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Effects of *HLA-DRB1/DQB1* Genetic Variants on Neuroimaging in Healthy, Mild Cognitive Impairment, and Alzheimer's Disease Cohorts

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Abstract Alzheimer's disease (AD) is the most common form of dementia and exhibits a considerable level of heritability. Previous association studies gave evidence for the associations of *HLA-DRB1/DQB1* alleles with AD. However, how and when the gene variants in *HLA-DRB1/DQB1* function in AD pathogenesis has yet to be determined. Here, we firstly investigated the association of gene variants in *HLA-DRB1/DQB1* alleles and AD related brain structure on magnetic resonance imaging (MRI) in a large sample from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We selected hippocampus, subregion, parahippocampus, posterior cingulate, precuneus, middle temporal, entorhinal cortex, and amygdala as regions of interest (ROIs). Twelve SNPs in *HLA-DRB1/DQB1* were identified in the dataset following quality control measures. In the total group hybrid population analysis, our study (rs35445101, rs1130399, and rs28746809) were associated with the smaller baseline volume of the left posterior cingulate and rs2854275 was associated with the larger baseline volume of the left posterior cingulate. Furthermore, we detected the above four associations in mild cognitive impairment (MCI) sub-group analysis, and two risk loci (rs35445101 and rs1130399) were also the smaller baseline volume of the left posterior cingulate in (NC) sub-group analysis. Our study suggested that *HLA-DRB1/DQB1* gene variants appeared to modulate the alteration of the left posterior cingulate volume, hence modulating the susceptibility of AD.

Keywords HLA · Genetics · Alzheimer's disease · Brain structure · Neuroimaging

Alzheimer's Disease Neuroimaging Initiative Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Introduction

Sporadic Alzheimer's disease (AD) is a complex neurodegenerative disease, caused by a combination of genetic, epigenetic, and environmental influences. *APOE* ε4 allele is the strongest known genetic risk factor [1], however, the effect of

APOE ε4 is less pronounced in older late-onset cases and up to 50 % of late-onset AD cases occur in the absence of the ε4 allele, suggesting there is the likelihood of additional susceptibility genes [2–4]. While more than 660 candidate genes for AD risk have been identified although the results are inconsistent between studies [5, 6], the increasing evidence that genetic risk factors for AD also impact the neuroimaging markers which appear to be shaped by genetic influences with heritability estimates as high as 80 % [7, 8]. Furthermore, these neuroimaging biomarkers are potent predictors of AD risk and progression. Therefore, a growing number of studies, which were using an intermediate phenotype approach such as structural brain imaging of hippocampal atrophy, as endpoints in genetic analyses of risk to further characterize complex genes associated with AD, were brought forth.

The human leukocyte antigen (*HLA*) genes within the major histocompatibility complex (MHC) at 6p21.3 in humans have been candidate risk genes for AD on the base of immune activation and possibly inflammation which are likely involved in AD. HLA-DR/DQ belongs to MHC II molecules, which are implicated in antigen presentation to T lymphocytes, by regulating T-cell responses against specific antigens. Abnormal proteins such as β-amyloid peptide (Aβ) (one of the hallmarks of the pathogenesis of AD) in the AD brains are first endocytosed by antigen-presenting cells, and then processed to fragments that are bound to MHC molecules, and presented to T lymphocytes. Antigen presentation can lead to B cell stimulation and subsequently to the production of specific autoantibodies. Excessive reactivity could have harmful side effects, and the immune-mediated neuroinflammation had been demonstrated to be involved in the pathogenesis of AD [9]. Moreover, the glia is the main cellular components of inflammation in the AD brain. The extensive HLA-DR immunoreactivity around neuritic plaques primarily localizes to reactive microglia, with equivocal localization to astrocytes [10, 11]. From that, the researchers hypothesize that *HLA-DRB1*/*DQB1* might contribute to AD pathogenesis. Previous association studies gave evidence for a linkage to *HLA-DRB1*/*DQB1* alleles (MHC class II genes) [10, 12, 13]. Seven brain regions, including the hippocampus, middle temporal area, entorhinal area, posterior cingulate, precuneus, parahippocampal area, and amygdala were selected as regions of interest (ROIs). These regions were known to be affected by AD, and their atrophy in AD has been previously validated via magnetic resonance imaging (MRI) studies [14].

Based on these finding, we attempt to explore the association of the variants in *HLA-DRB1*/*DQB1* alleles with AD-related brain structure on MRI in individuals enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI).

Methods

ADNI Dataset and Subjects

The publicly available ADNI data used in the preparation of our article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). ADNI is a large, multicenter, longitudinal neuroimaging study, launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations, as a $60 million, 5-year public-private partnership [15]. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The initial goal of ADNI is to recruit 800 subjects, but ADNI has been followed by ADNI-GO and ADNI-2. To date, the three protocols have covered more than 1500 adults, ages 55 to 90 years, to participate in the research, consisting of cognitively normal (CN) older individuals, people with early or late mild cognitive impairment (MCI), and people with early AD. Also, this study complied with the Declaration of Helsinki. Inclusion criteria for AD subjects is National Institute of Neurological and Communication Disorders/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria for probable AD, with a Mini Mental State Examination (MMSE) score between 20 and 26, a global Clinical Dementia Rating (CDR) of 0.5 or 1, a sum of boxes CDR of 1.0 to 9.0. However, only 812 participants, including 281 CN, 483 MCI, and 48 AD patients, were included in our study. The details concerning the ADNI cohort were reported elsewhere [15, 16]. The basic data of subjects in our analysis was downloaded from the ADNI website in 2015.

Genotype Data and SNP Selection

Genotypes from 15 SNPs spanning the *HLA-DRB1*/*DQB1* loci in the ADNI dataset were extracted and merged to create a single dataset as previously described [17]. All genetic analyses were performed using PLINK v1.07. We performed the quality control (QC) procedures using the PLINK software, and the inclusion criteria were as follows: minimum call rates >90 %, minimum minor allele frequencies (MAF) >0.01, and Hardy-Weinberg equilibrium test P >0.0001. Twelve SNPs in *HLA-DRB1*/*DQB1* passed QC (rs9269693, rs35445101, rs3830135, rs6689, rs1063355, rs9273448, rs1770,

rs9273471, rs2854275, rs2854272, rs1130399, rs28746809, rs3189152, rs1049056, rs1049053) (Table 1).

MRI Data

ADNI MRI scans were acquired at multiple sites and processed according to previously published methods using the FreeSurfer v4.5.0 (http://surfer.nmr.mgh.harvard.edu) software package [17]. This process mainly included motion correction and averaging of multiple volumetric T1 weighted images (when more than one is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) [18], intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. The technical details of these procedures are described in prior publications [19]. Seven neuroimaging measures were chosen for analysis on the basis of their established role in predicting AD risk and progression: hippocampal volume, parahippocampal volume, amygdala volume, posterior cingulate gyrus volume, precuneus volume, middle temporal lobe volume, and entorhinal cortex thickness. The MRI volumes of brain structures used in our study were from UCSF data in ADNI dataset (https://ida.loni.usc.edu/pages/access/studyData.jsp).

In the present analysis, there were 718 (NC = 257, MCI = 422, AD = 39) individuals included in the regional volume/thickness analysis.

Statistical Analysis

Differences in continuous variables (age, education years, cognitive scores, volume, etc.) were examined using one-way analysis of variance (ANOVA), and categorical data (gender, *ApoE* ε4 status) were tested using chi-square test. All statistical analyses were performed by R 3.12 and PLINK 8 (http://pngu.mgh.harvard.edu/wpurcell/plink/). Furthermore, we used a multiple linear regression model which considered age, gender, education years, and *ApoE* ε4 status as covariates to estimate coefficients for testing possible correlation between *HLA-DRB1*/*DQB1* loci genotypes and AD-related brain structures at baseline. Given that Bonferroni correction was inappropriate due to the nonindependence of these tests [17], the false discovery rate (FDR) based on the method developed by Hochberg and Benjamini [20] was used to control for multiple test. Statistical significance was considered for FDR-corrected $Pc < 0.05$. For these significant findings in total group (including CN, MCI, and AD), we further detected the correlation between these positive *HLA-DRB1*/*DQB1* loci and AD-related brain structures in subgroup (CN, MCI, and AD) analysis to identify that at which stage these variations impacted these pathological markers in the pathogenesis of AD. In addition, for these positive *HLA-DRB1*/*DQB1* loci, we further used Haploview (version 4.2) to estimate the linkage disequilibrium (LD) among the genotyped variants.

Table 1 The targeted *HLA-DRB1*/*DQB1* loci in the study

SNP	Chromosome	Physical position	Gene	Call rate (%)	Alleles	MAF	H-W (*P* value)
rs9269693	6	32546866	*HLA-DRB1*	94.1	C:T	0.262	0.1310
rs35445101	6	32546879	*HLA-DRB1*	93.8	A:G	0.328	0.4497
rs3830135	6	32548464	*HLA-DRB1*	92.7	C:T	0.109	0.7877
rs6689	6	32627700	*HLA-DQB1*	99.8	A:G	0.220	0.7612
rs1063355	6	32627714	*HLA-DQB1*	99.6	C:A	0.415	0.8762
rs9273448	6	32627747	*HLA-DQB1*	99.9	G:A	0.235	0.1279
rs1770	6	32627833	*HLA-DQB1*	95.6	A:G	0.423	0.1863
rs9273471	6	32628030	*HLA-DQB1*	99.6	G:A	0.413	0.1583
rs2854275	6	32628428	*HLA-DQB1*	99.9	G:T	0.115	0.2605
rs2854272	6	32629680	*HLA-DQB1*	98.9	A:G	0.399	0.4355
rs1130399	6	32629755	*HLA-DQB1*	99.4	C:T	0.194	0.6082
rs28746809	6	32633159	*HLA-DQB1*	100.0	T:C	0.185	0.6544
rs3189152	6	32634341	*HLA-DQB1*	100.0	A:G	0.410	0.3887
rs1049056	6	32634369	*HLA-DQB1*	95.2	C:A	0.157	0.2108
rs1049053	6	32634405	*HLA-DQB1*	99.9	T:C	0.236	0.1402

MAF minor allele frequency, *H-W* Hardy-Weinberg equivalent

Results

Characteristics of Included Subjects

The information about these included subjects is listed in Table 2. Totally, 281 cognitively normal (145 women, 74.51 ± 5.56 years), 483 MCI (201 women, 72.28 ± 7.45 years), and 48 AD patients (18 women, 75.51 ± 9.23 years) were recruited in this study. As expected, AD group had the highest frequency for the ε4 allele within *ApoE* gene (44.8 %), and CN group had the lowest frequency (14.9 %). Compared to CN and MCI subjects, AD dementia patients displayed the worst cognitive function based on these various neuropsychological scales (CDRSB, MMSE, ADAS-cog, etc.,). Likewise, AD group showed the most severely atrophy in hippocampus, middle temporal, and entorhinal cortex with MRI method.

Brain Structures and *HLA-DRB1*/*DQB1* Loci Genotypes in Total Group (CN + MCI + AD)

We firstly analyzed the association of these *HLA-DRB1*/*DQB1* loci with AD-related brain structures (hippocampus, parahippocampus, middle temporal, amygdala, posterior cingulate, precuneus, and entorhinal cortex) in a linear model which treated age, gender, education years, *APOE* ε4 status and intracranial volume (ICV), or thickness as covariates at baseline, using a multiple linear regression. Single nucleotide mutation rs35445101 (G allele) was demonstrated to be associated with the smaller baseline volume of left posterior cingulate for the total subjects in FDR test ($P = 9.49 \times 10^{-4}$, $Pc = 0.007$) (Fig. 1a; Supplementary Table 1). The variations at rs1130399 (T allele) were respectively associated with the smaller baseline volume of the left posterior cingulate ($P = 0.001$), right middle temporal ($P = 0.024$), and right parahippocampus ($P = 0.035$) at baseline in the overall group, however, only the association with the left posterior cingulate ($Pc = 0.011$) reached the statistically significant level after FDR test (Fig. 1b; Supplementary Table 1). Similarly, the variations at rs28746809 (C allele) were only associated with the smaller volume of the left posterior cingulate (rs28746809: $P = 0.006$, $Pc = 0.025$), and rs2854275 (T allele) were only associated with the larger volume of the left posterior cingulate (rs2854275: $P = 0.002$, $Pc = 0.011$) at baseline in the overall group after FDR test (Fig. 1c, d; Supplementary Table 1). Analysis of pairwise LD among the four positive variants showed that the three *HLA-DQB1* variants existed tightly in one block ($P < 0.05$, $D' = 1$) (Supplementary Fig. 1). A total of three common haplotypes were identified across the LD block, ranging in frequency from 69.1 to 11.5 % in total subjects.

Altogether, we can infer that the left posterior cingulate may be the pivotal region on which *HLA-DRB1*/*DQB1* gene variations target. Further, we selected the left posterior cingulate as our sole ROI and independently tested its association with these significant loci in the three subgroups, respectively.

Left Posterior Cingulate and *HLA-DRB1*/*DQB1* Loci Genotypes in the Subgroups

We then conducted subgroup analysis to ascertain whether *HLA-DRB1*/*DQB1* loci (rs35445101, rs1130399, rs2854275, and rs28746809) alter the volume of left posterior cingulate in

Table 2 The characteristics of the ADNI subjects at baseline

Characteristics	CN		MCI		AD		P^*
Age (years)	281	74.51 ± 5.56	483	72.28 ± 7.45	48	75.51 ± 9.23	–
Gender (male/female)	281	136/145	483	282/201	48	30/18	–
Education (years)	281	16.41 ± 2.66	483	15.98 ± 2.82	48	15.73 ± 2.62	0.08
APOE ε4 (0/1/2)	281	204/70/7	483	262/180/41	48	14/25/9	<0.01
CDR-SB	207	0.03 ± 0.13	406	1.44 ± 0.87	47	4.44 ± 1.69	<0.01
MMSE	281	29.07 ± 1.15	483	27.89 ± 1.69	48	22.96 ± 2.03	<0.01
ADAS-cog	281	9.06 ± 4.23	480	15.30 ± 6.65	48	29.80 ± 8.44	<0.01
RAVLT	280	44.83 ± 9.60	483	36.16 ± 10.86	47	22.32 ± 7.84	<0.01
FAQ	281	0.17 ± 0.66	481	2.85 ± 3.99	48	12.6 ± 7.14	<0.01
Hippocampus (mm^3)	257	7344 ± 895	422	6996 ± 1126	39	5757 ± 948	<0.01
Middle temporal (mm^3)	257	20,298 ± 2600	422	20,186 ± 2735	39	17,776 ± 3230	<0.01
Entorhinal (mm^3)	257	3803 ± 650	422	3610 ± 723	39	2919 ± 705	<0.01

Data are given as mean ± standard deviation unless otherwise indicated

* *P* values for continuous variables are from one-way analysis of variance (ANOVA). *P* values for categorical data are from chi-square test

CN cognitively normal, *MCI* mild cognition impairment, *AD* Alzheimer's disease, *CDR-SB* Clinical Dementia Rating sum of boxes, *ADAS-cog* Alzheimer's disease Assessment Scale Cognition, *MMSE* Mini-Mental State Exam, *RAVLT* Rey Auditory Verbal Learning Test, *FAQ* Functional Activities Questionnaire

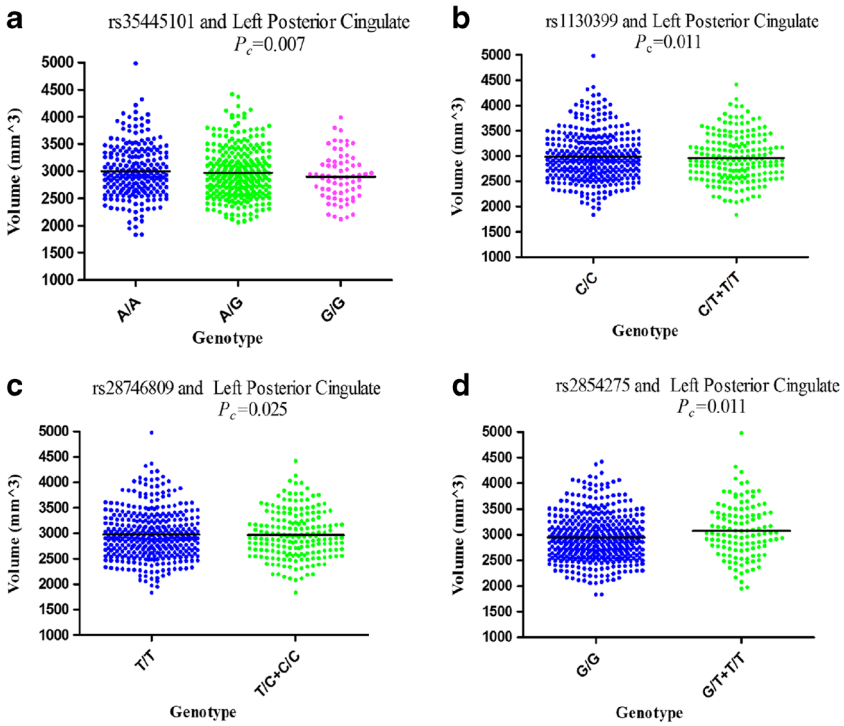

Fig. 1 The correlation between significant loci and AD specific brain structure on MRI in the total group. We identified four loci which associations were still significant after FDR correction in the total group. **a**–**c** Depicted that rs35445101 (G allele), rs1130399 (T allele), and rs28746809 (C allele) were respectively associated with smaller baseline volume of the left posterior cingulate in the total group; **d** depicted that rs2854275 (T allele) was associated with larger baseline volume of the left posterior cingulate in the total group

AD, MCI, or NC group at baseline and observed that G allele of rs35445101, T allele of rs1130399, and C allele of rs28746809 were respectively associated with the smaller volume of the left posterior cingulate, and T allele of rs2854275 was associated with the larger left posterior cingulate volume in MCI subgroup at baseline (rs35445101: $P=0.021$; rs1130399: $P=0.008$; rs28746809: $P=0.029$; rs2854275: $P=0.020$) (Fig. 2a–d; Supplementary Table 2), moreover, rs35445101 and rs1130399 were also respectively found to be associated with the smaller volume of the left posterior cingulate volume in NC subgroup at baseline (rs35445101: $P=0.014$; rs1130399: $P=0.033$) (Fig. 3a, b; Supplementary Table 2). The associations did not exist in the AD group, probably for inadequate sample sizes used to detect the effect of different genotypes.

Discussion

Our analysis in ADNI dataset showed three loci in *HLA-DRB1*/*DQB1* (rs35445101, rs1130399, and rs28746809) were associated with the smaller baseline volume of the left posterior cingulate, and rs2854275 was associated with the larger baseline volume of the left posterior cingulate, suggesting that *HLA-DRB1*/*DQB1* genetic variation might play a role in AD-related brain neurodegeneration. With reference to the website http://hla.alleles.org, both SNPs rs28746809 and rs1130399 existed in the *HLA-DQB1* 03:03* or *HLA-DQB1* 03:05* alleles in the Caucasians, and SNP rs2854275 exist in the *HLA-DQB1*02:01* alleles in the Caucasians, thus our results suggested that *HLA-DQB1* 03:03* or *HLA-DQB1* 03:05* alleles were related with the altered baseline volume of the left posterior cingulate, and *HLA-DQB1*02:01* alleles were also correlated to the altered baseline volume of the left posterior cingulate in ADNI subjects. Our study further revealed the potential pathways by which these genetic variations act in modulation of the susceptibility of AD.

Inflammation process has been identified to play an important role in AD and some identified genes involved in inflammation are found to be associated with the increased risk of developing AD, thereby lending increasing support for the roles of immune activation and possibly inflammation in the progression of AD [9]. Much earlier studies have showed the prediction and clinical course of the systemic inflammatory diseases rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), in which inflammatory mechanisms such as complement activation contribute to tissue destruction, are influenced by *HLA-DR* genotype [21, 22]. The researchers found that *HLA-DR4*-influenced glial activity in the AD hippocampus based on the finding that glial cells, which mediated inflammatory/immune mechanisms, were active around neuritic plaques in the AD brains [23]. Among the amyloid β-peptide (Aβ, a hallmark of AD) vaccination studies aimed at the clearance of Aβ, the researchers showed that the extent of Aβ T cell activation differed significantly among mouse strains bearing different MHC class II (MHC-II) haplotypes, and *HLA-DRB1*1501* allele had a high immunogenic role in Aβ autoimmunity [24]. Moreover, the possible associations of *HLA-DR1*, *2*, and *3* with increased AD risk and *DR4*, *6*, and *9* with decreased AD risk were detected [10, 12]. Recently, AD

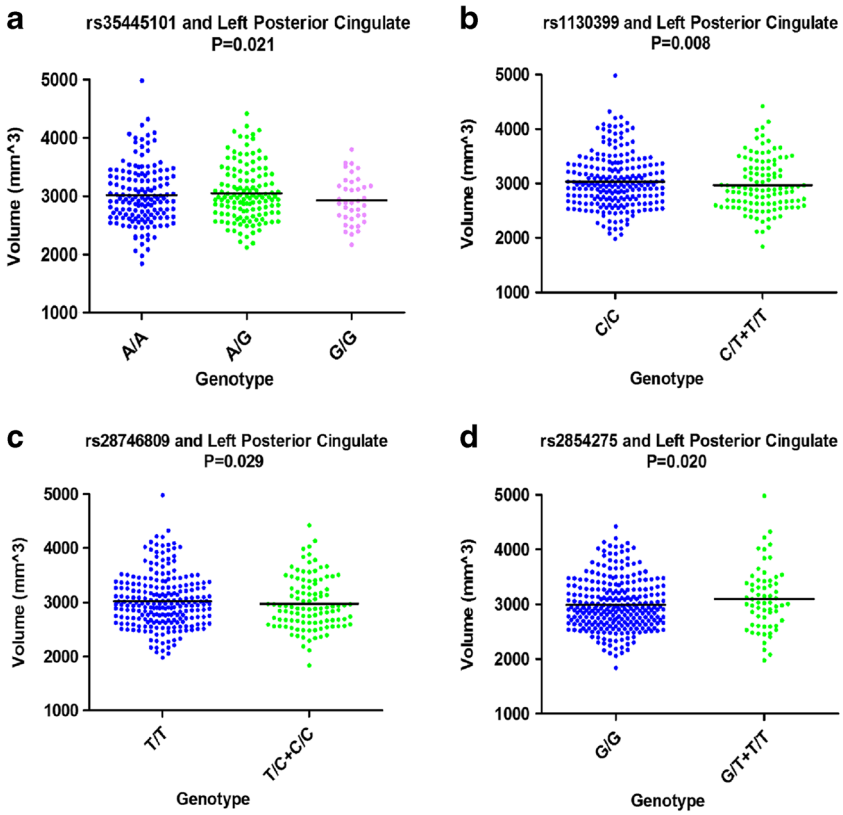

Fig. 2 The correlation between significant loci and the left posterior cingulate on MRI in the MCI group. We detected the associations of the four significant loci with the left posterior cingulate in the MCI group. **a**–**c** Depicted that rs35445101 (G allele), rs1130399 (T allele), and rs28746809 (C allele) were respectively associated with smaller baseline volume of the left posterior cingulate in the MCI group; **d** depicted that rs2854275 (T allele) was associated with larger baseline volume of the left posterior cingulate in the MCI group. *MCI* mild cognition impairment

in Tunisian patients was found to be associated with *HLA-DRB1**15, *DRB1**04, and *DQB1**06, as well as the haplotypes *DRB1**1501/*DQB1**0602 and *DRB1**04/*DQB1**0302 [13]. Altogether, from the simple analysis of the possible mechanism, *HLA-DR/DQ* genes are linked to AD.

Our study provided the first evidence linking *HLA-DRB1/DQB1* genetic variations with the altered baseline volume left posterior cingulate. However, the concrete mechanisms are still elusive. To determine when the four positive loci modulate risk for AD, we did the subgroup analysis to detect the associations of between the four loci and the MRI brain structure in three subgroups. The subgroup analysis results showed that the four SNPs were demonstrated to be associated with the altered baseline volume of the left posterior cingulate in the MCI group, and two risk loci (rs35445101 and rs1130399) were also demonstrated to be associated with the smaller baseline volume of the left posterior cingulate in the NC group, suggesting that the SNPs might play a role with an early start from the MCI or NC stage. Our study showed that the left posterior cingulate was the pivotal region on which four loci target. The result of the three variants in *HLA-DQB1* alleles tightly in one block and the fact that *HLA-DQB1* 03* allele and *HLA-DQB1*02* allele cannot coexist in the same chromosome provide further evidence for that *HLA-DRB1/DQB1* genetic variations target on the left posterior cingulate. Furthermore, it was previously reported that abnormalities in the posterior cingulate occurred in the early stage of AD [18, 19, 25–27]. It is thus inferred that *HLA-DRB1/DQB1* loci appear to influence the risk for AD by the action of altering the volume of the left posterior cingulate with the start from the NC or MCI stage.

On the other hand, the major contributors to brain atrophy include normal aging and abnormal pathological insults. As the stage further progresses (for example, from NC to MCI/AD), the role of abnormal pathologies is increasingly rising and would finally surpass that of normal aging. Given that normal aging might play a more important role in causing brain atrophy in the stage of NC than MCI/AD and abnormal pathologies would arise constantly as the stage progresses, it can be thus inferred that the potential pathways by which these variations act may be possibly associated with normal aging of the left posterior cingulate in the NC stage and possibly gradually turn to be associated with abnormal pathologies. More researches are warranted to validate these hypotheses. Furthermore, it has been widely accepted that at rest state, important brain areas—posterior cingulate cortex combines precuneus, lateral temporal cortex, medial prefrontal cortex, and inferior parietal lobule organized into a functionally relevant networks, the "default mode network" (DMN), which is a resting-state functional network and is particularly active in healthy people when they do not think about anything. Posterior cingulate area is a pivotal part of DMN. Several recent studies have revealed that DMN cortical hubs exhibit high amounts of Aβ deposits in AD, and their abnormalities

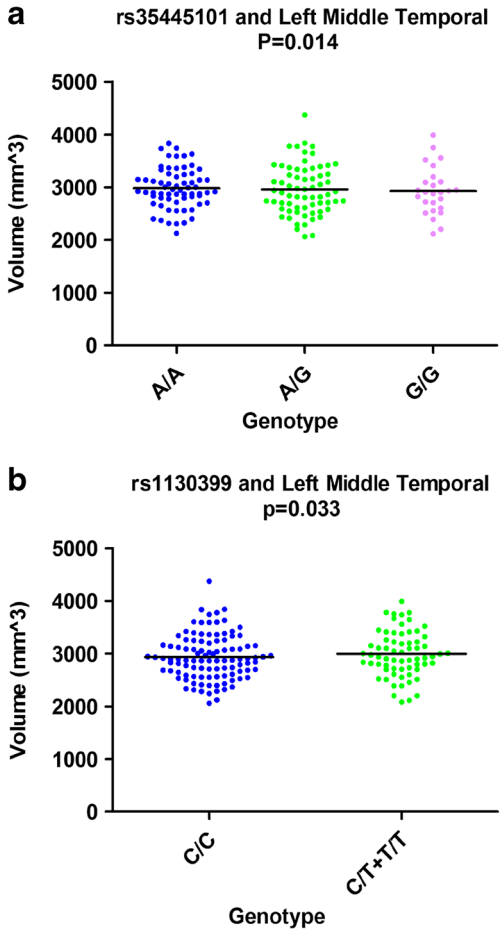

Fig. 3 The correlation between significant loci and the left posterior cingulate on MRI in the NC group. We detected the associations of the two significant loci with the left posterior cingulate in the NC group. **a**, **b** depicted that rs35445101 (G allele) and rs1130399 (T allele) were respectively associated with smaller baseline volume of the left posterior cingulate in the NC group. *NC* normal cognitive

are associated with memory deficits in AD [26, 28]. DMN was composed of large amounts of communication hubs named "synapses," and the normal operations of synapses are vitally important to maintain normal functions of this network [29]. Also, failure of DMN and abnormalities of synapses in the posterior cingulate both occurred in the early stage of AD [27, 28]. Therefore, we speculate that *HLA-DRB1*/*DQB1* loci might influence the operations of synapse by altering neurotransmitter delivering. Although rs2854275, rs1130399, and rs2854275 exist in HLA-DRB1, the allele which SNP rs2854275 exists in is different from the allele which both SNPs rs28746809 and rs1130399 exist in the Caucasians, and their effects on baseline volume of the left posterior cingulate were also opposite. We infer that the potential pathways by which these genetic variations act are different.

Our results are not in accordance with data obtained by the previous studies. The discordance may be due to distinct *HLA* variant frequencies in different cohorts, or to other interacting, genetic, or environmental factors, or to the heterogeneity of AD and MCI, or in some cases, to problems of study design. Additionally, another important reason is not ignored that the limited SNPs extracted from ADNI database cannot be used to type more *HLA-DRB1*/*DQB1* alleles to cover the previously studied alleles.

Like the previous researches on ADNI database [17, 30, 31], our quantitative traits (QTs) association study has some similar limitations, e.g., lack of part of QTs data available and confines of the Caucasian population. For our study, ADNI database can only provide a few loci out of thousands of genetic variants in *HLA-DRB1*/*DQB1* alleles, and these limited loci cannot be used to discriminate which *HLA-DRB1*/*DQB1* allele are associated with the MRI brain structures of interest.

In conclusion, our findings supported the claim that four loci in *HLA-DRB1*/*DQB1* (rs35445101, rs1130399, rs2854275, and rs28746809) modulated the alteration of the left posterior cingulate, suggesting that *HLA-DRB1*/*DQB1* gene variants appeared to participate in the neuronal degeneration of AD associated brain regions. Nevertheless, further neuropathological and biological studies of *HLA-DRB1*/*DQB1* and their loci may fully unveil their roles in AD, and further research in large independent samples with diverse ethnicity is required to confirm the effects of *HLA-DRB1*/*DQB1* on AD.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

References

1. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA et al (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. JAMA 278(16):1349–1356
2. National Institute on Aging/Alzheimer's Association Working Group (1996) Apolipoprotein E genotyping in Alzheimer's disease. Lancet 347(9008):1091–1095
3. Roses AD (1994) Apolipoprotein E affects the rate of Alzheimer disease expression: beta-amyloid burden is a secondary consequence dependent on APOE genotype and duration of disease. J Neuropathol Exp Neurol 53(5):429–437
4. Tysoe C, Galinsky D, Robinson D, Brayne C, Huppert FA, Dening T, Paykel ES, Easton DF et al (1998) Apo E and Apo CI loci are associated with dementia in younger but not older late-onset cases. Dement Geriatr Cogn Disord 9(4):191–198
5. Bertram A (2010) Medical image. Angina bullosa haemorrhagica. N Z Med J 123(1314):122
6. Tan MS, Jiang T, Tan L, Yu JT (2014) Genome-wide association studies in neurology. Ann Transl Med 2(12):124. doi:10.3978/j.issn.2305-5839.2014.11.12
7. Peper JS, Brouwer RM, Boomsma DI, Kahn RS, Hulshoff Pol HE (2007) Genetic influences on human brain structure: a review of brain imaging studies in twins. Hum Brain Mapp 28(6):464–473. doi:10.1002/hbm.20398
8. Zhang X, Yu JT, Li J, Wang C, Tan L, Liu B, Jiang T (2015) Bridging integrator 1 (BIN1) genotype effects on working memory, hippocampal volume, and functional connectivity in young healthy individuals. Neuropsychopharmacology : Off Publ Am Coll Neuropsychopharmacol 40(7):1794–1803. doi:10.1038/npp.2015.30
9. Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci 16(6):358–372. doi:10.1038/nrn3880
10. Curran M, Middleton D, Edwardson J, Perry R, McKeith I, Morris C, Neill D (1997) HLA-DR antigens associated with major genetic risk for late-onset Alzheimer's disease. Neuroreport 8(6):1467–1469
11. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T et al (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14(4):388–405. doi:10.1016/S1474-4422(15)70016-5
12. Neill D, Curran MD, Middleton D, Mawhinney H, Edwardson JA, McKeith I, Ballard C, Morris C et al (1999) Risk for Alzheimer's disease in older late-onset cases is associated with HLA-DRB1*03. Neurosci Lett 275(2):137–140
13. Mansouri L, Messalmani M, Klai S, Bedoui I, Derbali H, Gritli N, Mrissa R, Fekih-Mrissa N (2015) Association of HLA-DR/DQ polymorphism with Alzheimer's disease. Am J Med Sci 349(4):334–337. doi:10.1097/MAJ.0000000000000416
14. Simmons A, Westman E, Muehlboeck S, Mecocci P, Vellas B, Tsolaki M, Kloszewska I, Wahlund LO et al (2009) MRI measures of Alzheimer's disease and the AddNeuroMed study. Ann N Y Acad Sci 1180:47–55. doi:10.1111/j.1749-6632.2009.05063.x
15. Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack C, Jagust W, Trojanowski JQ, Toga AW et al (2005) The Alzheimer's disease neuroimaging initiative. Neuroimaging Clin N Am 15(4):869–877. doi:10.1016/j.nic.2005.09.008, **xi-xii**
16. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, Jack CR Jr, Jagust WJ et al (2010) Alzheimer's disease neuroimaging initiative (ADNI): clinical characterization. Neurology 74(3):201–209. doi:10.1212/WNL.0b013e3181cb3e25
17. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N, Salat D, Rosand J et al (2010) Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 67(6):677–685. doi:10.1001/archneurol.2010.108
18. Kiuchi K, Morikawa M, Taoka T, Nagashima T, Yamauchi T, Makinodan M, Norimoto K, Hashimoto K et al (2009) Abnormalities of the uncinate fasciculus and posterior cingulate fasciculus in mild cognitive impairment and early Alzheimer's disease: a diffusion tensor tractography study. Brain Res 1287:184–191. doi:10.1016/j.brainres.2009.06.052
19. Choo IH, Lee DY, Oh JS, Lee JS, Lee DS, Song IC, Youn JC, Kim SG et al (2010) Posterior cingulate cortex atrophy and regional cingulum disruption in mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 31(5):772–779. doi:10.1016/j.neurobiolaging.2008.06.015
20. Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat Med 9(7):811–818
21. Stastny P, Ball EJ, Khan MA, Olsen NJ, Pincus T, Gao X (1988) HLA-DR4 and other genetic markers in rheumatoid arthritis. Br J Rheumatol 27(Suppl 2):132–138
22. Wagner U, Kaltenhauser S, Sauer H, Arnold S, Seidel W, Hantzschel H, Kalden JR, Wassmuth R (1997) HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 40(2):341–351
23. Aisen PS, Luddy A, Durner M, Reinhard JF Jr, Pasinetti GM (1998) HLA-DR4 influences glial activity in Alzheimer's disease hippocampus. J Neurol Sci 161(1):66–69
24. Zota V, Nemirovsky A, Baron R, Fisher Y, Selkoe DJ, Altmann DM, Weiner HL, Monsonego A (2009) HLA-DR alleles in amyloid beta-peptide autoimmunity: a highly immunogenic role for the DRB1*1501 allele. J Immunol 183(5):3522–3530. doi:10.4049/jimmunol.0900620
25. Alves GS, O'Dwyer L, Jurcoane A, Oertel-Knochel V, Knochel C, Prvulovic D, Sudo F, Alves CE et al (2012) Different patterns of white matter degeneration using multiple diffusion indices and volumetric data in mild cognitive impairment and Alzheimer patients. PLoS One 7(12):e52859. doi:10.1371/journal.pone.0052859
26. Huang Y, Mucke L (2012) Alzheimer mechanisms and therapeutic strategies. Cell 148(6):1204–1222. doi:10.1016/j.cell.2012.02.040
27. Scheff SW, Price DA, Ansari MA, Roberts KN, Schmitt FA, Ikonomovic MD, Mufson EJ (2015) Synaptic change in the posterior cingulate gyrus in the progression of Alzheimer's disease. J Alzheimer's Dis : JAD 43(3):1073–1090. doi:10.3233/JAD-141518
28. Simic G, Babic M, Borovecki F, Hof PR (2014) Early failure of the default-mode network and the pathogenesis of Alzheimer's disease. CNS Neurosci Ther 20(7):692–698. doi:10.1111/cns.12260
29. Richiardi J, Altmann A, Milazzo AC, Chang C, Chakravarty MM, Banaschewski T, Barker GJ, Bokde AL et al (2015) BRAIN NETWORKS. Correlated gene expression supports synchronous activity in brain networks. Science 348(6240):1241–1244. doi:10.1126/science.1255905
30. Liu Y, Yu JT, Wang HF, Hao XK, Yang YF, Jiang T, Zhu XC, Cao L et al (2014) Association between NME8 locus polymorphism and cognitive decline, cerebrospinal fluid and neuroimaging biomarkers in Alzheimer's disease. PLoS One 9(12):e114777. doi:10.1371/journal.pone.0114777
31. Wang C, Tan L, Wang HF, Yu WJ, Liu Y, Jiang T, Tan MS, Hao XK et al (2015) Common variants in PLD3 and correlation to amyloid-related phenotypes in Alzheimer's disease. J Alzheimer's Dis : JAD 46(2):491–495. doi:10.3233/JAD-150110