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Association of TREM1 rs2234246 with miR-138-5p-dependent reporter regulation, TREM1 expression, and hippocampal volume in Alzheimer's disease

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Article

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Title: Association of TREM1 rs2234246 with miR-138-5p-dependent reporter regulation, TREM1 expression, and hippocampal volume in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder in which neuroinflammation contributes to disease pathogenesis. Triggering receptor expressed on myeloid cells 1 (TREM1) has been implicated in inflammatory responses, and the functional polymorphism rs2234246, located in the 3′-untranslated region (UTR) of TREM1, has been associated with TREM1 expression and brain amyloid deposition. We hypothesized that rs2234246 regulates *TREM1* expression through microRNA (miR)-138-5p and is associated with AD-related neurodegenerative changes. To test this hypothesis, we performed SNP functional analysis, dual-luciferase reporter assays, peripheral blood expression analyses o*f* membrane form (*mbTREM1*), soluble form (*TREM1sv*), and miR-138-5p, and magnetic resonance imaging (MRI)-based hippocampal volumetric analyses. The rs2234246 A allele was associated with increased *mbTREM1* mRNA expression but not *TREM1sv* expression. Dual-luciferase reporter assays demonstrated allele-dependent reporter repression consistent with an interaction between miR-138-5p and the TREM1 3′-UTR containing the A allele. In patients with AD, plasma TREM-1 protein concentration and miR-138-5p expression were significantly increased, whereas *TREM1sv* expression was significantly decreased. Furthermore, the rs2234246 A allele was associated with reduced right hippocampal volume in patients with AD. These findings provide complementary evidence of allele-dependent reporter regulation involving miR-138-5p, altered peripheral TREM1-related expression in AD, and an exploratory association between rs2234246 and right hippocampal volume. Further studies

are required to clarify the biological relationships among these findings and their relevance to AD pathogenesis.

Key words: Alzheimer's disease; TREM1; rs2234246; miR-138-5p; hippocampal volume; neuroinflammation

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease that is characterized by the extracellular deposition of amyloid beta-peptide in senile plaques [1,2] and progression by the accumulation of neurofibrillary tangles caused by the intracellular accumulation of hyperphosphorylated microtubule-associated protein tau [3]. As a result of the accumulation of senile plaques and neurofibrillary tangles, hippocampal atrophy is a critical feature of the morphological change in AD [4]. In the prevention of amyloid beta accumulation, microglia have an important role in the AD brain [5]. In fact, a functional single-nucleotide polymorphism (rs75932628) located in Triggering receptor expressed on myeloid cells 2 (TREM2) is strongly associated with the onset of AD [6], highly expressed in microglia in the human brain, and promotes phagocytosis of apoptotic neurons, misfolded proteins, and cellular debris [7]. In fact, loss of TREM2 function accelerated Aβ accumulation and neuronal loss in an AD mouse model [8].

TREM1, another TREM family member, is also associated with Aβ deposition and relevant to AD pathogenesis [9]. Our group has reported the elevated mRNA expressions of not only *TREM2*, but also *TREM1* in whole blood of AD patients [10,11]. *TREM1* has two major alternative splicing products (the membrane form (*mbTREM1*) and the soluble form (*TREM1sv*)), and they are translated into the proteins mbTREM1 and soluble TREM1, respectively [12]. The protein mbTREM1 is possibly regulated by rs2234246, located in the 3'-untranslated region (UTR) of *TREM1* [13]. A recent study

has reported that rs2234246 is associated with brain amyloid deposition in mild cognitive impairment and AD patients [14].

MicroRNAs (miRNAs), which are non-coding RNAs, suppress mRNA expression via binding the seed sequence located in the 3'-UTR and induce subsequent degradation of the mRNAs. Several reports have shown that some miRNAs are associated with AD pathogenesis in the brain [15] and are potential biomarkers in peripheral blood [16].

In this study, the aims were to (1) investigate whether rs2234246 changes the expressions of mRNA (*mbTREM1* and *TREM1sv*), (2) investigate allele-dependent regulation of the *TREM1* 3′-UTR by miR-138-5p using an in vitro reporter assay, (3) compare the miRNA expression that may regulate TREM1 expression between control participants and AD patients, and (4) investigate whether rs2234246 is related to hippocampal volume in control participants and AD patients.

Methods

Participants

From the Nakayama study (all older people aged > 65 years, [17]), 1) 93 control participants were evaluated for SNP functional analysis with *TREM1* expressions, 2) 40 control participants and 40 AD patients were included for mbTREM1 protein, *TREM1* mRNA, and miR-138-5p expression studies, and 3) 181 control participants and 53 AD patients were included in the MRI study. Patients with AD were diagnosed according to the National Institute on Aging–Alzheimer's Association (NIA-AA) diagnostic criteria by experienced neurologists and psychiatrists. Brain MRI was performed as part of the routine clinical assessment to support the diagnosis and exclude other structural brain diseases. Disease severity was assessed using the Mini-Mental State Examination (MMSE). Biomarker confirmation using amyloid PET or cerebrospinal fluid biomarkers was not available in the present cohort. In the present TREM1 mRNA study, mbTREM1 expression was evaluated to examine whether the finding observed in our previous study [11] could also be observed in the present cohort. There was no participant overlap between the molecular cohort in the present study and the cohort included in our previous study [11]; thus, the two cohorts were completely independent. All participants were unrelated, of Japanese origin, and signed written, informed consent forms approved by the institutional ethics committee of Ehime University Graduate School of Medicine (No. 161004). These analyses were performed in different participant cohorts derived from the Nakayama study and were intended

to provide complementary genetic, molecular, and neuroimaging evidence rather than sequential analyses within the same individuals. No statistical methods were used to predetermine sample size. Sample sizes were determined based on the availability of eligible participants and MRI data from the Nakayama study, and the availability of peripheral blood samples for the planned molecular analyses. All methods were performed in accordance with the relevant guidelines and regulations.

DNA isolation and genotyping of rs2234246

Venous blood was collected into potassium EDTA tubes. The plasma and blood cell fraction were separated by centrifugation (2,000 × g for 10 min at room temperature). Plasma samples were stored at −80°C for ELISA, and the blood cell fraction was stored at −80°C until DNA isolation. The frozen blood cell fraction was used for DNA isolation using a blood mini kit with QIAcube (Qiagen, Tokyo, Japan). The purified DNA was stored at 4 °C until genotyping. The genotyping of rs2234246 was conducted with the rhAmp assay (Integrated DNA Technologies, Coralville, IA, USA) using a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA). The genotyping probe was designed (CD.GT.PBVW7663.1). Then, 10 ng of gDNA were mixed with 2.5 μL of rhAmp Genotyping Master Mix, 0.25 μL of the probe, and 1.25 μL of deionized water.

RNA isolation and quantitative PCR

Venous blood was collected into PAXgene Blood RNA Systems tubes (BD, Tokyo, Japan). RNA was isolated by the manufacturer's protocol, and the concentration and quality were measured by a NanoDrop 1000 system (Thermo Fisher Scientific, Yokohama, Japan).

Then, 1.0 μg of RNA was used to synthesize 40-μL mixtures of complementary DNA (cDNA) using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The measurement of mRNA expression levels was assessed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) using a StepOnePlus Real-Time PCR System (Applied Biosystems). Three predesigned qPCR Assays were used (Hs.PT.58.40663161 for whole *TREM1* mRNA, Hs.PT.58.40064607 for *mbTREM1* mRNA, and Hs.PT.58.19948374 for *TREM1sv* mRNA expressions), as well as Hs.PT.39a.22214836 for *GAPDH* expression. RT-qPCR was conducted using the PrimeTime Gene Expression Master Mix (Integrated DNA Technologies, Inc.) in a final volume of 10 μL.

For miRNA analysis, 3.75 μL of total RNA solution was used in a 5-μL reverse-transcription reaction using the Mir-X miRNA First-Strand Synthesis Kit (Takara Bio Inc., Tokyo, Japan), according to the manufacturer's protocol. The resulting cDNA was diluted 10-fold and used for qPCR. The miRNA expression levels were measured in duplicate using a Mir-X miRNA qRT-PCR TB Green Kit (Takara Bio Inc.) and a StepOnePlus Real-Time PCR System (Applied Biosystems) with miRNA-specific primers (miR-138-5p: 5'- GGTGTTGTGAATCAGGCCGAAAA-3') according to the manufacturer's protocol. U6 included in the Mir-X miRNA qRT-PCR TB Green Kit was used as an internal standard.

The mRNA and miRNA expression levels were measured in duplicate. The same sample was used as a template for the calculation of the relative expression value using Livak's ΔΔCt method [18] to remove errors among plates. Outliers were identified using the Outlier Calculator (https://miniwebtool.com/outlier-calculator/) based on Tukey's fence method (1.5 × interquartile range [IQR]). Samples identified as outliers were excluded prior to statistical analyses.

In silico prediction for the seed sequence of miR-138-5p and dual luciferase reporter assay

The 3'-UTR sequence including rs2234246 (G/A) was subjected to custom prediction of miRDB (http://www.mirdb.org/) with the below parameters: species, human; submission type, mRNA target sequence.

The seed sequence of *TREM1* including rs2234246 (G/A) was cloned into a pmirGLO vector (Promega Corporation, Madison, WI, USA). The pmirGLO was digested by PmeI (New England Biolabs, Ipswich, MA, USA) and XbaI (New England Biolabs) according to the manufacturer's protocol. The cloned oligos (both alleles of rs2234246 [G/A]) are shown in **Table S1**, and they were annealed by annealing buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, and 100 mM $MgCl_2$) under the following conditions: 95 °C for 5 min and incubated at room temperature until they reached room temperature. Digested pmirGLO vector and annealed oligos were ligated with a Ligation-Convenience Kit: Nippon Gene (Tokyo, Japan). The plasmid was subjected to transformation of the *E. coli* DH5α,

and the completed plasmid sequence was confirmed by Sanger sequencing with the sequence primer shown in **Table S1**. For the luciferase reporter assay, luciferase reporter vectors and hsa-miR-138-5p mimic (C-300605-05-0002, Horizon Discovery Group, Cambridge, UK) were co-transfected into HEK293 (JCRB Cell Bank, Osaka, Japan) and SH-SY5Y cells (ECACC, Salisbury, UK) using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol and harvested 48 hours post-transfection. The dual-luciferase reporter assays were performed in two separate experimental batches in both HEK293 and SH-SY5Y cells. Each batch consisted of three independent experiments (n = 3 per group), and each independent experiment was measured in technical triplicate. The three technical measurements were averaged to obtain a single value for each independent experiment, and these independent experimental values were used for statistical analysis. The two experimental batches were analyzed separately to assess the reproducibility of the findings.

Measurement of plasma TREM-1 protein concentration

Plasma TREM-1 protein concentrations were measured using the Human TREM-1 Quantikine ELISA Kit (DTRM10C; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. This assay detects human TREM-1 in plasma but does not distinguish the molecular origin of circulating TREM-1; therefore, plasma TREM-1 protein concentrations were not interpreted as a direct measurement of membrane-bound TREM1 on intact cells.

Hippocampal volume assessed by magnetic resonance imaging

Brain MRI scans were acquired using a Philips Achieva 1.5T scanner (Philips Healthcare, Best, the Netherlands). For volumetric analysis, three-dimensional T1-weighted images (3D-T1WI; 3D gradient echo inversion recovery, sagittal acquisition) were obtained with the following parameters according to the protocol of brain MRI for the Alzheimer's Disease Neuroimaging Initiative study [19]: repetition time (TR) = shortest, echo time (TE) = 4 ms, flip angle = 8°, inversion time (TI) = 1000 ms, delay time (TD) = 2300 ms, field of view (FOV) = 240 mm, matrix size = 192 × 192, slice thickness = 1.2 mm, number of slices = 170, and SENSE factor = 1.

Hippocampal volumes were obtained using the automated subcortical segmentation procedure implemented in FreeSurfer (version 5.3; http://surfer.nmr.mgh.harvard.edu), which is based on a probabilistic atlas constructed from manually labeled training data [20]. In this study, only hippocampal volume and estimated total intracranial volume (eTIV) were used for analysis. To identify extreme volumetric outliers, hippocampal volume was regressed on age, squared age, and sex, and observations with residuals below −4 SD or above +4 SD were considered extreme outliers. Raw hippocampal volumes were used as dependent variables in the subsequent regression analyses.

Statistical analysis

SPSS version 22.0 (IBM Japan, Tokyo, Japan) was used for statistical analyses. The Shapiro–Wilk test was used to assess the normality of continuous variables. Differences in continuous variables between two groups were assessed using Student's t-test or the Mann–Whitney U test, as appropriate, and differences among three groups were assessed using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test according to the distribution of the data. Correlations between two continuous variables were assessed using Pearson's or Spearman's rank correlation coefficient, as appropriate. The chi-squared test or Fisher's exact test was used to assess differences in categorical variables, including sex, hyperlipidemia (HL), hypertension (HT), diabetes mellitus (DM), and rs2234246 genotype. Hardy–Weinberg equilibrium (HWE) for rs2234246 was evaluated in the control group of each cohort using the chi-squared goodness-of-fit test.

For the neuroimaging analyses, associations between rs2234246 and hippocampal volume were examined separately in patients with AD and control participants using multiple linear regression. Raw left and right hippocampal volumes were analyzed as dependent variables. The primary genetic model was an additive model, in which rs2234246 was coded according to the number of A alleles (G/G = 0, G/A = 1, and A/A = 2). A dominant model comparing A-allele carriers (G/A and A/A) with G/G homozygotes was additionally examined as a sensitivity analysis. All models were adjusted for age, sex, MMSE score, and eTIV. Unstandardized regression coefficients (B), standardized β coefficients, 95% confidence intervals (CIs), and two-sided P values were reported.

To evaluate whether the observed case–control differences in peripheral molecular measures were independent of total peripheral leukocyte count, additional multiple linear regression analyses were performed using diagnosis (AD vs. control) and white blood cell (WBC) count as independent variables.

A two-sided p value < 0.05 was considered nominally significant. Analyses were interpreted according to their hypothesis-driven or exploratory nature. Analyses examining the association of rs2234246 with TREM1 transcript expression and allele-dependent reporter activity were considered hypothesis-driven analyses. Case–control comparisons of peripheral molecular measures were considered supportive analyses, whereas genotype-stratified analyses of miR-138-5p expression, correlation analyses, and neuroimaging analyses were considered exploratory. For the exploratory correlation analyses presented in Table S3, Bonferroni correction was applied separately within each diagnostic group for 15 comparisons (five molecular measures × three demographic variables), resulting in a corrected significance threshold of $p < 0.0033$. Because the remaining analyses addressed distinct biological questions and were performed in separate experimental settings or participant cohorts, no single global correction for multiple testing was applied across all analyses. Accordingly, findings from exploratory analyses were interpreted cautiously and require confirmation in independent cohorts.

No statistical methods were used to predetermine sample size. No randomization was performed

because the study consisted of observational human analyses and predefined in vitro experiments. Investigators were not blinded during the in vitro experiments.

Results

SNP functional analysis

TREM1 mRNA has two splicing variants: *mbTREM1* (exons 1-4) and *TREM1sv* (exons 1, 2, and 4). To measure whole transcripts and these variants, the probes were set at the junction of exons 1 and 2 (whole *TREM1* mRNA), exons 2 and 3 (*mbTREM1* mRNA), and exons 2 and 4 (*TREM1sv* mRNA). The rs2234246 is located in the 3'-UTR of *TREM1* (**Figure 1A**). The demographic and biochemical data for the 93 participants are shown in **Table 1**. The genotype distribution of rs2234246 in the control subjects used for the SNP functional analysis was consistent with Hardy–Weinberg equilibrium (χ^2 = 0.176, P = 0.675). When gene expression was analyzed according to rs2234246 genotype, individuals carrying the A allele showed significantly higher whole *TREM1* (p = 0.026, **Figure 1B**) and *mbTREM1* mRNA expression (p = 0.002, **Figure 1C**) than those carrying the G allele, whereas *TREM1sv* expression did not differ among the genotypes (p = 0.286, **Figure 1D**).

When analyzed using the dominant model for the A allele (G/G vs. G/A + A/A), whole *TREM1* (p = 0.007, **Figure 1E**) and *mbTREM1* mRNA expression (p = 0.001, **Figure 1F**) remained significantly higher in A allele carriers, whereas *TREM1sv* expression did not differ between the groups (p = 0.206, **Figure 1G**). In addition, the white blood cell (WBC) count was significantly correlated with whole *TREM1* mRNA (p = 0.002, r = 0.322, **Figure 1H**), *mbTREM1* mRNA (p = 0.006, r = 0.294, **Figure**

1I), and *TREM1sv* mRNA (p = 0.028, r = 0.233, **Figure 1J**).

In silico prediction of miRNAs that regulate TREM1 mRNA expression and dual luciferase reporter assay

The 3'-UTR sequence of *TREM1* including rs2234246 was subjected to miRDB, and the results are shown in **Table S2**; miR-138-5p was predicted to interact with the *TREM1* 3′-UTR sequence containing the rs2234246 A allele, but not the G allele. A schematic overview of the dual-luciferase reporter assay and the predicted binding site of miR-138-5p within the TREM1 3′-UTR are shown in **Figure 2A**. The overexpression ratio of miR-138-5p was 180 times, which was confirmed by qPCR (data not shown). Dual-luciferase reporter assays were performed in two separate experimental batches using both SH-SY5Y and HEK293 cells, with each batch comprising three independent experiments measured in technical triplicate (**Figure 2B**). In the first batch, significant allele-dependent differences in luciferase activity were observed in HEK293 cells (overall ANOVA, p = 0.011) and SH-SY5Y cells (overall ANOVA, p = 0.018). Post hoc analysis demonstrated significantly lower luciferase activity in the rs2234246 A allele construct than in the vector control (HEK293, p = 0.016; SH-SY5Y, p = 0.022) and the rs2234246 G allele construct (HEK293, p = 0.020; SH-SY5Y, p = 0.037). In the second independent batch, significant allele-dependent differences were again observed in both SH-SY5Y cells (overall ANOVA, p = 0.015) and HEK293 cells (overall ANOVA, p = 0.008). Post hoc analysis

demonstrated significantly lower luciferase activity in the rs2234246 A allele construct than in the rs2234246 G allele construct in both SH-SY5Y ($p = 0.003$) and HEK293 cells ($p = 0.008$). In HEK293 cells, the rs2234246 A allele construct also showed significantly lower luciferase activity than the vector control ($p = 0.027$), confirming the reproducibility of the allele-dependent regulation.

Plasma TREM-1 protein concentration, *TREM1* mRNA, and miR-138-5p expression study

The demographic and biochemical data for this study are shown in **Table 2**. The MMSE score for AD patients was significantly lower ($p < 0.001$), and the genotype distribution for rs2234246 was significantly different between control participants and AD patients ($p = 0.034$). The genotype distribution in the control group also conformed to Hardy–Weinberg equilibrium ($\chi^2 = 0.124$, $P = 0.724$). The plasma TREM-1 protein concentration was significantly higher in AD patients than in control participants ($p = 0.039$, **Figure 3A**). In addition, the WBC count was significantly correlated with plasma TREM-1 protein concentration ($p = 0.019$, $r = 0.267$, **Figure S1**). In terms of mRNA expression levels, *TREM1sv* expression was significantly decreased ($p = 0.008$, **Figure 3D**), but no changes were found in whole *TREM1* and *mbTREM1* expressions (**Figure 3B and 3C**). Furthermore, the elevated *mbTREM1* expression was replicated with a different probe in AD patients of our previous report[11], but no change was found in whole *TREM1* and *TREM1sv* expressions (**Figure S2**). According to the predefined outlier detection method, three outliers were excluded from the plasma

TREM-1 protein analysis and two from the *TREM1sv* mRNA analysis, whereas no outliers were identified for *whole TREM1* or *mbTREM1* mRNA expression. To evaluate the potential influence of peripheral blood cell burden, we additionally performed analyses adjusting for WBC count. The overall results remained essentially unchanged after adjustment; plasma TREM-1 protein concentration remained significantly increased ($p = 0.016$), whereas *TREM1sv* mRNA remained significantly decreased ($p = 0.0057$) and miR-138-5p remained significantly increased ($p = 0.037$) in patients with AD. *Whole TREM1* and *mbTREM1* mRNA expression also remained unchanged after WBC adjustment.

The miR-138-5p expression in peripheral blood was higher in AD patients than in control participants ($p = 0.025$, **Figure 4A**). When considering the rs2234246 genotype in control participants, there were no changes in genotype ($p = 0.250$) and dominant model ($p = 0.106$), as shown in **Figure 4B**. However, significant changes were found in AD patients both in genotype ($p = 0.042$) and dominant model ($p = 0.011$), as shown in **Figure 4C**. According to the predefined outlier detection method, three outliers were excluded from the miR-138-5p analysis. Additional analyses adjusting for WBC count demonstrated that miR-138-5p expression remained significantly increased in patients with AD ($p = 0.037$). Plasma TREM-1 protein concentration, and miR-138-5p expressions were not correlated with demographic parameters after Bonferroni correction (**Table S3**).

MRI study

The demographic and biochemical characteristics of the MRI cohort are shown in **Table 3**. The genotype distribution in the MRI control group was consistent with Hardy–Weinberg equilibrium (χ^2 = 0.426, P = 0.514). The distributions of eTIV-normalized hippocampal volumes according to rs2234246 genotype are shown descriptively in **Figure 5**. In patients with AD, the additive model showed that increasing rs2234246 A-allele dosage was associated with smaller right hippocampal volume after adjustment for age, sex, MMSE score, and eTIV (B = −234.66 mm^3 per A allele, 95% CI = −455.05 to −14.27; standardized β = −0.247, P = 0.037). No association was observed with left hippocampal volume (B = 78.51 mm^3, 95% CI = −193.88 to 350.89; standardized β = 0.075, P = 0.565). In the dominant sensitivity model, the association with right hippocampal volume did not reach statistical significance (B = −237.80 mm^3, 95% CI = −517.81 to 42.20; standardized β = −0.201, P = 0.094), and no association was observed with left hippocampal volume (P = 0.415). In control participants, rs2234246 was not significantly associated with either right or left hippocampal volume in the additive model (right: standardized β = −0.091, P = 0.083; left: standardized β = −0.029, P = 0.559) or the dominant model (right: standardized β = −0.083, P = 0.112; left: standardized β = −0.024, P = 0.616). Detailed regression results are presented in **Table S4.**

Discussion

The present study yielded three complementary findings regarding *TREM1* rs2234246 in AD. First, the rs2234246 A allele was associated with higher peripheral *mbTREM1* mRNA expression, whereas *TREM1sv* expression did not differ according to genotype. Second, dual-luciferase reporter assays showed allele-dependent repression consistent with an interaction between miR-138-5p and the *TREM1* 3′-UTR containing the A allele. Third, the rs2234246 A allele was associated with smaller right hippocampal volume in patients with AD. In addition, peripheral miR-138-5p expression was increased in AD, whereas A-allele carriers within the AD group showed lower miR-138-5p expression. Because these findings were obtained from different experimental settings and participant cohorts, they should be interpreted as complementary observations rather than as evidence of a single sequential regulatory pathway.

Elevated *mbTREM1* expression in whole blood in rs2234246^{A} patients is concordant with previous research, which used peripheral blood mononuclear cells [13]. To elucidate how rs2234246 regulates *mbTREM1* expression, this study focused on miR-138-5p and conducted an in vitro experiment. In the reporter assay, miR-138-5p reduced luciferase activity of the construct containing the rs2234246 A allele compared with the G-allele construct. However, the dual-luciferase assay was designed to evaluate miR-138-5p-associated regulation of the isolated *TREM1* 3′-UTR under artificial in vitro conditions. Therefore, the assay demonstrates allele-dependent reporter repression consistent with an

interaction between miR-138-5p and the TREM1 3′-UTR but does not fully recapitulate the complex regulatory environment in vivo, where TREM1 expression is influenced by multiple transcriptional, post-transcriptional, and inflammatory regulatory mechanisms. Accordingly, the present findings support an allele-specific interaction between miR-138-5p and the TREM1 3′-UTR but do not establish a direct causal mechanism linking rs2234246 to increased mbTREM1 expression in vivo. Further studies using seed-mutant reporters, miR-138-5p inhibition or rescue experiments, and relevant cellular models are required to determine whether this interaction contributes to rs2234246-dependent TREM1 regulation in vivo. Interestingly, whole *TREM1*, *mbTREM1*, and *TREM1sv* mRNA expressions were positively correlated with WBC counts. Moreover, plasma TREM-1 protein concentration was also correlated with WBC counts. Compared with *TREM2* expression, *TREM1* was highly expressed in bone marrow and in monocytes among peripheral blood cells (https://www.proteinatlas.org/). In AD patients, changes of T cell and B cell counts have been reported, and a significantly increased CD4/CD8 ratio was observed compared with controls [21]. In addition, several reports have shown lymphocyte dysfunction [22,23]. Collectively, these findings should be interpreted with caution because gene expression measured in whole blood reflects both transcriptional regulation and differences in peripheral blood cell composition. Therefore, the observed correlations between WBC count and TREM1 expression may partly reflect differences in leukocyte abundance or composition rather than direct regulation of TREM1 expression.

In AD patients, miR-138-5p expression in whole blood was significantly higher than in control participants. A previous report already showed that miR-138-5p in plasma exosomes was elevated in AD patients [24]. Interestingly, miR-138-5p expression was significantly decreased in patients with the rs2234246^{A} allele only among AD patients. Concordant with our previous research that showed elevated *mbTREM1* expression, plasma TREM-1 protein concentration was also elevated in AD patients. Notably, the direction of the in vivo observations does not straightforwardly correspond to the reporter assay findings. Although the A-allele construct showed greater miR-138-5p-associated repression in the reporter assay, A-allele carriers showed higher peripheral mbTREM1 mRNA expression in the SNP-expression cohort and lower miR-138-5p expression within the AD group. Therefore, the present data do not establish a directional regulatory pathway from rs2234246 through miR-138-5p to mbTREM1 expression in vivo. The reporter assay, genotype–TREM1 association, and genotype–miR-138-5p association should instead be regarded as complementary observations obtained under different experimental and biological contexts. Additional mechanistic studies will be required to determine whether and how these observations are biologically connected. Although *mbTREM1* mRNA expression was not significantly altered in the present cohort, protein abundance is not always directly correlated with mRNA expression because it is influenced by post-transcriptional and post-translational regulatory mechanisms. Therefore, the increased plasma TREM-1 protein concentration may reflect differences in translational efficiency or protein stability, as well as

differences in cohort characteristics compared with our previous study [11]. In addition, differences in disease stage, sample processing procedures, normalization strategies, and peripheral blood cell composition may also have contributed to the discrepancy between the present study and our previous report. Because these factors were not systematically evaluated, the precise reason for the inconsistent findings remains unclear. TREM1 is mainly expressed on the surface of immune cells in peripheral blood [25,26]. When considering TREM1 function as an inflammatory process, the altered peripheral TREM1-related mRNA expression and increased plasma TREM-1 protein concentration may reflect the changes in peripheral blood cell profiles [21] and of inflammatory cytokines [27]. However, inflammatory cytokines and other neuroinflammatory markers were not measured in the present study. Future studies integrating cytokine profiling and other inflammatory biomarkers will be important to clarify the relationship between TREM1, miR-138-5p, and neuroinflammation in AD.

The rs2234246 variant has not been associated with AD risk in previous GWAS [28]. Therefore, the difference in rs2234246 genotype distribution between controls and patients with AD observed in the present study may represent a false-positive finding, particularly given the relatively small sample size. In the MRI study, increasing rs2234246 A-allele dosage was associated with smaller right hippocampal volume in patients with AD after adjustment for age, sex, MMSE score, and eTIV. However, this association was nominally significant in the primary additive model and did not reach statistical significance in the dominant sensitivity model. In addition, the A/A subgroup included only four

patients with AD. Therefore, the neuroimaging finding should be considered exploratory and requires replication in larger independent cohorts. Previous studies have reported the asymmetry of hippocampal atrophy in AD patients [29,30], with some studies demonstrating greater vulnerability of the right hippocampus than the left during disease progression[29,31]. The association observed for the right, but not the left, hippocampus in the present study may be consistent with previously reported asymmetric neurodegenerative changes in AD; however, a hemisphere-specific genetic effect cannot be established from the present data. Furthermore, several in vitro studies have shown that miR-138 was related to the promotion of tau phosphorylation in HEK293/tau [32] and acceleration of cell apoptosis in the SH-SY5Y AD cell model [33], and miR-138-5p was related to hippocampal neuroinflammation and cognitive dysfunction in a rat study [34]. A duplication of the miR-138 locus was reported to be associated with early-onset AD [35]. Furthermore, in a human postmortem study, increased expression of microglial markers was reported in a systematic review [36]. Although TREM1 is expressed in microglia and has been implicated in neuroinflammatory processes [37], the present study evaluated only peripheral blood samples. Therefore, the relationship between peripheral TREM1 regulation and central nervous system mechanisms remains speculative and requires validation using brain tissue or cell-type-specific analyses. These findings suggest that rs2234246 may be associated with right hippocampal volume in patients with AD, although this exploratory association requires replication in larger independent cohorts. Because the present study is cross-

sectional, longitudinal studies are required to determine whether this association is related to disease progression.

There are several limitations in this study. First, miR-138-5p and *TREM1* expression were evaluated only in peripheral blood, and their expression in the human brain was not examined. Therefore, the relationship between peripheral and central TREM1 regulation remains unclear. Furthermore, the SNP functional analysis, expression analyses, and MRI analyses were performed in different participant cohorts rather than in the same individuals. Therefore, the present study cannot directly establish a sequential relationship between rs2234246 genotype, miR-138-5p expression, TREM1 regulation, and hippocampal volume at the individual level. Instead, our findings should be interpreted as parallel associations, and future studies integrating genetic, molecular, and neuroimaging data within the same participants will be necessary to determine whether these observations are biologically interconnected. To the best of our knowledge, a change in *TREM1* expression has not been reported in the human hippocampus, but downregulated miR-138-5p expression in the entorhinal cortex, which is close to the hippocampus, has been reported in AD patients [38]. Second, the isolated RNA from PAXgene Blood RNA Systems tubes includes several cell types that have a nucleus, so it is unclear which cells contribute to the miR-138-5p and *TREM1* expression changes. Although *TREM1* is mainly expressed in monocytes among peripheral blood cells according to the database (https://www.proteinatlas.org/), its expression should be clarified based on each cell type to conduct functional research. Furthermore,

differential leukocyte counts were not available in the present study; therefore, adjustment for leukocyte composition was not possible. Consequently, the observed associations between WBC count and *TREM1* expression may partly reflect differences in peripheral blood cell composition rather than transcriptional regulation. Adjustment for total WBC count did not materially alter the observed case–control differences, suggesting that these findings were not explained by total leukocyte count alone. However, because differential leukocyte counts were unavailable, residual confounding by differences in leukocyte composition cannot be excluded. In addition, information regarding active inflammatory diseases, autoimmune diseases, infectious conditions, and immunomodulatory medications was not systematically collected; therefore, their potential influence on peripheral immune markers cannot be excluded. In addition, GAPDH and U6 were used as reference genes for mRNA and miRNA normalization, respectively. Although these reference genes are widely used, the stability of U6 expression was not independently validated in the present cohort. The use of multiple validated reference genes would further improve the robustness of gene expression analyses using whole blood. Third, *TREM1* mRNA expression changes differed between the present study (decreased *TREM1sv* expression) and our previous study (increased *mbTREM1* expression). This discrepancy may reflect differences in cohort characteristics, biological variability, or the assessment of different TREM1 transcript variants. Because the present study was not designed to investigate the mechanisms underlying these differences, the precise explanation remains unclear. Although mbTREM1 promotes

downstream inflammatory signaling [39], whereas soluble TREM1 translated from TREM1sv exerts inhibitory effects on the TREM1 signaling pathway [40], the interpretation that both findings indicate enhanced inflammatory activity remains speculative and requires further functional validation. Furthermore, although the principal analyses were hypothesis-driven, the relatively small sample size, particularly in subgroup analyses, may have limited the statistical power and increased the possibility of false-positive findings. Therefore, the present results should be interpreted with caution and require replication in larger independent cohorts. In addition, all participants enrolled in the present study were of Japanese origin. Therefore, the generalizability of these findings to other ethnic populations remains uncertain and should be validated in larger, multi-ethnic cohorts. Future studies integrating paired peripheral blood and postmortem brain samples will be required to determine whether peripheral *TREM1* and miR-138-5p expression reflects central molecular changes associated with AD. A schematic summary of the principal findings identified in the present study is shown in **Figure 6**.

In conclusion, the present study identified allele-dependent reporter regulation involving miR-138-5p at the *TREM1* 3′-UTR and alterations in peripheral TREM1-related and miR-138-5p measures in AD. In addition, rs2234246 showed an exploratory association with right hippocampal volume in patients with AD. Because the molecular and neuroimaging analyses were performed in different participant cohorts, these findings should be regarded as complementary observations and do not establish a sequential pathway linking miR-138-5p-mediated TREM1 regulation to hippocampal

volume differences. Further studies in larger independent cohorts and mechanistic models are required to determine the biological relationships among these findings.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

The study was approved by the Institutional Ethics Committee of Ehime University Graduate School of Medicine (No. 161004).

Consent to Participate

Written informed consent was obtained from all participants.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no competing interests.

References

1. Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256 (5054):184-185. doi:10.1126/science.1566067

2. Tanzi RE (2005) The synaptic Abeta hypothesis of Alzheimer disease. Nat Neurosci 8 (8):977-979. doi:10.1038/nn0805-977

3. Huang Y, Mucke L (2012) Alzheimer mechanisms and therapeutic strategies. Cell 148 (6):1204-1222. doi:10.1016/j.cell.2012.02.040

4. Katabathula S, Wang Q, Xu R (2021) Predict Alzheimer's disease using hippocampus MRI data: a lightweight 3D deep convolutional network model with visual and global shape representations. Alzheimers Res Ther 13 (1):104. doi:10.1186/s13195-021-00837-0

5. Zhao Y, Liu B, Wang J, Xu L, Yu S, Fu J, Yan X, Su J (2022) Abeta and Tau Regulate Microglia Metabolism via Exosomes in Alzheimer's Disease. Biomedicines 10 (8). doi:10.3390/biomedicines10081800

6. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe JS, Younkin S, Hazrati L, Collinge J, Pocock J, Lashley T, Williams J, Lambert JC, Amouyel P, Goate A, Rademakers R, Morgan K, Powell J, St George-Hyslop P, Singleton A, Hardy J, Alzheimer Genetic Analysis G (2013) TREM2 variants in Alzheimer's disease. N Engl J Med 368 (2):117-127. doi:10.1056/NEJMoa1211851

7. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ, Rujescu D, Hampel H, Giegling I, Andreassen OA, Engedal K, Ulstein I, Djurovic S, Ibrahim-Verbaas C, Hofman A, Ikram MA, van Duijn CM, Thorsteinsdottir U, Kong A, Stefansson K (2013) Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 368 (2):107-116. doi:10.1056/NEJMoa1211103

8. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Gilfillan S, Krishnan GM, Sudhakar S, Zinselmeyer BH, Holtzman DM, Cirrito JR, Colonna M (2015) TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell 160 (6):1061-1071. doi:10.1016/j.cell.2015.01.049

9. Replogle JM, Chan G, White CC, Raj T, Winn PA, Evans DA, Sperling RA, Chibnik LB, Bradshaw EM, Schneider JA, Bennett DA, De Jager PL (2015) A TREM1 variant alters the accumulation of Alzheimer-related amyloid pathology. Ann Neurol 77 (3):469-477. doi:10.1002/ana.24337

10. Ozaki Y, Yoshino Y, Yamazaki K, Sao T, Mori Y, Ochi S, Yoshida T, Mori T, Iga JI, Ueno SI (2017) DNA methylation changes at TREM2 intron 1 and TREM2 mRNA expression in patients with Alzheimer's disease. J Psychiatr Res 92:74-80. doi:10.1016/j.jpsychires.2017.04.003

11. Sao T, Yoshino Y, Yamazaki K, Ozaki Y, Mori Y, Ochi S, Yoshida T, Mori T, Iga JI, Ueno SI

(2018) TREM1 mRNA Expression in Leukocytes and Cognitive Function in Japanese Patients with Alzheimer's Disease. J Alzheimers Dis 64 (4):1275-1284. doi:10.3233/JAD-180418
12. Baruah S, Keck K, Vrenios M, Pope MR, Pearl M, Doerschug K, Klesney-Tait J (2015) Identification of a Novel Splice Variant Isoform of TREM-1 in Human Neutrophil Granules. J Immunol 195 (12):5725-5731. doi:10.4049/jimmunol.1402713
13. Aldasoro Arguinano AA, Dade S, Stathopoulou M, Derive M, Coumba Ndiaye N, Xie T, Masson C, Gibot S, Visvikis-Siest S (2017) TREM-1 SNP rs2234246 regulates TREM-1 protein and mRNA levels and is associated with plasma levels of L-selectin. PLoS One 12 (8):e0182226. doi:10.1371/journal.pone.0182226
14. Liu YS, Yan WJ, Tan CC, Li JQ, Xu W, Cao XP, Tan L, Yu JT, Alzheimer's Disease Neuroimaging I (2020) Common Variant in TREM1 Influencing Brain Amyloid Deposition in Mild Cognitive Impairment and Alzheimer's Disease. Neurotox Res 37 (3):661-668. doi:10.1007/s12640-019-00105-y
15. Reza-Zaldivar EE, Hernandez-Sapiens MA, Minjarez B, Gomez-Pinedo U, Sanchez-Gonzalez VJ, Marquez-Aguirre AL, Canales-Aguirre AA (2020) Dendritic Spine and Synaptic Plasticity in Alzheimer's Disease: A Focus on MicroRNA. Front Cell Dev Biol 8:255. doi:10.3389/fcell.2020.00255
16. Klyucherev TO, Olszewski P, Shalimova AA, Chubarev VN, Tarasov VV, Attwood MM, Syvanen S, Schioth HB (2022) Advances in the development of new biomarkers for Alzheimer's disease. Transl Neurodegener 11 (1):25. doi:10.1186/s40035-022-00296-z
17. Yoshino Y, Kumon H, Mori T, Yoshida T, Tachibana A, Shimizu H, Iga JI, Ueno SI (2021) Effects of AGXT2 variants on blood pressure and blood sugar among 750 older Japanese subjects recruited by the complete enumeration survey method. BMC Genomics 22 (1):287. doi:10.1186/s12864-021-07612-3
18. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (4):402-408. doi:10.1006/meth.2001.1262
19. Jack CR, Jr., Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, Borowski B, Britson PJ, J LW, Ward C, Dale AM, Felmlee JP, Gunter JL, Hill DL, Killiany R, Schuff N, Fox-Bosetti S, Lin C, Studholme C, DeCarli CS, Krueger G, Ward HA, Metzger GJ, Scott KT, Mallozzi R, Blezek D, Levy J, Debbins JP, Fleisher AS, Albert M, Green R, Bartzokis G, Glover G, Mugler J, Weiner MW (2008) The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 27 (4):685-691. doi:10.1002/jmri.21049
20. Fischl B (2012) FreeSurfer. Neuroimage 62 (2):774-781. doi:10.1016/j.neuroimage.2012.01.021
21. Huang LT, Zhang CP, Wang YB, Wang JH (2022) Association of Peripheral Blood Cell Profile

With Alzheimer's Disease: A Meta-Analysis. Front Aging Neurosci 14:888946. doi:10.3389/fnagi.2022.888946
22. Ahn JJ, Abu-Rub M, Miller RH (2021) B Cells in Neuroinflammation: New Perspectives and Mechanistic Insights. Cells 10 (7). doi:10.3390/cells10071605
23. Dai L, Shen Y (2021) Insights into T-cell dysfunction in Alzheimer's disease. Aging Cell 20 (12):e13511. doi:10.1111/acel.13511
24. Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, Hernandez AG, Smalheiser NR (2015) Plasma Exosomal miRNAs in Persons with and without Alzheimer Disease: Altered Expression and Prospects for Biomarkers. PLoS One 10 (10):e0139233. doi:10.1371/journal.pone.0139233
25. Bouchon A, Dietrich J, Colonna M (2000) Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 164 (10):4991-4995. doi:10.4049/jimmunol.164.10.4991
26. Colonna M (2003) TREMs in the immune system and beyond. Nat Rev Immunol 3 (6):445-453. doi:10.1038/nri1106
27. Park JC, Han SH, Mook-Jung I (2020) Peripheral inflammatory biomarkers in Alzheimer's disease: a brief review. BMB Rep 53 (1):10-19. doi:10.5483/BMBRep.2020.53.1.309
28. Bellenguez C, Kucukali F, Jansen IE, Kleineidam L, Moreno-Grau S, Amin N, Naj AC, Campos-Martin R, Grenier-Boley B, Andrade V, Holmans PA, Boland A, Damotte V, van der Lee SJ, Costa MR, Kuulasmaa T, Yang Q, de Rojas I, Bis JC, Yaqub A, Prokic I, Chapuis J, Ahmad S, Giedraitis V, Aarsland D, Garcia-Gonzalez P, Abdelnour C, Alarcon-Martin E, Alcolea D, Alegret M, Alvarez I, Alvarez V, Armstrong NJ, Tsolaki A, Antunez C, Appollonio I, Arcaro M, Archetti S, Pastor AA, Arosio B, Athanasiu L, Bailly H, Banaj N, Baquero M, Barral S, Beiser A, Pastor AB, Below JE, Benchek P, Benussi L, Berr C, Besse C, Bessi V, Binetti G, Bizarro A, Blesa R, Boada M, Boerwinkle E, Borroni B, Boschi S, Bossu P, Brathen G, Bressler J, Bresner C, Brodaty H, Brookes KJ, Brusco LI, Buiza-Rueda D, Burger K, Burholt V, Bush WS, Calero M, Cantwell LB, Chene G, Chung J, Cuccaro ML, Carracedo A, Cecchetti R, Cervera-Carles L, Charbonnier C, Chen HH, Chillotti C, Ciccone S, Claassen J, Clark C, Conti E, Corma-Gomez A, Costantini E, Custodero C, Daian D, Dalmasso MC, Daniele A, Dardiotis E, Dartigues JF, de Deyn PP, de Paiva Lopes K, de Witte LD, Debette S, Deckert J, Del Ser T, Denning N, DeStefano A, Dichgans M, Diehl-Schmid J, Diez-Fairen M, Rossi PD, Djurovic S, Duron E, Duzel E, Dufouil C, Eiriksdottir G, Engelborghs S, Escott-Price V, Espinosa A, Ewers M, Faber KM, Fabrizio T, Nielsen SF, Fardo DW, Farotti L, Fenoglio C, Fernandez-Fuertes M, Ferrari R, Ferreira CB, Ferri E, Fin B, Fischer P, Fladby T, Fliessbach K, Fongang B, Fornage M, Fortea J, Foroud TM, Fostinelli S, Fox NC, Franco-Macias E, Bullido MJ, Frank-Garcia A, Froelich L, Fulton-Howard B, Galimberti D, Garcia-Alberca JM, Garcia-Gonzalez P, Garcia-Madrona S, Garcia-Ribas G, Ghidoni R, Giegling I, Giorgio G, Goate AM, Goldhardt O, Gomez-Fonseca D, Gonzalez-Perez A, Graff C, Grande G,

Green E, Grimmer T, Grunblatt E, Grunin M, Gudnason V, Guetta-Baranes T, Haapasalo A, Hadjigeorgiou G, Haines JL, Hamilton-Nelson KL, Hampel H, Hanon O, Hardy J, Hartmann AM, Hausner L, Harwood J, Heilmann-Heimbach S, Helisalmi S, Heneka MT, Hernandez I, Herrmann MJ, Hoffmann P, Holmes C, Holstege H, Vilas RH, Hulsman M, Humphrey J, Biessels GJ, Jian X, Johansson C, Jun GR, Kastumata Y, Kauwe J, Kehoe PG, Kilander L, Stahlbom AK, Kivipelto M, Koivisto A, Kornhuber J, Kosmidis MH, Kukull WA, Kuksa PP, Kunkle BW, Kuzma AB, Lage C, Laukka EJ, Launer L, Lauria A, Lee CY, Lehtisalo J, Lerch O, Lleo A, Longstreth W, Jr., Lopez O, de Munain AL, Love S, Lowemark M, Luckcuck L, Lunetta KL, Ma Y, Macias J, MacLeod CA, Maier W, Mangialasche F, Spallazzi M, Marquie M, Marshall R, Martin ER, Montes AM, Rodriguez CM, Masullo C, Mayeux R, Mead S, Mecocci P, Medina M, Meggy A, Mehrabian S, Mendoza S, Menendez-Gonzalez M, Mir P, Moebus S, Mol M, Molina-Porcel L, Montrreal L, Morelli L, Moreno F, Morgan K, Mosley T, Nothen MM, Muchnik C, Mukherjee S, Nacmias B, Ngandu T, Nicolas G, Nordestgaard BG, Olaso R, Orellana A, Orsini M, Ortega G, Padovani A, Paolo C, Papenberg G, Parnetti L, Pasquier F, Pastor P, Peloso G, Perez-Cordon A, Perez-Tur J, Pericard P, Peters O, Pijnenburg YAL, Pineda JA, Pinol-Ripoll G, Pisanu C, Polak T, Popp J, Posthuma D, Priller J, Puerta R, Quenez O, Quintela I, Thomassen JQ, Rabano A, Rainero I, Rajabli F, Ramakers I, Real LM, Reinders MJT, Reitz C, Reyes-Dumeyer D, Ridge P, Riedel-Heller S, Riederer P, Roberto N, Rodriguez-Rodriguez E, Rongve A, Allende IR, Rosende-Roca M, Royo JL, Rubino E, Rujescu D, Saez ME, Sakka P, Saltvedt I, Sanabria A, Sanchez-Arjona MB, Sanchez-Garcia F, Juan PS, Sanchez-Valle R, Sando SB, Sarnowski C, Satizabal CL, Scamosci M, Scarmeas N, Scarpini E, Scheltens P, Scherbaum N, Scherer M, Schmid M, Schneider A, Schott JM, Selbaek G, Seripa D, Serrano M, Sha J, Shadrin AA, Skrobot O, Slifer S, Snijders GJL, Soininen H, Solfrizzi V, Solomon A, Song Y, Sorbi S, Sotolongo-Grau O, Spalletta G, Spottke A, Squassina A, Stordal E, Tartan JP, Tarraga L, Tesi N, Thalamuthu A, Thomas T, Tosto G, Traykov L, Tremolizzo L, Tybjaerg-Hansen A, Uitterlinden A, Ullgren A, Ulstein I, Valero S, Valladares O, Broeckhoven CV, Vance J, Vardarajan BN, van der Lugt A, Dongen JV, van Rooij J, van Swieten J, Vandenberghe R, Verhey F, Vidal JS, Vogelgsang J, Vyhnalek M, Wagner M, Wallon D, Wang LS, Wang R, Weinhold L, Wiltfang J, Windle G, Woods B, Yannakoulia M, Zare H, Zhao Y, Zhang X, Zhu C, Zulaica M, Eadb, Gr@Ace, Degesco, Eadi, Gerad, Demgene, FinnGen, Adgc, Charge, Farrer LA, Psaty BM, Ghanbari M, Raj T, Sachdev P, Mather K, Jessen F, Ikram MA, de Mendonca A, Hort J, Tsolaki M, Pericak-Vance MA, Amouyel P, Williams J, Frikke-Schmidt R, Clarimon J, Deleuze JF, Rossi G, Seshadri S, Andreassen OA, Ingelsson M, Hiltunen M, Sleegers K, Schellenberg GD, van Duijn CM, Sims R, van der Flier WM, Ruiz A, Ramirez A, Lambert JC (2022) New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat Genet 54 (4):412-436. doi:10.1038/s41588-022-01024-z

29. Barnes J, Scahill RI, Schott JM, Frost C, Rossor MN, Fox NC (2005) Does Alzheimer's disease

affect hippocampal asymmetry? Evidence from a cross-sectional and longitudinal volumetric MRI study. Dement Geriatr Cogn Disord 19 (5-6):338-344. doi:10.1159/000084560
30. Lindberg O, Walterfang M, Looi JC, Malykhin N, Ostberg P, Zandbelt B, Styner M, Paniagua B, Velakoulis D, Orndahl E, Wahlund LO (2012) Hippocampal shape analysis in Alzheimer's disease and frontotemporal lobar degeneration subtypes. J Alzheimers Dis 30 (2):355-365. doi:10.3233/JAD-2012-112210
31. Shi F, Liu B, Zhou Y, Yu C, Jiang T (2009) Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: Meta-analyses of MRI studies. Hippocampus 19 (11):1055-1064. doi:10.1002/hipo.20573
32. Wang X, Tan L, Lu Y, Peng J, Zhu Y, Zhang Y, Sun Z (2015) MicroRNA-138 promotes tau phosphorylation by targeting retinoic acid receptor alpha. FEBS Lett 589 (6):726-729. doi:10.1016/j.febslet.2015.02.001
33. Miao J, Jing J, Shao Y, Sun H (2020) MicroRNA-138 promotes neuroblastoma SH-SY5Y cell apoptosis by directly targeting DEK in Alzheimer's disease cell model. BMC Neurosci 21 (1):33. doi:10.1186/s12868-020-00579-z
34. Feng X, Hu J, Zhan F, Luo D, Hua F, Xu G (2021) MicroRNA-138-5p Regulates Hippocampal Neuroinflammation and Cognitive Impairment by NLRP3/Caspase-1 Signaling Pathway in Rats. J Inflamm Res 14:1125-1143. doi:10.2147/JIR.S304461
35. Boscher E, Husson T, Quenez O, Laquerriere A, Marguet F, Cassinari K, Wallon D, Martinaud O, Charbonnier C, Nicolas G, Deleuze JF, Boland A, Lathrop M, Frebourg T, Consortium F, Campion D, Hebert SS, Rovelet-Lecrux A (2019) Copy Number Variants in miR-138 as a Potential Risk Factor for Early-Onset Alzheimer's Disease. J Alzheimers Dis 68 (3):1243-1255. doi:10.3233/JAD-180940
36. Hopperton KE, Mohammad D, Trepanier MO, Giuliano V, Bazinet RP (2018) Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review. Mol Psychiatry 23 (2):177-198. doi:10.1038/mp.2017.246
37. Cai Y, Liu J, Wang B, Sun M, Yang H (2022) Microglia in the Neuroinflammatory Pathogenesis of Alzheimer's Disease and Related Therapeutic Targets. Front Immunol 13:856376. doi:10.3389/fimmu.2022.856376
38. Dobricic V, Schilling M, Schulz J, Zhu LS, Zhou CW, Fuss J, Franzenburg S, Zhu LQ, Parkkinen L, Lill CM, Bertram L (2022) Differential microRNA expression analyses across two brain regions in Alzheimer's disease. Transl Psychiatry 12 (1):352. doi:10.1038/s41398-022-02108-4
39. Gao S, Yi Y, Xia G, Yu C, Ye C, Tu F, Shen L, Wang W, Hua C (2019) The characteristics and pivotal roles of triggering receptor expressed on myeloid cells-1 in autoimmune diseases. Autoimmun Rev 18 (1):25-35. doi:10.1016/j.autrev.2018.07.008

40. Jolly L, Carrasco K, Salcedo-Magguilli M, Garaud JJ, Lambden S, van der Poll T, Mebazaa A, Laterre PF, Gibot S, Boufenzer A, Derive M (2021) sTREM-1 is a specific biomarker of TREM-1 pathway activation. Cell Mol Immunol 18 (8):2054-2056. doi:10.1038/s41423-021-00733-5

Figure legends

Figure 1. Summary of SNP functional analysis and *TREM1* expressions

(A) Schematic representation of TREM1 transcript variants and the location of rs2234246 in the 3′-UTR. *TREM1* mRNA expressions based on rs2234246 are shown below: genotype model of whole *TREM1* (B), *mbTREM1* (C), and *TREM1sv* mRNA expressions (D); and dominant model of whole *TREM1* (E), *mbTREM1* (F), and *TREM1sv* mRNA expressions (G). Correlations of WBC counts with whole *TREM1* (H), *mbTREM1* (I), and *TREM1sv* mRNA expressions (J). The mRNA expressions are shown as relative values, and WBCs are shown as count per μL. *P < .05. Abbreviations: mb, membrane form; r, correlation coefficient; sv, soluble form.

Figure 2. **In silico prediction of the seed sequence in the TREM1 gene and the luciferase assay**

(A) Schematic illustration of the dual-luciferase reporter assay and the predicted binding site of hsa-miR-138-5p within the TREM1 3′-UTR. The rs2234246 A/G polymorphism is located within the predicted miR-138-5p seed-matching sequence. (B) Dual-luciferase reporter assays performed in SH-SY5Y and HEK293 cells co-transfected with the hsa-miR-138-5p mimic and the pmirGLO vector containing the TREM1 3′-UTR harboring either the rs2234246 A or G allele. Independent first and second batches of experiments were performed on separate occasions to assess reproducibility. Each

experimental batch consisted of three independent experiments (n = 3 per group), with each independent experiment measured in technical triplicate. Technical replicates were averaged before statistical analysis, and data are presented as mean ± SD of the three independent experiments. *P < .05. Abbreviations: miR, microRNA; UTR, untranslated region.

Figure 3. Summary of plasma TREM-1 protein concentration and TREM1 mRNA expression in AD

(A) The expression changes of plasma TREM-1 protein concentration. The expression changes of whole *TREM1* (B), *mbTREM1* (C), and *TREM1sv* (D) in AD patients compared with control participants. *P < .05. Abbreviations: AD, Alzheimer's disease; mb, membrane form; sv, soluble form.

Figure 4. Summary of miR-138-5p expression in AD subjects

(A) The expression changes of miR-138-5p in AD patients compared with control participants. The expression changes of miR-138-5p when considering rs2234246 both in genotype and dominant models in control participants (B) and AD patients (C). *P < .05. Abbreviations: AD, Alzheimer's disease; miR, microRNA.

Figure 5. Hippocampal volume of control participants and AD participants by rs2234246

eTIV-normalized left and right hippocampal volumes are shown according to rs2234246 genotype (G/G, G/A, and A/A) and the dominant genetic model (G/G vs. G/A + A/A) in control participants (A) and patients with AD (B). Horizontal lines indicate the mean with SEM. These plots are presented for descriptive visualization of the data. Statistical associations between rs2234246 and raw hippocampal volume were evaluated using multiple linear regression adjusted for age, sex, MMSE score, and eTIV, with the additive model as the primary analysis and the dominant model as a sensitivity analysis; detailed results are presented in Table S4. AD, Alzheimer's disease; eTIV, estimated total intracranial volume; MMSE, Mini-Mental State Examination; SEM, standard error of the mean.

Figure 6. Schematic diagram summarizing the key concepts of the present study

Table 1. Demographic and biochemical data of SNP functional analysis

Parameters	Values
N	93
Age (years)	82.5 ± 2.7
Sex (male: female)	34: 59
Education	
1: < 6	1: 4
2: 7-9	2: 55
3: 10-12	3: 30
4: ≥ 13	4: 4
Hyperlipidemia	35
Hypertension	78
Diabetes Mellitus	11
MMSE	28.2 ± 1.4
WBC (count/ μ L)	6169.9 ± 2050.4
CRP (mg/dL)	0.11 ± 0.16
rs2234246 (G/G: G/A: A/A)	39: 41: 13

CRP, C-reactive protein; MMSE, Mini-Mental State Examination; WBC, white blood cell.

Table 2. Demographic and biochemical data of MbTREM1 protein, *TREM1* mRNA, and miR-138-5p expression study

Parameters	Control	AD	P-value
N	40	40	
Age (years)	81.7 ± 2.3	83.8 ± 4.8	0.050
Sex (male: female)	14: 26	14: 26	1.000
Education 1: < 6 2: 7-9 3: 10-12 4: ≥ 13	 1: 1 2: 25 3: 13 4: 1	 1: 6 2: 26 3: 8 4: 0	0.123
Hyperlipidemia	19	6	0.002
Hypertension	27	30	0.311
Diabetes Mellitus	9	7	0.412
MMSE	28.4 ± 1.5	18.7 ± 3.3	< 0.001
WBC (count/ μ L)	6145 ± 1534.4	5810 ± 1320.4	0.408
CRP (mg/dL)	0.12 ± 0.14	0.10 ± 0.12	0.303
rs2234246 (G/G: G/A: A/A)	25: 12: 2	14: 23: 3	0.034

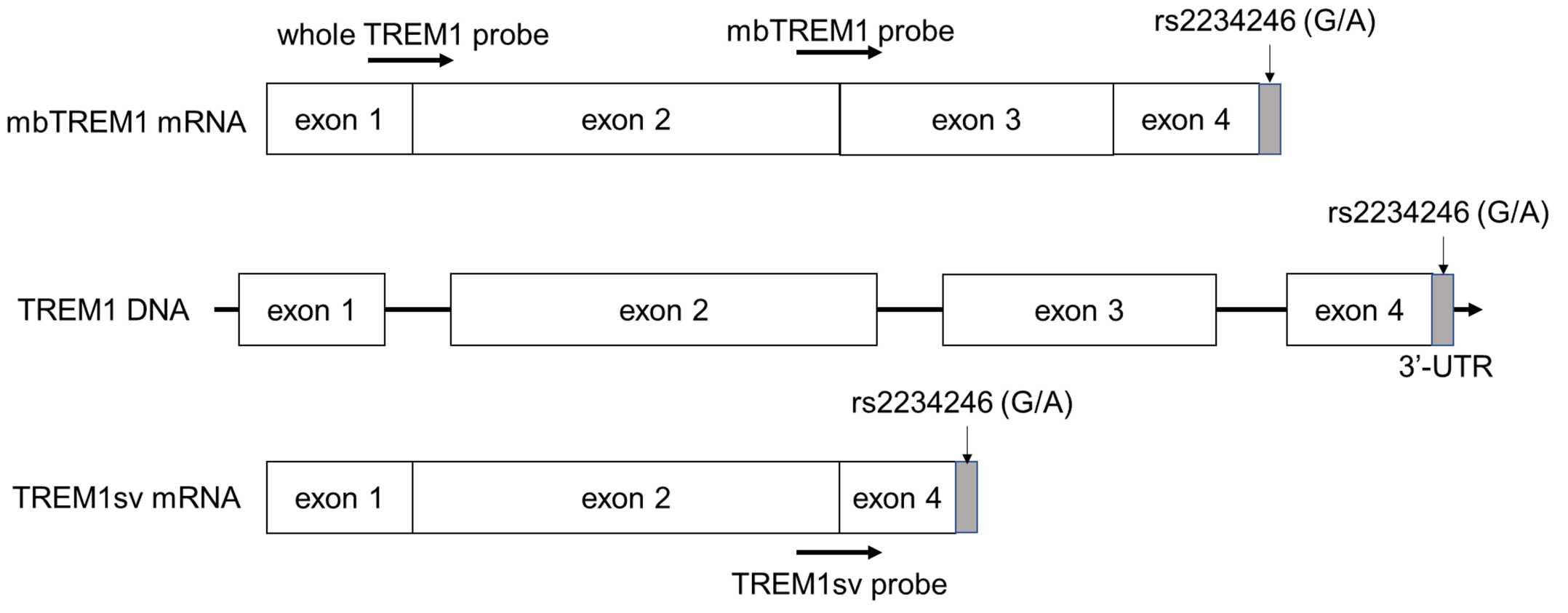
CRP, C-reactive protein; MMSE, Mini-Mental State Examination; WBC, white blood cell

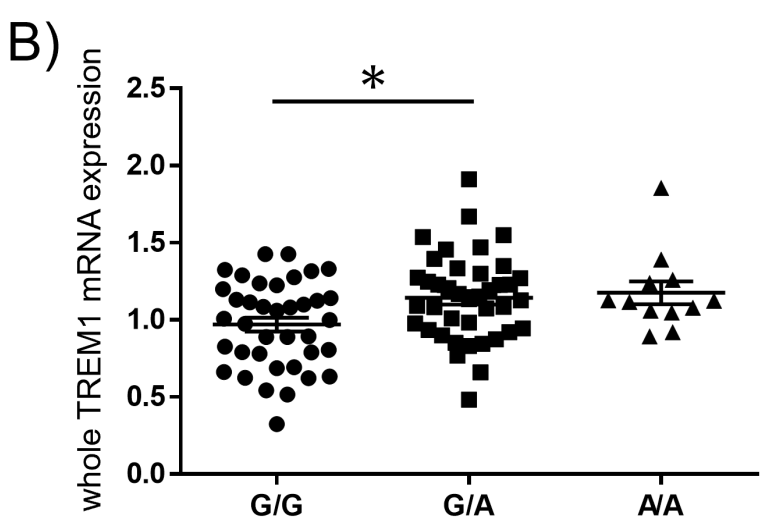
Table 3. Demographic and biochemical data of MRI study

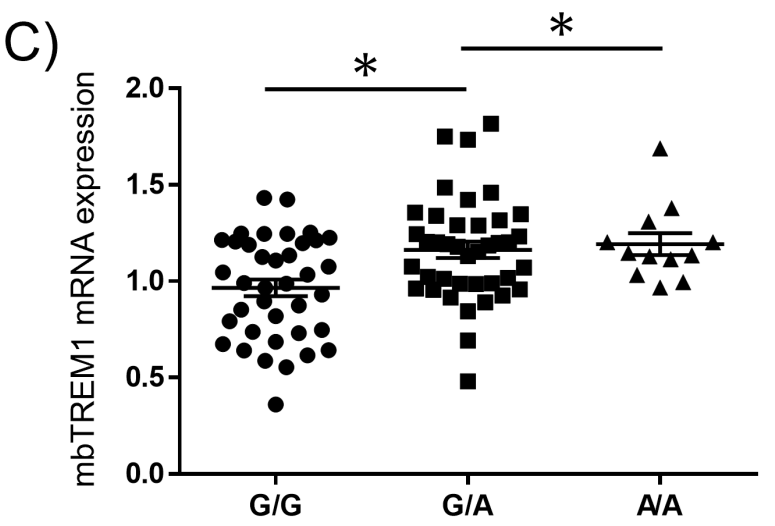
Parameters	Control	AD	P-value
N	181	53	
Age (years)	77.1 ± 7.0	84.6 ± 5.1	< 0.001
Sex (male: female)	71: 110	20: 33	0.845
BMI, kg/m^2	23.3 ± 3.1	23.1 ± 3.5	0.73
Education, ≤ 9 y, %	53.0	79.2	< 0.001
Hypertension, %	77.9	73.6	0.512
Diabetes Mellitus	12.7	15.7	0.581
Serum total cholesterol, mg/dL	197.1 ± 34.9	192.8 ± 30.0	0.490
History of brain attack, %	3.9	24.5	< 0.001
Current smoker, %	4.4	4.2	0.933
Current drinker, %	40.0	20.8	0.105
Chronic kidney disease, %	23.8	37.7	0.044
Regular exercise, %	37.0	28.3	0.242
ECG abnormalities, %	27.5	28.8	0.272
MMSE	28.5 ± 1.4	18.0 ± 4.3	< 0.001
rs2234246 (G/G: G/A: A/A)	77: 79: 25	19: 30: 4	0.200

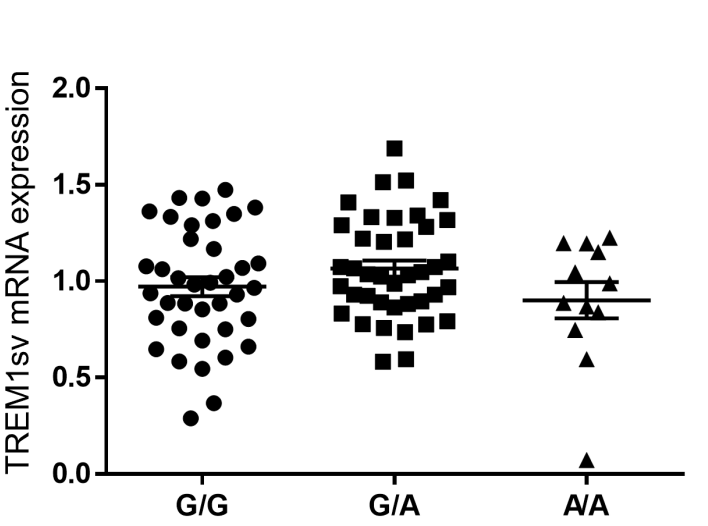
BMI, body mass index; ECG, Electrocardiogram; MMSE, Mini-Mental State Examination

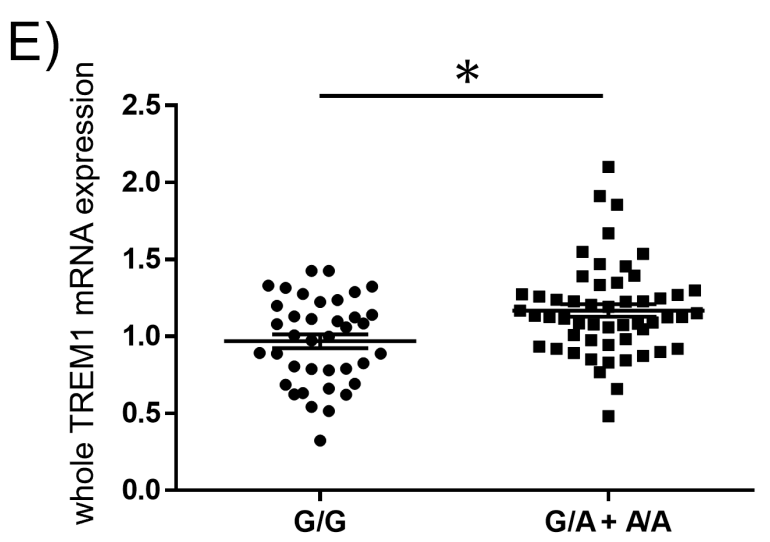
A)

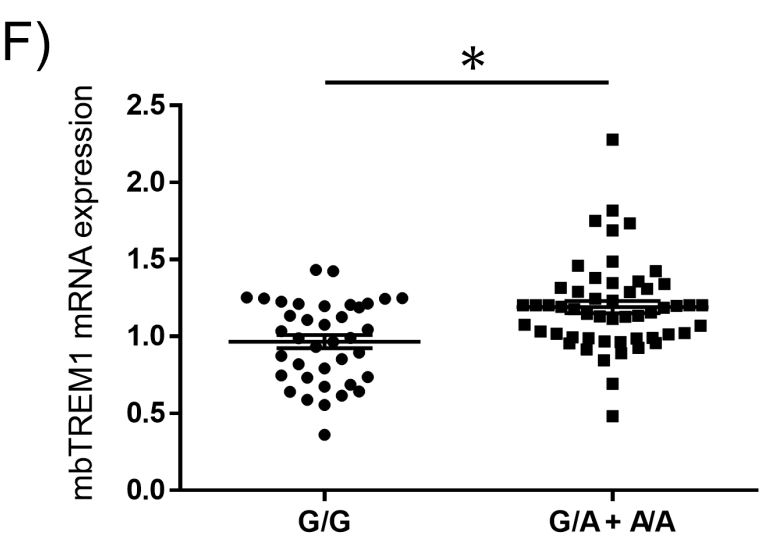

whole TREM1 probe
mbTREM1 probe
rs2234246 (G/A)
mbTREM1 mRNA
exon 1
exon 2
exon 3
exon 4
rs2234246 (G/A)
TREM1 DNA
exon 1
exon 2
exon 3
exon 4
3'-UTR
rs2234246 (G/A)
TREM1sv mRNA
exon 1
exon 2
exon 4
TREM1sv probe

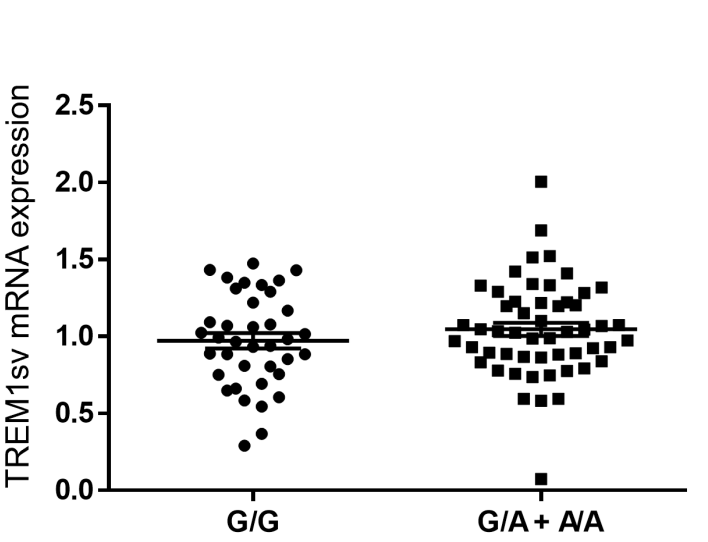

B)
whole TREM1 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
*
G/G
G/A
A/A

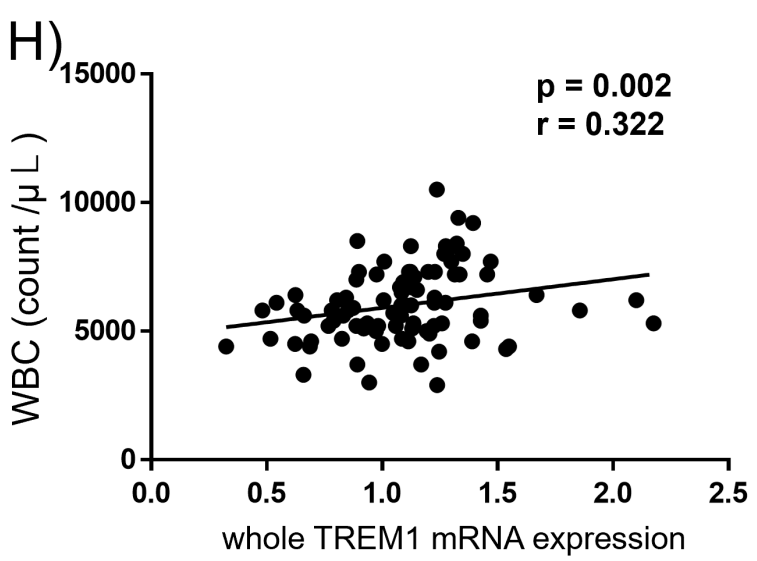

C)
mbTREM1 mRNA expression
2.0
1.5
1.0
0.5
0.0
*
*
G/G
G/A
A/A

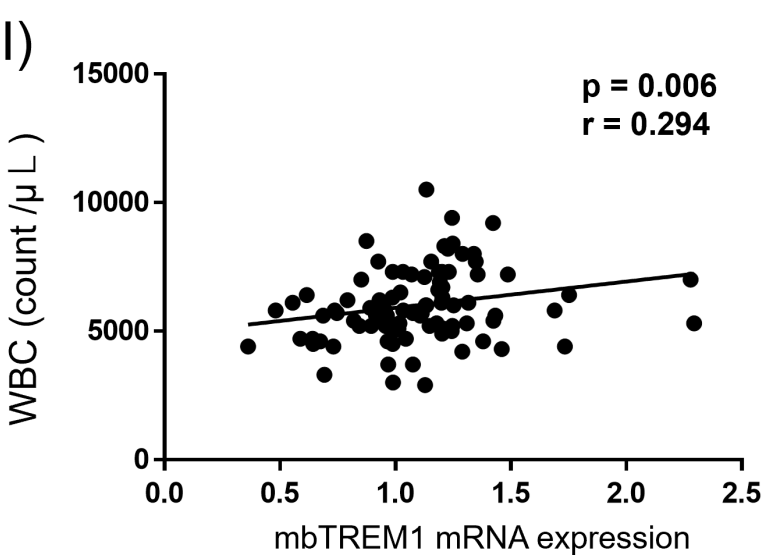

D)
TREM1sv mRNA expression
2.0
1.5
1.0
0.5
0.0
G/G
G/A
A/A

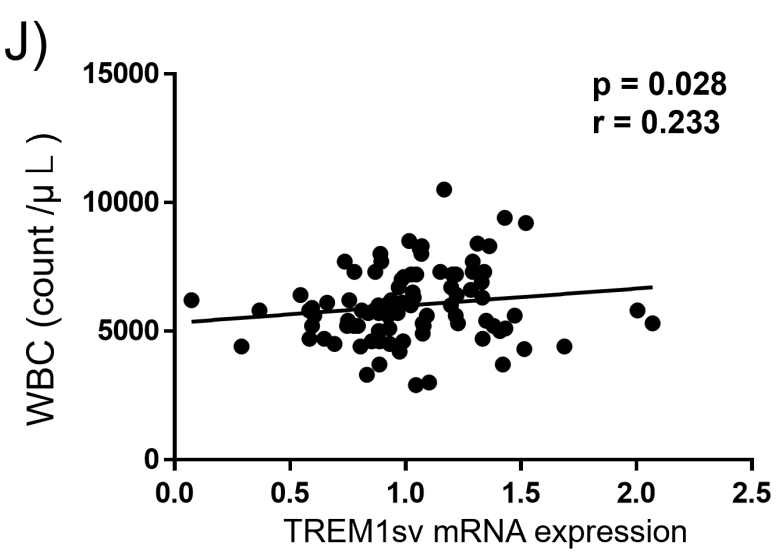

E)
whole TREM1 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
*
G/G
G/A + A/A


F)
mbTREM1 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
*
G/G
G/A + A/A

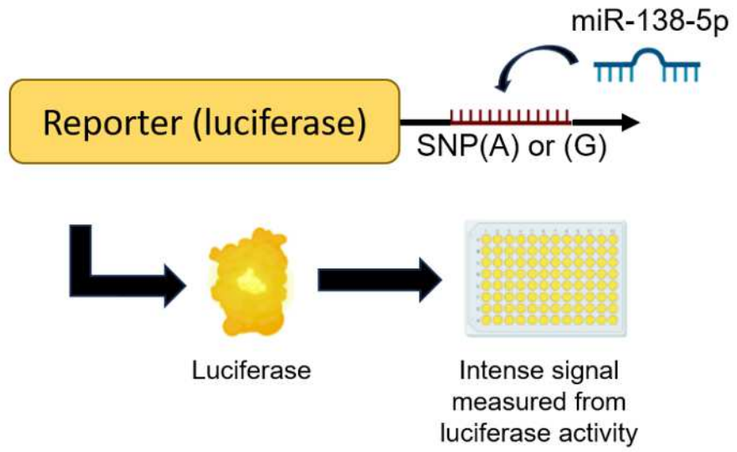

G)
TREM1sv mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
G/G
G/A + A/A


H)
WBC (count /μ L)
15000
10000
5000
0
p = 0.002
r = 0.322
0.0
0.5
1.0
1.5
2.0
2.5
whole TREM1 mRNA expression


I)
WBC (count /μ L)
15000
10000
5000
0
p = 0.006
r = 0.294
0.0
0.5
1.0
1.5
2.0
2.5
mbTREM1 mRNA expression


J)
WBC (count /μ L)
15000
10000
5000
0
p = 0.028
r = 0.233
0.0
0.5
1.0
1.5
2.0
2.5
TREM1sv mRNA expression

A)



Position 2035-2041 of *TREM1* 3'UTR

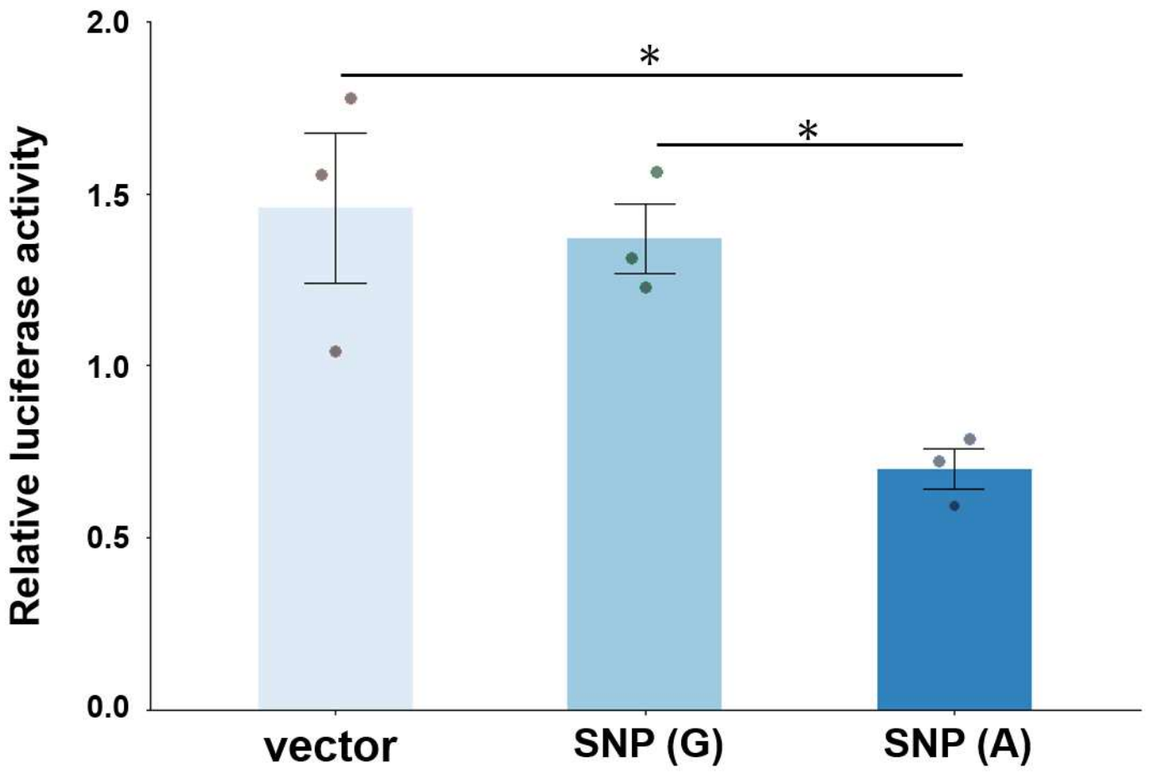
SNP (A)-*TREM1*	5'...AAUUAAAUGUGUAAUCACCAGCU... 3'
hsa-miR-138-5p	3'–GCCGGACUAAGUGUUGUGGUCGA– 5'
SNP (G)-*TREM1*	5'... AAUUAAAUGUGUAAUCACCGGCU... 3'

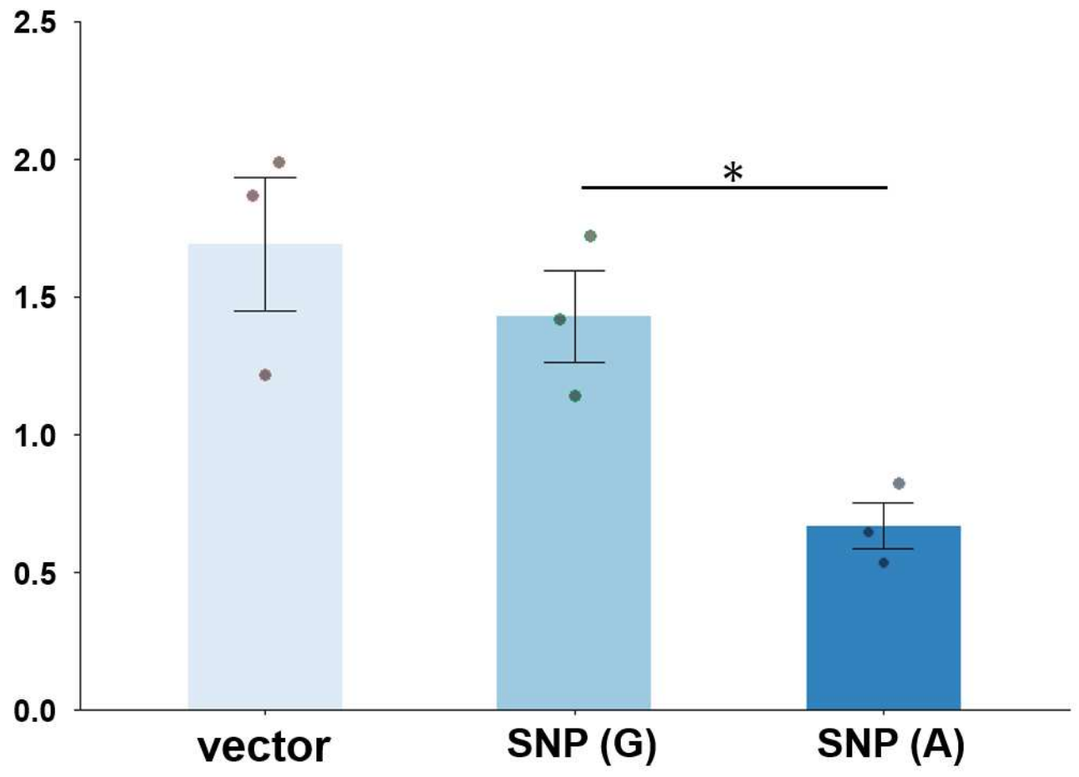
B)

1st batch

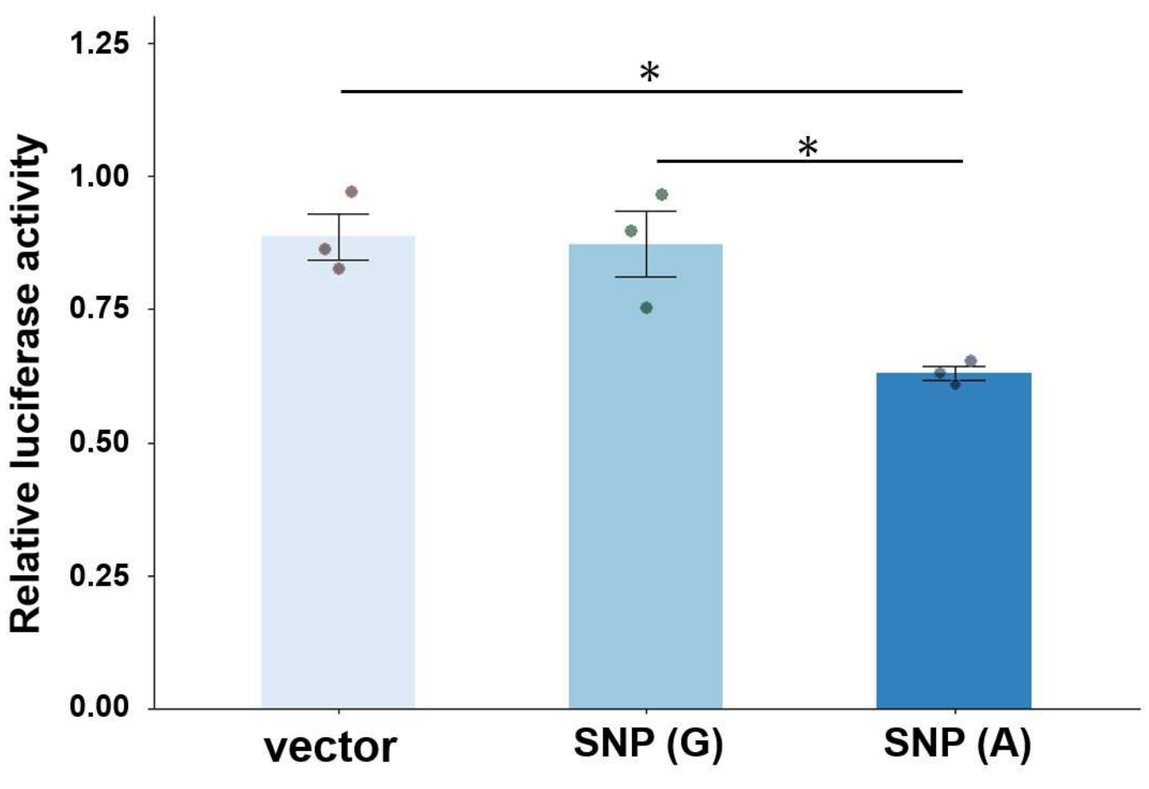
2nd batch

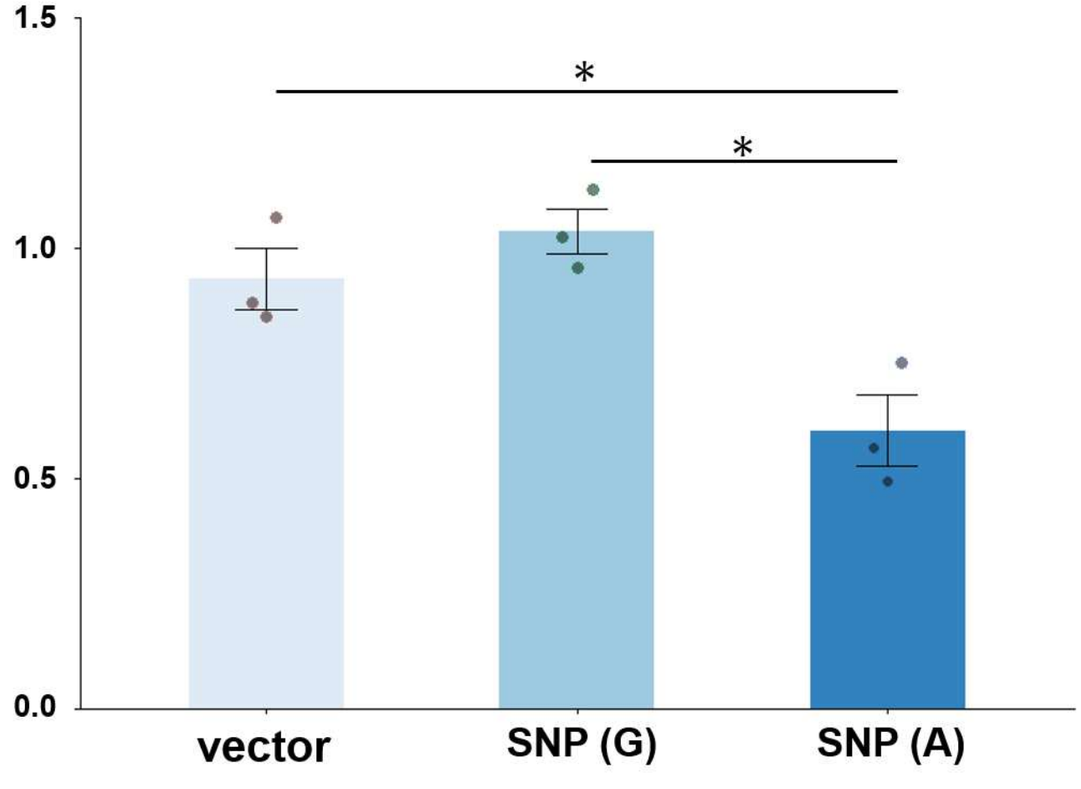
SH-SY5Y

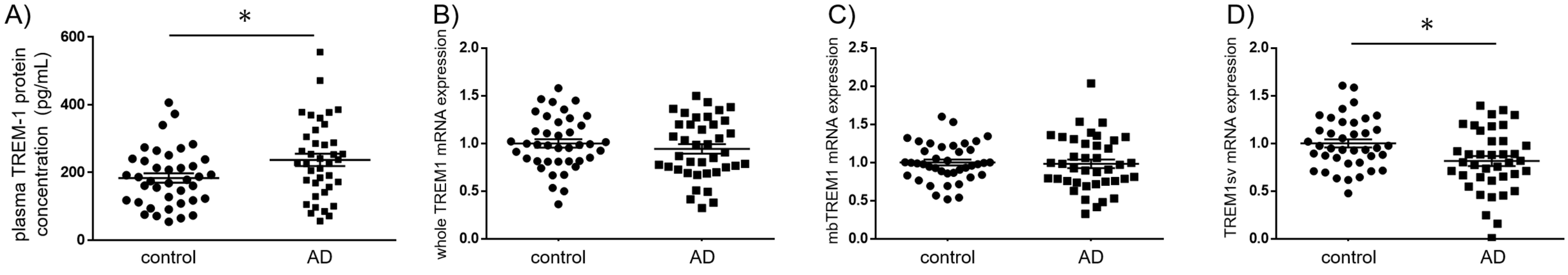


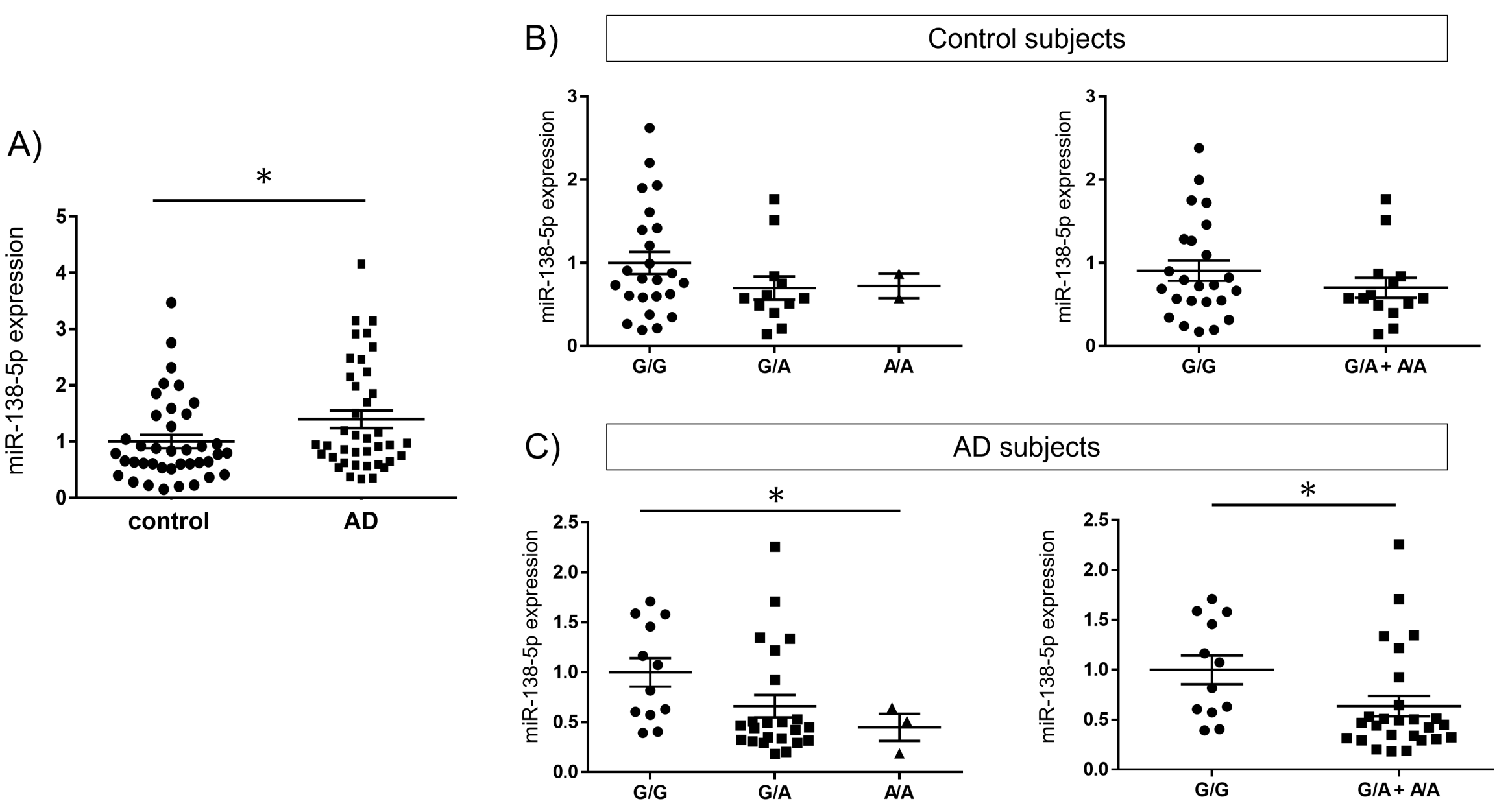


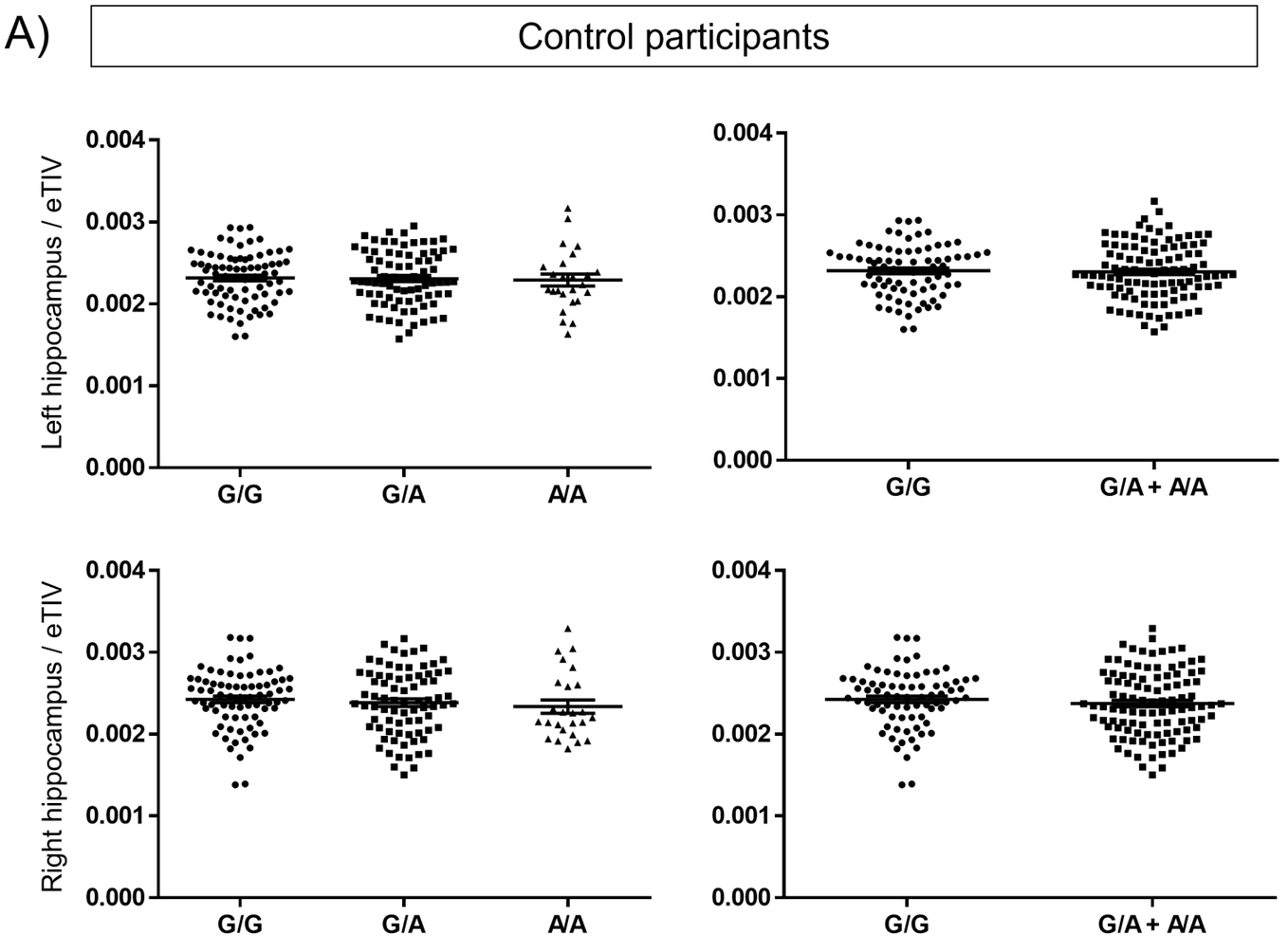
HEK293

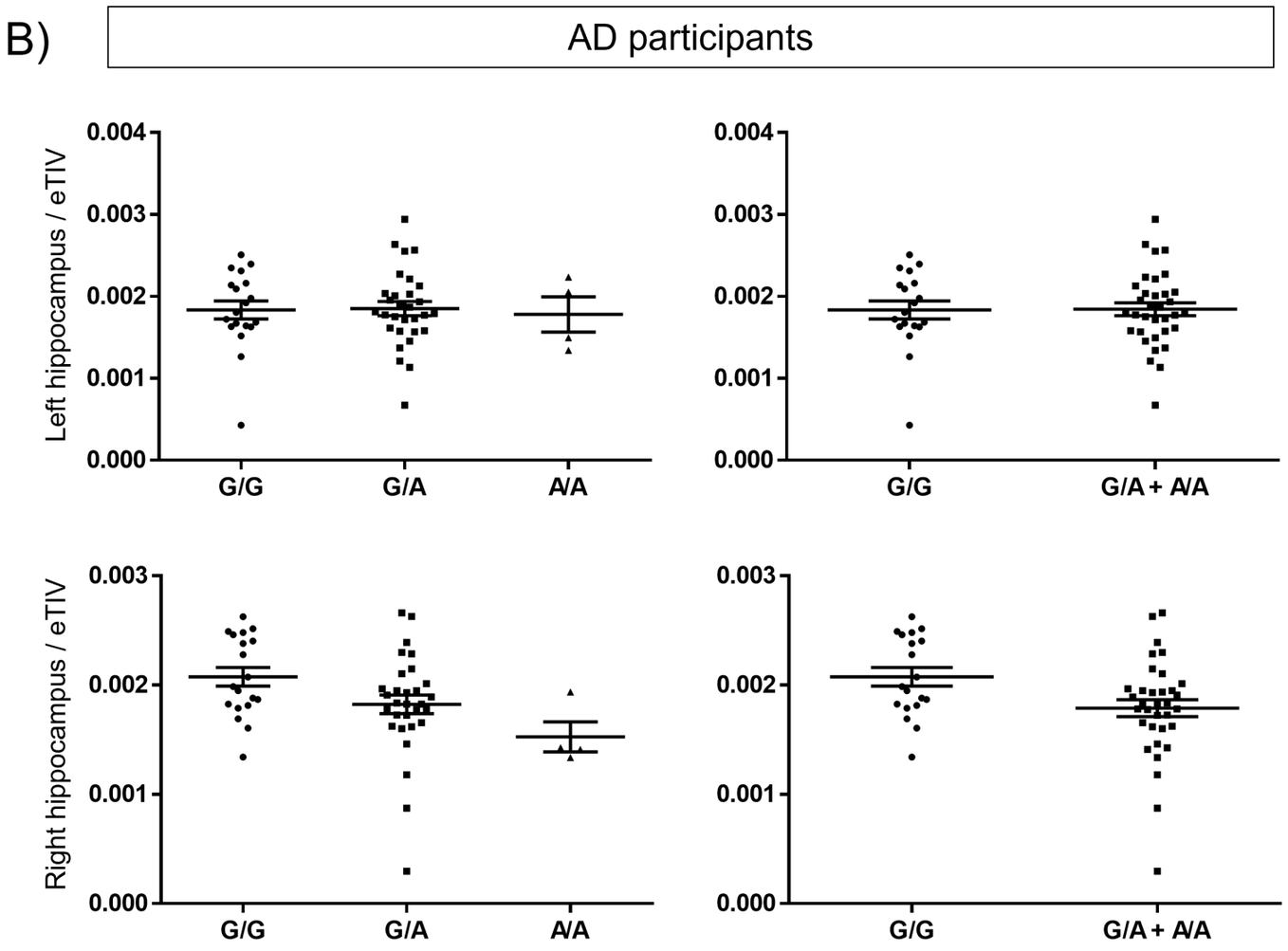


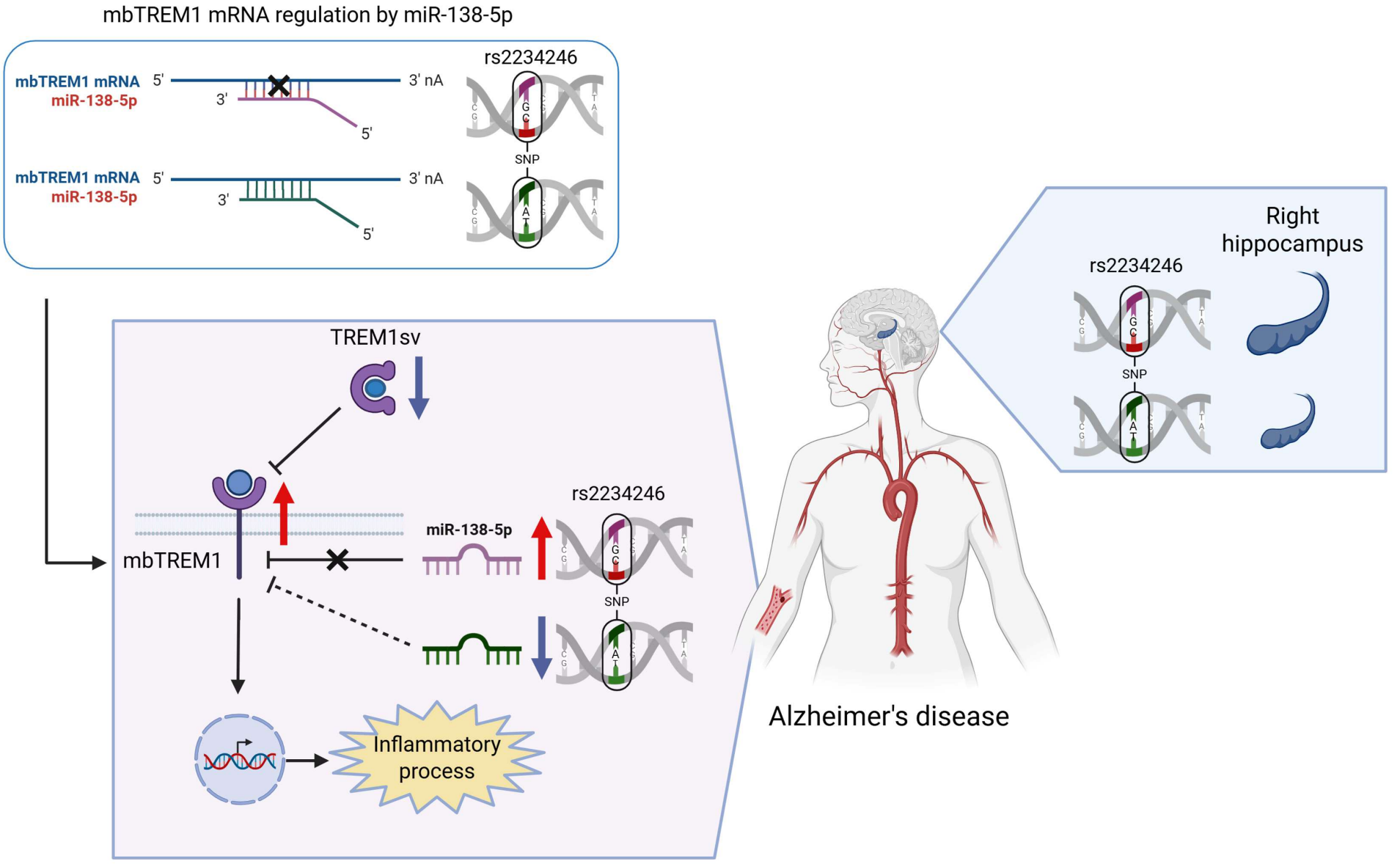



A)
plasma TREM-1 protein concentration (pg/mL)
600
400
200
0
*
control
AD
B)
whole TREM1 mRNA expression
2.0
1.5
1.0
0.5
0.0
control
AD
C)
mbTREM1 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
control
AD
D)
TREM1sv mRNA expression
2.0
1.5
1.0
0.5
0.0
*
control
AD


A)
*
miR-138-5p expression
0
1
2
3
4
5
control
AD
B)
Control subjects
miR-138-5p expression
0
1
2
3
G/G
G/A
A/A
miR-138-5p expression
0
1
2
3
G/G
G/A + A/A
C)
AD subjects
*
miR-138-5p expression
0.0
0.5
1.0
1.5
2.0
2.5
G/G
G/A
A/A
*
miR-138-5p expression
0.0
0.5
1.0
1.5
2.0
2.5
G/G
G/A + A/A


A)
Control participants
Left hippocampus / eTIV
Right hippocampus / eTIV
0.004
0.003
0.002
0.001
0.000
G/G
G/A
A/A
G/A + A/A


B)
AD participants
Left hippocampus / eTIV
Right hippocampus / eTIV
0.004
0.003
0.002
0.001
0.000
G/G
G/A
A/A
G/A + A/A


mbTREM1 mRNA regulation by miR-138-5p
mbTREM1 mRNA
miR-138-5p
5'
3'
3' nA
rs2234246
SNP
TREM1sv
mbTREM1
miR-138-5p
Inflammatory process
Alzheimer's disease
Right hippocampus

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