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Identification of a rare coding variant in *TREM2* in a Chinese individual with Alzheimer's disease

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Abstract

Rare variation in the *TREM2* gene is associated with a broad spectrum of neurodegenerative disorders including Alzheimer's disease (AD). *TREM2* encodes a receptor expressed in microglia which is thought to influence neurodegeneration by sensing damage signals and regulating neuroinflammation. Many of the variants reported to be associated with AD, including the rare R47H variant, were discovered in populations of European ancestry and have not replicated in diverse populations from other genetic backgrounds. We utilized a cohort of elderly Chinese individuals diagnosed as cognitively normal, or with mild cognitive impairment or AD to identify a rare variant, A192T, present in a single patient diagnosed with AD. We characterized this variant using biochemical cell surface expression assays and found that it significantly altered cell surface expression of the TREM2 protein. Together these data provide evidence that the A192T variant in *TREM2* could contribute risk for AD. This study underscores the increasingly recognized role of immune-related processes in AD and highlights the importance of including diverse populations in research to identify genetic variation that contributes risk for AD and other neurodegenerative disorders.

Keywords

TREM2; Alzheimer's disease; dementia; genetics; case report; Chinese; rare variant

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Introduction

The importance of immune factors and rare genetic variation in Alzheimer's disease (AD) is becoming increasingly appreciated, but the intersection of these two risk factors has remained understudied and may provide novel insights into AD pathogenesis. Similarly, most AD genetic analyses have focused on individuals of European descent, so studies which leverage the additional information available in diverse populations from around the globe may provide valuable perspectives on the genetic risk factors underlying neurodegeneration in AD.

TREM2 is one of the best known and most widely studied genes harboring rare (minor allele frequency [MAF] < 0.01) variation associated with neurodegenerative diseases. In the human brain, TREM2 is a receptor of the innate immune system and is expressed primarily in microglia (Zhang et al., 2014). It is involved in sensing particular lipids and damage signals, promoting microglial survival, and regulating central nervous system inflammation (Colonna & Wang, 2016; Kleinberger et al., 2014; Wang et al., 2015). *TREM2* has been implicated in multiple neurodegenerative diseases such as Nasu–Hakola disease, frontotemporal dementia (FTD), Parkinson's disease, amyotrophic lateral sclerosis, and AD (Borroni et al., 2014; Kiialainen, Hovanes, Paloneva, Kopra, & Peltonen, 2005; Painter et al., 2015). More recently, the R47H variant in *TREM2* has been associated with risk for AD in populations of European descent (Guerreiro, Wojtas, et al., 2013; Jonsson et al., 2013), and is thought to act by altering amyloid plaque morphology and promoting axonal dystrophy (Colonna & Wang, 2016; Yuan et al., 2016).

The R47H finding has not yet been replicated in East Asian (Huang et al., 2015; Ma et al., 2014; Miyashita et al., 2014; Yu et al., 2014) nor other diverse populations (Jin et al., 2015). However, recent studies have identified rare variants other than R47H in *TREM2* that were statistically associated with risk for AD (Jiang et al., 2016) in Han Chinese individuals. Our study aimed to replicate and discover new variants in *TREM2* associated with AD in a cohort of elderly Chinese individuals living in the San Francisco Bay Area. In addition to variant discovery, this study aimed to validate the functional relevance of *TREM2* risk variants found in Chinese individuals with AD using cell surface expression analysis.

Methods

Study participants and assessment

Eighty-eight Chinese individuals living in the San Francisco Bay Area visited the University of California, San Francisco (UCSF) Memory and Aging Center (MAC) as part of studies of healthy aging and dementia (Chao et al., 2011, 2014; Yokoyama et al., 2015). Each participant underwent a language-appropriate, multistep screening process requiring at least one in-person visit to the MAC. Participants received a neurologic exam, underwent cognitive assessment, and medical history (Rankin, Kramer, & Miller, 2005). A study partner was interviewed by the evaluation team and provided insight into the participant's functional abilities. A multidisciplinary team composed of a neurologist, neuropsychologist, and nurse determined a consensus diagnosis based on published criteria (Albert et al., 2011; Mckhann et al., 2011).

Sequence data acquisition, quality control, and post-processing

The cohort was screened using targeted sequencing of genes previously implicated in neurodegenerative dementia, including *TREM2* and the most common causative genes for Mendelian forms of AD and FTD. Exonic regions of these genes were captured using a custom Nimblegen SeqCap EZ Choice (Roche) library. The samples were sequenced on an Illumina HiSeq2500 at the UCLA Neuroscience Genomics Core (Los Angeles, CA). The GRCh37/hg19 reference genome was used to map sequence reads and GATK was used to interactively joint-call variants according to the developers' recommendations (https://www.broadinstitute.org/gatk/(McKenna et al., 2010)).

The resulting variants were filtered following previously published guidelines (Carson et al., 2014). Briefly, variants with a genotype quality (GQ) score higher than 20 and read depth (DP) score greater than 8 were included in the study. We used the Variant Effect Predictor tool in Ensembl to annotate the filtered variants in all target genes, including *TREM2*. The predicted effect of each variant was determined using PolyPhen and SIFT. Prior to analysis, we used PLINK (Purcell et al., 2007) to remove individuals with genotyping rates below 95% and single nucleotide polymorphisms (SNPs) with genotyping rates below 95%.

Genetic evaluation

Exonic SNPs in *TREM2* with MAF < 0.05 and classified as missense or nonsense variants were included in this evaluation because they represent the pool of variants most likely to contribute biological risk for disease. Variants were extracted from the dataset using PLINK and examined to see whether any of the genotyped SNPs segregated in AD cases versus controls.

Antibodies

The HA.11 monoclonal antibody from Covance was used to detect HA-tagged TREM2. The transferrin receptor (TfR) monoclonal antibody was from Invitrogen.

Molecular biology

Human TREM2 cDNA was obtained from R&D Systems, amplified by PCR and inserted into the pEGFP-N1 vector after removing the EGFP coding sequence. An HA epitope tag and linker sequence identical to that used in Kleinberger et al. (2014) were inserted after the TREM2 signal peptide using the Phusion high-fidelity DNA polymerase (NEB) system for site-directed mutagenesis. The *TREM2* variants used in this study were also generated using Phusion, with the HATREM2 construct serving as the template DNA. The constructs were verified by sequencing at the UC Berkeley DNA Sequencing Facility.

Cell culture

HEK-293T cells were maintained at the UC Berkeley Cell Culture Facility under standard conditions. The cells were transiently transfected using Lipofectamine 2000 (ThermoFisher) following the manufacturer's specifications. Culture medium was changed 4 h after transfection and experiments were carried out the following day.

Immunoblotting

Cells were harvested on ice by washing with cold PBS followed by lysing in a buffer containing 100 mM NaCl, 10 mM Tris-Cl, pH 7.6, 1% (v/v) Triton X-100 and *Complete* protease inhibitor cocktail (Roche). Material that was not Triton-soluble was sedimented by centrifugation at 20,000 g for 10 min at 4°C. Supernatants were mixed with 5× SDS-PAGE sample buffer supplemented with DTT and heated at 55°C for 10 min prior to running in 4–20% acrylamide gradient gels (Life Technologies). Following SDS-PAGE, the proteins were transferred onto PVDF membranes (EMD Millipore) and blocked in 5% non-fat milk (dissolved in PBS containing 0.1% Tween-20). The proteins were probed with HA and TfR antibodies at 1:2,500 and 1:10,000, respectively. Blots were developed using enhanced chemiluminescence and imaged on a ChemiDoc digital imager (Bio-Rad). ImageJ (NIH) was used to quantify protein signals. For overall TREM2 expression analysis, the TREM2 signals derived from cell lysates were first normalized to the corresponding TfR signal and then calculated as a fraction of the wild type (WT) signal.

Cell surface biotinylation

Cell surface biotinylation was completed using procedures outlined in Kleinberger et al. (2014). Briefly, cells were washed at room temperature with PBS and labeled with the EZ-Link Sulfo-NHS-SS-Biotin reagent (ThermoFisher) at 1 mg/ml in PBS for 15 min. Following this, the cells were placed on ice and washed with cold Tris-buffered saline to quench the biotin reagent. The cells were then washed with cold PBS after which they were lysed and clarified as described above. *Strep*-Tactin resin (IBA) was added to the lysates to capture biotinylated proteins and the mixtures rotated at 4°C for 1 h. The resin was pelleted and washed multiple times with lysis buffer. 2× SDS-PAGE sample buffer supplemented with DTT was added to the washed resin. The samples were then vortexed, heated, and prepared for immunoblotting as described above. For the analysis of surface-labeled TREM2, we quantified the entire surface-labeled signal (including mature and immature bands) by densitometry and normalized the signal of individual variants to the WT signal.

Results

Cohort composition

Of the 88 individuals included in this study, 37 were diagnosed as cognitively normal, 20 with mild cognitive impairment (MCI), and 31 with AD. Demographic and clinical information is summarized in Table 1.

Variant discovery in a Chinese individual with amnestic Alzheimer's disease

After variant quality control and filtering, one variant in *TREM2*, rs150277350, was available for analysis. rs150277350 was found in one patient with AD and is predicted to be a missense mutation resulting in an alanine-to-threonine change at position 192 (A192T) in TREM2. Polyphen predicted the variant to be "benign" and SIFT predicted it to be "tolerated." rs150277350 was previously reported as a potential modifier of AD risk in a recent report on a Chinese cohort screened for *TREM2* variants (Jiang et al., 2016), but was

not significant after multiple testing correction and was not functionally characterized. This patient's clinical description is provided in detail in the Case Report section.

The A192T variant in TREM2 shows altered cell surface expression

In addition to variants such as the early onset FTD-associated mutation Y38C (Guerreiro, Bilgic, et al., 2013) that strongly reduce surface expression (Kleinberger et al., 2014; Park et al., 2015), other variants such as R136W that may be associated with AD risk have been suggested to alter cell surface expression in previous work (Jin, 2014). Thus, we used cell surface expression analysis to evaluate the effects of the A192T variant of TREM2. Two point variants were generated using site-directed mutagenesis: A192T and the Y38C variant mentioned above, which we used as an internal control. The variants were successfully transfected into HEK-293T cells and their expression was evaluated using immunoblotting (Figure 1(a)). As expected, the Y38C variant showed impaired protein maturation as well as reduced overall and cell surface expression (Figure 1(a,b)). The A192T variant showed apparently normal protein maturation and displayed a trend toward lower overall expression, although this did not reach significance. On the other hand, the A192T variant showed a significant reduction in cell surface expression compared with WT ($p < 0.009$ relative to WT by unpaired, two-tailed *t*-test; Figure 1(a,b)).

Case report

The individual with the A192T variant of TREM2 was an 84-year-old Chinese man who presented a 1–2-year history of personality changes and declining memory function. The patient's informant noted instances of short-term memory loss, getting lost in familiar environments, and increasing irritability and rigidity in his routines. He was originally born in Taiwan but immigrated to the US approximately 40 years prior to our evaluation.

The general neurological examination was normal. The cranial nerves were fully tested and normal with the exception to the pupils, which were minimally reactive due to a previous surgical operation. The motor exam revealed normal bulk and tone throughout, with no pronator drive, normal fine finger movements and foot taps, and full power to confrontation throughout. The sensory examination was normal, with sensation to light touch preserved throughout. Coordination testing revealed a normal finger to nose test bilaterally. Deep tendon reflexes in the upper extremities were normal and symmetric; the lower extremity reflexes were absent with the exception of a right patellar reflex. Toes were downgoing bilaterally. Gait testing revealed a normal gait, normal toe and heel walking, and a normal tandem gait. The Romberg test was negative.

The patient's Mini-Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) score was 24/30, missing one point for date of the month, one point for name of the location, one point for the floor, and three points for recall. On the Benson Complex Figure Copy task (Possin, Laluz, Alcantar, Miller, & Kramer, 2011), his score for the copy portion was 13/17, and he recalled 2/17 elements after 10 min. On an eight-item word list task, he committed to memory a maximum of six words during encoding and had a free recall of zero words and cued recall of four words after 10 min.

Outside laboratory tests revealed normal values on a metabolic panel, liver function tests, hemoglobin A1C test, and lipid profile. TSH, RPR, and vitamin B12 levels were also normal. The patient had no remarkable family history for neurologic diseases. Medical history revealed a family history of cancer.

Shortly after his first visit at UCSF, the patient underwent an MRI outside of our research center. The T1 sequence was read as showing mild symmetric cortical atrophy, with hippocampi showing minimal to mild atrophy bilaterally. On FLAIR and T2 sequences, there were a few small punctate areas of hyperintensity in the subcortical white matter. The attending physician judged the burden of these hyperintensities as a minimally contributing, if at all, source of the patient's clinical symptomatology.

After a thorough review of the patient's exams, blood testing, and imaging results, other sources of cognitive impairment such as vitamin deficiencies, normal pressure hydrocephalus, and cerebrovascular disease were ruled out. Given this, the patient was diagnosed with dementia likely due to AD (Mckhann et al., 2011).

The patient underwent genetic screening for risk variants through ongoing research studies. His *APOE* genotype was ε3/ ε4. The patient was revealed to be heterozygous for the A192T variant in *TREM2* as described above. None of the patient's family members were available for testing for the A192T variant. The patient has been followed in our clinic since his initial visit. The patient has maintained a stable diagnosis of AD and has shown increasing memory impairment and irritability. His MMSE score decreased from24 at age 84 to 23 at age 86 and was 18 at age 89.

Discussion

We present the case of an elderly Chinese man diagnosed with AD who carried the A192T variant in *TREM2*. His case, combined with cellular expression assays, suggests a possible role for the A192T variant in *TREM2* as a contributor to risk for AD. In addition to providing support for the role of the A192T variant in AD risk, this case underscores the increasingly appreciated role of the immune system in AD and the impact of rare variation in *TREM2* on risk for AD in diverse populations.

The frequency of this variant in our Chinese cohort was 0.57%, and frequency specifically in the Chinese MCI and AD cases was 0.98%. This is higher than the cohort-wide frequency of 0.043% and case frequency of 0.10% reported in a cohort of 2,342 Han Chinese individuals (Jiang et al., 2016) and likely due to our small sample size. In East Asian populations, the ExAC database frequency of rs150277350 is 0.069% (Lek et al., 2016). In all other defined populations, the frequency of rs150277350 is less than 0.02%. Given the low frequency of this variant in all noted populations, large studies will be required to statistically confirm the A192T *TREM2* variant as a contributor to AD risk and additional empirical research will be required to elucidate the mechanisms by which the A192T variant might confer risk for AD.

Unlike most of the *TREM2* variants associated with AD risk in previous studies, the A192T variant does not produce an amino acid change in the extracellular domain of TREM2 (Jiang et al., 2016; Jin, 2014; Jin et al., 2014). Rather, the A192T variant is near the end of the

transmembrane portion of TREM2, and thus may alter cell surface expression and AD risk by a distinct mechanism yet to be determined. The variant's position may also explain why the A192T variant was not predicted to be deleterious by Polyphen and SIFT, yet proved to be significantly involved in cell surface expression of TREM2. Whether the A192T *TREM2* variant and the single copy of *APOE* ε4 interact to confer AD risk remain a question for broader population-based and molecular studies.

Our study benefits from its combined use of genetic, clinical, and molecular techniques to identify and characterize a single patient's polymorphismin *TREM2*. As this is a single case, further studies will be required to confirm the clinical relevance of this variant in Chinese and other diverse populations. A weakness of our study is that we were not able to pathologically confirm that the patient had AD and thus cannot definitively rule out the possibility that cerebrovascular disease and/or other neurodegenerative processes were responsible for the patient's clinical presentation. Further, we were not able to genotype the patient's family members. It remains to be determined whether or not the risk variant segregates within families. Further studies will be required to quantify the effect size of any AD risk conferred by the A192T variant.

As a proof of concept, our study demonstrates that combining an individual's genetic information with functional cellular characterization can provide supportive evidence for a novel variant contributing risk for AD. This "personalized" approach suggests that, in the future, clinicians and scientists may be able to work together to optimize treatment for a specific patient by rapidly translating and validating single genetic risk candidates for biologically relevant changes in protein function that may directly contribute to disease. Ultimately, this type of biological validation could allow for targeting aberrant molecular pathways in neurodegenerative disease as therapeutic interventions become available.

In summary, we highlight the A192T variant in *TREM2* as a potential risk factor for AD in diverse populations. This case underscores the growing role that the immune system and rare variation play in AD and demonstrates how combining individual genetic variants with functional characterization has the potential to provide more rapid identification of novel risk-conferring variants in neurodegenerative disease.

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Disclosure statement

Takeda Pharmaceutical Company Limited provided funding for genotyping participants, but played no role in the design, execution, or interpretation of this study's results.

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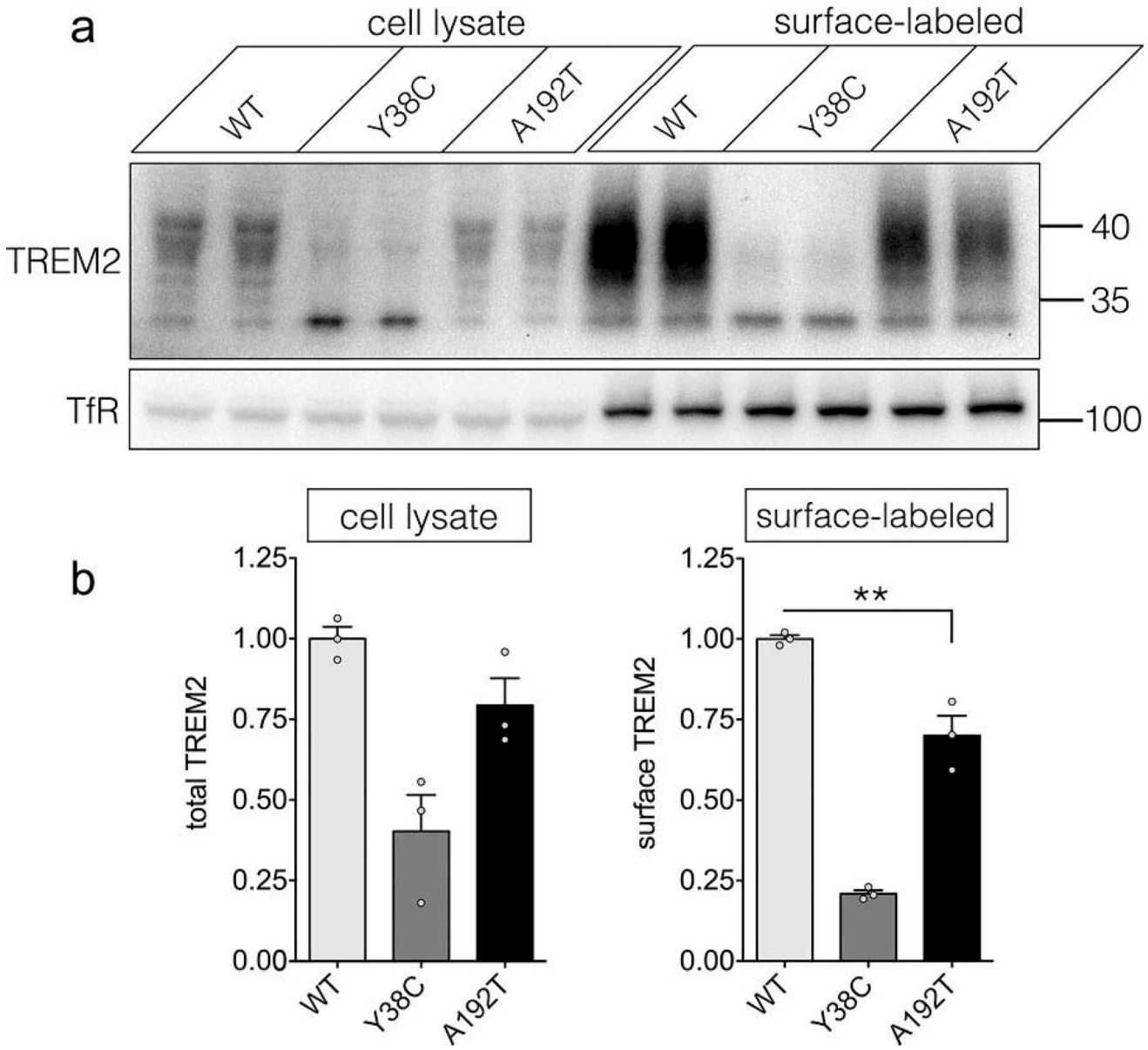

Figure 1.

Overall and cell surface expression of TREM2 variant A192T. The A192T variant was expressed transiently in HEK-293T cells alongside cells expressing wild type (WT) TREM2 or the Y38C variant. The A192T variant displayed normal protein maturation, but showed significantly reduced cell surface expression ($p < 0.009$ relative to WT by unpaired, two-tailed *t*-test). The overall and surface expression results were quantified (lower panels) from three separate transfections. Transferrin receptor (TfR) was used to control for loading and cell surface labeling.

Table 1

Demographic and clinical information for the cohort is provided for each diagnostic category.

	Diagnosis		
Diagnosis	**NC**	**MCI**	**AD**
N	37	20	31
Age (mean ± *SE*)	68.2 ± 1.6	76.2 ± 2.0	81.8 ± 1.4
Sex (M/F)	14/23	8/12	15/16
Education (mean ± *SE*)	16 ± 0.5	12.9 ± 0.9	12.8 ± 1.0
CDR (mean ± *SE*)	0.03 ± 0.02	0.4 ± 0.04	1.3 ± 0.1

NC: normal control; MCI: mild cognitive impairment; AD: Alzheimer's disease; M: male; F: female; *SE* standard error.