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Missense variant in *TREML2* protects against Alzheimer's disease

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[2] Data used in the preparation of this article were obtained from the Genetic and Environmental Risk for Alzheimer's disease (GERAD1) Consortium. As such, the investigators within the GERAD1 consortia contributed to the design and implementation of GERAD1 and/or provided data but did not participate in analysis or writing of this report. A full list of GERAD1 investigators shall be included in either supplementary content or acknowledgements.

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ABSTRACT

TREM and TREM-like receptors are a structurally similar protein family encoded by genes clustered on chromosome 6p21.11. Recent studies have identified a rare coding variant (p.R47H) in *TREM2* that confers a high risk for Alzheimer's disease (AD). In addition, common single nucleotide polymorphisms in this genomic region are associated with cerebrospinal fluid biomarkers for AD and a common intergenic variant found near the *TREML2* gene has been identified to be protective for AD. However, little is known about the functional variant underlying the latter association or its relationship with the p.R47H. Here, we report comprehensive analyses using whole-exome sequencing data, cerebrospinal fluid biomarker analyses, meta-analyses (16,254 cases and 20,052 controls) and cell-based functional studies to support the role of the *TREML2* coding missense variant p.S144G (rs3747742) as a potential driver of the meta-analysis AD-associated genome-wide association studies signal. Additionally, we demonstrate that the protective role of *TREML2* in AD is independent of the role of *TREM2* gene as a risk factor for AD.

1. Introduction

Genome-wide association studies (GWAS) are a very powerful approach for identification of novel loci associated with disease status or other complex traits. However, these single nucleotide polymorphisms (SNPs) are usually not the functional variants driving the association and, in many cases, regional linkage disequilibrium (LD) prevents identification of a single candidate gene in the region. Often, additional studies are required to demonstrate unambiguously that the gene and/or variant implicated in disease risk is functionally related to pathogenesis.

Recently, the International Genomics of Alzheimer's Project (IGAP) identified 11 new loci ($p < 10^{-8}$) associated with risk for Alzheimer's disease (AD), and 13 additional suggestive loci (p value between10^{-6} and 10^{-8}) (Lambert et al., 2013). Among the latter group, there is an inter-genic SNP (rs9381040; $p < 6.3 \times 10^{-7}$) located 5.5 Kb downstream from *TREML2* and 24 Kb upstream from *TREM2*. The *TREM* and *TREM-like receptor* genes clustered on chromosome 6p21.1 (Ford and McVicar, 2009) have different patterns of LD among them (Cruchaga et al., 2013). This genomic region has previously been implicated in genetic risk for AD (Benitez et al., 2013; Bertram et al., 2013; Cruchaga et al., 2013; Guerreiro et al., 2013; Jonsson et al., 2012; Reitz and Mayeux, 2013). A low frequency missense variant in *TREM2* (p.R47H, minor allele frequency = 0.003) was reported to substantially increase risk for AD (Benitez et al., 2013; Guerreiro et al., 2013). SNPs in this region were also found to be associated with a cerebrospinal fluid (CSF) biomarker for AD (phospho-tau$_{181}$ levels) (Cruchaga et al., 2013). Because of the design of the IGAP study (a meta-analysis) and the low frequency of the *TREM2* variant, it was not possible to determine whether the GWAS signal of this variant (rs9381040) was independent of the *TREM2*-p.R47H variant. In this study, we used exome-sequencing data to identify the most likely functional variant in *TREML2* responsible for the GWAS signal and to determine whether this signal is independent of *TREM2*-p.R47H (rs75932628) variant.

2. Methods

2.1. Exome sequencing Knight-Alzheimer's Disease Research Center (ADRC)

Enrichment of coding exons and flanking intronic regions was performed using a solution hybrid selection method with the SureSelect human all exon 50 Mb kit (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's standard protocol on 46 unrelated AD cases and 39 unrelated controls from the Knight-ADRC. This was performed by the Genome Technology Access Center at Washington University in St Louis (https://gtac.wustl.edu/). The captured DNA was sequenced by paired-end reads on the HiSeq 2000 sequencer (Illumina, San Diego, CA, USA). Raw sequence reads were aligned to the reference genome National Center for Biotechnology Information (NCBI) 36/hg18 by using Novoalign (Novocraft Technologies, Selangor, Malaysia). Base and/or SNP calling was performed using SNP SAMtools (Li et al., 2009). SNP annotation was carried out using version 5.07 of SeattleSeq Annotation server (see URL) (Benitez et al., 2011). On average, 95% of the exome had fold coverage >8.

2.2. UK-National Institute on Aging (UK-NIA) Dataset

A description of the UK-NIA dataset can be found in Guerreiro et al. (2013). Briefly, this dataset includes whole-exome sequencing data from 143 AD cases and 183 controls (Table 1).

2.3. Alzheimer's disease genetic consortium methods

Data used in the preparation of this article were obtained from the Alzheimer's disease genetic consortium (ADGC). A description of the samples included in the study as well as the methods used can be found in Naj et al. (2011). Imputed data from 10,067 AD cases and 9606 controls from the ADGC were used in this study (Naj et al., 2011). Genome-wide imputation was performed per cohort using MACH software with HapMap phase 2 (release 22) CEPH Utah pedigrees reference haplotypes and genotype data passing quality control as inference. Imputation quality was determined as r^2 and only SNPs imputed with $r^2 \geq 0.50$ were included in the analysis. A multivariate logistic regression was performed to evaluate the association between genetic markers and risk for late-onset AD (LOAD) adjusting for age, gender, population substructure, and study-specific effects.

2.4. For use of genetic and environmental risk for Alzheimer's disease genotype data from "the 610 group"

Data used in the preparation of this article were obtained from the Genetic and Environmental Risk for Alzheimer's disease (GERAD) Consortium. The imputed GERAD sample comprised 3177 AD cases and 974 healthy elderly (age >70) control subjects with available age and gender data. Cases and elderly screened control subjects were recruited by the Medical Research Council (MRC) Genetic Resource for AD (Cardiff University; Institute of Psychiatry, London; Cambridge University; Trinity College Dublin), the Alzheimer's Research UK Collaboration (University of Nottingham; University of Manchester; University of Southampton; University of Bristol; Queen's University