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Investigation of *TREM2* variant in the Wisconsin Registry for Alzheimer's Prevention

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Abstract

Recent studies have found an association between a variant in *TREM2* (rs75932628-T) and both Alzheimer's disease (AD) and cognitive function in individuals age 80–100. The role of *TREM2* in younger, asymptomatic individuals is unknown. We examined this variant in 1,148 participants from the Wisconsin Registry for Alzheimer's Prevention, a longitudinal study of middle-aged adults enriched for a parental history of AD. Thirteen individuals carried the T risk allele. Carriers were more likely to have a parental history of AD (100% of carriers versus 70% of non-carriers; $p=0.01$) and, among the parental history subset, families with a *TREM2* carrier had a younger maternal age of AD onset than non-carriers (67.9 versus 75.6 years; $p=0.03$). There was no significant association between *TREM2* carrier status and cognitive function or decline. In conclusion, the association between *TREM2* and both parental history of AD and younger maternal age of AD onset provide additional support for the role of *TREM2* in AD and illustrate the importance of considering family history in AD study design.

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The data contained in this manuscript have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at *Neurobiology of Aging*.

This study was conducted with the approval of the University of Wisconsin Institutional Review Board and all subjects provided signed informed consent before participation. No animals were used in the study.

All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

Keywords

TREM2; family history; Alzheimer's disease; memory; cognition; longitudinal

1. Introduction

Currently, over 5 million Americans have Alzheimer's disease (AD) and that number is expected to increase to nearly 14 million by 2050 (Alzheimer's Association, 2013). AD is the sixth leading cause of death in the United States and the only of the top 10 causes of death with no way to prevent, cure, or impede its progression (Alzheimer's Association, 2013). The earliest symptoms of AD are declines in anterograde memory and executive function. Having a family history of AD is a well-established risk factor; however, confirmed genetic risk factors for AD, such as apolipoprotein E (*APOE*) ϵ 4 carrier status, do not fully account for the increased risk. Recently, two large consortia found an association between a low frequency variant in *TREM2* (triggering receptor expressed on myeloid cells 2) and AD in individuals of European descent (Guerreiro, et al., 2012, Jonsson, et al., 2012). The association has since been replicated in four additional case-control samples of European descent (Benitez, et al., 2013, Giraldo, et al., 2013, Gonzalez Murcia, et al., 2013, Pottier, et al., 2013). The minor (T) allele codes for an amino acid change from arginine to histidine and is associated with a 3- to 5-fold increase in the risk for AD. Moreover, Jonsson et al. (2012) found that elderly (age 80–100) carriers of this variant who were free from AD had poorer cognitive function than non-carriers. However, no published study has examined this association in middle-aged individuals. The primary purpose of this study was to examine the effect of the *TREM2* variant (rs75932628) on cognitive performance in a longitudinal study of middle-aged adults, enriched for a parental history of AD.

2. Methods

2.1. Study population

Study participants were from the Wisconsin Registry for Alzheimer's Prevention (WRAP), a longitudinal study of initially asymptomatic middle-aged adults enriched for a parental history of AD (i.e., a biological parent with either autopsy-confirmed or probable AD as defined by NINCDS-ADRDA research criteria (McKhann, et al., 1984)). Details of the study design and methods have been previously described (Engelman, et al., 2013, La Rue, et al., 2008, Sager, et al., 2005). Baseline recruitment began in 2001 and follow-up assessments are ongoing with approximately four years between the baseline and second visits and two years between subsequent visits. WRAP participants were included in the current analyses if they were self-reported non-Hispanic white and passed the genotyping quality control, and excluded if they reported neurological diseases or comorbidities that might be expected to influence cognitive test performance (e.g., multiple sclerosis, Parkinson's disease, stroke, epilepsy/seizures, or meningitis) or developed AD on or before the second visit. A total of 1,148 participants met these inclusion/exclusion criteria.

2.2. Neuropsychological assessment

The WRAP cognitive test battery and factor analysis have been previously described (Dowling, et al., 2010, Engelman, et al., 2013, Sager, et al., 2005). Cognitive factor scores for episodic memory (Verbal Learning and Memory and Immediate Memory) and executive function (Working Memory and Speed and Flexibility) obtained at up to four visits were examined in the current study (Supplementary Table 1). These domains were selected

because they are the first to show decline in the early stages of AD. Baseline full-scale intelligence quotient (FSIQ) was included for describing the sample.

2.3. Genotyping and quality assurance

Genotyping of the *TREM2* variant, rs75932628, and a panel of 100 validated European ancestry informative markers was performed by PreventionGenetics (Marshfield, WI) using small volume PCR reactions on ArrayTape™ technology (Douglas Scientific, Alexandria, MN) and the InvaderPlus® assay (Third Wave Technologies, Madison, WI). Duplicate quality control samples from 62 individuals were placed randomly throughout each of the 96-well plates. The genotype discordance rate was <0.1%. All discordant genotypes were set to missing. Genotype quality assurance checks were performed using the PLINK software v1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>) (Purcell, et al., 2007). Samples were excluded if they had call rates <80%, self-reported gender inconsistent with the gender marker (rs25601), or a missing *TREM2* genotype. The *TREM2* variant was coded as T allele carrier versus non-carrier in subsequent analyses because no individuals were homozygous for the T allele.

2.4. Statistical analysis

Sample characteristics of the *TREM2* carriers and non-carriers were compared using t-tests or Fisher's exact tests. In the subset of participants with a parental history of AD, maternal and paternal age of AD onset in the carrier and non-carrier groups were also compared via t-test. *TREM2* associations with cognitive performance and longitudinal change were tested for each of the cognitive factors using linear mixed models. The four cognitive factor scores were standardized ($\sim N[0, 1]$) continuous variables. In primary analyses, covariates included age, gender, and the top two principal components of ancestry (described in Engelman et al. (2013), as well as random effects to account for within subject correlations. For each cognitive factor, we tested for a *TREM2* association with longitudinal cognitive decline by including a *TREM2**age interaction term in the model; non-significant interactions were removed and the model rerun before examining *TREM2* main effects on cognition. Given the small number of *TREM2* carriers and the significant association between *TREM2* and *APOE* $\epsilon 4$ carrier status, *APOE* was included as a covariate in secondary analyses. Since all *TREM2* carriers had a parental history of AD, secondary analyses also included re-running primary analyses on the subset with a parental history of AD. All analyses were performed using SAS v9.3 and used an alpha of 0.05.

3. Results

Characteristics of the 1,148 participants, according to *TREM2* carrier status, are shown in Table 1. Thirteen individuals were carriers of the T risk allele. While the two groups did not differ in terms of baseline age, gender, or FSIQ, *TREM2* carrier status was significantly associated with a positive parental history of AD, *APOE* $\epsilon 4$ carrier status, and having less than a BA or BS degree. In the subset with a parental history of AD, families with a *TREM2* carrier also had a significantly lower maternal age of AD onset than non-carriers (67.9 years versus 75.6 years; $p=0.03$), but not a significantly different paternal age of AD onset ($p=0.20$).

In linear mixed models adjusting for age, gender, and the top two principal components of ancestry, there were no significant *TREM2* main effects or *TREM2**age interactions for any of the four cognitive factor scores. The results were consistent when *APOE* $\epsilon 4$ was included in the model as a covariate. The results were also consistent when the primary analyses were repeated in the AD parental history subset. In this subset, as in the entire sample, the strongest effect of *TREM2* on cognitive outcomes was seen for the Working Memory factor

score with adjusted mean Working Memory z-scores of -0.28 and 0.071 for *TREM2* carriers and non-carriers, respectively ($p=0.19$, Cohen's $d=0.31$). Given the small number of *TREM2* carriers, a post-hoc power analysis estimated that there would have to be at least 65 *TREM2* carriers, from a sample of nearly 6,000 people, to have adequate statistical power to detect such an effect size.

4. Discussion

Recent case-control studies have reported an association between a low frequency, nonsynonymous variant in *TREM2* (rs75932628) and AD. We examined this variant in middle-aged, asymptomatic individuals. Our study population was intentionally enriched for individuals with a parental history of AD. While the minor allele frequency (MAF) in the parental history positive participants was 0.008, the MAF in the participants without a parental history of AD was 0, illustrating the importance of considering family history in the study design. All of the individuals in our study who had a copy of the *TREM2* variant had a parent with AD, compared to only 70% in the *TREM2* non-carriers ($p=0.01$). Moreover, the maternal age of onset was almost eight years younger in *TREM2* carriers than in non-carriers ($p=0.03$). This younger maternal age of onset is in line with the younger age of onset seen with each increasing *APOE* $\epsilon 4$ allele (Slooter, et al., 1998, Thambisetty, et al., 2013). In addition to the risk for AD seen in previous studies (Benitez, et al., 2013, Giraldo, et al., 2013, Gonzalez Murcia, et al., 2013, Guerreiro, et al., 2012, Jonsson, et al., 2012, Pottier, et al., 2013), carriers of the *TREM2* variant may experience an earlier age of AD onset. The lack of association between *TREM2* carrier status and paternal age of AD onset was likely due to small sample size since only four *TREM2* carriers had a father with AD.

In our primary analyses, we did not find a significant association between *TREM2* carrier status and cognitive function or decline. However, we did see a modest effect of *TREM2* on Working Memory and Speed and Flexibility scores, with lower performance in the *TREM2* carriers (Cohen's $d=0.31$ and 0.29 , respectively). Both of these factors contain cognitive tests related to executive function. Our inability to detect a significant association between this confirmed AD risk variant and cognitive function or decline could be due to inadequate power with only 13 *TREM2* carriers. Alternately, it may be that our participants are too young to show significant cognitive decline related to *TREM2*. Our participants had a mean age of just over 50 at baseline and approximately 60 years at the fourth visit. Therefore, our participants are approaching, but have not yet reached, the age of AD onset of the parents of *TREM2* carriers from our study. WRAP visits are ongoing, with follow-up every two years. Analyses in WRAP over the next several years, as well as results from other longitudinal studies, will help clarify the role of *TREM2* in cognitive function and decline, and age of AD onset.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1Sample characteristics by *TREM2* carrier status (*N*=1,148).

Characteristic	Mean (SD) or <i>n</i> (%)		<i>p</i> -value ^{<i>a</i>}
	<i>TREM2</i> non-carrier (<i>n</i> =1,135)	<i>TREM2</i> carrier (<i>n</i> =13)	
Baseline age	53.7 (6.6)	52.0 (5.2)	0.37
Female	784 (69.1)	11 (84.6)	0.37
Education: BA/BS or higher	703 (61.9)	7 (53.9)	0.03
FSIQ ^{<i>b</i>}	113.4 (9.4)	109.5 (10.5)	0.13
<i>APOE</i> ε4 carrier	443 (39.0)	9 (69.2)	0.04
Parental history of AD	798 (70.4)	13 (100)	0.01

^{*a*} Fisher's exact test or t-test^{*b*} FSIQ, full-scale intelligence quotient