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TREM2 is associated with risk of Alzheimer disease in Spanish population

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Abstract

Two recent studies have reported the association of rs75932628-T in the *TREM2* gene with risk for Alzheimer's disease (AD). Rs75932628-T is a rare non-synonymous variant (p. R47H) that confers a high risk of AD with an effect size similar to that of the *APOE* ε4 allele. However, this association has not been replicated in any independent studies to date. The allelic frequency of rs75932628 varies according to the population from 0.02% to 0.63% among healthy controls. In an attempt to replicate the association between rs75932628-T and AD risk, we genotyped rs75932628 in a cohort of 504 AD subjects and 550 healthy controls from a Spanish population. Rs75932628-T showed a minor allele frequency (MAF) of 0.3% among this cohort. Interestingly, in our study rs75932628-T was found exclusively in 1.4% of AD cases (7/504), including four EOAD cases, and none of the controls (n=0/550). Here, we report the first positive replication study in a Spanish population and confirm that *TREM2* rs75932628-T is associated with risk for AD.

1. Introduction

Homozygous loss-of-function mutations in the triggering receptor expressed on myeloid cells 2 protein (*TREM2,* MIM 605086) were initially associated with an autosomal recessive form of early-onset dementia, Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; MIM 221770), from Swedish and Norwegian families (Paloneva, et al., 2003). Subsequently, mutations in the *TREM2* gene were found worldwide in PLOSL patients from different countries and ethnic origins (Klunemann, et al., 2005, Numasawa, et al., 2011, Paloneva, et al., 2003, Soragna, et al., 2003). Interestingly, PLOSL

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patients carrying *TREM2* mutations exhibit a similar clinical phenotype with respect to the neurologic and skeletal abnormalities (Klunemann, et al., 2005, Numasawa, et al., 2011, Paloneva, et al., 2003, Soragna, et al., 2003). However, the clinical spectrum associated with *TREM2* mutations was broadened after the identification of three siblings from a Lebanese family carrying the *TREM2* homozygous (c.40+3delAGG) mutation and exhibiting early-onset dementia without skeletal symptoms (bone cysts) (Chouery, et al., 2008). Moreover, mutations in *TREM2* (homozygous nonsense mutation p.Q33*) were also found in three Turkish probands with frontotemporal-like dementia without any bone-associated symptoms (R.J. Guerreiro, et al., 2012). Interestingly, patients carrying the same *TREM2* mutation (p.Q33*) but from different genetic backgrounds, exhibit different clinical phenotypes. These results suggest the presence of genetic/environmental modifiers of disease expressivity.

Previous efforts to link T*REM2* to AD or frontotemporal lobar degeneration (FTLD) in an Italian population did not identify allelic variants in the *TREM2* coding region in patients with AD (n=100) and FTLD (n=56), or in age-matched (n=80) and younger control subjects (n=60) (Fenoglio, et al., 2007). However, two independent studies recently reported that a non-synonymous variant in *TREM2*, rs75932628 (encoding R47H), is strongly associated with AD (R. Guerreiro, et al., 2012, Jonsson, et al., 2012). Rs75932628-T is a rare variant that expresses a different minor allele frequency (MAF) across healthy individuals from different populations. For example, in Iceland rs75932628 has a MAF of 0.63%, but in the USA the MAF is 0.12% (Jonsson, et al., 2012). In addition, it has been reported that the MAF of rs75932628-T among European or North American descent AD cases also varies between the European Alzheimer's Disease Initiative Consortium (EADI) (MAF=0.9%), the Genetic and Environmental Risk for Alzheimer's Disease Consortium (GERAD) (MAF=1%) and AddNeuroMed (ANM)(MAF=2%) (R. Guerreiro, et al., 2012). The exome variant server (EVS) database (http://evs.gs.washington.edu/EVS/) reports that rs75932628-T exhibits wide variability in frequency presenting much higher (MAF=0.26%) for European Americans (EA) than for African Americans (AA) (MAF= 0.02%). All together, these results suggest that the MAF of rs75932628 varies according to population.

In this study, we analyzed whether heterozygous variants in *TREM2* can increase the risk of AD in Spanish population.

2. Methods

2.1. Study subjects

DNA was collected from individuals of Spanish descent recruited between 2003 and 2012 from the Memory Disorders Unit, Department of Neurology, Clínica Universidad de Navarra, School of Medicine (Pamplona, Spain). The diagnosis of probable AD was made according to NINCDS-ADRDA criteria (McKhann, et al., 1984). Prior to their participation, a written informed consent was obtained from family members. The Institutional Review Board (IRB) at the University of Navarra, Pamplona, Spain approved the study. Demographic information for all 180 participants with early-onset AD (EOAD) has been published and each was screened for *amyloid-beta precursor protein (APP), presenilin (PSEN1), presenilin 2 (PSEN2), granulin* (*GRN*) and *microtubule-associated protein tau* (*MAPT*) genes (Jin, et al., 2012). Briefly, the cohort included 15 familial and 165 sporadic EOAD cases. EOAD cases were 38% male with an average age at onset (AAO) of 58.6 ± 3.2 (29–65). Additionally, 324 late-onset AD (LOAD) samples were included with 40% males and an AAO of 74.1 ± 5.3 (65–91). Controls (n=550) were 40% male, and the mean age at the latest assessment was 66.5 ± 11.5 (13–107) [mean ± SD (range)]

2.2. *TREM2* variants genotyping

The genotyping of rs104894002 (p. Q33*), rs142232675 (p. D87N), rs143332484 (p. R62H) and rs75932628 (p. R47H) was performed as previously published (Benitez, et al., 2011, Cruchaga, et al., 2012). Rs104894002, rs142232675 and rs143332484 were genotyped in EOAD cases (n=180) and all controls (n=550). Two different genotyping technologies were used to verify the results: MassARRAY SNP (Sequenom, Inc) and KASPar (KBioscience). MassARRAY is a PCR-based system wherein different size products are analyzed by SEQUENOM MALDI-TOF mass spectrometry. The KBioscience Competitive Allele-Specific PCR genotyping system (KASPar) is a FRET-based endpoint-genotyping technology, v4.0 SNP. The concordance rate between them was 99% andthe percentage of SNPs with genotype call rates was >98%.

2.3. Statistics

Fisher's exact test was used to compare the distribution of allelic and genotype frequency in case control series. All statistics were 2-tailed and significance was set at $p < 0.05$.

3. Results

The p. R47H variant was directly genotyped in 180 patients with EOAD, 324 LOAD participants and in 550 controls. From a total of 1,054 individuals, rs75932628-T exhibits a MAF=0.3%. Rs75932628-T was found in 1.4% (7/504) of AD cases and in none of the controls (0/550) (p=0.009). Four p. R47H carriers were EOAD patients (AAO mean=57.2 ± 1.3 years) and three were LOAD patients (AAO mean=74 ± 3.5 years). None of EOAD and p. R47H carriers have pathogenic mutations in *APP, PSEN1, PSEN2, MAPT* and *GRN* genes (Jin, et al., 2012) Among the carriers, four are females and three are males. Three were *APOE* ε3/ε4 positive and four were *APOE* ε3/ε3. However, due to the sample size we could not test the interaction with *APOE*. Three EOAD also reported a family history of AD-type dementia but there was not DNA available from relatives to perform a segregation analysis. Rs104894002 (p.Q33*) was not found in any screened individual (n=180 cases and 550 controls). Rs142232675 (p D87N) was found in one healthy 77 year old control (n=550) but none of the EOAD cases (n=180). Rs143332484 was found in 2.7% of the EOAD cases (6/180) and 2.5% of the controls (14/550), but its frequency did not reach statistical significance (p=0.6). This is the first replication study of the AD risk associated with *TREM2* in Spanish population.

4. Discussion

Rs75932628-T is a rare non-synonymous variant that confers a risk of AD with an effect size that is similar to that of the *APOE* ε4 allele (R. Guerreiro, et al., 2012, Jonsson, et al., 2012). To date, these findings have not been independently replicated in populations beyond those already reported (R. Guerreiro, et al., 2012, Jonsson, et al., 2012). Our replication study confirmed the association of rs75932628-T with AD in a group of Spanish AD patients, validating recent reports from independent cohort studies (R. Guerreiro, et al., 2012, Jonsson, et al., 2012). Across these studies, rs75932628-T has shown different MAF among controls and AD patients. Here, we report a global allelic frequency of 0.3% for rs75932628-T. We found that all 7 carriers were AD patients, and we identify no carriers among 550 healthy individuals from the same genetic background. These findings show that the frequency of rs75932628-T is lower in the Spanish population than previously reported among other European populations (R. Guerreiro, et al., 2012, Jonsson, et al., 2012). It has also been reported that *TREM* p.Q33X carriers show early onset dementia with clinical FTLD symptoms (R.J. Guerreiro, et al., 2012). Here, we did not find any p.Q33x carriers in our EOAD cases. p.R47H reduces the AAO of LOAD by an average 3.18 years in carriers compared to noncarriers (Jonsson, et al., 2012). Here, we report that the p. R47H variant is

present not only in LOAD (n=3) but also in EOAD (n=4). To date, this is the first report of EOAD carrying p.R47H. All EOAD carriers matched the diagnosis of probable AD according to standard clinical research criteria (McKhann, et al., 1984). Due to this small proportion of cases, it is not possible perform analysis on the effect on AAO. These findings suggest that *TREM2* variants can also be a risk factor for EOAD.

This is the first positive replication study in a Spanish population and confirms that *TREM2* rs75932628-T is associated with risk for AD.

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