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Genetic Analysis of *TREM2* Variants in Tunisian Patients with Alzheimer's Disease

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Significance of the Study

- Rare variants within exon 2 of the *TREM2* gene increase the risk of Alzheimer's disease in Caucasian populations. This is the first case-control study to assess the association between these variants and the risk of Alzheimer's disease in a North African population. We sequenced exon 2 of the *TREM2* gene in a cohort of Tunisian patients with late-onset Alzheimer's disease and healthy individuals and identified 5 variants, none of which was associated with the risk of Alzheimer's disease. Our study does not support a major role for *TREM2* in the pathogenesis of late-onset Alzheimer's disease in the Tunisian population.

Keywords
TREM2 gene · Variants · Alzheimer's disease · North-African population · Case-control study

Abstract
Objective: Rare variants in the *TREM2* gene have been reported to significantly increase the risk of Alzheimer's disease in Caucasian populations. Hitherto, this association was not studied in North African populations. In this work, we aimed to study the association between *TREM2* exon 2 variants and the risk of late-onset Alzheimer's disease (LOAD) in a Tunisian population. ***Subjects and Methods:*** We sequenced exon 2 of *TREM2* in a Tunisian cohort of 172 LOAD patients and 158 control subjects. We used the Fisher exact test to compare the distribution of allelic frequencies between the two groups. ***Results:*** We identified 4 previously reported nonsynonymous variants (p.Asp39Glu, p.Arg62His, p.Thr96Lys, and p.Val126Gly) and 1 novel synonymous variant (p.Gln109Gln), none of which was significantly associated with the risk of Alzheimer's disease. Moreover, the rare *TREM2* variant (p.Arg47His), which was considered to be a risk factor for Alzheimer's disease in European descent populations, was not detected in our cohort. ***Conclusion:*** These findings do not support a major role for *TREM2* in the pathogenesis of LOAD in the Tunisian population.

Introduction

TREM2 gene encodes the triggering receptor expressed on myeloid cells 2, which is highly expressed in microglia of the central nervous system [1] and is involved in regulating the immune system by promoting phagocytosis

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Table 1. Demographic characteristics of subjects

	LOAD patients	Controls	*p* value
Subjects, *n*	172	158	
Sex ratio (male:female)	0.5 (58:114)	0.64 (62:96)	0.297
Mean age at examination ± SD, years	75.84±9.64	74.27±4.17	0.06
Mean age at onset ± SD, years	68.69±10.05		
ApoE ε4/ApoE ε4, *n* (%)	27 (15.7)	3 (1.9)	1.3 10e-5
ApoE ε3/ApoE ε4, *n* (%)	73 (42.4)	45 (28.5)	0.008
ApoE ε3/ApoE ε3, *n* (%)	72 (41.9)	110 (69.6)	0.03

LOAD, late-onset Alzheimer's disease; SD, standard deviation.

and regulating the inflammatory response. The rare missense mutation p.Arg47His (rs75932628) within *TREM2* increases the risk of neurodegenerative disorders such as Parkinson's disease [2], essential tremor [3], and late-onset Alzheimer's disease (LOAD). This mutation was identified as a rare risk factor for LOAD in several European descent cohorts with an odds ratio similar to that for apolipoprotein E epsilon 4 (ApoE ε4) [4, 5].

TREM2 variants associated with Alzheimer's disease (AD) have been screened in various populations worldwide; however, no studies have been carried-out in North African populations. Most of the aforementioned variants were observed in exon 2 of the *TREM2* gene. Therefore, the aim of this work was to evaluate the association of *TREM2* exon 2 variants with risk of AD in a sample of the Tunisian population.

Subjects and Methods

Subjects

One hundred seventy-two Tunisian patients with LOAD were recruited from the Neurology Department of Razi Hospital, Manouba, Tunisia. Clinical diagnosis of LOAD was done according to the criteria of the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [6]. In addition, 158 unrelated control subjects were recruited from different primary care clinics. None of the control group subjects had cognitive impairment or personal or familial history of neurological and psychiatric disorders. Written informed consent was obtained from each individual prior to enrollment in the genetic study. Research protocols were approved by the Medical Ethics Committee of Razi Hospital and conformed to the guidelines of the Declaration of Helsinki.

ApoE Genotyping and TREM2 *Sequencing*

Blood samples were collected from LOAD patients and controls. Genomic DNA was extracted from blood by the salting-out method [7]. ApoE genotyping was performed as previously described [8]. Exon 2 of *TREM2* gene was amplified by polymerase chain reaction (PCR) from genomic DNA using two primers: 5′-TGAATGAATGTCTCCTCCCCAG-3′ and 5′-CAGCCACTGCCCACTCA-3′, under the following reaction conditions: denaturation at 95 °C for 5 min followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final cycle of 7-min extension at 72 °C. PCR products were purified and sequenced using the BigDye Terminator Cycle Sequencing Kit v1.1 on a 3,500xl Genetic Analyzer DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed using the SeqScape software (Applied Biosystems) and compared to the *TREM2* GenBank reference sequence (NM_018965).

In silico Tools

The impact of *TREM2* variations was predicted using SIFT (Sorting Intolerant From Tolerant; http://sift.jcvi.org/www/SIFT_chr_coords_submit.html), PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/) and PredictSNP2 (http://loschmidt.chemi.muni.cz/predictsnp2/) [9]. Frequencies of variants were reported from exome database (1,000G; http://browser.1000genomes.org/index.html), ExAC (http://exac.broadinstitute.org/), EVS (http://evs.gs.washington.edu/EVS/), and Great Middle East (GME) Variome Project database (http://igm.ucsd.edu/gme/data-browser.php) including 508 samples from North Africa.

Statistical Analysis

We used Fisher's exact test to compare the distribution of allelic frequencies between LOAD patients and control groups. ApoE genotype and sex distributions were compared using the χ^2 test and mean age was compared using the *t* test. All analyses were two-tailed and a *p* value of 0.05 or less was considered statistically significant. Odds ratios and 95% confidence intervals were calculated using SPSS.

Results

Demographic characteristics and ApoE genotypes of Tunisian LOAD patients ($n = 172$) and controls ($n = 158$) are summarized in Table 1. Both groups had similar sex ratios and similar mean ages. ApoE genotyping showed a

Table 2. Variants of *TREM2* after sequencing of exon 2 in LOAD patients and controls

Variant	db SNP ID	Position (GRCh37)	Exonic function	LOAD patients (carriers, MAF)	Controls (carriers, MAF)	*p* value	OR (95% CI)	Population frequency data			
								1,000G	gnomAD	EVS	GME
p.Asp39Glu	rs200392967	g.41129275G>C	nonsyn	1 (0.005)	0	1	NA	0	6.5 e-5	7.6 e-5	0
p.Arg62His	rs143332484	g.41129207C>T	nonsyn	2 (0.011)	0	0.49	NA	0.005	0.008	0.007	0
p.Thr96Lys	rs2234253	g.41129105G>T	nonsyn	5 (0.029)	6 (0.037)	0.76	1.3 (0.39-4.36)	0.041	0.012	0.039	0
p.Gln109Gln	NA	g.41129065C>T	syn	5 (0.029)	0	0.06	NA				
p.Val126Gly	rs121908402	g.41129015A>C	nonsyn	1 (0.005)	0	1	NA	0	8.2 e-6	0	0

LOAD, late-onset Alzheimer's disease; db SNP, single nucleotide polymorphism database; MAF, minor allele frequency; syn, synonymous; nonsym, nonsynonymous.

Table 3. Characteristics of individuals carrying the *TREM2* variants

Variant	Carrier	Gender	Age at examination/ Age at onset, years	ApoE genotype	Family history of neurodegenerative disease
p.Asp39Glu	patient 1	F	86/82	ε4/ε4	yes
p.Arg62His	patient 2	M	79/71	ε3/ε3	yes
	patient 3	F	78/71	ε3/ε3	yes
p.Thr96Lys	patient 4	M	79/66	ε4/ε4	yes
	patient 5	M	69/67	ε3/ε3	yes
	patient 6	F	72/65	ε3/ε4	yes
	patient 7	F	80/75	ε3/ε4	yes
	patient 8	F	89/79	ε3/ε3	yes
	control 1	M	71/–	ε3/ε3	no
	control 2	M	68/–	ε3/ε4	no
	control 3	F	70/–	ε3/ε3	no
	control 4	M	68/–	ε3/ε3	no
	control 5	F	70/–	ε3/ε4	no
p.Gln109Gln	patient 9	M	85/70	ε3/ε4	yes
	patient 10	F	75/65	ε4/ε4	yes
	patient 11	F	72/67	ε3/ε3	no
	patient 12	F	85/68	ε3/ε4	no
	patient 13	F	77/72	ε3/ε3	no
p.Val126Gly	patient 14	M	82/72	ε3/ε4	yes

significant difference in genotype distributions between the two groups; as expected, the ApoE ε4 allele was over-represented in LOAD patients compared to the control group (36.9 vs. 16.1%; $p < 0.05$). The ApoE ε2 allele was not detected in patients and controls.

Sequencing of exon 2 of the *TREM2* gene revealed 5 variants in 14 LOAD patients and 1 variant in 5 controls. As shown in Table 2, 4 previously reported nonsynonymous variants (p.Asp39Glu, p.Arg62His, p.Thr96Lys, and p.Val126Gly) and 1 novel synonymous variant (p. Gln109Gln) were observed in patients. The variant p. Thr96Lys was also identified in controls. All variants were present in the heterozygous state and were detected in separate individuals (no individuals carried 2 or more variants). Among the 14 patients carrying the *TREM2* variants, 11 had a positive family history of AD (Table 3).

The frequencies of each variation in the population's exome sequencing database are detailed in Table 2. No common variants having minor allele frequency (MAF) >5% were found, and 2 variants (p.Val126Gly and p.Asp-39Glu) were rare, with MAF <1%. Three variants were predicted to have a probable damaging effect (p.Asp39G-lu, p.Thr96Lys, and p.Val126Gly; Table 4). The association analysis of all the identified *TREM2* variants and AD

Table 4. In silico prediction of *TREM2* missense variants

Variant	db SNP ID	Position (GRCh37)	SIFT	Polyphen2	PredictSNP2
p.Asp39Glu	rs200392967	g.41129275G>C	tolerated	possibly damaging	deleterious
p.Arg62His	rs143332484	g.41129207C>T	tolerated	benign	neutral
p.Thr96Lys	rs2234253	g.41129105G>T	damaging	probably damaging	neutral
p.Val126Gly	rs121908402	g.41129015A>C	damaging	Probably damaging	deleterious

Table 5. Summary of all variant screening studies of *TREM2* in patients with Alzheimer's disease (AD)

Ethnicity	AD patients	*TREM2* variants in AD patients		
		exon 2	exon 3	exon 4
Caucasian	256 European + 836 North American [5]	Q33X, Y38C, R47H, R62H, T66M, D87N, T96K, R98W	R136Q, H157Y	–
	117 Norwegian + 944 Dutch + 517 German + 3,759 Icelandic + 399 US [4]	R47H	–	–
	726 French [13]	Q33X, R47H, R62H, R62C, D87N, T96K	–	–
	427 American [14]	R47H	–	–
	504 Spanish [15]	R47H, R62H	–	–
	3,172 Spanish [16]	R47H	–	–
	1,216 Belgian [11]	D39G, D39E, R47H, G58A, R62H, D87N, T96K	L133L, H157Y	S162R, L211P, T223I
	2,082 US [10]	Q33X,R47H, R52H, R62H, T66M, D87N, T96K	R136W, R136Q, H157Y	W191X, E202D, L211P, H215Q, T223I
	210 North American [17]	R47H, D87N,	H157Y	L205P, G219C
East Asian	1,133 Chinese [18]	V34V, C110C, H114H, G115S	–	–
	360 Chinese [19]	A130V	–	–
	988 Chinese [20]	-	H157Y	S183C, A192T
	400 Korean [21]	-	H157Y	A192T
	2,190 Japanese [22]	R47H,	H157Y	L211P
AfricanAmerican	899 African American [23]	R47H, R62H, D87N	E151K,	W191X, L211P
Others	131 Iranian [24]	R47H, G55R, R62H, R62C	–	–

Variations significantly associated with AD are underlined.

was not statistically significant (Table 2). Even the rare variants p.Val126Gly and p.Asp39Glu were not significantly associated with AD ($p > 0.05$).

Discussion

This is the first investigation into the possible association between *TREM2* variants and risk of AD in a North African population. In this study, we sequenced exon 2 of the *TREM2* gene in a cohort of Tunisian patients with LOAD and healthy individuals, and identified 5 variants (p.Asp39Glu, p.Arg62His, p.Thr96Lys, p.Gln109Gln, and p.Val126Gly). The p.Arg47His mutation was not found in our cohort. Association analysis revealed that none of the identified variants were associated with AD risk. Therefore, we could not test the interaction of the ApoE ε4 allele with these variants.

A summary of the reported screening studies for *TREM2* mutations to date, including AD patients, is presented in Table 5. *TREM2* p.Arg62His was previously observed in AD patients and controls in Spanish, Belgian,

and French populations (Table 5), and did not show a nominally significant association with AD. However, a previous case-control study of large European American descent cohorts showed that in addition to p.Arg47His [10], p.Arg62His is a risk factor for AD. The variant rs2234253 (p.Thr96Lys), detected in Belgian, French, and Japanese populations (Table 5), was not shown to enhance AD risk. In our study, p.Thr96Lys was the unique variant detected in controls and LOAD patients with equivalent MAF. According to public exome databases, this variant was more common in African populations (MAF = 14.5%, 1,000G) than other populations, but was not present in North African populations of the GME (Table 2).

Two rare variants predicted to be deleterious, p.Asp39Glu and p.Val126Gly, were identified in our study. The p.Asp39Glu was previously reported in 2 AD patients at the heterozygous state [11], while homozygous p.Val126Gly was reported in 2 patients with Nasu-Hakola disease [12]. Among the 5 variants, variant Gln109Gln was not found in any of the exome databases. However, this variant is synonymous and does not change the amino acid sequence of TREM2 protein.

The previously reported rare variant rs75932628 (p.Arg47His), identified to be a risk factor for AD in Caucasian populations (Table 5), was not found in our cohort. Our findings are in agreement with published reports of African American, Iranian, and East Asian populations (Table 5) which showed that the p.Arg47His mutation could not be linked to an increased risk of AD. In our study, the lack of association of the p.Arg47His variant may be due to its very low MAF in the Tunisian population. In the GME database, this genetic polymorphism had a global MAF of 0.15 and 0.1% in North African populations. Moreover, this variant is considered to be a risk factor according to the ethnicity of the population. AD risk in the Tunisian population may be influenced by genetic and/or environmental factors which might reduce the effect of this variant. However, the limitation of our work is the small cohort size, which could explain the lack of statistical significance.

Conclusion

This study is the first to explore the possibility of an association of *TREM2* with the risk of AD in North Africa, particularly in Tunisia. This population seems to be closer to the African American or East Asian populations than to the European ones regarding the AD risk factor p.Arg47His. We hypothesize that variations in exon 2 of *TREM2* may not play a major role in the pathogenesis of LOAD in the Tunisian population. However, further studies on larger cohorts of North African populations are needed to confirm this hypothesis.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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