Neurobiology of Aging 36 (2015) 2443.e21–2443.e26

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

More evidence for association of a rare *TREM2* mutation (R47H) with Alzheimer's disease risk

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ARTICLE INFO

Article history:
Received 25 February 2015
Accepted 19 April 2015
Available online 25 April 2015

Keywords:
TREM2
Rare variants
LOAD

ABSTRACT

Over 20 risk loci have been identified for late-onset Alzheimer's disease (LOAD), most of which display relatively small effect sizes. Recently, a rare missense (R47H) variant, rs75932628 in *TREM2*, has been shown to mediate LOAD risk substantially in Icelandic and Caucasian populations. Here, we present more evidence for the association of the R47H with LOAD risk in a Caucasian population comprising 4567 LOAD cases and controls. Our results show that carriers of the R47H variant have a significantly increased risk for LOAD (odds ratio = 7.40, $p = 3.66E-06$). In addition to Alzheimer's disease risk, we also examined the association of R47H with Alzheimer's disease–related phenotypes, including age-at-onset, psychosis, and amyloid deposition but found no significant association. Our results corroborate those of other studies implicating *TREM2* as an LOAD risk locus and indicate the need to determine its biological role in the context of neurodegeneration.

1. Introduction

Late-onset Alzheimer's disease (LOAD) is a fatal neurodegenerative disorder affecting more than 5 million Americans (Alzheimers Association:http://www.alz.org/alzheimers_disease_facts_and_figures.asp#quickFacts, [accessed 12 Jan 2015]). Alzheimer's patients exhibit progressive deficits in cognitive function thought to be caused by the combination of abnormal aggregation of beta-amyloid (Aβ) and hyperphosphorylation of the microtubule-stabilizing tau protein in the brain. The full molecular mechanism of disease has yet to be determined. Previously, 21 risk loci—*APOE, CR1, BIN1, INPP5D, MEF2C, CD2AP, HLA-DRB1/HLA-DRB5, EPHA1, NME8, ZCWPW1, CLU, PTK2B, PICALM, SORL1, CELF1, MS4A4/MS4A6E, SLC24A4/RIN3, FERMT2, CD33, ABCA7*, and *CASS4* —have been identified for LOAD in large genome-wide association studies (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2013; Naj et al., 2011; Seshardi et al., 2010). These loci are varied in genomic location, biological function, and cellular localization and expression. Rare mutations affecting LOAD risk also have been identified with sequencing methods in the *APP* and *PLD3* genes (Bamne et al., 2014; Cruchaga et al., 2014; Jonsson et al., 2012; Kero et al., 2013).

Triggering receptor expressed on myeloid cells 2 (*TREM2*) is differentially expressed by microglia among different brain regions (Schmid et al., 2002). Two independent groups found a rare missense variant, rs75932628, in exon 2 of *TREM2* (Guerreiro et al., 2013a; Jonsson et al., 2013). This substitution of histidine for arginine at residue 47 (R47H) increases LOAD risk at a magnitude similar to that of *APOE*4*. Other groups have found similar associations in Caucasian populations from both Europe and North America (Benitez et al., 2013; Giraldo et al., 2013; Gonzalez Murcia et al., 2013; Roussos et al., 2014). This variant has also been associated with early-onset Alzheimer's disease in a case and/or control study of Caucasian individuals of French descent (Pottier et al., 2013). An association between R47H carriers with both history of parental LOAD and earlier maternal age-at-onset (AAO) in a cohort of middle-aged unaffected individuals has been described as well (Engelman et al., 2014).

In addition to its association with LOAD, variants in *TREM2* have been shown to cause autosomal recessive Nasu-Hakola disease (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy), a disease which counts among its features

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http://dx.doi.org/10.1016/j.neurobiolaging.2015.04.012

Table 1
Association of R47H variant with LOAD risk

Genotype				MAF	OR (95% CI)	*p*
	CC	CT	TT			
Controls	2919	8	0	0.0027	—	—
Cases	1584	29	0	0.018	7.40 (3.171–17.26)	3.66E-06
Total	4503	37	0	0.008		

Key: LOAD, late-onset Alzheimer's disease; MAF, minor allele frequency; OR, odds ratio.

early-onset neurodegeneration (Paloneva et al., 2002; Sorgana et al., 2003). Risk for other neurodegenerative diseases, including frontotemporal dementia (Cuyvers et al., 2014, Guerreiro et al., 2013b, 2013c; Rayaprolu, 2013), Parkinson's disease (Rayaprolu et al., 2013), and sporadic amyotrophic lateral sclerosis (Cady et al., 2014), also appears to be mediated by *TREM2* variation. Network analysis revealed connections of other known AD genes to *TREM2*, as well as genes with known functions in other neurological diseases, suggesting a microglial link among these diseases (Forabosco et al., 2013).

Neuroinflammation is thought to play a key role in LOAD pathogenesis (reviewed by Latta et al., 2014). *TREM2* is expressed primarily by microglial cells of the brain, is involved in neuroinflammatory responses, and complexes with DAP12 (aka TYROBP) for intracellular signaling (reviewed by Ma et al., 2015). Ligands for TREM2 have yet to be identified, although one study shows an increase of these unknown ligands on apoptotic cells, including neurons (Hsieh et al., 2009). An examination of TREM2 in senescence-accelerated mice showed TREM2 levels increase with age. The same study demonstrated decreased levels of TREM2 are responsible for increased expression of proinflammatory cytokines, tumor necrosis factor–α and interleukin-6 and decreased expression of anti-inflammatory cytokine, interleukin-10 (Jiang et al., 2014). The aim of this study is to replicate the association of the *TREM2* R47H variant in a large AD case and/or control sample. Additionally, the associations of this variant with psychosis (a common LOAD endophenotype), fibrillar Aβ deposition determined by Pittsburgh Compound-B (PiB) positron emission tomography, and age-at-onset are examined.

2. Methods

2.1. Study population

The study population and informed consent procedures have been described previously (DeKosky et al., 2008; Kamboh et al., 2012). Briefly, 4885 individuals from 2 cohorts, the University of Pittsburgh Alzheimer's Disease Research Center (ADRC) and Gingko Evaluation of Memory (GEM) study, were used in this study. The ADRC cohort was comprised of 1283 cases (mean AAO 72.8 ± 6.5, 63% female, 25% autopsy confirmed) and 996 controls (mean age 75.6 ± 6.4, 64% female). The GEM cohort consisted of 338 cases (48% female) and 1950 controls (mean age 78.3 ± 3.1, 44% female). Diagnosis of LOAD in cases for both cohorts was determined based on Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) criteria. After quality control procedures and removal of non-Caucasian samples, 4567 samples remained for analysis. These samples were comprised of 1621 cases (59.8% female) and 2946 controls (51% female).

Table 2
Association of R47H variant with age-at-onset[a]

Genotype	N	Mean AAO (SD)
CC	1253	72.8 (6.51)
CT	25	73.0 (6.29)
Total	1278	$p = 0.94$

Key: AAO, age-at-onset; SD, standard deviation.
[a] Age-at-onset only available for ADRC samples.

Table 3
Association of R47H variant with amyloid deposition

Genotype	N	Mean PIB value (SD)
CC	314	1.89 (0.58)
CT	7	2.10 (0.99)
Total	321	$p = 0.287$

Key: PIB, Pittsburg Compound-B; SD, standard deviation.

2.2. Genotyping

Genotyping was performed with a custom designed TaqMan assay for *TREM2*/rs75932628 according to the manufacturer's protocol. (Life Technologies, Grand Island, NY, USA). For each cohort 10% replicates were included for quality control. Two control samples that are heterozygous for the R47H variant (courtesy of Dr. Carlos Cruchaga, Washington University) also were included on each plate. Assayed plates were read using a 7900 HT Fast Real Time PCR (Applied Biosystems). Genotyping calls were made using Applied Biosystems TaqMan Genotyper Software (v1.0.1, Life Technologies, Inc, 2010). Automated calls were manually reviewed for discrepancies in replicate samples. There were no discrepancies for the ADRC cohort and a discrepancy of rate of 0.0042 for the GEM cohort. We removed those discrepant samples before analysis. Twenty-seven samples failed genotyping, bringing the effective sample size for association analysis to 4540.

2.3. Determination of psychosis in AD patients

We evaluated the association of psychosis in 1204 ADRC participants with AD who had been genotyped for the *TREM2* 'T' allele, 1069 of whom were characterized during life for cognition and for psychotic symptoms by the Clinical Core of the ADRC, as previously used successfully to examine antemortem (DeMichele-Sweet et al., 2011; Hollingworth et al., 2011) and postmortem correlates of AD + P (Murray et al., 2012). The presence or absence of delusions and hallucinations are indicated as part of the semi-structured examinations and ratings scored on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Behavioral Rating Scale (Tariot et al., 1995). Delusions are defined as a false belief, not attributable to membership in a social or cultural group, based on incorrect inference about external reality. Delusions are differentiated from confabulations due to cognitive impairment by their persistence and their resistance to persuasion or contrary evidence. Hallucinations are defined as sensory perceptions for which there is no reality basis. Hallucinations occurring when the subject is not fully awake (i.e., hypnagogic or hypnapompic) are not considered hallucinations for the purpose of diagnosis or ratings of psychopathology.

Table 4
Association of R47H variant with psychosis in AD

Genotype	AD + P	AD − P
CC	533	352
CT	14	4
Total	547	356
		$p = 0.13$

Key: AD, Alzheimer's disease.