Original Research

TREM2 coding variants in Slovak Alzheimer's disease patients

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Abstract

Background: Triggering receptor expressed on myeloid cells 2 (TREM2) is an important modulator of innate immune responses. In the human brain, TREM2 is primarily expressed on microglia and is involved in cell survival, phagocytosis, and regulation of inflammation. TREM2 dysfunction has been linked to the pathogenesis of various neurodegenerative diseases including Alzheimer's disease (AD). Rare coding variants of the *TREM2* gene have been reported to modulate AD risk in several populations, however, data on their association with susceptibility to AD in the Slovak population have been missing. **Methods**: We have analyzed 10 non-synonymous coding variants located in *TREM2* exon 2 by direct sequencing in 270 late-onset Alzheimer's disease (LOAD) patients and 331 controls. **Results**: Four out of 10 *TREM2* mutant variants have been identified in the analyzed groups, namely rs75932628 C > T (R47H), rs142232675 C > T (D87N), rs143332484 C > T (R62H), and rs2234253 G > T (T96K). R47H was found only in the AD group, while T96K was present only in the controls. Although no significant association between *TREM2* coding variants and LOAD susceptibility has been detected, the observed odds ratio (OR) of 3.69 for R47H carriers suggests an increased risk of LOAD for this variant in the Slovak population. Moreover, we also found a higher OR for the rs143332484-T allele in *APOE ε*4 non-carriers (1.99) when compared to *APOE ε*4 carriers (0.62). **Conclusions**: Our results suggest an impact of specific *TREM2* rare coding variants on AD risk in the Slovak population.

Keywords: Alzheimer's disease; *TREM2* variants; apolipoprotein E; case-control study; sequencing

1. Introduction

Triggering Receptor Expressed on Myeloid cells 2 (TREM2) is a pattern recognition receptor present on dendritic cells, monocytes, and tissue-specific macrophages [1–3]. This 230 amino acid long transmembrane glycoprotein consists of an extracellular immunoglobulin-like V type domain, a transmembrane domain, and a cytoplasmic tail [4]. Because of the short cytoplasmic tail, TREM2 acts through the intracellular adaptor molecule DNAX-activation protein 12 (DAP12), also known as TYRO protein tyrosine kinase binding protein (TYROBP) [5]. *TREM2* binds to anionic lipids, high- and low-density lipoproteins, and to several apolipoproteins such as APOA1, APOA2, APOB, APOE, and APOJ (clusterin) [6–9]. The molecule is implicated in a wide array of functions including cell maturation, survival, proliferation, activation, phagocytosis, and the regulation of inflammation [10]. Anti-inflammatory properties of TREM2 after TLR stimulation have been confirmed in several *in vitro* and *in vivo* studies [11–13].

In the human brain, TREM2 is primarily expressed on microglia and is involved in the phagocytosis of apoptotic neurons, and modulation of inflammation [10,14–16]. As revealed in microglia, the reduction of TREM2 signaling increases TNF and NO synthase-2 (NOS2) transcription, while overexpression of TREM2 decreases transcription of TNF, IL1β, and NOS2 [14].

Enhanced expression of TREM2 was found in various neurodegenerative disorders such as Parkinson's disease [17], amyotrophic lateral sclerosis [18], stroke [19, 20], traumatic brain injury [21], and Alzheimer's disease [22–25]. In Alzheimer's disease (AD) subjects, increased TREM2 expression has been associated with the recruitment of microglia to amyloid plaques [24,26]. In various *in vitro* and *in vivo* models, TREM2 has been associated with Aβ40 and Aβ42 uptake by microglia suggesting its effect on Aβ clearance in AD models [27–31]. TREM2 can also increase microglial cell number, proliferation, and survival resulting in clustering of microglia to amyloid plaques [27,32,33].

Several *TREM2* genetic variants have been identi-

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fied that increase the risk of late-onset Alzheimer's disease (LOAD). The most-well studied variant is rs75932628 C > T, a single nucleotide polymorphism (SNP) in exon 2 encoding an arginine to histidine missense substitution at amino acid 47 (R47H). Two independent studies in 2013 found for the first time that heterozygous form of the *TREM2* R47H is associated with LOAD risk [34,35]. This rare *TREM2* variant can increase AD risk by 2–4 fold which is comparable to the increased risk associated with one copy of the known *APOE ε*4 risk allele [36,37]. The association of the *TREM2* R47H variant with AD has been observed in European [38–46], and Afro-American populations [47,48]. However, the R47H variant does not seem to be associated with LOAD risk in the Asian population [49–54]. Other *TREM2* variants suggested as risk factors for developing LOAD include D87N, R62H, T96K, Y38C, L211P, and H157Y [34,38,47,55–60]. Most variants are associated with decreased cell-surface expression of *TREM2* (R47H, Y38C, R136Q) or by impaired *in vitro* interactions with ligands like APOE, LDL, and clusterin (R47H, R62H and D87N) [27,61,62]. R47H carriers in AD patients also demonstrated upregulation of IFN type I response and pro-inflammatory cytokines [63]. Moreover, the R47H variant of sTREM2 is less able to bind and disaggregate oligomeric Aβ, which leads to Aβ protofibril formation and neurotoxicity [64].

The aim of our study was to perform an association analysis between *TREM2* coding variants and risk of LOAD in the Slovak population. We have analyzed 10 non-synonymous rare variants in exon 2 of the *TREM2* by means of direct sequencing. To our knowledge, no such analysis has been performed in the Slovak population until now.

2. Materials and methods

2.1 Study groups

The case-control study involved 270 late-onset AD patients (99 men and 171 women, mean age: 78.56 ± 6.18 years). The diagnosis of probably AD was established by physicians according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) diagnostic criteria [65]. The clinical examination included personal medical and family history assessment, neurologic examination, neuropsychological assessment, and neuroimaging. The clinicians estimated age at onset (AAO) using standardized methodology; it is the age, at which the patient started to show significant symptoms of memory loss and cognitive impairment with a progressive clinical course. Unrelated cases with early-onset AD (age at onset <65 years) and having other neurologic or psychiatric diseases were excluded from the study. All AD patients have been recruited in the period from 2016 until 2020 via several psychiatric clinics throughout Slovakia. The mean age at disease onset was 75.50 ± 6.38 years. The reference cohort in our case-control study com-

prised 331 unrelated volunteers (133 males and 198 females with mean age of 76.05 ± 7.64 years). Montreal Cognitive Assessment (MoCA) was selected as the screening test for cognitive impairment in this study [66]. The cut-off score of 26 from 30 has been considered for normal cognition. All control subjects were without any personal or family history of AD and they were recruited from a matched group of a larger population sample. All patients and controls were Caucasians of Slovak descent. *APOE ε*4 allele as a known genetic risk factor for AD was evaluated in both study groups and implemented as a stratification factor in further analyses. Detailed parameters of the study groups are summarized in Table 1. This case-control study was approved by the Independent Ethical Committee of the Bratislava Municipality under the No. 05440/2021/HF. All the investigations were carried out in accordance with the International Ethical Guidelines and the Declaration of Helsinki. From all patients or their representatives, as well as from control subjects, informed written consent for enrolling in the study and for personal data management was obtained.

2.2 Sequence analysis

Genomic DNA was isolated from ethylenediamine tetraacetic acid (EDTA)-treated whole blood samples (2 mL) by a modified salting-out procedure [67]. *TREM2* exon 2 region from 6472 to 7004 bp was amplified using forward primer 5′-TCCTTCAGGGCAGGATTTTT-3′ and reverse primer 5′-AGTGGGTGGTTCTGCACAC-3′. A 25 μL PCR reaction mixture contained 50 ng of template DNA, 0.2 mM of each dNTP (Thermo Fisher Scientific Inc., Lot. Nr. 00654321, MA, USA), 1 U of Taq DNA polymerase (Thermo Fisher Scientific Inc., Lot Nr. 1817560, MA, USA), 1.5 mmol MgCl2 (Thermo Fisher Scientific Inc., Lot Nr. 1817560, MA, USA), and 10 pmol of each specific primer. The PCR conditions consisted of initial denaturation at 94 °C for 3 min, followed 30 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 30 s, and elongation at 72 °C for 1 min. The final elongation at 72 °C for 10 min completed the reaction. The PCR products were run in 1.0% agarose gel for 20 min and then visualized under UV light. A fragment size of 533 bp was confirmed using the 100 bp DNA ladder (Solis BioDyne Inc., Lot. Nr. 07110000, Estonia, Europe). To perform sequence analysis, the PCR products were purified using an EXO SAP-IT kit according to the manufacturer's recommendations (USB Inc., Lot. Nr. 00123457, WI, USA) and then sequenced (approximately 100 ng) using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Lot Nr. 00567001, MA, USA). Direct sequencing was performed individually on both strands using forward and reverse PCR primers. The sequencing reaction was run on a 3130 ABI PRISM Genetic analyzer (Applied Biosystems Inc., MA, USA) and the sequence data were analyzed by SeqScape software (version 3.0, Applied Biosys-

Table 1. Characteristics of the study groups.

Parameter	LOAD patients (n = 270)	Controls (n = 331)	*p* value
Age at examination, y; mean $\times$ SD (age range)	78.56 $\times$ 6.18 (65–95)	76.05 $\times$ 7.64 (65–95)	<0.0001
Age at onset, y; mean $\times$ SD (age range)	75.50 $\times$ 6.38 (65–95)	-	-
Sex, n; female/male, (% female)	171/99 (63.33%)	198/133 (59.82%)	0.38
MoCA score; mean $\times$ SD	14.53 $\times$ 5.78	27.53 $\times$ 1.53	<0.0001
APOE ε4 status, n; positive/negative (% positive)	134/136 (49.63%)	63/268 (19.03%)	<0.0001

Differences in age and MoCA score between the two groups were examined by the Mann-Whitney unparametric test. Differences in sex and *APOE* ε4 status were assessed using the Pearson χ^2 test. $p < 0.05$ is considered statistically significant. CI, confidence interval; LOAD, late-onset Alzheimer's disease; MoCA, Montreal Cognitive Assessment; n, number; OR, odds ratio; SD, standard deviation; y, years.

tems Inc., MA, USA). The reference sequence was reported on the website http://www.ebi.ac.uk/imgt/hla/align.html.

Genotyping of the *APOE* ε4 risk allele was performed by the determination of rs429358 (C > T) and rs7412 (T > C) polymorphisms in the fourth exon using direct sequencing as described previously [68].

2.3 Statistical analysis

Allele and genotype frequencies were determined by direct counting. Genotypes were tested for their fit to Hardy-Weinberg equilibrium using the chi-squared goodness-of-fit test. Statistical differences in allele and genotype frequencies between AD patients and the control group were evaluated by the Pearson chi-squared test using the InStat statistical software (version 3.10, Graph-Pad Software Inc., CA, USA). The *p* values, odds ratios (OR), and 95% confidence intervals (95% CI) were calculated in the codominant inheritance model. The multivariate logistic regression analysis adjusted for sex, age (age at onset in patients and age at the examination in controls), and *APOE* ε4 carrier status as possible modifying factors was performed by the SNPstats web software available at http://bioinfo.iconcologia.net/SNPstats [69]. Regression analysis and synergy factor (SF) measurement were also performed to assess the significance and size of interaction between *TREM2* variants and the *APOE* ε4 allele, as previously described [70]. The *p* value of <0.05 was considered as statistically significant.

3. Results

3.1 Characteristics of the study groups

The demographic and clinical characteristics of the study groups are shown in Table 1. The study included 270 AD patients and 331 unrelated controls. There was no statistically significant difference between the AD group and controls in relation to gender ($p = 0.38$), with females having a higher prevalence in both AD patients (63.33%) and controls (59.82%). The mean age at examination was significantly higher in the AD group than in controls (78.56 versus 76.05 years; $p < 0.0001$). Concerning the MoCA assessment, there was a significantly lower MoCA score in AD patients compared to controls (14.53 versus 27.53; $p <$

0.0001). A significantly higher prevalence of the *APOE* ε4 risk allele was found in the AD group compared to controls (49.63% vs 19.03%, $p < 0.0001$).

3.2 Association of TREM2 variants with LOAD risk

Ten non-synonymous rare variants in *TREM2* exon 2 have been analyzed in 270 AD patients and 331 controls: rs75932628 C > T (R47H), rs147564421 G > A (R98W), rs142232675 C > T (D87N), rs201258663 G > A (T66M), rs797044603 A > G (Y38C), rs104894002 G > A (Q33X), rs143332484 C > T (R62H), rs2234252 G > A (A28V), rs2234253 G > T (T96K), and rs374851046 C > G (R52H). Allele and genotype frequencies of the *TREM2* variants observed in the analyzed groups are shown in Table 2. Four out of 10 *TREM2* coding variants have been identified in the analyzed groups, namely R47H, D87N, R62H and T96K. Genotype frequencies of *TREM2* variants fit the Hardy-Weinberg equilibrium in AD patients ($p = 0.98$ for R47H; $p = 0.93$ for D87N, $p = 0.80$ for R62H, $p =$ NA for T96K) as well as in controls ($p =$ NA for R47H, $p = 0.87$ for D87N, $p = 0.82$ for R62H, $p = 0.98$ for T96K).

The carriage of the rs75932628-T allele (R47H) was identified in one of the AD cases (0.19%), while this allele was missing in the control group. Univariate chi-square analysis revealed that the carrier of the minor T allele had a 3.69-fold increased risk to develop AD compared to non-T carriers. Regarding other *TREM2* variants, the D87N variant was identified in 3 AD cases (0.56%) and 6 controls (0.91%), R62H was identified in 8 AD cases (1.48%) and 7 controls (1.06%) and T96K variant was identified in one control subject (0.15%). No statistically significant differences in either *TREM2* mutant allele or genotype frequencies were found between the AD group and controls ($p > 0.05$, Table 2). Multivariate analysis of the association between the *TREM2* variants and LOAD risk adjusted for age, sex and *APOE* ε4 status as potential confounding variables revealed no changes in comparison with the univariate analysis ($p > 0.05$, Table 2). Logistic regression analysis also revealed no statistically significant *p* value for the interaction of *TREM2* variants with *APOE* ε4 allele dosage ($p > 0.05$, Table 2).

Table 2. Association between *TREM2* coding variants and LOAD risk.

TREM-2	LOAD (n = 270) a frequency	Controls (n = 331) a frequency	*a* vs *A*-crude analysis p	OR (95% CI)	*Aa* vs *AA*-crude analysis p	OR (95% CI)	*Aa* vs *AA*-adjusted analysis* p	OR (95% CI)	Interaction *p* values† TREM-2× APOE ε4
6671 C > T (R47H)	1 (0.19%)	0 (0.00%)	0.27	3.68 (0.15–90.69)	0.21	3.69 (0.15–91.02)	0.38	-	NA
6823 G > A (R98W)	0 (0.00%)	0 (0.00%)	NA	NA	NA	NA	NA	NA	NA
6790 C > T (D87N)	3 (0.56%)	6 (0.91%)	0.48	0.61 (0.15–2.46)	0.48	0.61 (0.15-2.46)	0.62	0.69 (0.16–3.03)	0.75/0.72
6728 G > A (T66M)	0 (0.00%)	0 (0.00%)	NA	NA	NA	NA	NA	NA	NA
6645 A > G (Y38C)	0 (0.00%)	0 (0.00%)	NA	NA	NA	NA	NA	NA	NA
6628 G > A (Q33X)	0 (0.00%)	0 (0.00%)	NA	NA	NA	NA	NA	NA	NA
6716 C > T (R62H)	8 (1.48%)	7 (1.06%)	0.51	1.41 (0.51–3.91)	0.51	1.41 (0.51–3.95)	0.73	1.21 (0.41–3.59)	0.27/0.32
6614 G > A (A28V)	0 (0.00%)	0 (0.00%)	NA	NA	NA	NA	NA	NA	NA
6818 G > T (T96K)	0 (0.00%)	1 (0.15%)	0.37	0.41 (0.02–10.04)	0.27	0.41 (0.02–10.04)	0.31	-	NA
6686 C > G (R52H)	0 (0.00%)	0 (0.00%)	NA	NA	NA	NA	NA	NA	NA

*Logistic regression analysis adjusted for age (age at onset in patients and age at examination in controls), sex and *APOE* ε4 status.
†Crude/adjusted analysis (for age and sex) of interaction between *TREM-2* mutant allele and *APOE* ε4 allele. $p < 0.05$ is considered statistically significant. -: OR values couldn't be calculated by software program. *a*, mutant allele; *A*, wild-type allele; CI, confidence interval; LOAD, late-onset Alzheimer's disease; n, number; NA, not available; OR, odds ratio.

Table 3. Association between *TREM2* rs143332484 C > T (R62H) variant and LOAD risk in subjects stratified by *APOE* ε4 status.

	LOAD T frequency	Controls T frequency	T vs C-crude analysis p	OR (95% CI)	CT vs CC-crude analysis p	OR (95% CI)	CT vs CC-adjusted analysis* p	OR (95% CI)
APOE ε4 positive individuals	4 (1.49%)	3 (2.38%)	0.53	0.62 (0.14–2.82)	0.54	0.62 (0.13–2.84)	0.58	0.64 (0.14–3.02)
APOE ε4 negative individuals	4 (1.47%)	4 (0.75%)	0.33	1.99 (0.49–8.00)	0.34	2.00 (0.49–8.12)	0.34	1.99 (0.48–8.21)

*Logistic regression analysis adjusted for age (age at onset in patients and age at examination in controls) and sex. $p < 0.05$ is considered statistically significant. CI, confidence interval; LOAD, late-onset Alzheimer's disease; OR, odds ratio.

Table 4. Combined effects of *TREM2* rs143332484-T (R62H) and *APOE* ε4 allele carriage in LOAD risk.

TREM2 rs143332484-T	APOE ε4	LOAD	Controls	p	OR (95% CI)	SF (p value)
−	−	132 (48.89%)	264 (79.76%)	-	reference	0.31 (0.27)
+	−	4 (1.48%)	4 (1.21%)	0.32	2.00 (0.49–8.13)	-
−	+	130 (48.15%)	60 (18.13%)	<0.0001	4.33 (2.99–6.28)	-
+	+	4 (1.48%)	3 (0.91%)	0.19	2.67 (0.59–12.09)	-

Note: − = no copies of the allele; + = one or two copies of the allele. *p*, OR and 95% CI values were obtained by χ^2 analysis. SF was calculated as follows: 2.67/(2.00 × 4.33). $p < 0.05$ is considered statistically significant. CI, confidence interval; LOAD, late-onset Alzheimer's disease; OR, odds ratio; SF, synergy factor.

3.3 Analysis of TREM2 variants in relation to APOE ε4 carrier status

Association between the *TREM2* variants and LOAD risk in subjects stratified by *APOE* ε4 carrier status was also performed. Analyses in *APOE* ε4-positive and *APOE* ε4-negative groups revealed no statistically significant differences in the distribution of *TREM2* variants between AD patients and the control group ($p > 0.05$ for R62H variant, Table 3). However, we found a somewhat higher odds ratio for rs143332484-T (R62H) in *APOE* ε4 non-carriers (1.99) than in *APOE* ε4 carriers (0.62).

To further determine the genetic interaction between the *APOE* ε4 allele and the *TREM2* variants, we assessed the combined gene effects of *TREM2* rs143332484-T (R62H) and the *APOE* ε4 alleles on LOAD risk (Table 4). When compared to subjects without both alleles, carriers of rs143332484-T, but no *APOE* ε4 were at two-fold increased odds of developing LOAD ($p = 0.32$), while the OR in subjects with at least one copy of *APOE* ε4, but no *TREM2* rs143332484-T (R62H) was 4.33 ($p < 0.0001$). In subjects with both alleles, the OR was reduced to 2.67 ($p = 0.19$). This observed combined effect size of the two alleles was markedly lower than the predicted joint OR assuming independent effects of both rs143332484-T and *APOE* ε4 (OR = 8.66), however, the difference was not significant ($p = 0.27$). The calculated SF value of 0.31 indicates antagonism between *TREM2* rs143332484-T (R62H) and *APOE* ε4 alleles in LOAD risk (Table 4).

4. Discussion

TREM2 is a pattern recognition receptor expressed on myeloid cells involved in the modulation of the innate immune response [3]. In the human brain, TREM2 is primarily expressed on microglia, and is involved in cell survival, chemotaxis, phagocytosis, and regulation of inflammation [10,14–16]. It was found that TREM2 can protect against AD by binding with $A\beta40$ and $A\beta42$ monomers [27–31,33]. Moreover, the soluble form of TREM2 is involved in the inhibition of $A\beta$ aggregation thus it prevents the formation of pathological amyloid plaques [64].

Rare coding variants in *TREM2* were identified as risk factors for Alzheimer's disease in several populations. In 2013 two independent studies described for the first time the association of R47H with LOAD risk [34,35]. Since then the R47H variant has been consistently reported to increase the risk for AD across ethnicities as stated in meta-analyses [37,46,54,59,60] and large-scale GWAS analyses [71,72].

In this study, we examined the contribution of *TREM2* rare variants on risk for LOAD in the Slovak population. We have analyzed 10 non-synonymous coding variants located in *TREM2* exon 2 encoding the ectodomain. Four out of 10 *TREM2* mutant variants have been identified in analyzed groups, namely R47H, D87N, R62H and T96K. No statistically significant differences in the distribution of the *TREM2* R47H variant were found between AD patients and the control group. This finding is due to the low frequency of the risk allele in the study population and agrees with other reports [39,55]. In our study, the R47H variant was identified in one AD case (0.19%), but it was absent in the control group. The OR for R47H was 3.69 what is comparable with previous studies on this variant in Caucasian populations with pooled OR of 3.93 [37].

The distribution of R47H variant in AD patients seems to differ across ethnicities. The rs75932628-T was found in 0.6 to 1.4% of AD cases in the Spanish population [38,40], 1.6 to 2.1% in the French population [73,74], 0.74% of AD cases in the Belgian population [55], 0.4% in the Polish population [58], 1.4 to 2.5% in UK [34,41,42], 1.8% in the Icelandic population [43], 2.3% in the Iranian population [75], 1.8% to 6% in North Americans [56,76–78], 0.2% in Afro-Americans [47], 1.7% in the Colombian population [48], 0.98% in the Argentinian population [59], and in 0.05% in the Japanese population [51].

Regarding other *TREM2* variants in the Slovak population, D87N and R62H were found in both AD patients and controls, while T96K was identified in one control subject. No statistically significant differences in the distribution of *TREM2* mutant variants were found between AD patients and the control group, as reported in other studies [34,47,55].

We are aware of several limitations of the current study. First, the relatively small sample sizes may reduce the power of the study to detect associations between *TREM2* gene variants and the risk of late-onset Alzheimer's disease. A larger number of LOAD patients would be desirable for replication, especially with regard to the low prevalence of most TREM variants. We assume our present-day results as useful for sample size planning in future investigations on this topic. Secondly, the estimation of age at onset may be biased by different factors such as the significance of clinical symptoms as experienced by patients.

A significant association of R47H with the risk of AD in the Caucasian population (OR = 3.93, 95% CI: 3.15–4.90, $p < 0.001$) was confirmed in the latest meta-analysis examining 22,175 AD cases and 33,049 controls [37]. Furthermore, meta-analysis of R62H and D87N in Caucasians identified no significant association of these *TREM2* variants with AD susceptibility (R62H: OR = 1.17, 95% CI: 0.90–1.52, $p = 0.231$; D87N: OR = 1.62, 95% CI: 0.94–2.82, $p = 0.084$). However, a three-stage case-control study of 48,343 AD cases and 36,790 controls revealed significant association between the R62H variant and AD risk in Caucasians ($p = 1.55 \times 10^{-14}$, OR = 1.67) [79]. A most recent meta-analysis in Caucasians also indicated that H157Y carriers were more predisposed to AD (OR = 4.22, 95% CI: 1.93–9.21, $p < 0.001$) as compared to controls [37]. In contrast to the Caucasian population, the R47H variant has a lower frequency in the East Asian population (0.03% vs 0.08%) and thus no association of the R47H variant with AD risk has been identified [37,52]. Similarly, no association of H157Y or L211P with AD in the East Asian population was found [52]. Concerning other *TREM2* variants, S31F, R47C, G55R, L205P, and G219C were identified as novel variants in the AD patient group [56,75,77]. Moreover, *TREM2* rs187370608 A > G was significantly associated with AD susceptibility as revealed in GWAS using combined AD-by-proxy approach and case-control study ($p = 1.45 \times 10^{-16}$, $p = 1.26 \times 10^{-25}$) [80,81].

The diminished effect of *TREM2* rare coding variants on protein function has been confirmed by biochemical analyses. The most studied R47H variant showed decreased cell-surface expression and impaired ligand-binding [9,77]. R47H variant also affects TREM2 maturation [82]. Finally, the R47H variant of soluble TREM2 is less capable of binding and disaggregating oligomeric $A\beta$, thus promoting $A\beta$-induced neurotoxicity [64]. As a consequence of loss of TREM2 function, R47H carriers showed reduced myeloid cell responses to amyloid deposition and more frequent α-synucleinopathy [45,83]. Some studies found that R47H carriers had a trend toward a decrease in the age of AD onset [41,45], however, others revealed no significant association [40,43].

Other *TREM2* rare variants associated with decreased cell-surface receptor expression include R136Q, R136W, Y38C, T66M, S31F, R47C, and E151K [77]. R62H and D87N showed impaired interactions with ligands, however, the T96K variant increased TREM2 affinity to their ligands [61,84]. Y38C variant exhibited impaired TREM2 maturation and folding leading to changes in microglia morphol-

ogy, loss of synaptic proteins, and reduced hippocampal synaptic plasticity in mouse models [85].

As *APOE* ε4 allele belongs to known risk factors for susceptibility to AD [36,86,87], we also examined the combined effect of *APOE* ε4 and *TREM2* variants on LOAD risk. Analyses in *APOE* ε4-positive and *APOE* ε4-negative groups revealed no statistically significant differences in the distribution of *TREM2* variants between AD patients and the control group. However, we found a somewhat higher OR for rs143332484-T (R62H) in *APOE* ε4 non-carriers (1.99) than in *APOE* ε4 carriers (0.62). A recent meta-analysis in non-*APOE* ε4 carriers based on whole-exome sequencing data identified 4 candidate variants with strong statistical power on AD risk. They include *APOE*/rs7412 coding for the *APOE* ε2 allele, (OR = 0.40; $p = 5.46 \times 10^{-24}$), *TOMM40*/rs157581 (OR = 1.49; $p = 4.04 \times 10^{-7}$) and *TREM2*/rs75932628 (R47H) (OR = 4.00; $p = 1.15 \times 10^{-7}$). The fourth significant variant, *NSF*/rs199533 (K702K), was found on chromosome 17 (OR = 0.78; $p = 2.88 \times 10^{-7}$). These candidate gene variants showed either protective or negative effects on AD risk [88].

In our study we also calculated the synergy factor value that has predicted an antagonism between *TREM2* rs143332484-T (R62H) and *APOE* ε4 alleles in LOAD risk. It is not clear whether the observed antagonistic interaction between *TREM2* rs143332484-T (R62H) and *APOE* ε4 alleles correlates with the biological events in AD subjects. *APOE* ε4 has been associated with impaired binding and clearance of Aβ leading to deposition of amyloid plaques in the brain [89,90]. Moreover, *APOE* ε4 has been involved in impaired synaptic integrity and plasticity [91]. Carriers of at least one *APOE* ε4 allele exhibited an earlier disease onset, faster disease progression, and increased brain atrophy [36,92,93].

The APOE- TREM2 relationship has been studied by several authors [6–8,94]. It was found that TREM2 is binding to APOE to enhance the phagocytosis of apoptotic neurons [6]. Regarding the *TREM2* R62H variant its decreased ligand affinity has been observed [9]. It can be hypothesized that impaired ligand affinity of R62H affects phagocytosis of APOE-bound apoptotic cells by microglia contributing to AD pathology.

5. Conclusions

In our case-control study, we assessed the contribution of *TREM2* rare variants on risk for LOAD in the Slovak population. We have analyzed 10 non-synonymous coding variants located in *TREM2* exon 2 encoding for the extracellular domain. Four out of 10 *TREM2* mutant variants have been identified in both analyzed groups, namely R47H, D87N, R62H, and T96K. R47H substitution was found only in the AD group, while T96K was present only in the controls. The OR of 3.69 in *TREM2* R47H carriers suggests an increased risk of this variant for LOAD also in the Slovak population.

Abbreviations

AD, Alzheimer's disease; APO, apolipoprotein; Aβ, amyloid β; DAP12, DNAX-activation protein 12; EDTA, ethylenediamine tetraacetic acid; GWAS, genome wide association studies; LDL, low density lipoproteins; LOAD, late-onset Alzheimer's disease; LPS, lipopolysaccharide; MoCA, Montreal Cognitive Assessment; NA, not available; OR, odds ratio; SNP, single nucleotide polymorphism; sTREM2, soluble TREM2; TLR, toll-like receptor; TNF, tumor necrosis factor; TREM2, triggering receptor expressed on myeloid cells 2; TYROBP, TYRO protein tyrosine kinase binding protein.

Author contributions

VD and IS—study design and manuscript writing. ZP, BV and IK—sample collection. GM and RP— analysis by direct sequencing. JJ—data analysis and interpretation. VD and AO—PCR assays. SS—data collection.

Ethics approval and consent to participate

The study was approved by the Independent Ethical Committee of the Bratislava Municipality under the No. 05440/2021/HF. Informed written consent was obtained from all participants.

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Conflict of interest

The authors declare no conflict of interest.

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