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Associations Between TREML2 Gene Variants and Alzheimer's Disease: Biomarkers, Neuroimage, and Cognition

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Abstract.
Background: Recent genetic research identified a protective factor against late-onset Alzheimer's disease (AD) in Caucasians, a variant called rs3747742-C in the *TREML2* gene. However, the roles of other *TREML2* variants in AD have not been fully explored.
Objective: We conducted a focused analysis of 16 *TREML2* variants, examining their connection to AD by studying their correlation with cerebrospinal fluid (CSF) proteins, neuroimage, and cognition in the Alzheimer's Disease Neuroimaging Initiative database (ADNI).
Methods: A multiple linear regression model was utilized to estimate potential associations between *TREML2* genotypes and various endophenotypes in the entire ADNI sample at baseline, with age, gender, years of education, and *APOE* ε4 status included as covariates. To examine changes in clinical outcomes over time, linear mixed-effects models were employed.
Results: We found that the SNP rs17328707-A was associated with higher ADNI-VS scores, smaller ventricles, and larger middle temporal volume at baseline. The SNP rs6915083-G was linked to lower CSF t-tau and p-tau levels, and higher CSF Aβ levels. The SNP rs9394766-G was associated with a smaller hippocampus and larger ventricles at baseline. In longitudinal cohorts, the rs6915083-G SNP was associated with changes in ADNI-MEM and ADNI-EF scores, as well as the rate of hippocampal and middle temporal atrophy.
Conclusions: Our findings reveal that *TREML2* gene variants have different effects on AD. Two variants are protective, while one may be a risk factor. This enhances our understanding of AD genetics and could guide future research and personalized treatments.

Keywords: Alzheimer's disease, cognition, gene, single nucleotide polymorphisms, TREML2

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[2]Data used in preparation of this paper were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://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 in the writing of this paper. 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.

INTRODUCTION

Alzheimer's disease (AD) is a prevalent neurodegenerative disorder, impacting around 5% of the global population over 65 years [1]. It is characterized by presence of intracellular tau tangles and extracellular amyloid-β plaques in the brain [2]. AD is typically diagnosed in individuals over 65 years, referred to as late-onset AD (LOAD), with only a 1% incidence of early onset cases (before the age of 65) [3]. The heritability of LOAD ranges from 60% to 80% based on evidence from twin studies [4]. Previous studies have identified many genetic variants to be associated with the process of AD pathology [5]. However, a large fraction of genetic variants remains unidentified.

Triggering receptor expressed on myeloid cells like 2 (T*REML2*) is a protein-coding gene expressed on lymphoid and myeloid/granuloid cells, which has recently been linked to AD susceptibility [6]. Its expression is elevated in neutrophils, macrophages, and microglia in response to inflammatory signals [7–9]. TREML2 rs3747742 was identified as a protective factor against AD in both Caucasians [10] and Han Chinese population [11]. Moreover, the missense variant has been linked to lower cerebrospinal fluid (CSF) levels of total tau (t-tau) [12] and phosphorylated tau (p-tau) [10], as well as the volume of both right hippocampus CA1 subfield [13] and white matter hyperintensities [14]. These findings suggest that *TREML2* may reduce AD risk by mitigating process of neurodegeneration. To date, there is no comprehensive study on the correlation between *TREML2* gene and AD.

In the present study, we conducted a targeted analysis of 16 *TREML2* variants using tagger methods and analyzed the roles of *TREML2* variants in AD pathogenesis by investigating the correlation of these variants with CSF proteins, neuroimaging biomarkers and cognition in the Alzheimer's Disease Neuroimaging Initiative database (ADNI).

MATERIALS AND METHODS

ADNI dataset

We undertook cross-sectional and longitudinal analyses of participants enrolled in the ADNI database (http://adni.loni.usc.edu). The ADNI, established in 2003 as a public-private partnership, is a large, multicenter, longitudinal neuroimaging study, aimed to evaluate the combination of magnetic resonance imaging (MRI), positron emission tomography scans, biological markers, and clinical assessments for measuring the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information on ADNI, see http://www.adni-info.org. ADNI was approved by the institutional review boards of all participating centers and written informed consent was obtained from all participants or authorized representatives. We utilized the latest version of sequencing data from ADNI-1/2,GO/3 cohort.

Genotyping

For this version, GenomeStudio v2009.1 (Illumina), an updated version of BeadStudio, was used to reprocess the array data for all samples. We extracted *TREML2* genotypes from the ADNI PLINK data format. Filtering criteria applied to individuals and single nucleotide polymorphisms (SNPs) were as follows: minimum call rates > 90%, minimum minor allele frequencies (MAF) > 0.05, and Hardy-Weinberg equilibrium test $p > 0.001$. Finally, using tagger methods in Haploview 4.2 platform, we extracted other 15 common variants (Table 1). We downloaded genotype data separately from the ADNI-1, ADNI-2, ADNI-GO, and ADNI-3 databases. Subsequently, we extracted the genotypes for the 15 common variants using the PLINK software from the respective databases mentioned above. Among them, four loci were found to have available sequencing data in ADNI.

CSF proteins

Methods for collection and processing of CSF sample were reported in previous study [15]. The CSF proteins, including $A\beta_{1-42}$, T-tau, and P-tau, were calculated using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit–based reagents. Additional analysis details and quality control procedures are showed at site (http://adni.loni.ucla.edu). The measurements of CSF biomarker for this article were cross-sectional from the baseline evaluation.

Cognition

General cognition was assessed by Mini-Mental State Examination (MMSE). Composite scores for executive functioning (ADNI-EF) and mem-

Table 1
The characteristics of tagger SNPs

SNP	Location	Position	Allele change	MAF	H-W (p)
rs6915083	6 : 41196267	Intron variant	A⟶C	0.46	0.0036
rs9394766	6 : 41192063	UTR variant 3 prime	A⟶G	0.233	0.2044
rs7453883	6 : 41199357	Intron variant	C⟶G	0.0783	0.154
rs62396355	6 : 41198411	Intron variant	A⟶G	0.064	0.0522
rs6929995	6 : 41193744	Intron variant	A⟶C	0.0824	0.422
rs62396356	6 : 41200025	Intron variant	A⟶G	0.077	0.1981
rs56302558	6 : 41197533	Intron variant	G⟶A	0.215	0.4454
rs17328707	6 : 41191415	UTR variant 3 prime	G⟶A	0.077	0.0962
rs6902672	6 : 41198102	Intron variant	T⟶C	0.228	0.2425
rs3800342	6 : 41191794	UTR variant 3 prime	C⟶A	0.333	0.5211
rs11759347	6 : 41199359	Intron variant	C⟶A	0.154	0.0783
rs34346157	6 : 41198572	Intron variant	G⟶A	0.387	0.0021
rs4714431	6 : 41200599	Intron variant	A⟶C	0.337	0.3712
rs4711657	6 : 41194977	Intron variant	G⟶A	0.387	0.0713
rs13207171	6 : 41198081	Intron variant	C⟶T	0.108	0.0407

SNP, single nucleotide polymorphism; MAF, minimum minor allele frequencies; H-W, Hardy-Weinberg equilibrium test; UTR, Untranslated Regions.

ory (ADNI-MEM) using data from the ADNI neuropsychological battery using item response theory (IRT) methods. IRT was used to create composite scores for ADNI-MEM, ADNI-EF, and ADNI-LAN from ADNI neuropsychological data. It considers item difficulty and discrimination to better estimate individuals' latent abilities. By modeling responses to various items and their characteristics, IRT offers a nuanced assessment of cognitive abilities and memory, enhancing the validity and reliability of composite scores. The ADNI-EF composite score computation model encompassed contributions from various cognitive tasks: Category Fluency-animals (6.63%), Category Fluency-vegetables (6.84%), Trails A (9.92%) and B (14.44%), Digit Span Backwards (5.43%), WAIS-R Digit Symbol Substitution (14.74%), as well as five Clock Drawing items (circle, symbol, numbers, hands, time) (41.98%). The development of ADNI-MEM was influenced by the utilization of distinct word lists in the Rey Auditory Verbal Learning Test (RAVLT) and the ADAS-Cog, as well as the deliberate absence of data in Logical Memory I. The final model for ADNI-LAN (language) included the following components from the ADNI neuropsychological battery: Category Fluency–Animals, Category Fluency–Vegetables, and Boston Naming (Total). Additionally, language-related tasks from the MMSE included Repeating a sentence, reading a sentence, writing a sentence, and following a Series of Instructions (items 4 and 5). Furthermore, the Montreal Cognitive Assessment contributed six language items: Letter F Fluency, three animal naming items, and two sentence repetition tasks. There are seven items related to VS (visuospatial functioning) Neuropsychological Battery: Clock copy–Circle, Symmetry, Numbers, Hands, Time; ADAS- Cognitive Behavior: Constructional praxis; MMSE: Copy design.

Brain structures on MRI

The MR acquisition protocol used in the ADNI subjects has been described in detail in [13]. In brief, structural MRI was performed using a Siemens Trio 3.0 T scanner (n 5 507) or Vision 1.5 T scanner (n 5 131) (GE, Siemens, and Philips). Regional volume estimates were processed using Free-surfer software package version 4.3 and 5.1 image processing framework for the 1.5 and 3.0 T MRI images, respectively. Regions of interest (ROIs) included the hippocampus, entorhinal, middle tempol and ventricles.

Statistical analysis

All statistical analyses were conducted using R 4.2.3 and PLINK 1.9. The Kruskal-Wallis test was used to assess differences across different genotypes for continuous variables, while chi-squared tests were employed for categorical data. A multiple linear regression model was utilized to estimate potential associations between TREML2 genotypes and various endophenotypes in the entire ADNI sample at baseline, with age, gender, years of education, and *APOE* ε4 status included as covariates. Genotypes were treated as continuous variables, where they were converted into a scale of "0, 1, 2". The genotype corresponding to the rarer allele frequency was assigned

Table 2
Baseline Clinical Characteristics

Characteristic	rs6915083 and rs4714431			rs9394766 and rs17328707		
	CN ($n=615$)	MCI ($n=780$)	AD ($n=241$)	CN ($n=281$)	MCI ($n=484$)	AD ($n=47$)
Age, y	73.25 (6.12)	73.09 (7.61)	75.26 (8.07)	74.51 (5.56)	72.30 (7.46)	75.36 (9.27)
Gender, Male, n (%)	279 (45.4)	469 (60.1)	134 (55.6)	136 (48.4)	283 (58.5)	29 (61.7)
Education, y	16.56 (2.54)	15.89 (2.85)	14.95 (3.08)	16.41 (2.66)	15.98 (2.82)	15.72 (2.65)
APOE4 (0/1/2)	428/167/20	401/302/77	77/117/46	204/70/7	263/180/41	13/25/9
MMSE	29.12 (1.09)	27.59 (1.81)	23.15 (2.05)	29.07 (1.15)	27.89 (1.69)	22.85 (1.91)
CSF Aβ, pg/ml	1015.28 (389.75)	839.42 (351.99)	637.18 (266.67)	1037.23 (389.12)	872.99 (348.38)	691.23 (318.44)
CSF p-Tau, pg/ml	22.31 (9.07)	27.26 (13.51)	36.40 (14.73)	22.33 (8.95)	26.28 (13.24)	36.69 (13.87)
CSF Tau, pg/ml	241.97 (89.38)	282.01 (120.03)	362.92 (128.40)	243.57 (89.63)	274.13 (118.46)	370.02 (125.62)
Hippocampus, mm^3	7461.26 (881.71)	6809.99 (1143.15)	5647.26 (1076.01)	7331.91 (878.43)	6965.58 (1110.61)	5768.31 (986.12)

The data in the table is presented in the form of means and standard deviations.

a value of 2, while the genotype corresponding to the higher allele frequency was assigned a value of 0. To examine changes in clinical outcomes over time, linear mixed-effects models were employed. All variables entering the regression models were standardized by Z-scale beforehand using the "scale" function in R software, where $z=(x-u)/s$, u represents the sample mean and s represents the sample standard deviation. To control for multiple hypothesis testing, the false discovery rate (FDR) method developed by Hochberg and Benjamini was applied. Statistical significance was defined as FDR-corrected $p<0.05$.

RESULTS

Table 2 presents the characteristics of the subjects included in the study. The SNP rs6915083 and rs4714431 data were extracted from three cohorts: ADNI-1, ADNI-2/GO, and ADNI-3. These cohorts consisted of a total of 615 individuals with CN, 780 individuals with MCI, and 241 individuals with AD. The average age of the participants was 73.48 (± 7.19) years, and 882 (53.91%) of them were males. Furthermore, 84.94% of the participants had more than 12 years of education. Differences were observed in age, gender ratio, years of education, *APOE* ε4 carriers ratio, CSF biomarkers, and volume of ROIs among the different diagnosis groups. The SNP rs9394766 and rs17328707 data were extracted specifically from the ADNI-2/GO cohort, which included 281 CN individuals, 484 MCI individuals, and 47 AD individuals. The mean age of the participants in this cohort was 73.24 (± 7.07) years, with 448 (55.17%) of them being males. Similarly, 85.61% of the participants had more than 12 years of education. Apart from the years of education, there were differences in age, gender ratio, *APOE* ε4 carriers ratio, CSF biomarkers, and ROIs volume among the different diagnosis groups.

In cross-sectional cohorts, we observed that the SNP rs17328707-A was correlated with higher ADNI-VS scores ($\beta=0.23$, $p=0.001$), smaller ventricles ($\beta=-0.13$, $p=0.04$), and larger middle temporal ($\beta=0.16$, $p=0.03$) volume at baseline. The SNP rs6915083-G was linked to lower levels of CSF t-tau ($\beta=-0.11$, $p=0.01$) and p-tau ($\beta=-0.11$, $p=0.02$), as well as higher levels of CSF Aβ ($\beta=0.11$, $p=0.04$). Additionally, the SNP rs9394766-G was associated with a smaller hippocampus ($\beta=-0.13$, $p=0.02$) and larger ventricles ($\beta=0.11$, $p=0.02$) at baseline (Fig. 1 and Supplementary Table 1).

In longitudinal cohorts, we discovered that the rs6915083-G SNP was linked to changes in ADNI-MEM ($\beta=0.01$, $p=0.03$) and ADNI-EF scores ($\beta=0.01$, $p=0.05$). It was also associated with the rate of hippocampal ($\beta=0.009$, $p=0.01$) and middle temporal ($\beta=0.01$, $p=0.02$) atrophy (Fig. 2 and Supplementary Table 2).

DISCUSSION

Our study explores the association of *TREML2* rs17328707, rs6915083, rs9394766 and rs4714431 with CSF protein levels, neuroimaging biomarkers and cognitive function in total participants. We found that the SNP rs17328707-A was associated with higher ADNI-VS scores, smaller ventricles, and larger middle temporal volume at baseline. The SNP rs6915083-G was linked to lower CSF t-tau and p-tau levels, and higher CSF Aβ levels. The SNP rs9394766-G was associated with a smaller hippocampus and larger ventricles at baseline. In longitudinal cohorts, the rs6915083-G SNP was associated with changes in ADNI-MEM and ADNI-EF

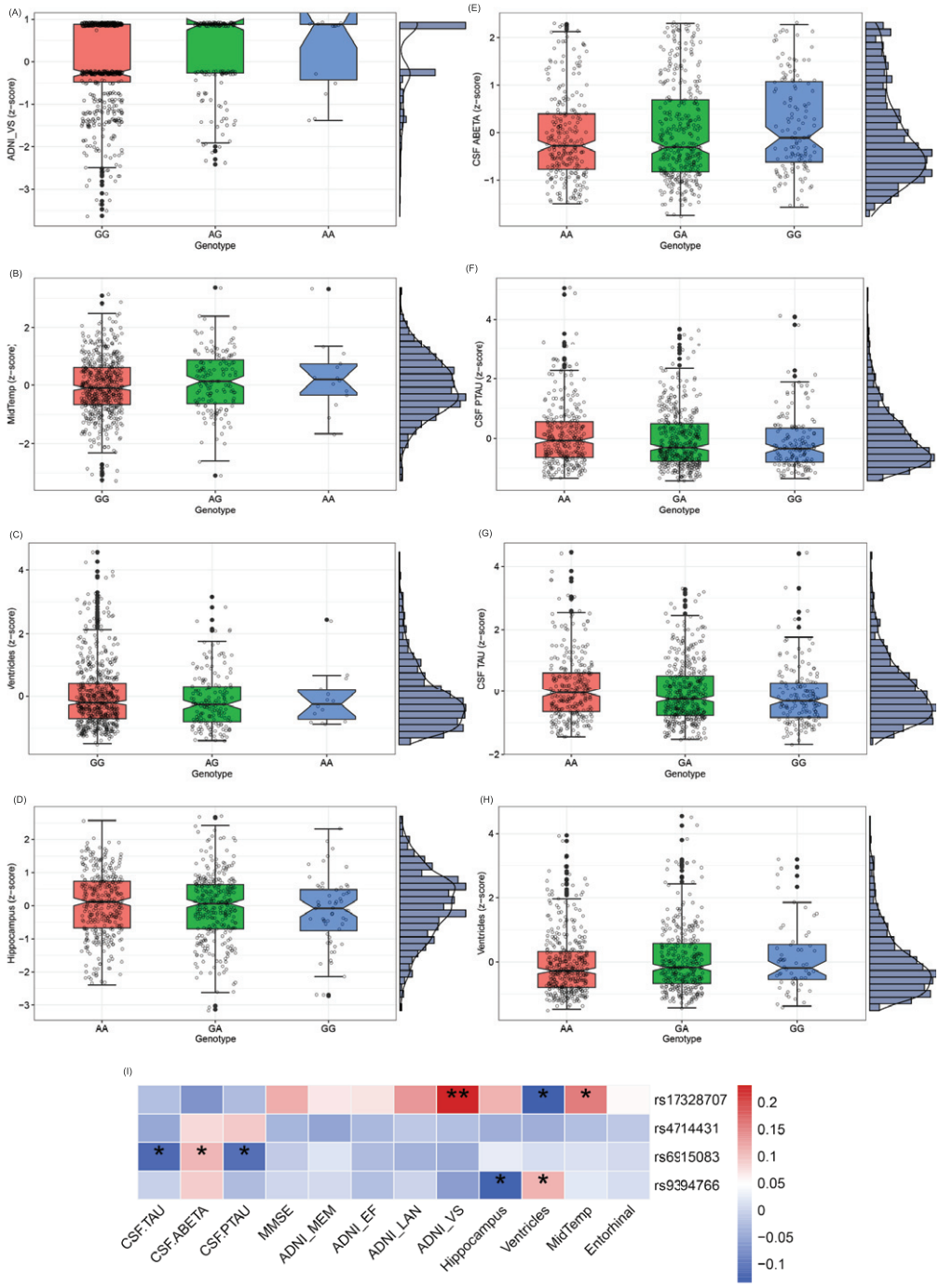

Fig. 1. Distributions of clinical indicators across various genotypes. A) The distribution of ADNI_VS across different genotypes of SNPrs17328707. B) The distribution of middle temporal lobe volume across different genotypes of SNPrs17328707. C) The distribution of ventricular volume across different genotypes of SNPrs17328707. D) The distribution of hippocampal volume across different genotypes of SNPrs9394766. E) The distribution of CSF Aβ across different genotypes of SNPrs6915083. F) The distribution of CSF p-tau across different genotypes of SNPrs6915083. G) The distribution of CSF t-tau across different genotypes of SNPrs6915083. H) The distribution of ventricular volume across different genotypes of SNPrs9394766. I) The heat map showed correlations of clinical indicators and various genotypes at baseline, with colors representing the correlation coefficients (β) of multiple linear regressions. The color bar represents the range of β values. Models were adjusted for age, gender, and education. Controlling for multiple comparisons was performed with false-discovery-rate (FDR) method of Benjamini and Hochberg. Significance: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, -$p \geq 0.05$.

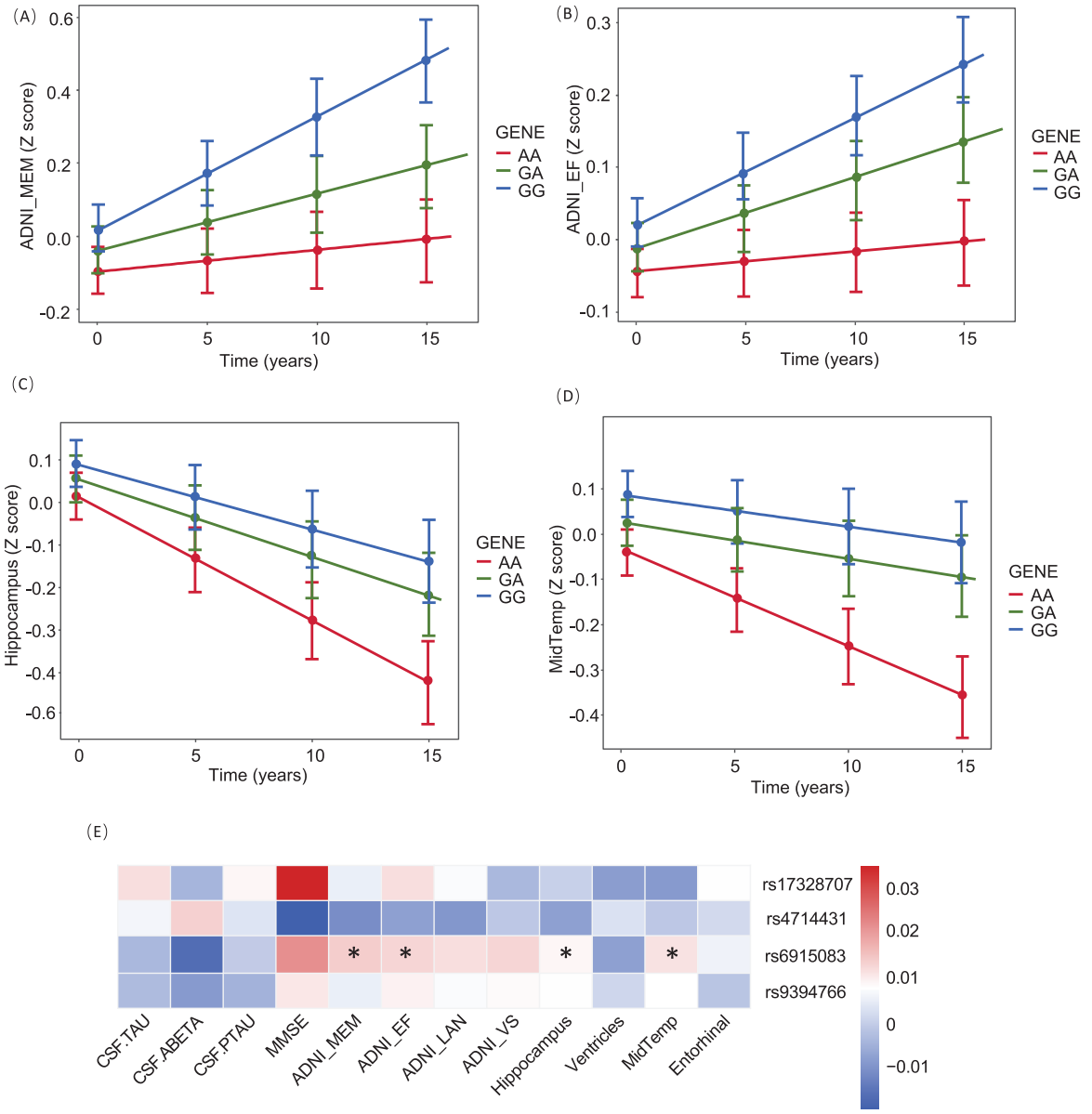

Fig. 2. The longitudinal variation of different clinical indicators across various genotypes. A) The longitudinal variation of ADNI-MEM in the rs6915083 genotype. B) The longitudinal variation of ADNI-EF in the rs6915083 genotype. C) The longitudinal variation of hippocampal volume in the rs6915083 genotype. D) The longitudinal variation of middle temporal lobe volume in the rs6915083 genotype. E) The heat map showed correlations of clinical indicators and various genotypes longitudinal, with colors representing the correlation coefficients (β) of multiple linear regressions. The color bar represents the range of β values. Models were adjusted for age, gender, and education. Controlling for multiple comparisons was performed with false-discovery-rate (FDR) method of Benjamini and Hochberg. Significance: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, -$p \geq 0.05$.

scores, as well as the rate of hippocampal and middle temporal atrophy. These findings suggest that different loci on the TREML2 gene may have varying effects on AD, with two tagger SNPs acting as protective factors and one as a risk factor.

TREML2, mainly expressed by microglia [8, 10, 16], is thought to play a crucial role in the modulation of immune functions [7, 17]. As for the correlation between *TREML2* and AD pathogenesis, evidence from animal models showed that treatment of microglia with interleukin-1β (IL-1β) increased expression of TREML2 in primary mice microglia [10]. Consistent with previous findings, Zheng et al. found that lipopolysaccharide stimulation signifi-

cantly increased *TREML2* expression in mice brain, and knock-down of *TREML2* resulted in a reduction of proinflammatory responses in microglia [8]. In light of these findings, it appears that in the context of AD, *TREML2* might induce neuroinflammation by activate pro-inflammatory responses, facilitate the proliferation of microglia and involve in the pathogenesis and progression of AD. Our study revealed an association between *TREML2* variants and Aβ levels, which has never been reported before. The association between *TREML2* and Aβ might be achieved through the modulation of microglial functions. Recent findings show that microglia act as a shield around amyloid deposits, compressing them into a potentially less harmful form, which can prevent new Aβ from sticking to existing plaques and decrease damage to nearby neuropil [18]. Research involving *TREM2*, a substrate necessary for microglial phagocytosis, demonstrated microglia's safeguarding role against toxic Aβ accumulation and AD development: soluble Aβ clearance, insoluble fibrillar Aβ phagocytosis, activation state and chemotaxis induction, and amyloid plaques compression and corralling [19]. On the other hand, aggregated Aβ triggers the activation of microglia, which in turn results in elevated production and release of multiple cytokines [20]. Microglia surrounding amyloid deposits endeavor to engulf and break down the insoluble amyloid, ultimately leading to its degradation [21, 22]. The exhaustion of microglial immune response in turn could cause aggregation of Aβ and contribute to pathogenesis of AD. As for the correlation between neuroinflammation and tau protein, studies have shown that proinflammatory cytokines released by activated microglia can trigger the pathological modification of tau protein [23–25], which can lead to neurodegeneration. Furthermore, microglia-mediated neuroinflammation can disrupt intracellular mitochondrial function, thereby causing damage and even deaths to neurons [26]. Additionally, tau proteins within cells might be released into the extracellular space after neurodegeneration [27]. Soluble extracellular tau can enhance neurotoxicity [28, 29] and trigger the release of proinflammatory cytokines [30]. The interplay among Aβ, tau protein, microglial immune responses, and neurodegeneration contribute to the development of AD pathology, and *TREML2* can expand the immune-related neuroinflammatory phase to exacerbate this pathological process.

Besides evidence from animal models, the relationship between *TREML2* and AD pathogenesis was further consolidated in cohort studies. The 2018 NIA-AA research framework proposes a classification system with Aβ deposition, pathologic tau, and neurodegeneration (ATN) for diagnosis and staging of AD [31]. In our study, we found that *TREML2* variants exhibited significant associations with all the ATN biomarkers including CSF Aβ (A), p-tau (T), t-tau (N) and brain structures (N). Previous studies have observed that *TREML2* missense variant rs3747742-C was significantly associated with decreased risk of AD in both Caucasians and Han Chinese [10, 11]. Moreover, *TREML2* rs3747742-C was observed to be associated with CSF p-tau and t-tau levels [10, 12], which is in line with our findings. Wang et al. explored the possible association between *TREML2* rs3747742 and volumes of AD-related brain structures including entorhinal cortex, middle temporal gyrus, parahippocampal gyrus, amygdala, and hippocampus in ADNI cohort, and found that TREML2 rs3747742-C was associated with a larger right hippocampal CA1 sub-field volume [13]. Consistent with the previous findings, Kühn and his colleagues found that rs3747742-C was significantly associated with a reduced AD-related brain atrophy and AD scores [32]. While in our study, *TREML2* variants were observed to be associated with AD-related brain structures including hippocampus and middle temporal volume, as well as AD scores. Despite the consistent findings of association between *TREML2* and AD biomarkers, the specific mechanism by which *TREML2* variants affects the expression of related proteins and volume of AD-related brain structures needs further research.

There are limitations in our study. Firstly, while we examined 19 tagger SNPs in our study, we were only able to extract data for 4 of them from the ADNI database. This implies that we could not comprehensively analyze all potential functions of the TREML2 gene. Secondly, our analysis was conducted exclusively within the Caucasian population, which may impose limitations on the generalizability of our findings. Therefore, we strongly recommend further research in other populations to validate and expand upon our findings, in order to obtain a more comprehensive understanding of cognitive and neuroimaging outcomes related to *TREML2* variants.

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CONFLICT OF INTEREST

The authors declare no competing interests. Jin-Tai Yu is an Editorial Board Member of this journal but was not involved in the peer-review process nor had access to any information regarding its peer-review.

DATA AVAILABILITY

The data supporting the findings of this study are openly available in at https://ida.loni.usc.edu. These data were derived from the following resources available in the public domain: https://ida.loni.usc.edu.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-230936.

REFERENCES

[1] Alzheimer's Association (2016) 2016 Alzheimer's disease facts and figures. *Alzheimers Dement* **12**, 459-509.

[2] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356.

[3] DeTure MA, Dickson DW (2019) The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener* **14**, 32.

[4] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, Fiske A, Pedersen NL (2006) Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry* **63**, 168-174.

[5] Wightman DP, Jansen IE, Savage JE, Shadrin AA, Bahrami S, Holland D, Rongve A, Borte S, Winsvold BS, Drange OK, Martinsen AE, Skogholt AH, Willer C, Brathen G, Bosnes I, Nielsen JB, Fritsche LG, Thomas LF, Pedersen LM, Gabrielsen ME, Johnsen MB, Meisingset TW, Zhou W, Proitsi P, Hodges A, Dobson R, Velayudhan L, Heilbron K, Auton A, andMe Research T, Sealock JM, Davis LK, Pedersen NL, Reynolds CA, Karlsson IK, Magnusson S, Stefansson H, Thordardottir S, Jonsson PV, Snaedal J, Zettergren A, Skoog I, Kern S, Waern M, Zetterberg H, Blennow K, Stordal E, Hveem K, Zwart JA, Athanasiu L, Selnes P, Saltvedt I, Sando SB, Ulstein I, Djurovic S, Fladby T, Aarsland D, Selbaek G, Ripke S, Stefansson K, Andreassen OA, Posthuma D (2021) A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. *Nat Genet* **53**, 1276-1282.

[6] Wang SY, Gong PY, E Y, Zhang YD, Jiang T (2020) The role of TREML2 in Alzheimer's disease. *J Alzheimers Dis* **76**, 799-806.

[7] Thomas KA, King RG, Sestero CM, Justement LB (2016) TREM-like transcript 2 is stored in human neutrophil primary granules and is up-regulated in response to inflammatory mediators. *J Leukoc Biol* **100**, 177-184.

[8] Zheng H, Liu CC, Atagi Y, Chen XF, Jia L, Yang L, He W, Zhang X, Kang SS, Rosenberry TL, Fryer JD, Zhang YW, Xu H, Bu G (2016) Opposing roles of the triggering receptor expressed on myeloid cells 2 and triggering receptor expressed on myeloid cells-like transcript 2 in microglia activation. *Neurobiol Aging* **42**, 132-141.

[9] Wang SY, Fu XX, Duan R, Wei B, Cao HM, Yan E, Chen SY, Zhang YD, Jiang T (2023) The Alzheimer's disease-associated gene TREML2 modulates inflammation by regulating microglia polarization and NLRP3 inflammasome activation. *Neural Regen Res* **18**, 434-438.

[10] Benitez BA, Jin SC, Guerreiro R, Graham R, Lord J, Harold D, Sims R, Lambert JC, Gibbs JR, Bras J, Sassi C, Harari O, Bertelsen S, Lupton MK, Powell J, Bellenguez C, Brown K, Medway C, Haddick PC, van der Brug MP, Bhangale T, Ortmann W, Behrens T, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD, Haines JL, Turton J, Braae A, Barber I, Fagan AM, Holtzman DM, Morris JC; 3C Study Group; EADI consortium; Alzheimer's Disease Genetic Consortium (ADGC); Alzheimer's Disease Neuroimaging Initiative (ADNI); GERAD Consortium; Williams J, Kauwe JS, Amouyel P, Morgan K, Singleton A, Hardy J, Goate AM, Cruchaga C (2014) Missense variant in TREML2 protects against Alzheimer's disease. *Neurobiol Aging* **35**, 1510 e1519-1526.

[11] Jiang T, Wan Y, Zhou JS, Tan MS, Huang Q, Zhu XC, Lu H, Wang HF, Chen Q, Tan L, Zhang YD, Tan L, Yu JT (2017) A missense variant in TREML2 reduces risk of Alzheimer's disease in a Han Chinese population. *Mol Neurobiol* **54**, 977-982.

[12] Song YN, Li JQ, Tan CC, Wang HF, Tan MS, Cao XP, Yu JT, Tan L, Alzheimer's Disease Neuroimaging Initiative (2019) TREML2 mutation mediate Alzheimer's disease risk by altering neuronal degeneration. *Front Neurosci* **13**, 455.

[13] Wang SY, Xue X, Duan R, Gong PY, E Y, Jiang T, Zhang YD, Alzheimer's Disease Neuroimaging Initiative (2020) A TREML2 missense variant influences specific hippocampal subfield volumes in cognitively normal elderly subjects. *Brain Behav* **10**, e01573.

[14] Lin H, Satizabal C, Xie Z, Yang Q, Huan T, Joehanes R, Wen C, Munson PJ, Beiser A, Levy D, Seshadri S (2017) Whole blood gene expression and white matter hyperintensities. *Mol Neurodegener* **12**, 67.

[15] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, Dean R, Siemers E, Potter W, Lee VM, Trojanowski JQ, Alzheimer's Disease Neuroimaging Initiative (2009) Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* **65**, 403-413.

[16] ElAli A, Rivest S (2016) Microglia in Alzheimer's disease: A multifaceted relationship. *Brain Behav Immun* **55**, 138-150.

[17] Halpert MM, Thomas KA, King RG, Justement LB (2011) TLT2 potentiates neutrophil antibacterial activity and chemotaxis in response to G protein-coupled receptor-mediated signaling. *J Immunol* **187**, 2346-2355.

[18] Condello C, Yuan P, Schain A, Grutzendler J (2015) Microglia constitute a barrier that prevents neurotoxic protofibrillar Abeta42 hotspots around plaques. *Nat Commun* **6**, 6176.

[19] Hansen DV, Hanson JE, Sheng M (2018) Microglia in Alzheimer's disease. *J Cell Biol* **217**, 459-472.

[20] Streit WJ, Khoshbouei H, Bechmann I (2021) The role of microglia in sporadic Alzheimer's disease. *J Alzheimers Dis* **79**, 961-968.

[21] Paresce DM, Chung H, Maxfield FR (1997) Slow degradation of aggregates of the Alzheimer's disease amyloid beta-protein by microglial cells. *J Biol Chem* **272**, 29390-29397.

[22] Fiala M, Lin J, Ringman J, Kermani-Arab V, Tsao G, Patel A, Lossinsky AS, Graves MC, Gustavson A, Sayre J, Sofroni E, Suarez T, Chiappelli F, Bernard G (2005) Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *J Alzheimers Dis* **7**, 221-232; discussion 255-262.

[23] Li Y, Liu L, Barger SW, Griffin WS (2003) Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. *J Neurosci* **23**, 1605-1611.

[24] Quintanilla RA, Orellana DI, Gonzalez-Billault C, Maccioni RB (2004) Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. *Exp Cell Res* **295**, 245-257.

[25] Gorlovoy P, Larionov S, Pham TT, Neumann H (2009) Accumulation of tau induced in neurites by microglial proinflammatory mediators. *FASEB J* **23**, 2502-2513.

[26] Wilkins HM, Swerdlow RH (2016) Relationships between mitochondria and neuroinflammation: implications for Alzheimer's disease. *Curr Top Med Chem* **16**, 849-857.

[27] Gomez-Ramos A, Diaz-Hernandez M, Cuadros R, Hernandez F, Avila J (2006) Extracellular tau is toxic to neuronal cells. *FEBS Lett* **580**, 4842-4850.

[28] Gomez-Ramos A, Diaz-Hernandez M, Rubio A, Diaz-Hernandez JI, Miras-Portugal MT, Avila J (2009) Characteristics and consequences of muscarinic receptor activation by tau protein. *Eur Neuropsychopharmacol* **19**, 708-717.

[29] Gomez-Ramos A, Diaz-Hernandez M, Rubio A, Miras-Portugal MT, Avila J (2008) Extracellular tau promotes intracellular calcium increase through M1 and M3 muscarinic receptors in neuronal cells. *Mol Cell Neurosci* **37**, 673-681.

[30] Kovac A, Zilka N, Kazmerova Z, Cente M, Zilkova M, Novak M (2011) Misfolded truncated protein tau induces innate immune response via MAPK pathway. *J Immunol* **187**, 2732-2739.

[31] Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R, Contributors (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.

[32] Kuhn AL, Frenzel S, Teumer A, Wittfeld K, Garvert L, Weihs A, Homuth G, Prokisch H, Bulow R, Nauck M, Volker U, Volzke H, Grabe HJ, Van der Auwera S (2022) TREML2 gene expression and its missense variant rs3747742 associate with white matter hyperintensity volume and Alzheimer's disease-related brain atrophy in the general population. *Int J Mol Sci* **23**, 13764.