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TREM2 R47H variant and risk of essential tremor: A cross-sectional international multicenter study

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Conflict of interest statements

All authors declare that they do not have any conflict of interest.

Contributors

SOC and PP were responsible for design, clinical data acquisition, analysis, interpretation and manuscript writing. OLB, EL, OAR, IW, DB, CM, MS, RK, JGA, FJJ, AG, JG, ME, MJM, JIL, KCY, ZKW, LB, GA and MAP collaborated with the acquisition, analysis and interpretation of data and critical revision of the manuscript for important intellectual content. OAR, RK, JGA, FJJ, JC, ME, JIL, LB, GA, MAP and PP obtained funding for the study.

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Abstract

Introduction—Essential tremor (ET) is the most frequent movement disorder in adults. Its pathophysiology is not clearly understood, however there is growing evidence showing common etiologic factors with other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (AD, PD). Recently, a rare p.R47H substitution (rs75932628) in the TREM2 protein (triggering receptor expressed on myeloid cells 2; OMIM: *605086) has been proposed as a risk factor for AD, PD and amyotrophic lateral sclerosis (ALS). The objective of the study was to determine whether TREM2 p.R47H allele is also a risk factor for developing ET.

Methods—This was a cross-sectional multicenter international study. An initial case-control cohort from Spain (n = 456 ET, n = 2715 controls) was genotyped. In a replication phase, a case-control series (n = 897 ET, n = 1449 controls) from different populations (Italy, Germany, North-America and Taiwan) was studied. Owing to the rarity of the variant, published results on p.R47H allele frequency from 14777 healthy controls from European, North American or Chinese descent were additionally considered. The main outcome measure was p.R47H (rs75932628) allelic frequency.

Results—There was a significant association between TREM2 p.R47H variant and ET in the Spanish cohort (odds ratio [OR], 5.97; 95% CI, 1.203–29.626; p = 0.042), but it was not replicated in other populations.

Conclusions—These results argue in favor of population-specific differences in the allelic distribution and suggest that p.R47H (rs75932628) variant may contribute to the susceptibility of ET in Spanish population. However, taking into account the very low frequency of p.R47H, further confirmatory analyses of larger ET series are needed.

Keywords

TREM2; Essential tremor; p.R47H; Risk; Genetics

Introduction

Recently, a rare missense variant (rs75932628, p.R47H) in TREM2 (triggering receptor expressed on myeloid cells 2; OMIM: *605086) has been identified as a risk factor for several neurodegenerative diseases such as Alzheimer disease (AD) [1] and [2], Parkinson disease (PD) [3], frontotemporal dementia (FTD) [4], and amyotrophic lateral sclerosis (ALS) [5]. Although it is still uncertain how the p.R47H substitution promotes neurodegeneration, it has been hypothesized that dysfunction of TREM2 can potentially impair its neuroprotective function in microglia and cause an inefficient cleanup of cellular debris [6]. These events would ultimately lead to malfunction and death of neurons resulting in the onset of neurological symptoms.

Essential tremor (ET [MIM 190300]) is characterized by postural and/or action 4–12 Hz tremor that mainly affects the arms and head. It is considered the most frequent adult-onset movement disorder, with an increased age-dependent prevalence of up to 21.7% in the very old (95 years old and over). ET was originally classified as a monosymptomatic non-degenerative movement disorder; however, over the last two decades, evidence in favor of a neurodegenerative origin in some ET cases has been accumulating. From a clinical point of view, some subjects with ET can develop worsening of tremor years after the disease onset without spontaneous remission. Moreover, some ET patients develop slight parkinsonian and cerebellar signs over time, suggesting additional involvement of the extrapyramidal and cerebellar systems [7]. Cognitive and neuropsychiatric disturbances have also been reported to be associated with ET, and an increased risk of developing dementia among older subjects with ET has been described [8]. In fact, the association between ET and late-onset PD has been consistently reported, and this holds to a lesser extent with AD as well [7], [9] and [10].

The main neuropathological findings, although still surrounded by some controversy, also support the notion of a neurodegenerative etiology for ET. In brains from ET patients the following findings have been described: cerebellar atrophy with Purkinje cell loss, depletion of pigmented neurons in locus coeruleus and brainstem pathology with loss of brainstem neurons, which directly synapse with Purkinje cells. In addition, the presence of Lewy bodies in the brainstem has been reported in 24% of ET cases, suggesting that abnormal protein processing could play a role in ET, as suggested for PD and AD [11].

Together, these data reinforce a neurodegenerative hypothesis, and support the notion of a common shared etiology with AD and PD, for at least a proportion of ET subjects. These links may be explained by shared genetic predisposing factors. As TREM2 p.R47H variant has been recently proposed as a “global” risk factor for neurodegeneration, we investigated whether p.R47H is also a risk factor for ET.

Subjects and methods

A Spanish case-control cohort made up of 456 patients with ET and 2715 healthy subjects was initially studied. ET patients were specifically recruited through a multicenter collaborative effort across the country. The minor allele frequency and demographic

characteristics for controls were obtained from in-house data ($n = 546$) and previously published series [$n = 2169$; Table 1[2] and [3]].

In a replication phase, an international multicenter case-control cohort consisting of 897 patients with ET and 1449 healthy subjects was analyzed. Its geographical origin was as follows: 275 patients and 299 controls from Germany, 257 American patients and 693 controls, 265 patients and 357 controls from Italy; and finally, 100 patients and 100 controls from Taiwan. Finally in a third step, an additional control group of 14777 individuals from European (Spanish, Italian, German), North American or Chinese populations was obtained from published data (Table 1 and Table 2), to increase the power of the replication analysis, owing to the rarity of the variant.

All patients were diagnosed with either “definite” or “probable” ET by a movement disorder specialist according to the current research criteria [12]. In subjects with familial ET, only one affected subject from each family was included. Prior to their participation, written informed consent was obtained from all individuals. The study was approved by the respective local ethics committees.

Genomic DNA was extracted from peripheral blood using standard methods. TREM2 p.R47H (rs75932628) variant was genotyped using KASPar® or TaqMan® allele-specific polymerase chain reaction custom assays ($n = 546$ and $n = 2809$, respectively) and analyzed using allelic discrimination software (7300 System SDS 1.3.1.21 Applied Biosystems Software). The genotype call rate was 99.7% in cases ($n = 1349$) and 99.15% in controls ($n = 1989$). The p.R47H carriers genotyped by KASPar® SNP (KBioscience) assay were validated by using BigDye® Terminator v3.1 Cycle Sequencing Kit (InvitrogenLife). In the case of p.R47H carriers genotyped by TaqMan® SNP (Life Technologies) a positive (heterozygous) and a negative control (homozygous non-mutated) were included in all genotyping plates.

The association between TREM2 p.R47H and ET condition was analyzed using odds ratios (OR) and the Fisher exact test based on allelic frequencies. In the first stage, the comparison was carried out in the Spanish cohort. In a second phase, we examined the replication in the international multicenter case-control cohort.

In a third stage, the additional control group from published data was taken into account to increase the power of the analysis. All p-values were two-tailed and significance threshold was set at $p < 0.05$, without correction for multiple hypotheses. All analyses were carried out using SPSS 15.0 software for Windows (SPSS Inc., Chicago, Illinois, USA).

Statistical power was calculated by using the “Genetic Power Calculator” (<http://pngu.mgh.harvard.edu/~purcell/gpc/>) [13] considering an allelic OR of 2.5 [the lowest 95% confidence OR bound obtained in the original association of TREM2 p.R47H variant with AD [14]], a minor allele frequency (MAF) of 0.002 (the MAF available in 1000genomes dataset), $\alpha = 0.05$ and a disease prevalence of 4% [15]. For the Spanish dataset, the power to detect association was 44% and considering all series grouped, the power was >90% (Supplementary Table 1).

Results

The Spanish cohort included 339 familial and 117 sporadic ET patients. Up to 48% of cases and 58% of the controls were women. The mean age at onset was 49.86 ± 20.11 (4–89) (mean $\pm$ standard deviation [range]) years. The mean age of the patients was 66.11 ± 15.97 (16–98) years. Controls had an average age at the last assessment of 73.51 ± 10.35 (30–99) years (Table 1). In the Spanish series, the missense variant p.R47H was identified in 3 of 456 (0.65%) patients, and 3 of 2715 controls (0.11%) showing a statistically significant difference between the number of p.R47H carriers in ET versus controls [odds ratio (OR), 5.97; 95% CI, 1.203–29.626; $p = 0.042$; Table 2].

In an attempt to replicate the association between p.R47H and ET, we also genotyped this variant in 4 additional patient-control series from different populations. Demographic characteristics are described in Table 1. In these series p.R47H (rs75932628) was present in 3 of 897 patients (0.33%; 1 patient from Germany and 2 from North America) and 7 out of 1449 controls (0.48%; 3 Italian patients and 4 from North America). This analysis showed no statistically significant association between p.R47H and ET in any of the replication cohorts (Table 2).

In addition, considering previously published data of healthy subjects from the same geographical regions and ancestral origin, we found no significant association between p.R47H (rs75932628) and ET in any of the replication cohorts (Supplementary Table 2). In fact, the analysis of all (Spanish and non-Spanish) series showed no statistically significant differences.

TREM2 p.R47H ET carriers' mean age at onset was 59.20 ± 30.74 (range: 5–81) years old compared with 51.95 ± 19.16 (range: 4–93) for non-carriers (Supplementary Table 3). No statistically significant differences in age at onset were found between p.R47H ET carriers and noncarriers. The p.R47H ET carriers were 33.4% female and 3 had family history of ET. A sister of one of the p.R47H carriers, who was also diagnosed with ET, did not carry the p.R47H variant. Since the p.R47H variant has been associated with other neurological disorders, we specifically reviewed the family history of PD, AD and ALS among the p.R47H variant carriers. Data regarding family history of dementia and/or PD were available for five of the six p.R47H allele carriers. Family history of PD was not reported among any of the carriers. Two of them (40%) reported a family history of dementia. A brother of one of the p.R47H ET carriers was diagnosed with vascular dementia but was found not to carry the p.R47H substitution.

TREM2 p.R47H healthy allele carriers had a mean age of 67.4 ± 9.96 years (range: 47–84). Among these, five (50%) were female. None of them had family history of dementia, ET or PD.

Conclusions

As far as we know this is the first study assessing the role of the rare genetic variant TREM2 p.R47H in ET. Our findings suggest that p.R47H (rs75932628) may be associated with ET in the Spanish population. However, this association was not confirmed in the other

populations considered in this study. This discrepancy could be attributed to an undetected population stratification in the Spanish cohort, which unfortunately could not be ruled out since we do not have additional genetic data. In order to detect subpopulation differences, allele frequency analysis of p.R47H of the Spanish sample according to its geographical origin (Southern and Northern Spain) showed no significant differences suggesting the presence of an homogeneous distribution the p.R47H allele frequency across the country (Supplementary Table 5). The analysis of further independent ET samples from Spain would be very helpful to elucidate the role of p.R47H in ET risk.

The lack of replication in the non-Spanish series may be explained, at least partially, by the very low MAF of the p.R47H allele in other populations. Thus, genotyping of larger samples is needed to increase the statistical power in order to elucidate the association of TREM2 p.R47H with ET (Supplementary Table 1).

To interpret our results correctly, we have to consider that p.R47H MAF varies worldwide across different populations from a MAF of 0% in China to a MAF of 0.63% in Iceland, a variation which was also observed in our study (Table 2; Supplementary Table 3). This variability of TREM2 p.R47H frequency observed worldwide suggests that not only large case-control association studies, but also ethnically-matched case-control cohorts, need to be analyzed to understand the clinical relevance of this factor in different populations.

Another point to consider is that the TREM2 p.R47H allele frequency also varies across the different neurodegenerative diseases for which a significant association has been reported (Supplementary Table 4). Indeed, the association between p.R47H allele and AD has not been replicated in Italian or Chinese populations to date (Supplementary Table 4), as was also the case in the present study, probably owing to the p.R47H low allelic frequency in such populations. In the case of the German population, Jonsson et al. described a significant association, although they used an uncorrected p-value of 0.04 [1]. The inconsistent results concerning p.R47H allelic frequency across populations has also been reported for FTD [4], for which p.R47H association with FTD risk has only been described in North Americans (Supplementary Table 4), although an association with FTD was reported by Borroni and colleagues when considering all TREM2 exon 2 rare variants found (Supplementary Table 4).

Given that ET has a high prevalence worldwide (about 0.9%), genetic risk factors other than p.R47H are probably involved. Even assuming an OR near 6 for p.R47H allele in the Spanish population, this would still mean that it has a relatively small effect on the risk of ET, given the very low prevalence of the variant (Table 2). Therefore, our study does not rule out the presence of other rare high-risk variants for ET in TREM2. Indeed, in African American AD patients, the strongest TREM2 single-marker association in a genome-wide meta-analysis was located at a different marker rs7748513 ($p < 0.001$) [16]. Thus, future sequencing of TREM2 gene in ET case-control series may be useful to assess the role of TREM2 risk variants in ET. Such analyses would help to clarify whether TREM2 dysfunction could play a role in ET and might be partially responsible for the shared etiology with other neurological conditions such as AD or PD [9].

Another hypothesis could be that p.R47H ET carriers are at higher risk of developing other neurodegenerative diseases such as PD and/or AD, due to the co-occurrence described between ET and these two diseases. However, to confirm this hypothesis it would be necessary to perform longitudinal studies of the p.R47H ET allele carriers. Another option would be to perform retrospective studies of the PD and AD p.R47H carriers to see if they were initially diagnosed with ET.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- This is the first study assessing the role of TREM2 p.R47H variant in ET.
- The total case-control cohort was composed of 1353 ET and 5168 healthy controls.
- Our sample included cases and healthy controls from Spain, Italy, Germany, America and Taiwan.
- There was a significant association between TREM2 p.R47H and the risk for ET in the Spanish cohort (OR 5.97; $p = 0.042$).
- This association was not confirmed in other populations.

Table 1

Clinical-epidemiological data of the ET-control series analyzed.

	Spain[2,3]	Italy	Germany	Taiwan	America	Total
Controls						
N	2715	357	299	100	697	4168
Female, N. (%)	1595 (58.8)	153 (42.9)	121 (40.5)	50 (50)	412 (58.9)	2331 (55.9)
Current age, y	73.51	68.18	66.36 (5.45)	64.96	72.87	72.22
Mean (SD)	(10.35)	(7.69)	[57–85]	(10.55)	(13.24)	(11.32)
[Range]	[30–99] ^a	[35–86]		[41–90]	[30–98]	[30–99]
Cases^b						
N	456	265	275	100	257	1353
Gender, N. (%)	216 (48.3)	106 (40)	112 (40.7)	50 (50)	137 (53.3)	621 (46.2)
Age at onset, y	49.86	55.49	48.33	54.70	50.66	51.44
Mean (SD)	(20.11)	(15.80)	(20.01)	(17.29)	(19.98)	(19.15)
[Range]	[4–89]	[7–80]	[4–83]	[7–86]	[5–88]	[4–89]
Current age, y	66.11	67.44	67.94	64.96	73.42	68.43
Mean (SD)	(15.97)	(10.46)	(14.06)	(12.56)	(11.73)	(13.82)
[Range]	[16–98]	[25–95]	[21–96]	[20–87]	[35–98]	[16–98]

N: number of individuals; SD: standard deviation.

^aRange data only available from in-house genotyping data [3].^bET cases have been defined according to the current diagnostic criteria published by Deuschl et al. [12].

Table 2
Association between the TREM2 p.R47H variant and ET frequency in the present study.

Cohort	No. of participants	No. of p.R47H carriers (%)	MAF, %	OR (95% CI)	P-value ^a
Spain					
Controls	2715	3 (0.11)	0.05	5.970 (1.203–29.626)	0.042
Cases	456	3 (0.65)	0.33		
Germany					
Controls	299	0 (0)	0.00	1.002 (0.998–1.005)	0.479
Cases	275	1 (0.36)	0.18		
Italy					
Controls	357	3 (0.84)	0.42	0.996 (0.991–1.001)	0.266
Cases	265	0 (0)	0.00		
North-America					
Controls	693	4 (0.57)	0.29	1.350 (0.246–7.391)	0.664
Cases	257	2 (0.77)	0.39		
Taiwan					
Controls	100	0 (0)	0.00	N.a.	N.a.
Cases	100	0 (0)	0.00		

ET: essential tremor; MAF, minor allele frequency.
^aUncorrected p-values are shown from Fisher exact test.