individuals with AD dementia will be 13.8 million by 2050 [1]. This prediction, coupled with a

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lack of disease-modifying treatments, is estimated to have cumulative costs of more than $20 trillion by 2050 for the care of AD patients [2]. To date, extensive efforts have been made to delineate a set of risk factors that affect the development of AD. In particular, genome-wide association studies (GWASs) have been used to identify genetic variants that may contribute to AD. Most GWASs of AD have focused on the detection of single-nucleotide polymorphisms (SNPs) that are associated with the susceptibility of developing AD [3–8]. The early onset of AD is known to result from mutations in one of three genes: the amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2) [9,10]. The inheritance of the ε4 allele of the apolipoprotein E (*APOE*) has a substantial impact on the late onset of sporadic AD [11,12]. Recent GWASs have identified additional AD-related genes, including clusterin (CLU), complement receptor 1 (CR1), and phosphatidylinositol-binding clathrin assembly protein gene (PICALM) [13,14], whereby those genes alter production of the amyloid β peptide (Aβ) [15–17].

In the present study, we aimed to detect genetic common variants associated with accelerated cognitive decline after the conversion of mild cognitive impairment (MCI) to AD. MCI is a clinical syndrome characterized by insidious onset and progression of cognitive impairments. It is often considered as a transitional stage between normal aging and AD, because approximately 50% of MCI patients develop AD in 5 years from diagnosis [18]. Critically, therapeutic interventions and disease-modifying drugs appear to be more effective during the MCI or early stage of AD than at the more severe stages of AD [19–21]. As such, it is an ongoing quest to delineate a set of risk factors that affect conversion from MCI to AD. Recent GWASs have focused on the following phenotypes related to MCI-AD progression: binary outcome indicating MCI-AD conversion, time to conversion from MCI to AD, and cognitive decline. For example, Hu et al. (2011) found novel loci to be associated with longitudinal cognitive changes of MCI patients [22] and those loci were also associated with time to conversion from MCI to AD. Here, we only focus on an MCI-AD conversion group to identify genetic common variants contributing to rapid cognitive decline after an MCI patient develops AD.

We analyzed the Alzheimer's Disease Neuroimaging Initiative (ADNI; ADNI-1, ADNI-2, and ADNI-GO) cohort data. Among them, there remained 245 participants who converted to AD at follow-up. Longitudinal Alzheimer's Disease Assessment Scale-Cognition (ADAS-Cog) scores were used to measure cognitive function including memory, language, praxis, and orientation domain scores indicated greater cognitive impairment. To identify SNPs associated with longitudinal cognitive changes, we tested interaction effects between individual SNPs and AD diagnosis trajectory (MCI = 0, AD = 1) on the longitudinal ADAS-Cog scores. To account for individual variability of (1) baseline ADAS-Cog score and (2) the effect size of AD diagnosis trajectory, random effects for intercept and slope of AD diagnosis trajectory were incorporated in our model.

2. Materials and methods

2.1. Alzheimer's Disease Neuroimaging Initiative

The study population was obtained from the ADNI database (www.loni.usc.edu/ADNI). The ADNI study has aimed to detect and monitor the early stage of AD by investigating serial magnetic resonance imaging, positron emission tomography, genetic, biochemical biomarkers, and neuropsychological and clinical assessment. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California, San Francisco. The ADNI began in 2004 and recruited 400 subjects with MCI, 200 subjects with early AD, and 200 cognitively normal elderly from more than 50 sites across the United States and Canada. This multisite, longitudinal study was financially supported as $67 million by National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, and 13 private pharmaceutical companies. This initial phase, called ADNI-1, was extended with ADNI-GO in 2009. ADNI-GO investigated the existing ADNI-1 cohort and included 200 participants diagnosed as having early MCI. In 2011, ADNI-2 began to study participants from the ADNI-1/ADNI-GO and added 150 elderly control subjects, 100 early MCI participants, 150 late MCI participants, and 150 MCI patients. For up-to-date information, see www.adni-info.org.

2.2. Data description

In the sample of this study, we considered White participants who had developed AD from MCI at the baseline. During 120 months of follow-up, 245 MCI patients progressed to AD before study completion and the remaining 338 MCI patients did not convert to AD before study end. We considered only 245 patients who developed AD by their follow-up. The data included basic demographic and clinical information at the baseline: age, education length, and gender. Their ADAS-Cog scores were recorded about every 9 months in average with the mean follow-up duration of 48 months. Genotyping for the ADNI-1, ADNI-GO, and ADNI-2 data was performed using the Human 610-Quad BeadChip, Illumina Human Omni Express BeadChip, Illumina Omni 2.5 M (whole-genome sequencing [WGS] Platform), respectively. It was completed on all ADNI participants using the genotyping protocol whose details are described in [23] and http://adni.loni.usc.edu/methods/genetic-data-methods.

2.3. Quality control and genotype imputation

We performed quality control (QC) steps on the raw genotype data to ensure that only high-quality data were included in the final analysis. QC procedures included (1) call rate check per subject and per SNP marker, (2) gender check, (3) sibling pair identification, (4) the Hardy-Weinberg equilibrium test, (5) marker removal by the minor

allele frequency, and (6) population stratification. We also calculated an inbreeding coefficient (F) that represents the expected percentage of homozygosity. There were no subjects with excessive heterozygosity ($|F| > 0.15$) [53]. The second line preprocessing steps included removal of SNPs with (1) more than 5% missing values, (2) minor allele frequency smaller than 5%, and (3) Hardy-Weinberg equilibrium P value $< 10^{-6}$. The previously mentioned procedures were carried out in PLINK version 1.9 [24] with visualization performed in R (http://www.r-project.org/) using the qqman package (http://cran.r-project.org/web/packages/qqman/).

Genotype imputation was conducted to estimate unobserved genotypes. MaCH software [25] was used with NCBI 1000 Genomes build 37 (UCSC hg19) as the reference panel. We used different imputation quality metric R^2 for different minor allele frequency categories by following the recommendations given in Beecham et al. [26]. After the imputation step, 242 subjects and 8,092,642 SNPs remained in the present study. Strict QC procedures were followed: (1) removal of individuals with 10% missing genotypes, removal of SNPs with (2) more than 2% missing values, (3) minor allele frequency smaller than 5%, and (4) Hardy-Weinberg equilibrium P value $< 10^{-3}$. After the further QC, 242 subjects and 5,908,215 SNPs remained. Their demographic information is summarized in Supplementary Table 1.

2.4. Statistical analysis

To identify the SNPs that were associated with ADAS-Cog score changes over time after AD conversion from MCI, we ran linear mixed-effects models with ADAS-Cog score trajectory measured across time as longitudinal responses. Fixed main effects included gender (1 = male; 0 = female), length of education, age at the baseline, follow-up time trajectory, AD diagnosis trajectory (0 = MCI; 1 = AD), and each SNP. We included a random intercept to account for individual variability of baseline ADAS-Cog scores. A random slope of the AD diagnosis trajectory was added to take into account its variability across subjects. We tested a (fixed) interaction effect between each SNP and the AD diagnosis trajectory. To test the interaction effect, we used an approximate F-test based on the Kenward-Roger approach [27]. The first five PC scores from all the 22 chromosomes were also included to address the population stratification issue [28]. The principal component analysis was conducted using PLINK package. Quantile-quantile (QQ) plots and Manhattan plots were produced using the qqman package in R. Regional plots (see Supplementary Figs. 2–6) were generated to graphically show the GWAS results within a given genomic region using Locus Zoom 1.1 [29]. All SNPs within 200–400 kb of identified SNPs by our GWAS were plotted with their $-\log_{10}(P)$ value. Their degree of linkage disequilibrium (LD) with each SNP was color-coded.

We ran sensitivity analyses to examine if our results were robust to model specification. We compared the estimation and test results for the top meaningful SNPs (Table 1) by changing the included covariates. We considered two reduced models: all covariates (1) without the length of education and (2) without the length of education and gender. We also conducted a sensitivity analysis using the full model on a subsample consisting of subjects whose baseline ages were greater than 60.

Table 1
Meaningful single-nucleotide polymorphisms (SNPs) associated with accelerated cognitive decline and the corresponding regression coefficients for the interaction effects. Minor allele frequency (MAF) is the frequency where the second most common allele occurs in a population

SNP	Chr	Gene	MAF	Coefficient	SE	F value	P value
rs17090219	18		0.093	5.15	0.93	30.54	9.48×10^{-8}
rs3936289	3	*ST6GAL1*	0.256	3.34	0.64	26.83	4.92×10^{-7}
rs56378310	13	*RAB20*	0.424	−2.98	0.60	24.40	1.52×10^{-6}
rs192470679	13	*PDS5B*	0.300	2.89	0.59	24.36	1.55×10^{-6}
rs11121365	1	*SPSB1*	0.062	5.76	1.18	24.00	1.92×10^{-6}
rs2484	3	*BDH1*	0.095	4.53	0.94	23.03	2.83×10^{-6}
rs10903488	10	*ADARB2*	0.083	4.58	0.96	22.80	3.24×10^{-6}

NOTE. The SNPs mentioned in table have been reported to be related to Alzheimer's disease (AD) directly/indirectly. The regression coefficient, SE (standard error), and F-test statistic value are shown for the interaction effect of each of the SNPs and the AD diagnosis trajectory. A positive coefficient value implies that cognitive decline gets accelerated after AD conversion as the number of minor alleles of the corresponding SNP increases.

3. Result

3.1. Participant characteristics

To avoid the possible bias caused by population stratification, this study was restricted to White participants from the ADNI-1 and ADNI-2/ADNI-GO cohorts. There were 242 White MCI-AD converters after the QC steps. Supplementary Table 1 lists the characteristics of the study participants.

3.2. SNPs associated with the time to conversion

After running a set of linear mixed-effects models for each SNP, we examined if there existed population stratification. The genomic inflation factor λ is estimated as 1.000, which suggests that there is no evidence of population stratification. The QQ plot (Supplementary Fig. 1) shows no evidence of population stratification, as most of the observed P values do not deviate from the expected line (red line). The plot implies that identified SNPs by our longitudinal GWAS do not have a spurious association with the ADAS-Cog score trajectory. Also, as the Manhattan plot shows in Fig. 1, we observed that 56 SNPs had P values that were smaller than a suggestive significance level in GWAS ($P < 1 \times 10^{-5}$). Within the same gene, we only present one of the most significant genotyped SNPs in Supplementary Table 2. Supplementary Figs. 2–6 and Fig. 2 present the regional plots of the identified SNPs in Table 1. Sensitivity analyses (Supplementary Table 3) showed that estimated coefficient values and the corresponding P values in the primary

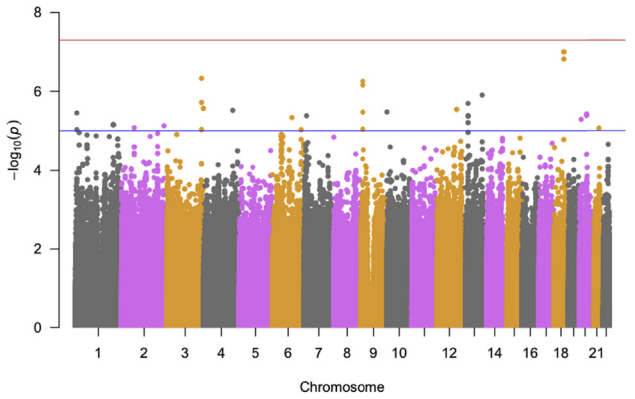

Fig. 1. The Manhattan plot shows the individual $-\log_{10}(P$ values) against base pair positions of 5,908,215 SNPs. Horizontal red and blue lines indicate genome-wide ($P < 5 \times 10^{-8}$) and suggestive ($P < 1 \times 10^{-5}$) significance levels, respectively. Abbreviation: SNPs, single-nucleotide polymorphisms.

analysis were similar to those in the reduced models and the full model on the subsample (age $>$ 60).

There were eight SNPs, including rs17090219 in chromosome 18, that had the most significant interaction effect with the presence of AD measured at every visit ($P = 9.48 \times 10^{-8}$). The corresponding estimated coefficient is 5.16 (Table 1), which suggests that the cognitive decline tends to be accelerated after AD conversion as the number of minor allele copies in rs17090219 increases. To visualize the mean ADAS-Cog score trend for different SNP groups (the number of copies of minor allele is 0, 1, or 2), we plotted fitted locally polynomial regression (loess) curves. Fig. 3 shows loess plots of three different groups: 0, 1, or 2 for rs17090219. Fig. 3A suggests that cognitive performance rapidly decreases as time goes by for subjects with two copies of the minor allele in rs17090219. Other subject groups had similar ADAS-Cog score trends to each other. Fig. 3B shows

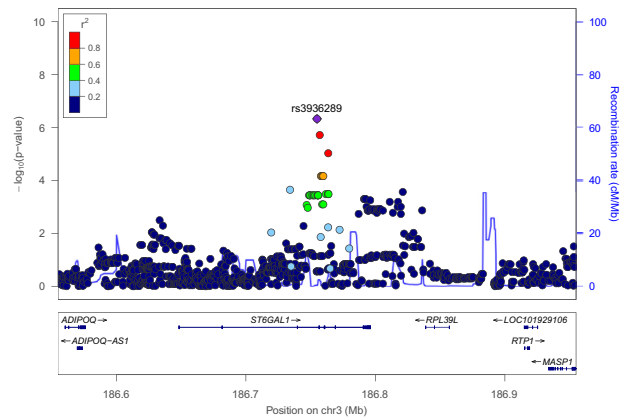

Fig. 2. A regional plot for association with cognitive decline. The purple dot is the most significant SNP rs3936289 among SNPs in the region surrounding it within 200 kb. Dots are colored according to the range r^2 to show their degree of LD with rs3936289. The blue line shows the estimated recombination rate. Abbreviations: LD, linkage disequilibrium; SNP, single-nucleotide polymorphism.

that cognitive dysfunction is accelerated after AD conversion for the two copies group. The main and interaction effects for rs17090219 suggest that the mean ADAS-Cog score increases by 6.64 for an additional copy of the minor allele, for any fixed change in the other covariates. This is a novel locus that has not been previously identified in other AD-related articles showing direct/indirect association with AD.

Our GWAS identified 11 SNPs located within the PDS5 cohesin associated factor B (*PDS5B*) gene. One of the significant SNPs within the *PDS5B* gene was rs192470679 ($P = 1.55 \times 10^{-6}$) with the corresponding estimated coefficient 2.89 (Table 1). Fig. 4 shows the corresponding loess plots of the three groups: 0, 1, or 2 for rs192470679. Fig. 4A shows that cognitive decline is accelerated as time goes by, especially for subjects who have two copies of the minor allele in rs192470679. Inspection of the bottom figure shows a clear difference among the mean ADAS-Cog score trajectories on the relative time to AD conversion. For subjects having zero or one copy of the minor allele, cognitive decline is increased until AD conversion and it tends to plateau after the conversion. ADAS-Cog scores keep rapidly increasing in their changes for subjects who have two copies of the minor allele. In particular, the additional copy of the minor allele of rs192470679 is associated with a 3.28 increase in the average ADAS-Cog score.

Also, there was an SNP rs10903488 ($P = 3.24 \times 10^{-6}$) within a gene known as adenosine deaminase, RNA-specific, B2 (*ADARB2*). Its regression coefficient was estimated as 4.58 (Table 1), which implies a greater rate of cognitive decline as the number of minor alleles of rs10903488 increases. Fig. 5A shows that although cognitive deficit increases for all participants over time, the rate of deficit accrual is faster for participants with at least one copy of the minor allele. There is a clear difference in the ADAS-Cog score trend between one and two copies of the minor allele groups on the relative time to conversion domain. Cognitive decline tends to plateau after AD conversion in the zero copy group, whereas the one or two copies groups have accelerated progression of cognitive dysfunction. The average ADAS-Cog score increases by 5.61 as the number of minor alleles of rs10903488 increases by 1.

4. Discussion

In this GWAS of ADNI-1, ADNI-2, and ADNI-GO data, we identified significant SNPs associated with ADAS-Cog score changes over time after AD conversion from MCI at the 1×10^{-5} suggestive significance level. We found six genes including *BDH1*, *ST6GAL1*, *RAB20*, *PDS5B*, *ADARB2*, and *SPSB1* that were directly or indirectly related to AD.

There are two genes, *PDS5B* and *ADARB2*, that have direct association with AD. The *PDS5B* gene has been found to be significantly associated with brain atrophy and episodic memory scores [30,31] for cognitively normal, MCI, and AD subjects. *ADARB2* is one of the genes that influences

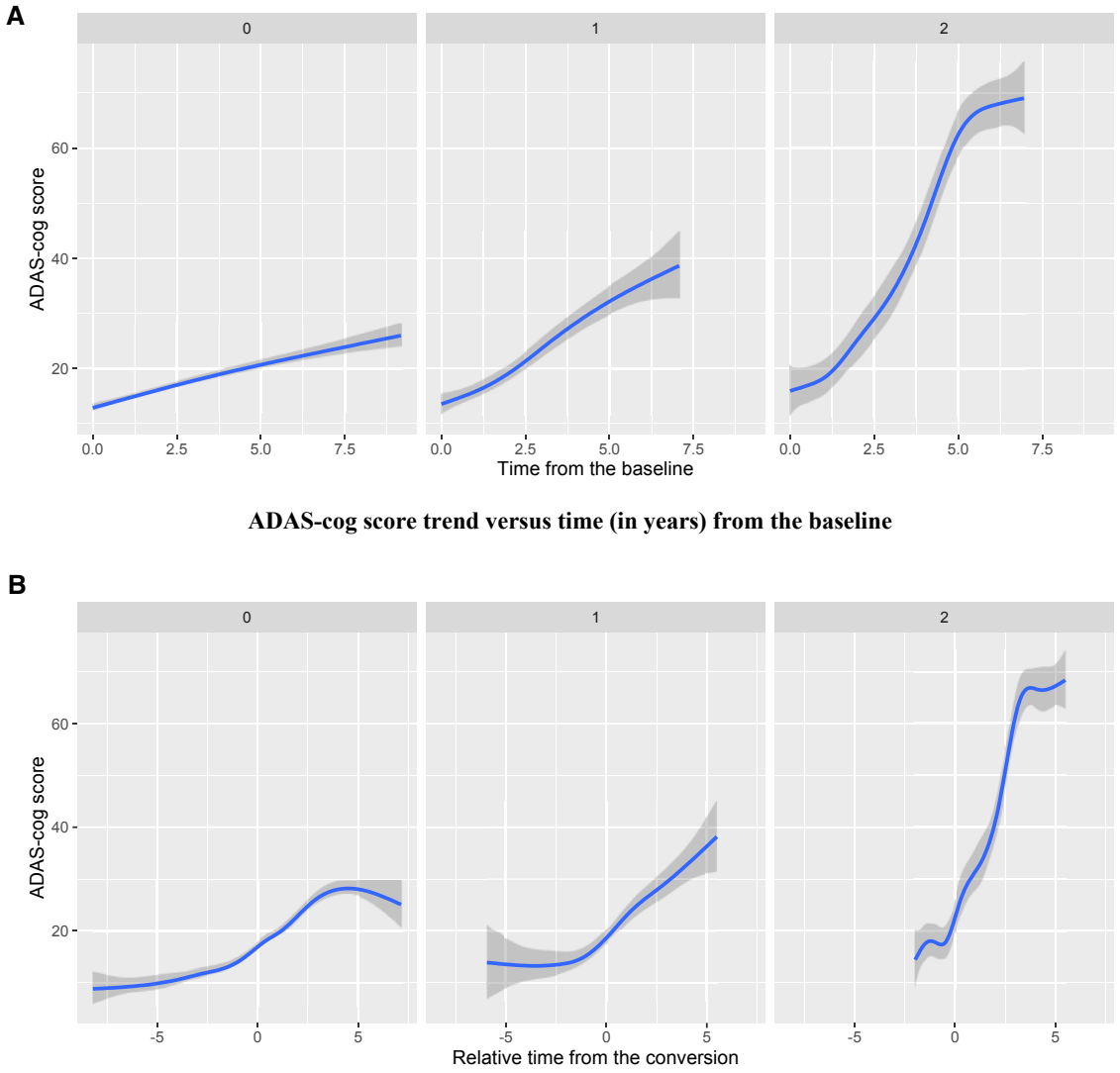

Fig. 3. (A) The mean trend of ADAS-Cog score was plotted against time (in years) from the baseline for each group (the number of minor alleles of *rs17090219* = 0, 1, or 2). (B) The mean trend of ADAS-Cog score was plotted against relative time (in years) from the AD conversion for each group. Abbreviation: ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognition.

magnetic resonance imaging–derived temporal lobe volume measures [32], where temporal lobe volume significantly changes in aging and in AD [33]. Together these studies demonstrate that the two genes affect cognitive deficit of MCI patients through brain atrophy and brain volume changes when the MCI patients develop AD.

There are four genes, *BDH1*, *ST6Gal1*, *RAB20*, and *SPSB1* that are associated with AD indirectly.

Our GWAS identified two SNPs located within the 3-hydroxybutyrate dehydrogenase, type 1 (*BDH1*) gene. Shi et al. [34] identified that the *BDH1* gene was related to aging. Aging is an important factor in AD studies, because risks of age-related diseases, such as hypertension, diabetes mellitus, heart attack, stroke, can be decreased in offspring of long-lived parents [35]. The *BDH1* gene has effects on both human aging and cognitive decline within the MCI patients.

Aβ, the main component of amyloid plaques, plays an important role in developing AD. Sequential cleavage of APP forms Aβ through β-secretase (BACE1, β-site APP-cleaving enzyme 1) and γ-secretase [36–40]. We found three SNPs within the gene ST6 beta-galactosamide alpha-2,6-sialyltranferase 1 (*ST6Gal1*). It has been demonstrated that cleavage and secretion of *ST6Gal1* is affected by BACE1 and overexpression of *ST6Gal1* increases soluble APP secretion [40,41]. It suggests that *ST6Gal1* plays an important role in the APP pathway by affecting Aβ generation and being produced within the process of Aβ formation.

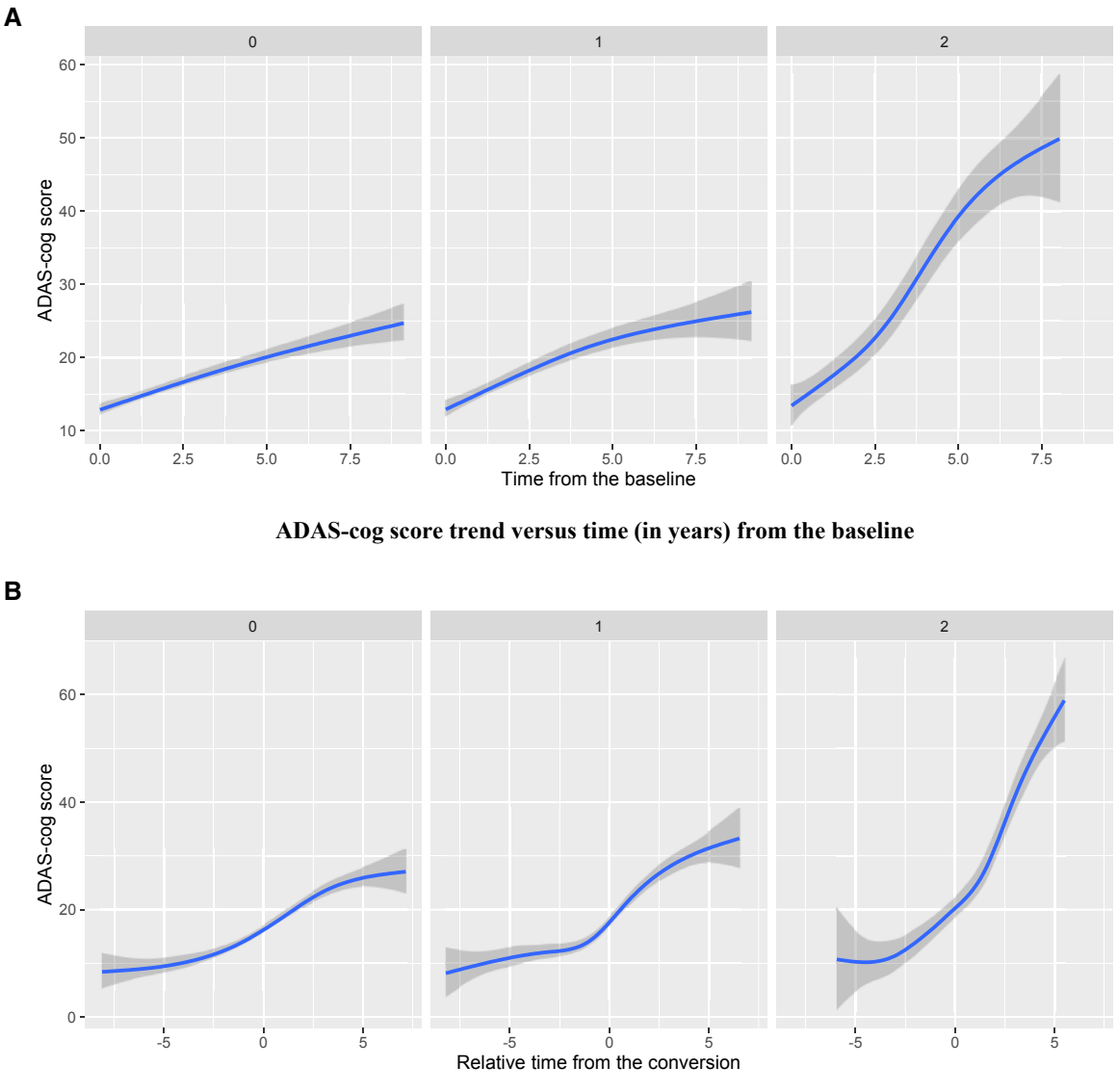

Fig. 4. (A) The mean trend of ADAS-Cog score was plotted against time (in years) from the baseline for each group (the number of minor alleles of *rs192470679 within PDS5B* = 0, 1, or 2). (B) The mean trend of ADAS-Cog score was plotted against relative time (in years) from the AD conversion for each group. Abbreviations: AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognition.

Wyss-Coray et al. [42] demonstrated that certain inflammatory mediators strongly drive AD by their mouse models. Genetic studies in mouse models suggest that multiple inflammatory mediators have great effects on AD-like pathogenesis. More reviews can be found in Cummings et al. [42]. One SNP, rs56378310, in a gene *RAB20* (*RAB20*, member Ras oncogene family) was identified at the suggestive level ($P < 1 \times 10^{-5}$). Liang et al (2012) found that *RAB20* and *RAB32* (member Ras oncogene family) were strongly upregulated in the acute phase of inflammation in mice, which suggested that these *RAB*s might participate in subsequent inflammatory responses in the brain [43].

Our GWAS identified two SNPs including rs11121365 and rs12069701 located in a gene called, SplA/ryanodine receptor domain and SOCS box containing 1 (*SPSB1*). *SPSB1*, *SPSB2*, and *SPSB4* regulate ubiquitination and proteasomal degradation of inducible nitric oxide (NO) synthase in activated macrophages [44]. They, in result, negatively control NO production and limit cellular toxicity. Togo et al. (2004) suggest that NO pathways contribute to pathogenesis of neurodegeneration in AD and other neurodegenerative dementias by involving in microvasculopathy and neuroinflammation [45]. A large amount of NO is produced by the inducible NO synthase [46], which thus has been linked to AD, asthma, cancer, cerebral infarction, inflammatory bowel disease, arthritis, and endotoxin shock [47,48]. It shows that *SPSB1* contributes to the development of AD through NO pathways.

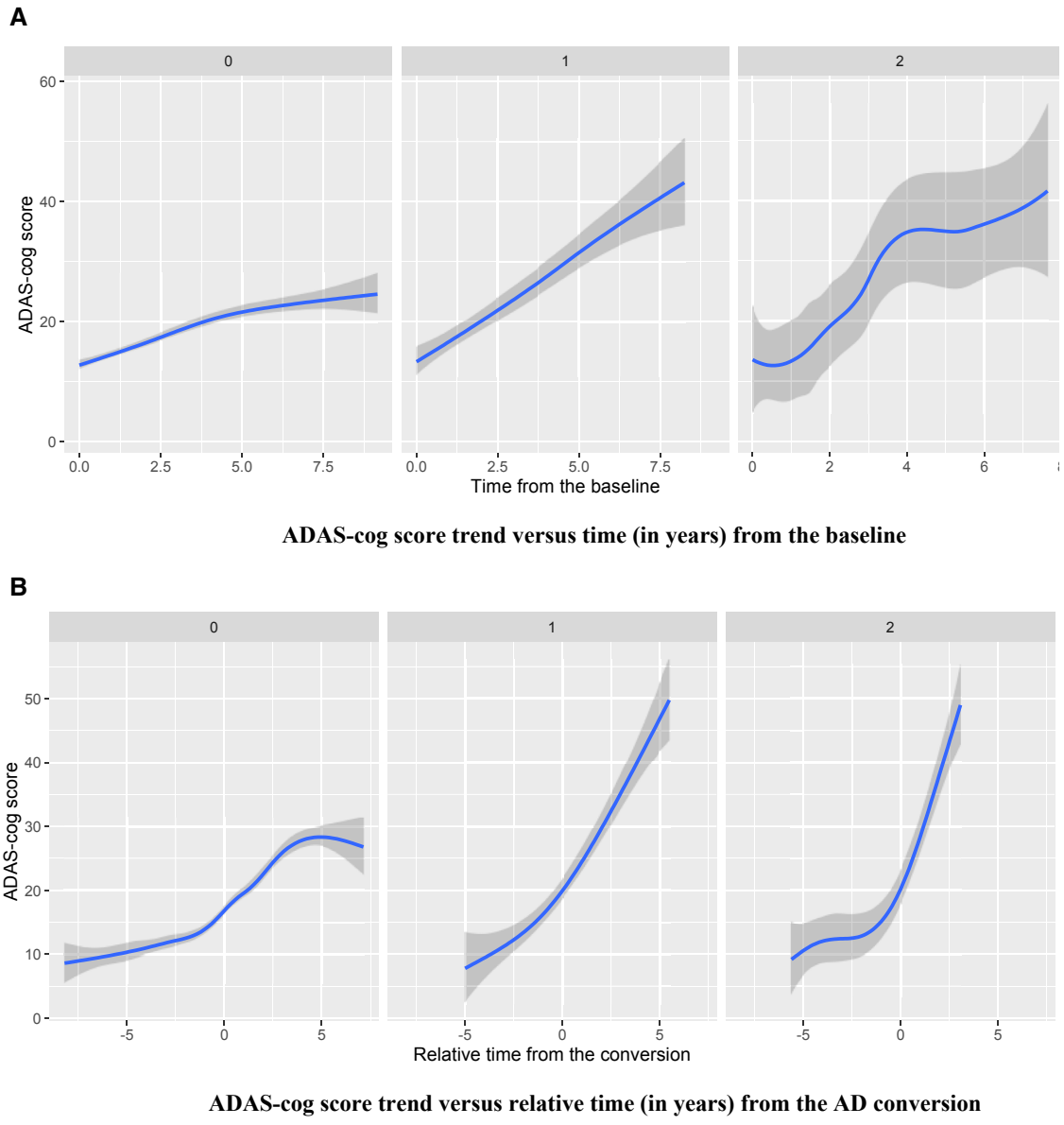

Fig. 5. (A) The mean trend of ADAS-Cog score was plotted against time (in years) from the baseline for each group (the number of minor alleles of *rs10903488 within ADARB2* = 0, 1, or 2). (B) The mean trend of ADAS-Cog score was plotted against relative time (in years) from the AD conversion for each group. Abbreviations: AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognition.

Other than the identified SNPs at the 1×10^{-5} significance level in a region of LD of rs3936289 (see Fig. 2), two SNPs including rs114805931 have *P* values as 1.25×10^{-5}. Fig. 2 shows regional association for the regions around rs3936289 within ST6GAL1. Their *P* values are plotted based on the −log 10 scales, and the color represents the range in r^2 to show their degree of LD with rs3936289. They are located within the gene *CACNA1D* whose disruption caused by copy number variations may result in a dysregulation in Ca^{2+} homeostasis and affect the development of AD [49]. Also, Kim and Rhim (2011) found that *CACNA1D* was upregulated by $A\beta_{25-35}$, which suggested that CaV1.3 had an impact on the pathogenesis of AD [50].

In 2011, a GWAS in South Asians identified that SNPs at ST6GAL1 might increase risk to develop type II diabetes by being involved in post-translational modification of cell-surface components by glycosylation, glycosylation through addition of sialic acid residues is reported to influence both insulin action and cell surface trafficking [51]. Whitmer et al. (2009) found that among older patients with type II diabetes, a history of severe hypoglycemic episodes was associated with a greater risk of dementia [52]. It suggests that ST6GAL1 may be related to the pathogenesis of AD through type II diabetes.

After identifying the relevant genes to cognitive decline, we examined if the genes are associated with the risk of MCI by running logistic regression using the

baseline diagnosis of ADNI-1, ADNI-GO, and ADNI-2 subjects. We included age, gender, length of education, and ADAS-Cog score at the baseline as covariates. Among the identified SNPs, 11 SNPs within the *PDS5B* gene were associated ($P < .05$) with the risk of MCI with/without considering the presence of *APOE* ε4 allele as an additional covariate. In particular, the *P* values of rs192470679 were .024 and .028, respectively, for the *APOE* ε4 allele excluded and included models. It suggests that the *PDS5B* gene is associated with cognitive dysfunction in both early and transition stages of MCI.

In summary, we detected six genes that putatively associated with accelerated cognitive decline of MCI subjects after they developed AD. The genes had been implicated in other AD or AD-related disease studies. This GWAS sheds light on what genetic factors have impact on the ADAS-Cog score changes within MCI-AD conversion subjects. It allows better understanding of the underlying mechanism and pathology of accelerated cognitive impairment of AD patients within their transition period from MCI, which further may help to develop future treatments targeting the identified genetic loci. Although the associations were significant at the 1×10^{-5} suggestive level, a bigger sample size could ensure more significant results. Also, a follow-up study is recommended as future works to examine if the identified genes are replicated in other samples.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dadm.2017.04.004.

RESEARCH IN CONTEXT

1. Systematic review: We used PubMed and Google Scholar to review the literature related to genetic association analyses of Alzheimer's disease (AD). Also, we used them to search for articles showing any interesting relationship between our significant loci to AD and AD-related diseases.
2. Interpretation: We identified new six genes that could be associated with accelerated cognitive decline of mild cognitive impairment (MCI) subjects after their conversion to AD. The genes had been implicated in other AD or AD-related disease studies. Our study suggests that the genetic factors have affected the ADAS-Cog score changes within MCI-AD conversion subjects. It helps to understand the underlying mechanism of accelerated cognitive impairment of AD patients within their transition period from MCI.
3. Future directions: A follow-up study should be taken as future works to examine if the identified genes are replicated in other and bigger samples.

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