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Genome-wide association study identifies Alzheimer's risk variant in MS4A6A influencing cerebrospinal fluid sTREM2 levels

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ABSTRACT

The triggering receptor expressed on myeloid cells 2 (*TREM2*) gene has been reported to increase the risk of Alzheimer's disease (AD). The soluble TREM2 protein (sTREM2) in cerebrospinal fluid (CSF) was also associated with AD. However, the role of sTREM2 in AD and its genetic modifiers remain unclear. We carried out a genome-wide association study for CSF sTREM2 levels using participants from the Alzheimer's Disease Neuroimaging Initiative and validated the significant association in an independent cohort from Chinese Alzheimer's Biomarker and LifestylE study. rs7232 in membrane spanning 4-domains A6A (*MS4A6A*) gene was associated with CSF sTREM2 levels at genome-wide significance ($p = 1.42 \times 10^{-15}$). The locus influences CSF sTREM2 levels especially in nondemented individuals. And the association was replicable in the validation cohort from Chinese Alzheimer's Biomarker and LifestylE study ($p = 0.0106$). Besides, the expressions of *MS4A6A* and *TREM2* were correlated in brain regions ($p < 2 \times 10^{-16}$). The findings of our study suggest that the AD risk variant in the *MS4A6A* gene participates in the regulation of sTREM2.

1. Introduction

Heterozygous mutations in the triggering receptor expressed on myeloid cells 2 (*TREM2*) gene have been demonstrated to be associated with increased risk of Alzheimer's disease (AD). The functional *p*.R47H variant increases susceptibility to late-onset AD in Caucasians with an odds ratio similar to that of apolipoprotein E (*APOE*) ε4 allele (Guerreiro et al., 2013; Jonsson et al., 2013; Neumann et al., 2013). Besides, *p*.H157Y in *TREM2* exon 3 was also associated with increased risk of AD in Han Chinese population (Jiang et al., 2016a). It has been suggested that TREM2 protein also plays a role in AD-related pathology, including amyloid-β (Aβ) deposition and tau hyperphosphorylation (Gao et al., 2017). TREM2 protein is an innate immune receptor primarily expressed on the surface of microglia. TREM2 has functions of suppressing inflammatory responses and regulating phagocytic pathways (Bajramovic et al., 2011; Hsieh et al., 2009). Loss of function of TREM2 could impair microglial clearance of Aβ (Frank et al., 2008).

A soluble form of TREM2 (sTREM2) which is released by ectodomain cleavage of TREM2 can be measured in the cerebrospinal fluid (CSF) (Piccio et al., 2008). It has been found that TREM2 is shed predominantly at the H157-S158 peptide bond where the risk variant *p*.H157Y has been discovered in Han Chinese population (Schlepckow et al., 2017; Thornton et al., 2017). sTREM2 was considered a potential biomarker implicated in AD (Heslegrave et al., 2016; Piccio et al., 2016; Suarez-Calvet et al., 2016b). The levels of CSF sTREM2 are correlated with the levels of CSF AD-related biomarkers t-tau and p-tau (Alosco et al., 2018; Heslegrave et al., 2016). It is also associated with gray matter volume in the early stage of AD (Gispert et al., 2016). In addition, sTREM2 could induce inflammatory responses and increase microglial survival (Zhong et al., 2017). And it is

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[2] 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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reported that microglia activation that precedes the onset of AD could be reflected by higher levels of CSF sTREM2 (Hamelin et al., 2016; Suarez-Calvet et al., 2016b). These findings suggested that sTREM2 may be involved in AD pathology (Heslegrave et al., 2016; Piccio et al., 2016). However, the potential role of sTREM2 in AD and its genetic modifiers remain unclear.

In our study, we performed a genome-wide association study (GWAS) and a subgroup analysis for CSF sTREM2 to discover potential genetic factors involved in sTREM2, which demonstrated that AD risk variants in membrane spanning 4-domains A6A (*MS4A6A*) gene could influence CSF sTREM2 levels, especially in nondemented elders.

2. Materials and methods

2.1. Participants

In this study, 449 and 179 non-Hispanic white individuals whose data met all quality control (QC) criteria were included from the Alzheimer's Disease Neuroimaging Initiative (ADNI) 1/GO/2 cohorts and ADNI-1 cohort, respectively. The demographic information for participants and description of their CSF sTREM2 levels are shown in Table 1.

The full cohort with both sTREM2 and genotype data included 508 participants from ADNI-1/GO/2 cohorts and 281 participants from ADNI-1 cohort. There are some overlaps between the participants of ADNI-1/GO/2 cohorts and ADNI-1 cohort. Eight-four individuals in both ADNI-1/GO/2 cohorts and ADNI-1 cohort were removed from ADNI-1 cohort in our study. To reduce potential bias due to population stratification, all the subjects were restricted to non-Hispanic Caucasians. This step removed 48 subjects from ADNI-1/GO/2 cohorts and 18 subjects from ADNI-1 cohort. Population substructure and cryptic relatedness were tested with multidimensional scaling components and genomic identity by descent. This step removed 2 participants from ADNI-1/GO/2 cohorts who were cryptically related and clustering separately from the other samples (024_S_2239 and 024_S_4084, $\pi = 0.423$).

2.2. ADNI data set

Data used in the preparation for this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see www.adni-info.org.

2.3. IGAP data set

International Genomics of Alzheimer's Project (IGAP) is a large two-stage study based on GWASs in individuals of European ancestry. In stage 1, IGAP used genotyped and imputed data on 7,055,881 single-nucleotide polymorphisms (SNPs) to meta-analyze four previously published GWAS data sets consisting of 17,008 AD cases and 37,154 controls (The European Alzheimer's disease Initiative; the Alzheimer Disease Genetics Consortium; the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium; and the Genetic and Environmental Risk in AD consortium). In stage 2, 11,632 SNPs were genotyped and tested for association in an independent set of 8572 AD cases and 11,312 controls. Finally, a meta-analysis was performed combining results from stages 1 and 2.

2.4. Braineac data sets

The Braineac is a web-based tool to access the expression quantitative trait loci (eQTLs) data sets from the UK Brain Expression Consortium (Ramasamy et al., 2014). The data sets are available online for the exploration of the regulatory significance of genetic variants in the human brain (http://www.braineac.org/). The Braineac includes 10 eQTLs data sets covering 10 human brain regions including cerebellar cortex, frontal cortex, hippocampus, medulla (specifically inferior olivary nucleus), occipital cortex, putamen, substantia nigra, temporal cortex, thalamus, and intralobular white matter. More detailed information is described in the original study (Ramasamy et al., 2014).

2.5. CSF sTREM2 measurements and QC

CSF sTREM2 measurements were carried out with the enzyme-linked immunosorbent assay (ELISA) protocol previously established by the Haass' group with minor changes. The assay is based

Table 1
Demographic information

Baseline diagnosis	AD	MCI	HC	Total	*p*
ADNI-1/GO/2 cohorts					
N	27	270	152	449	—
Age, mean ± SD y	75.2 ± 10.5	71.7 ± 7.3	74.7 ± 5.6	72.9 ± 7.1	0.09
M/F, n	17/10	163/107	81/71	261/188	0.32
APOE ε4 carrier, %	66.7	45.6	21.7	38.8	<0.001
CSF sTREM2, mean ± SD pg/mL	4865.7 ± 2718.5	4009.7 ± 1894.9	4178.6 ± 1947.8	4118.4 ± 1989.8	0.09
ADNI-1 cohort					
N	69	80	30	179	—
Age, mean ± SD y	74.3 ± 8.3	75.2 ± 7.5	76.2 ± 6.1	75.0 ± 7.6	0.25
M/F, n	36/36	47/33	18/12	101/78	0.48
APOE ε4 carrier,%	68.1	55.0	30.0	55.3	0.002
CSF sTREM2, mean ± SD pg/mL	4111.1 ± 1602.7	4023.8 ± 1790.3	4621.7 ± 1903.3	4156.7 ± 1743.3	0.30
CABLE cohort					
N	—	130	247	377	—
Age, mean ± SD y	—	64.3 ± 9.8	60.9 ± 10.8	62.1 ± 10.5	0.01
M/F, n	—	70/60	140/107	210/167	0.68
CSF sTREM2, mean ± SD pg/mL	—	16,303.6 ± 6824.1	15,600.7 ± 6846.4	15,712.0 ± 6843.2	0.23

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; CSF, cerebrospinal fluid; CABLE, Chinese Alzheimer's Biomarker and LifestylE; HC, healthy control; MCI mild cognitive impairment, SD; standard deviation; sTREM2, soluble TREM2 protein.

on the MSD platform as previously described (Suarez-Calvet et al., 2016a, b). The assay consists of a biotinylated polyclonal goat anti-human TREM2 antibody as a capture antibody, a monoclonal mouse anti-human TREM2 antibody as a detection antibody, and a SULFO-TAG–labeled anti-mouse secondary antibody. Recombinant human TREM2 protein was used as a standard (62.5–8000 pg/mL). All CSF samples were distributed randomly across plates and measured in duplicate. And all the antibodies and plates were from a single lot to exclude variability between batches. The experiment was performed by operators experienced in running the assays and blinded to the clinical information. The mean intraplate coefficient of variation was 3.1%. All duplicate measures had a coefficient of variation $< 15\%$.

Mean and standard deviation (SD) of CSF sTREM2 measures were calculated to reduce the potential influence of extreme outliers on statistical results. Participants who had a value which was 4-fold SD greater or smaller than the mean value (4118.35 pg/mL for ADNI-1/GO/2 cohorts and 4157.67 for ADNI-1 cohort) were removed from the analysis. This step removed 3 additional participants from ADNI-1/GO/2 cohorts. The CSF sTREM2 levels were measured by two research groups. There was a strong correlation between the corrected values of sTREM2 in the two groups. And individuals with CSF sTREM2 levels that fell outside the prediction intervals (based on 98% tolerance) were removed. This step removed 6 participants from ADNI-1/GO/2 cohorts.

2.6. Genotyping and QC

The ADNI-1/GO/2 samples were genotyped with the Omni 2.5 M BeadChip (Illumina, Inc, San Diego, CA). The ADNI-1 samples were genotyped with the Human 610-Quad BeadChip (Illumina, Inc, San Diego, CA). Genome-wide complex trait analysis was used to calculate principal component factors for each sample (Price et al., 2006). The 1000 Genome Data and BEAGLE software (version 5.0) were used for imputating the individual genotypes of ADNI-1/GO/2 cohorts (Browning and Browning, 2007). Following the standard procedure, imputation was conducted using the Beagle software (version 5.0) with the HapMap GRCh37 as reference. SNPs with a Beagle $R^2 < 0.8$, a call rate $<95\%$, a minor allele frequency (MAF) <0.2, and Hardy-Weinberg equilibrium test $p < 0.001$ were removed. The individual genotypes of ADNI-1 cohort were not imputed in our study because they were mapped based on GRch36. The genetic association analyses were performed using the PLINK software (version 1.07) with the following criteria: call rate for SNPs $> 95\%$, call rate for individuals $> 90\%$, MAF > 0.20, and Hardy-Weinberg equilibrium test $p > 0.001$. APOE alleles which were defined by rs7421 and re429358 were genotyped separately by an APOE genotyping kit (Kim et al., 2011). Finally, a total of 3,263,912 imputed and genotyped SNPs passed filters and QC were included for analyses in ADNI-1/GO/2 cohort. And 319,112 SNPs passed filters and QC were included for analyses in ADNI-1 cohort.

2.7. Replication in CABLE study

Three hundred and seventy-seven elders who were northern Han Chinese in origin were derived from Chinese Alzheimer's Biomarker and LifestylE (CABLE) study as an independent replication cohort.

CABLE is a large-scale cohort study mainly focusing on Alzheimer's risk factors and biomarkers in Chinese Han population. CABLE aimed to determine the genetic and environmental modifiers of AD biomarkers and their utility in AD early diagnosis. Identification of lifestyle factors that may influence the risk of AD in Chinese Han population was also one of the aims of CABLE. The samples in CABLE study were recruited at Qingdao Municipal Hospital, consisting of cognitively normal elders and individuals with MCI or AD.

The diagnosis of AD was made by experienced clinicians according to the criteria of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. The general cognitive function was assessed by an adapted Chinese version of Mini–Mental State Examination and Montreal Cognitive Assessment.

All participants were Han Chinese in origin and aged between 50 to 90 years. The exclusion criteria were (1) central nervous system infection, head trauma, epilepsy, multiple sclerosis, or other major neurological disorders; (2) major psychological disorders (e.g., depression); (3) severe systemic diseases (e.g., malignant tumors) that may affect CSF or blood levels of AD biomarkers including Aβ and tau; (4) family history of genetic disease. All participants underwent clinical and neuropsychological assessments, biochemical testing, as well as blood and CSF sample collection. Demographic information, AD risk factor profile, and medical history were also collected by a comprehensive questionnaire and an electronic medical record system.

CSF sTREM2 concentrations were determined with the ab224881 Human TREM2 SimpleStep ELISA kit from Abcam at the Qingdao University. CSF samples were diluted 4-fold. SNPs for the validation stage were chosen on the basis of the genome-wide study in the initial analysis. Genotyping of these SNPs was performed using SNaPshot Multiplex System (Applied Systems) following the manufacture's protocol. Participants who had a value which was 4-fold SD greater or smaller than the mean value (15,677.15 pg/mL) were removed from the analysis. In total, CSF sTREM2 levels and genotyping of rs7232 were obtained from 377 participants. Data analysis was performed with linear regression implemented in R, accounting for age, sex, and diagnosis as covariates.

2.8. Standard protocol approvals, registrations, and patient consents

The ADNI study was approved by the institutional review board at each of the participating centers, and all participants provided written informed consent.

The CABLE study was conducted in accordance with the Helsinki declaration, and the protocol for this study was approved by the Institutional Ethics Committees of Qingdao Municipal Hospital. Written informed consent was obtained from all study participants directly or from their caregivers.

2.9. Statistical analyses

Associations between CSF sTREM2 levels and the genetic variants were determined using PLINK (version 1.07) with the additive genetic model, with each SNP represented as a count of the number of minor alleles. Age, sex, diagnosis, and the two principal component factors from population stratification analysis were included as covariates. To adjust for multiple testing, the thresholds of $p < 1 \times 10^{-5}$ and $p < 5 \times 10^{-8}$ were used for suggestive and genome-wide significant associations, respectively (Risch and Merikangas, 1996). The effects of genotypes on CSF sTREM2 were examined with a multiple linear regression implemented in R, accounting for age and sex as covariates. The potential associations between SNPs and gene expression were examined by a linear regression analysis under an additive genetic model using data from Braineac data set. Regional associations were visualized with the LocusZoom web tool (http://locuszoom.org/).

3. Results

3.1. Characteristics of included subjects

A total of 449 individuals from ADNI-1/GO/2 cohorts and 179 individuals from ADNI-1 cohort were included in our GWAS (Table 1). In summary, 27 AD (75.2±10.5 years), 270 MCI (71.7±7.3 years), and 179 healthy control (HC) (74.7±5.6 years) subjects from ADNI-1/GO/2 cohort and 69 AD (74.3±8.3 years), 80 MCI (75.2±7.5 years), and 30 HC (76.2±6.1 years) subjects from ADNI-1 cohort were included in this study. The AD group had the highest frequency of ε4 allele within the *APOE* gene in both ADNI-1/GO/2 (66.7%) cohorts and ADNI-1 cohort (68.1%). CSF sTREM2 levels are positively correlated with age in all participants ($p = 9.28 \times 10^{-9}$). However, there is no significant difference among the CSF sTREM2 levels of three diagnostic groups ($p = 0.09$ in ADNI-1/GO/2 cohort, $p = 0.30$ in ADNI = 1 cohort, after ANOVA and Tukey's multiple comparisons test).

3.2. rs7232 in MS4A6A is associated with CSF sTREM2 levels

After adjusting for age, gender, diagnosis, and the two principal component factors, rs7232 in *MS4A6A* was identified as the top SNP that was significantly associated with CSF sTREM2 levels ($p = 1.42 \times 10^{-15}$) in ADNI-1/GO/2 cohorts (Fig. 1A). The minor allele (A) of rs7232 was associated with higher CSF sTREM2 levels in a dose-dependent manner. The nearby SNPs and their linkage disequilibrium (LD) patterns with rs7232 are shown in Fig. 1B. Several SNPs in LD with rs7232 showed association with CSF sTREM2 levels with the $p < 0.01$. However, no SNPs were still strongly associated with CSF sTREM2 levels after controlling for the rs7232 genotype (Fig. 1C), indicating that the nearby SNPs were driven by rs7232.

In the ADNI-1 cohort, rs17602572 in *MS4A6A*, which was in LD with rs7232 ($r^2 = 0.75$), was identified as the only SNP associated with CSF sTREM2 levels at genome-wide significance ($p = 3.30 \times 10^{-8}$; Supplementary Fig. S1A). Regional association analysis showed that no SNPs were still associated with CSF sTREM2 levels after controlling for rs17602572 (Supplementary Fig. S1B and C). rs7232 was not included in the genotype data of ADNI-1 cohort because of the different gene chips used for genotyping. However, rs17602572 was included in the genotype data of ADNI-1/GO/2 cohorts after imputation. rs17602572 was associated with CSF sTREM2 levels with p value of 1.31×10^{-12} in ADNI-1/GO/2 cohorts (Fig. 1B). And the association between rs17602572 and CSF sTREM2 levels was not significant ($p = 0.8281$) after controlling for rs7232 genotype (Fig. 1C), suggesting that the association was also driven by rs7232.

SNPs of the TREM gene cluster were also tested for association. However, no SNPs of the TREM gene cluster were strongly associated with CSF sTREM2 levels at genome-wide significance level or regional Bonferroni-adjusted significance level. *APOE* ε4 was not associated with CSF sTREM2 levels either ($p = 0.342$).

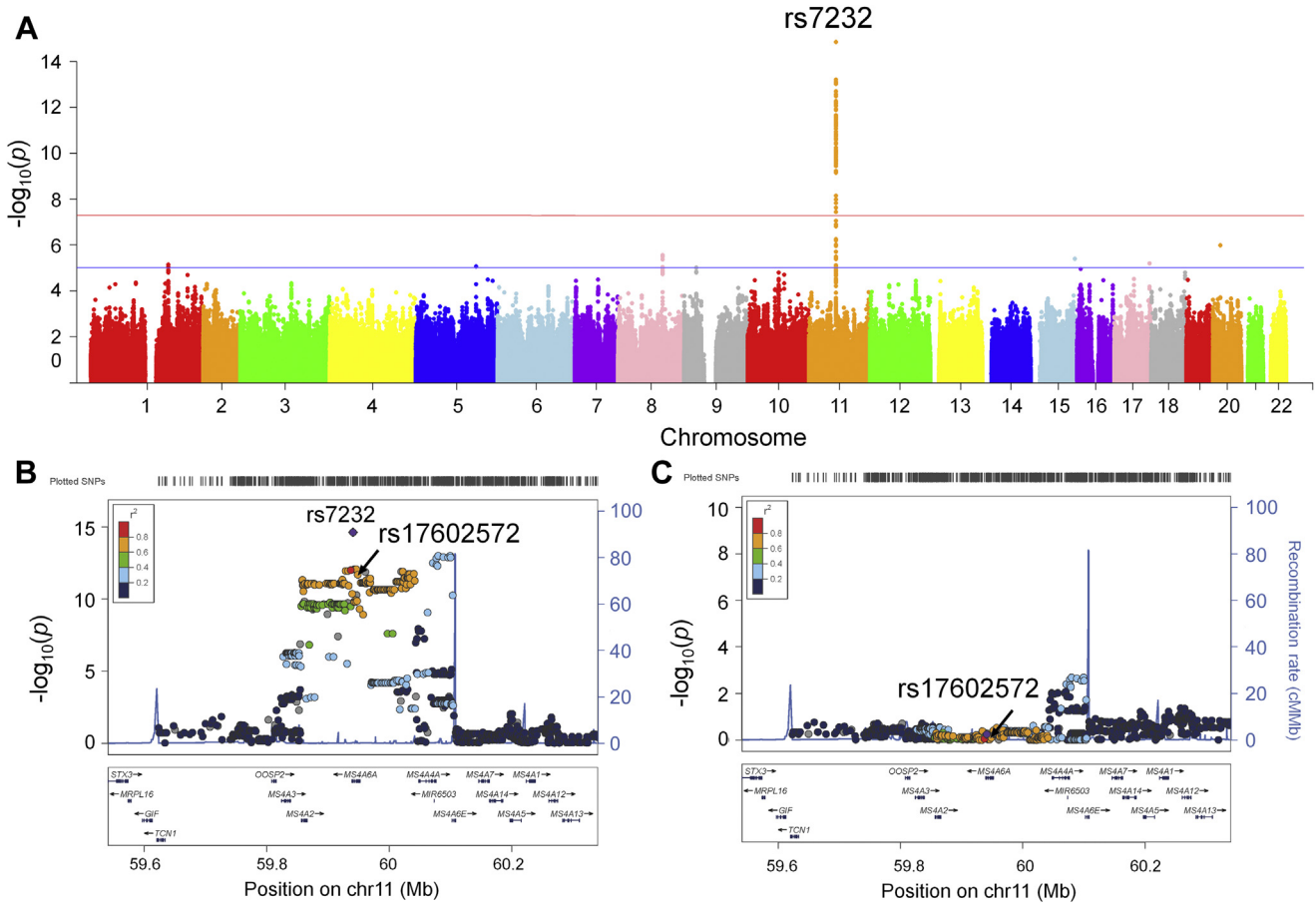

Fig. 1. Manhattan and regional plots for associations with CSF sTREM2 in ADNI-1/GO/2 cohorts. (A) Manhattan plots for associations with CSF sTREM2 levels in ADNI-1/GO/2 cohorts. (B) Regional association results for the *MS4A6A* region of chromosome 11. (C) Association results for chromosome 11: 57831716–61079651 controlling for rs7232. The association between rs17602572 and CSF sTREM2 levels was not significant ($p = 0.8281$) after controlling for rs7232 genotype. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; sTREM2, soluble TREM2 protein; *MS4A6A*, membrane spanning 4-domains A6A.

3.3. Subgroup analyses in different diagnostic groups

Subgroup analyses were conducted to find out the association between the SNPs and CSF sTREM2 levels in different diagnostic groups. As shown in Fig. 2, the minor allele (A) of rs7232 was associated with higher CSF sTREM2 levels in a dose-dependent fashion in HCs and MCI individuals ($p = 4.32 \times 10^{-10}$ for HC, $p = 2.55 \times 10^{-7}$ for MCI). However, in the AD group, results did not show significant differences in CSF sTREM2 among three genotypes in both multiple linear regression analysis ($p = 0.109$) and ANOVA ($p = 0.172$). In nondemented individuals (HC + MCI), rs7232 is still the most significant SNP associated with CSF sTREM2 levels ($p = 5.49 \times 10^{-15}$). The same pattern was also found in ADNI-1 cohort (Supplementary Fig. S2). The association between rs17602572 and CSF sTREM2 levels was much more significant in the HC and MCI groups ($p = 4.33 \times 10^{-5}$ for HC; $p = 4.80 \times 10^{-5}$ for MCI) than that in the AD group ($p = 0.030$). And rs17602572 was still the top SNP associated with CSF sTREM2 levels in nondemented individuals from ADNI-1 cohort (1.59×10^{-7}).

3.4. Replication in an independent CABLE study

As the association between rs7232 and CSF sTREM2 was significant especially in nondemented elders, we measured CSF sTREM2 levels and genotyped rs7232 in an independent Chinese Han cohort of 377 nondemented individuals from CABLE study to validate our findings. Among them, there are 247 cognitive normal individuals and 130 individuals who were diagnosed with MCI (Table 1). In summary, the age of participants ranged from 40 to 88 years (mean 61.8 years; SD, 10.4 years). There are 167 male and 133 female participants included in the cohort. The mean CSF sTREM2 concentration was 14,602.4 pg/mL with an SD of 6808.1 pg/mL. The minor allele (A) of rs7232 was also significantly associated with higher CSF sTREM2 levels ($p = 0.$, 0.0106) after adjustment for age, sex, and diagnosis (Fig. 3).

We also performed a meta-analysis including ADNI and CABLE samples. There was no significant heterogeneity in the meta-analysis of ADNI and CABLE study ($I^2 = 0\%$). The meta-analysis showed that rs7232 was associated with CSF sTREM2 levels ($p_{meta} = 1.59 \times 10^{-11}$, Fig. 4).

3.5. Associations between the associated SNPs in MS4A6A and risks of AD

The IGAP research included 2 stages, a discovery step and a replication step. Two data sets are available from IGAP including the results from stage 1 and the results from stage 2 with the combined stage 1/stage 2 *p*-values (Lambert et al., 2013). The two SNPs (rs7232 and rs17602572) associated with CSF sTREM2 levels were available in IGAP data set. And both rs7232 and rs17602572 were identified as protective loci for AD in combined data set of IGAP stages 1 and 2 (rs7232: OR, 0.90; 95% CI, 0.87–0.92; $p = 2.621 \times 10^{-14}$; rs17602572: OR, 0.90; 95% CI 0.88–0.93; $p = 1.418 \times 10^{-14}$).

3.6. Expression quantitative trait loci analysis

Large-scale blood eQTLs data sets were used to determine whether rs7232 was reported eQTLs (Westra et al., 2013). The associations between rs7232 and gene expression in human brain tissues were analyzed by a linear regression analysis with the Braineac data sets (Ramasamy et al., 2014). We found that rs7232 could not regulate the expression of genes in *MS4A* cluster in whole blood. Besides, rs7232 could not regulate the gene expression of *MS4A6A* or *TREM2* in brain regions after Bonferroni correction. The association between the expressions of *MS4A6A* and *TREM2* were also investigated. The expressions of *MS4A6A* and *TREM2* are highly correlated in the whole brain ($p < 2 \times 10^{-16}$, Fig 5) and in all the 10 brain regions (Supplementary Fig. S3).

4. Discussion

We present a GWAS of CSF sTREM2 levels in the ADNI cohort and demonstrate that rs7232 in *MS4A6A* gene could influence CSF sTREM2 levels. The minor allele (A) of rs7232 in *MS4A6A* was associated with higher CSF sTREM2 levels in a dose-dependent fashion, especially in nondemented individuals. And the association was also observed in our replication cohort. Furthermore, the expressions of *MS4A6A* and *TREM2* are highly correlated.

The role of *TREM2* has been studied in the context of AD since the rare variant *p*.R47H in *TREM2* was first discovered in 2013 (Guerreiro et al., 2013; Jonsson et al., 2013). Several AD-related *TREM2* variants were found to affect the efficiency of TREM2 signaling, suggesting that altered TREM2 function participates in the development of AD (Ulrich et al., 2017). *MS4A6A* has also been demonstrated to be associated with AD risk in previous studies. Our group has reported that a common variant rs610932 in *MS4A6A* is strongly associated with decreased risk of AD (Tan et al., 2013). Rare variants in *MS4A6A* have also been identified to be associated with AD (Vardarajan et al., 2015). But the association between rare variants in *MS4A6A* and AD is

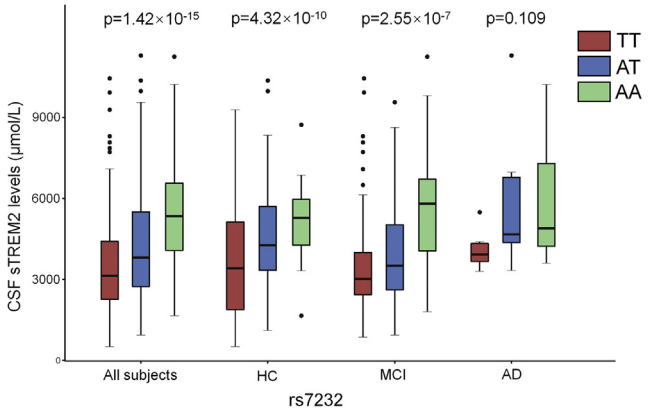

Fig. 2. CSF sTREM2 levels in different diagnostic groups and genotypes. The minor allele (A) of rs7232 showed significant association with higher CSF sTREM2 levels in a dose-dependent fashion in all diagnostic groups except the AD group. Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; sTREM2, soluble TREM2 protein; HC, healthy control; MCI, mild cognitive impairment.

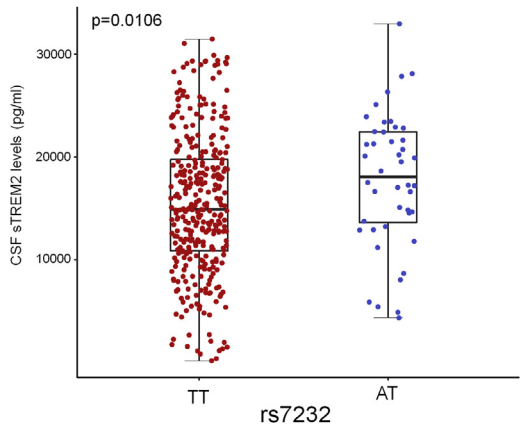

Fig. 3. CSF sTREM2 levels in a replication cohort. Increased dose of the minor allele of rs7232 was significantly associated with increased CSF sTREM2 levels in an independent Chinese Han cohort of 377 participants ($p = 0.0106$). Abbreviations: CSF, cerebrospinal fluid; sTREM2, soluble TREM2 protein.

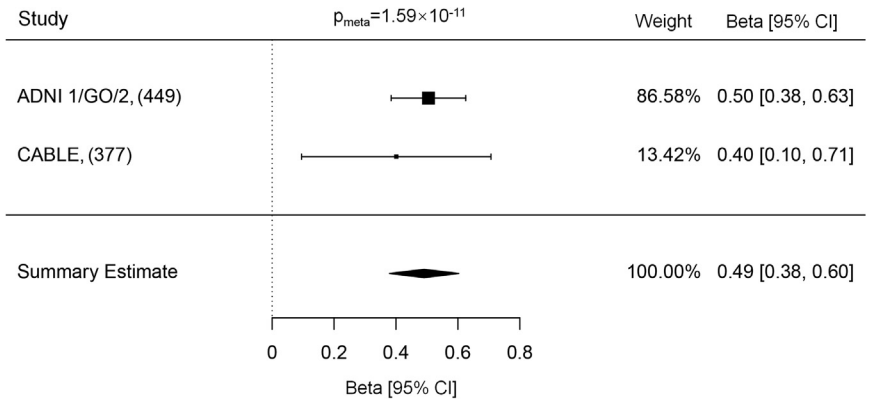

Fig. 4. Forest plot of the meta-analysis of the association between rs7232 and CSF sTREM2 levels. The meta-analysis showed that rs7232 was strongly associated with CSF sTREM2 levels ($p_{meta} = 1.59 \times 10^{-11}$). Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; CABLE, Chinese Alzheimer's Biomarker and LifestylE; CSF, cerebrospinal fluid; sTREM2, soluble TREM2 protein.

not clear as it has been reported that some rare deleterious variants in *MS4A* cluster were more frequent in controls than in patients with AD (Ghani et al., 2016). The expression of *MS4A6A* is upregulated in AD brains and associated with clinical dementia rating and Braak tangle score (Karch et al., 2012). But the underlying mechanism of *MS4A6A* involved in AD is still unknown. It was found in the present study that rs7232 which is an AD risk variant in *MAS4A6A* could influence CSF sTREM2 levels, suggesting that *MS4A6A* may influence AD risk through the modification of sTREM2 levels. Similar results have been reported recently that *MS4A* gene cluster might be a regulator of sTREM2 (Deming et al., 2018). The SNP they identified in association with sTREM2 (rs1582763) was located between *MS4A4A* and *MS4A6E*. And the SNP was also identified in our study with *p* value of 1.24×10^{-12}. However, the association between rs1582763 and sTREM2 was not significant after controlling for rs7232. The participants in their study included much more AD cases than normal controls. But in our study, we have found that rs7232 was associated with sTREM2 levels especially in HC subjects and patients with MCI. This might be the reason for the different SNPs identified in their study. Which SNP is the key regulator of CSF sTREM2 needed further study.

The AD risk variant *p*.H157Y in *TREM2* gene could enhance TREM2 shedding, which increases the generation of sTREM2 (Schlepckow et al., 2017; Thornton et al., 2017). Levels of CSF sTREM2 have been reported to change following Aβ deposition. And CSF sTREM2 levels are correlated with CSF tau levels (Alosco et al., 2018; Suarez-Calvet et al., 2016a, b). But the potential role and functions of sTREM2 in AD pathology remain unclear. CSF sTREM2

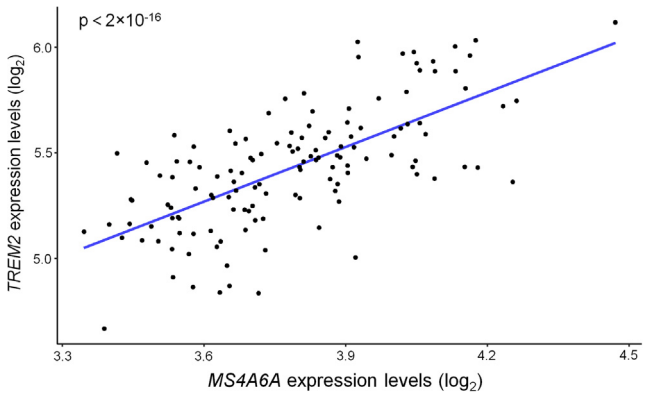

Fig. 5. Correlation between expression levels of *MS4A6A* and *TREM2*. The expressions of *MS4A6A* and *TREM2* are highly correlated in the whole brain ($p < 2 \times 10^{-16}$). Abbreviations: *MS4A6A*, membrane spanning 4-domains A6A.

could be a cause or a consequence of AD progression. It has been suggested that sTREM2 may have dual roles of promoting the production of inflammatory cytokines and enhancing microglial cell viability at the same time (Zhong et al., 2017). The result of our study that minor allele of rs7232 was associated with increased CSF sTREM2 levels and decreased AD risk is in favor of a protective role of sTREM2, although the mechanisms of sTREM2 involved in AD still need further study.

Subgroup analyses in the present study demonstrated that the SNPs in *MS4A6A* identified from GWAS mainly influence CSF sTREM2 levels in nondemented individuals including cognitively normal subjects and individuals with MCI. This is consistent with previous findings that sTREM2 was associated with the early stage of AD. In dominantly inherited patients with AD, CSF sTREM2 levels increased five years before the expected symptom onset (Suarez-Calvet et al., 2016a). Besides, sTREM2 could reflect the change of the microglial activation status, which occurs in the early stage of AD (Suarez-Calvet et al., 2016b). Besides, they have found that the CSF sTREM2 levels were higher in patients with MCI than those in normal controls and patients with AD. However, the change of CSF sTREM2 levels in AD is controversial. Although the sTREM2 levels were found to be increased in patients with AD in several studies (Piccio et al., 2016; Suarez-Calvet et al., 2016a, b), we and other groups found no significant difference in sTREM2 levels between different diagnostic groups (Deming et al., 2018; Henjum et al., 2016). Studies with larger sample size might address the question how sTREM2 levels changed during AD continuum and whether the SNPs in *MS4A6A* influence sTREM2 levels mainly in the early stage of AD. Previous gene coexpression network analysis has suggested that *TREM2* is a hub gene within an immune-gene-enriched module that contains *MS4A6D,* which is also in MS4A gene cluster (Matarin et al., 2015). Besides, the expression of caspase-4, which is an important part of the innate immune response, was strongly correlated with TREM2, MS4A6A, and the TREM2 adaptor protein TYROBP (Kajiwara et al., 2016). These results suggested that *TREM2* and *MS4A6A* expressions might play an important role in the immune response to AD pathology. Although the exact role of *MS4A6A* in AD is still unclear, it may have similar effects with *TREM2,* which participates in the regulation of microglia function (Jiang et al., 2016b). In our study, we also found that the expressions of *MS4A6A* and *TREM2* are highly correlated in the brain. sTREM2 was generated by the shedding of TREM. Both MS4A6A and TREM2 are expressed mainly on microglia in the brain (Darmanis et al., 2015). Thus, we think that MS4A6A may also play a role in the cleavage of TREM2. The increased expression of *MS4A6A* may enhance the cleavage of TREM2 and then increase CSF sTREM2 levels. And the

changes in *TREM2* expression and CSF sTREM2 levels both contribute to the AD pathology.

There are some potential limitations in this study. First, the sample size in replication cohort is limited. The MAF of rs7223 is 0.32 in ADNI and 0.06 in CABLE study. Only 44 samples carried a minor allele A of rs7232 and no one carried homozygous A of rs7232 in the whole 377 samples from CABLE. This might be the reason for the discrepancy of *p* values between discovery and replication analyses. Power analysis showed that the estimated sample size is 4006 to achieve similar significant association in CABLE on 80% power. So larger sample size may better demonstrate the association between *MS4A6A* and CSF sTREM2 in Chinese because of the low MAF of rs7232 in Han population. Besides, larger cohorts for stratified analyses for each diagnostic group are needed to investigate the change in CSF sTREM2 during AD progression. Second, combined joint analysis of ADNI samples and our samples cannot be conducted because of the different ELISA kits used for the measurement of CSF sTREM2. Further studies are needed for the understanding of mechanisms whereby *MS4A6A* influences CSF sTREM2 levels.

In summary, we demonstrated that rs7232, an AD risk variant in *MS4A6A*, is associated with CSF sTREM2 levels especially in non-demented individuals. The expression of *MS4A6A* is highly correlated with the expression of *TREM2* in the brain. Our findings suggest that *MS4A6A* gene may participate in the regulation of sTREM2.

Disclosure

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

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