Assessing the role of the *TREM2* p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia

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ABSTRACT

A non-synonymous genetic rare variant, rs75932628-T (p.R47H), in the *TREM2* gene has recently been reported to be a strong genetic risk factor for Alzheimer's disease (AD). Also, rare recessive mutations have been associated with frontotemporal dementia (FTD). We aimed to investigate the role of p.R47H variant in AD and FTD through a multi-center study comprising 3,172 AD and 682 FTD patients and 2,169 healthy controls from Spain. We found that 0.6% of AD cases carried this variant compared to 0.1% of controls (odds ratio [OR]=4.12, 95% confidence interval [CI]: 1.21-14.00, *P*=0.014). A meta-analysis comprising 32,598 subjects from four previous studies demonstrated the large effect of the p.R47H variant in AD risk (OR=4.11, 95% CI: 2.99-5.68, $P=5.27 \times 10^{-18}$). We did not find an association between p.R47H and age of onset of AD or family history of dementia. Finally, none of the FTD patients harbored this genetic variant. These data strongly support the important role of p.R47H in AD risk and suggest that this rare genetic variant is not related to FTD.

Key Words: Alzheimer's disease; Frontotemporal dementia; TREM2; Genetic association; p.R47H; Rare variant

1. Introduction

The rapid population growth in recent centuries and the evolutionary forces that shape allelic variation over time have led to a large number of rare genetic variants in the human genome, and they seem to vastly exceed the number of common alleles (Tennessen et al. 2012). A substantial fraction of these rare variants may have functional consequences and therefore can contribute to the allelic architecture of complex diseases (Pritchard, 2001). A very recent example of a rare genetic variant with large individual effect is the non-synonymous amino acid substitution p.R47H (rs75932628) in the gene encoding the triggering receptor expressed on myeloid cells 2 (*TREM2*) and its association with the risk for sporadic Alzheimer's disease (AD) (Guerreiro et al. 2013; Jonsson et al. 2013). This association has also been replicated in a cohort of French patients with an early-onset form of AD (EOAD, age of onset ≤65 years) (Pottier et al. 2013), and in a Spanish study with a series of patients that comprised both late and EOAD (Benitez et al. 2013). Interestingly, homozygous mutations in *TREM2* have been described in patients with an unusual early-onset frontotemporal dementia (FTD) from consanguineous families living in the Anatolian region of Turkey (Guerreiro et al. 2013) and in a Colombian family (Giraldo et al. 2013), thus broadening the clinical heterogeneity spectrum caused by mutations in this particular locus. In addition, homozygous mutations in *TREM2* are also the cause of Nasu-Hakola disease, which is mainly characterized by pre-senile FTD and cystic bone lesions (Paloneva et al. 2002).

Since (i) the *TREM2* p.R47H variant has been related to the broad spectrum of AD, including EOAD, (ii) no specific assessment of this variant has been performed in FTD, and (iii) allele frequency of the p.R47H varies across populations, we aimed to evaluate how this non-synonymous amino acid change contributes to the risk of early-

and late-onset forms of AD or FTD by studying a large cohort of patients and controls from Spanish origin.

2. Methods

2.1. Study subjects

A total of 3,172 AD patients (mean age at onset 74.8 ± 9.9 years, 68.5% women), 682 FTD patients (mean age at onset 64.8 ± 9.9 years, 46.8% women), and 2,169 healthy controls (mean age at clinical assessment 75.4 ± 10.3 years, 58.2% women) were collected through a collaborative effort involving 11 specialized centers across the country. All individuals were Spanish and of European origin. Among AD patients, 466 were classified as early-onset cases (EOAD) (cut-off age of onset being 65 years). Patients were diagnosed using established clinical criteria for AD (McKhann et al. 1984) or FTD (Neary et al. 1998). All participants or their families provided written informed consent, and the study was approved by the respective ethics committees.

2.2. Genotyping

Genotyping of the rs75932628 (p.R47H) variant was performed using three approaches: TaqMan SNP Genotyping Assays (Applied Biosystems, Foster city, California), High Resolution Melting (Eco-PCR, Illumina, San Diego, California), and KASPar (KBioscience, Berlin). A human DNA sample carrying a T-allele (rs75932628-T) in heterozygous state was distributed to all genotyping centers and was included as a positive control in all genotyping plates.

2.3. Statistical analysis

Genotype frequency comparisons were performed by Fisher's exact test. Differences between groups were analyzed by Student's t-test for continuous data. Multiple logistic regression models were used to adjust for covariates, such as age, gender and *APOE*-ε4 status. Data were analyzed using the Statistical Package for the Social Sciences, version 19.0.0 (SPSS Inc., Chicago, IL). Genetic interactions (epistasis) were assessed by the synergy factor analysis (Cortina-Borja et al. 2009). Meta-analysis was conducted using the inverse variance method (fixed effects model) in Episheet excel application according to Fleiss (Fleiss 1993). Fixed and random effects meta-analyses were automatically generated by Episheet. The weighting of each study was calculated using the estimated standard errors. The bona fide genome-wide significant *P*-value threshold was established at $P<5x10^{-8}$ (de Bakker et al. 2008). Final meta-analysis results and Forest plot for TREM2 p.R47H showing association results in all available data were derived using OpenMeta (Wallace et al. 2009). Power calculations were performed with the PS software (Version 2.1.30).

3. Results

A total of 6,023 individuals were genotyped for the p.R47H polymorphism, and distribution of genotype frequencies across phenotypes is presented in Table 1. The T allele at the rs75932628 biallelic polymorphism was carried by 0.6% of AD cases and 0.1% of controls (*P*=0.014), and yielded an odds ratio (OR) of 4.12 [95% confidence interval [CI]: 1.21-14.00], almost identical to the risk conferred by the *APOE*-ε4 allele (4.2, Table 1). Although not statistically significant, age at onset of AD was slightly lower in rs75932628-T carriers than noncarriers (70±6.0 and 74.8±9.9 years, respectively, *P*=0.08). To effectively detect whether the association between

rs75932628-T variant and AD was modified by age, the sample was stratified into EOAD cases (age of onset ≤65 years old, n=466) and late-onset cases (>65 years old, n=2,375). The rs75932628-T association was replicated within the late-onset AD series (OR=4.28; 95% CI: 1.23-14.92, *P*=0.014). However, no significant association was found for the EOAD series (OR=3.12; 95% CI: 0.52-18.68, *P*=0.217). Data regarding family history of dementia were available for 14/18 AD patients carrying the rs75932628-T allele. Of these patients, only five (35.7%) reported a positive family history of dementia. This percentage was almost identical to the prevalence of family history of dementia in rs75932628-T noncarriers (35.3%, *P*=0.974). To further address the association between the p.R47H variant and the risk of AD, and to compare the observed effect size of p.R47H among different populations, we conducted a meta-analysis using our data and the available data from the literature (Benitez et al. 2013; Guerreiro et al. 2013; Jonsson et al. 2013; Pottier et al. 2013). In total, we analyzed the genotypes of 32,598 subjects comprising 12,967 patients and 19,631 controls. The summary OR under a fixed effect model showed that individuals with the p.R47H variant were 4.11 times more likely to develop AD than noncarriers (95% CI: 2.99-5.68, $P=5.27\text{x}10^{-18}$, Figure 1). No evidence of heterogeneity between studies was detected (Q=2.17, *P*>0.98 with nine degrees of freedom).

We next assessed the effect of the *APOE*- ε4 allele on the association between rs75932628-T and the risk of AD. The risk of the p.R47H variant was no longer significant after adjustment for the *APOE*- ε4 allele (*P*=0.34). Since there were significant differences between the frequency of rs75932628-T in carriers of the *APOE*- ε4 allele compared to noncarriers (*P*=0.007), we then evaluated whether the *TREM2* association was indeed confounded by the presence of *APOE*- ε4 in rs75932628-T carriers through a meta-analysis that included two previous studies with six populations

(Jonsson et al. 2013; Pottier et al. 2013). This analysis showed no difference in the frequency of rs75932628-T carriers according to *APOE*- ε4 status (*P*=0.28, Suppl. Fig. 1). No significant interaction between *APOE*- ε4 and rs75932628-T resulted from the logistic-regression model (*P*=0.577). Evaluation of this interaction by the synergy factor (*SF*) analysis, which measures both the size and significance of genetic interactions (Cortina-Borja et al. 2009), corroborated the lack of synergy between *APOE*- ε4 and rs75932628-T (*SF*=1.31, *P*=0.43).

The study of the p.R47H variant in the FTD group (n=682) did not disclose any carrier of this genetic variant.

4. Discussion

The role of rare genetic variants in common disorders has been fueled in the last few years by the appearance of next-generation sequencing technologies. Two independent works have recently demonstrated the power of these techniques. After a large scale analysis of sequencing data, they identified the rare p.R47H substitution as a major genetic risk factor for AD (Guerreiro et al. 2013; Jonsson et al. 2013). In the present work we were able to replicate this finding by analyzing a large case-control cohort comprising more than 6,000 individuals. The effect size of the *TREM2* rs75932628-T rare variant was consistent with those reported previously (Guerreiro et al. 2013; Jonsson et al. 2013; Benitez et al. 2013), and reached the same magnitude as the *APOE*-ε4 allele, the most important genetic risk factor for AD. Our meta-analysis comprising > 32,000 subjects strongly supports the important role of the p.R47H non-synonymous variant in AD risk.

We were not able to replicate the recently reported association of rs75932628-T and EOAD risk in a French population (Pottier et al. 2013), despite the frequency of

EOAD patients carrying the p.R47H variant being slightly higher than controls (0.4% and 0.1%, respectively). A possible explanation for this lack of replication is that the frequency of the rs75932628-T variant in the Spanish population is much lower than that of the French population (0.14% and 0.5%, respectively in controls, 0.4% and 2.0% in patients). Therefore, even though our sample size was almost double that of Pottier et al., the much lower frequency of the rs75932628-T in our population could have led to an insufficient statistical power. The present study had a power of 46% (alpha=0.05) to detect a risk equal to that reported in the French analysis. It is important to note, however, that patients carrying the rs75932628-T variant had a slightly, although not significantly, earlier age of onset, thus raising the question as to whether this genetic variant could modulate the age of AD onset.

We did not find any interaction between the ε4 allele of the *APOE* gene and the p.R47H variant. Nevertheless, the *APOE*-ε4 adjusted risk of the rs75932628-T variant resulted in a loss of statistical significance. The high prevalence of the APOE-E4 isoform in AD patients, in conjunction with its prominent effect on AD risk may explain this somewhat unexpected outcome. However, our meta-analysis to evaluate the effect of *APOE*- ε4 on the association of rs75932628-T with AD strongly indicates the independence of both genes in the risk of AD. Therefore, co-occurrence of both alleles (rs75932628-T and *APOE*- ε4) in our AD series was not confirmed in other populations and might be due to random effects.

As far as we know, this is the first study to assess the role of the p.R47H variant in FTD patients. Our results strongly suggest that being a heterozygous carrier of this variant does not contribute to the risk of common forms of FTD and emphasize the clinical heterogeneity associated with *TREM2.* In-depth analysis of this locus in FTD is warranted.

Disclosure statement

The authors declare no potential conflicts of interest.

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Figure legends

Figure 1:

Forest plot with the rs7593268-T allelic odds ratios from published studies and overall odds ratio.

Suppl. Figure 1:

Forest plot showing the effect of the *APOE*-ε4 allele on the association of p.R47H variant with Alzheimer's disease in different populations and overall OR comparing *APOE*- ε 4 carriers and non-carriers.

Table 1. Frequency of *TREM2* p.R47H and *APOE-ε4* carriers across phenotypes.

	TREM2 p.R47H N (frequency %)	*APOE-ε4* N (frequency %)
Controls	3 (0.14)	257 (16.1)
AD	18 (0.57)	1,264 (44.8)
FTD	-	64 (24.5)
OR [95%CI] (AD vs C)	4.12 [1.21-14.00], $P = 0.014$	4.23 [3.61-4.93], $P = 6.9 \times 10^{-83}$
OR [95%CI] (FTD vs C)	-, $P = 1$	1.69 [1.24-2.31], $P = 0.001$