Lack of association between triggering receptor expressed on myeloid cells 2 polymorphism rs75932628 and late-onset Alzheimer’s disease in a Chinese Han population
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Introduction
Alzheimer’s disease (AD) is a genetically heterogeneous disorder characterized by the coexistence of monogenic and genetically complex forms. It is a major cause of dementia among the elderly. A twin study showed that the heritability of late-onset Alzheimer’s disease (LOAD) is ~60–80% (Gatz et al., 2006). Increasingly more attention is being paid to the gene mutations of AD, especially to LOAD. Until 2009, apolipoprotein E (ApoE) was the only established susceptibility marker for LOAD (Gatz et al., 2006). To identify the other genes for LOAD, efforts were focused on carrying out genome-wide association studies. Since 2009, several large genome-wide association studies and meta-analyses have identified significant associations of LOAD with single-nucleotide polymorphisms, such as CLU, CRI, and PICALM (Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010; Lambert et al., 2013).

The role of rare genetic variants in common disorders has been gaining attention in the last few years. Triggering receptor expressed on myeloid cells 2 (TREM2) has only recently been added to the list of associated LOAD genes. So far, several studies have shown that rs75932628-T (predicted to cause an R47H substitution) increases susceptibility to AD (Benitez et al., 2013; Giraldo et al., 2013; Guerrero et al., 2013; Jonsson et al., 2013; Pottier et al., 2013; Cuyvers et al., 2014; Jin et al., 2014; Roussos et al., 2014; Ruiz et al., 2014; Slattery et al., 2014). The rare R47H variant was found in both the Icelandic population (Jonsson et al., 2013) and an international cohort of European descent (Guerreiro et al., 2013), and this association has been replicated in a cohort of French patients with an early-onset form of AD (age of onset <65 years) (Pottier et al., 2013) and in a Spanish study with a series of patients including both LOAD and early-onset AD patients (Benitez et al., 2013). Subsequently, other further studies have also reported the association of P.R47H with AD in American, Belgian, and British populations (Cuyvers et al., 2014; Jin et al., 2014; Roussos et al., 2014; Slattery et al., 2014). A meta-analysis of these studies and others reports an odds ratio of 2.7 (Lu et al., 2015), further strengthening the case for the rare p.R47H substitution as a major genetic risk factor for AD. However, recent studies in Japanese and Chinese populations showed that mutations in exon 2 of TREM2 were unlikely to play a key role in the susceptibility of LOAD (Ma et al., 2014; Miyashita et al., 2014; Yu et al., 2014). Given the conflicting roles of TREM2 in LOAD, it would be of interest to investigate the link between the TREM2 polymorphism and LOAD in our study group of Han Chinese patients.

Participants and methods
Study participants
A total of 786 LOAD patients (mean age at onset 72.3 ± 11.1 years, 51.8% men) and 803 healthy controls...
(mean age at clinical assessment 71.9 ± 10.7 years, 51.3% men) were included in the current study. From a power analysis on the basis of the study by Guerreiro et al. (2013) on Whites (risk allele frequency in the AD group = 0.010, risk allele frequency in the control group = 0.002), this sample set was estimated to be large enough to detect risk alleles using PASS 11 software (NCSS, Kaysville, Utah, USA) ($\alpha = 0.05$, power = 80.8%). AD patients were recruited from the Memory Clinic, Huashan Hospital, and healthy adults were recruited from the community; they were matched for age, sex, and education with the AD patients. All the AD patients were recruited after they had completed the laboratory tests and cranial computed tomography/MRI scan and were found to have no clinically significant abnormalities in vitamin B12, folate acid, thyroid function (free triiodothyronine, free tetraiodothyronine, thyroid-stimulating hormone), rapid plasma regain, or Treponema pallidum particle agglutination.

AD was diagnosed as probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA/NINCDS-AIREN) criteria.

### Extraction of genomic DNA

Genomic DNA was isolated from fresh peripheral blood using the QIAamp DNA Blood Kit (Qiagen, Valencia, California, USA) according to the manufacturer’s instruction.

### High-resolution melting analysis

PCR cycling was performed on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA) and high-resolution melting (HRM) was performed on Rotor-Gene 6000 TM (Corbett Research, Mortlake, New South Wales, Australia). The primer sequences were designed to amplify a small fragment surrounding the rs75932628 polymorphism and to avoid the presence of other sequence variations. The primers for the 120-bp amplification that spanned the rs75932628 polymorphism were designed to amplify a small fragment surrounding the rs75932628 polymorphism and to avoid the presence of other sequence variations. The primers for the 120-bp amplification that spanned the rs75932628 polymorphism were AAG GAC AGC AGC CAC AAG TT and reverse primer TGT TTG CCC CTA TGA CTC CA. PCR was performed in a 20 μl volume containing 10 μl Premix Taq Hot Start Version, 0.5 μmol/l of each primer, 5 μmol/l of SYTO 9, 100 ng of genomic DNA, and PCR-grade water using Premix Taq Hot Start Version (TaKaRa Biotechnology Co. Ltd, Dalian, People’s Republic of China). Each reaction was performed in triplicate. The PCR cycling conditions were as follows: one cycle of 95°C for 2 min, 40 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s, one cycle of 72°C for 5 s, followed by an HRM step of 95°C for 2 min, 60°C for 2 min, and continuous acquisition from 87°C to 93°C at one acquisition per 0.1°C. Fluorescence acquisition was set as recommended by the manufacturer. The potential samples of the TREM2 rs75932628-T mutation were found by analyzing the PCR-product melting curves using the accompanying Rotor-Gene 6000 1.7 software (Corbett Research, Mortlake, New South Wales, Australia).

### Sequencing

A total of 46 potential mutations identified with HRM were subjected to direct sequencing. Amplicons were gel-purified using the QIAquick gel purification kit (Qiagen). DNA sequencing analysis was carried out in an ABI PRISM 310 genetic analyzer (Applied Biosystems).

### Ethical compliance

This study was approved by the ethics committee of Shanghai Huashan Hospital Fudan University. All participants or their families provided written informed consent.

### Statistical analyses

Descriptive statistics are presented as mean values (SD). Comparison of means was performed using an unpaired Student’s t test. ApoE ε4 genotype and sex distribution were compared using the $\chi^2$-test. P values less than 0.05 were considered statistically significant.

### Results and discussion

The characteristics of the study population are summarized in Table 1. No statistically significant differences were observed in age (age at onset for patients with LOAD compared with age at examination for control participants; $P = 0.435$), education ($P = 0.342$), and sex ($P = 0.433$) between patients with LOAD and the control participants. As expected, the Mini-Mental State Examination scores were significantly lower in patients with LOAD than in the control participants ($P = 0.000$). The number of patients with any ApoE ε4 in LOAD was more than that among the normal controls ($P = 0.000$).

The rare variant rs75932628-T, reported previously in European populations, was not detected in our cohorts. All patients and controls in our study had the TREM2 rs75932628 CC genotype, and none showed CT or TT genotypes. Our results, combined with other five studies consisting of 7286 Chinese participants (Feng et al., 2014; Ma et al., 2014; Yu et al., 2014; Li et al., 2016; Tan et al., 2016), indicate that the minor allele frequency of the

### Table 1 Demographic features of the participants

|                | NC ($n = 803$) | LOAD ($n = 786$) | $P$ value (two sided) |
|----------------|---------------|-----------------|-----------------------|
| Age (years)    | 71.9 (10.7)   | 72.3 (11.1)     | 0.435                 |
| Education (years) | 12.5 (3.4)   | 12.3 (3.1)     | 0.342                 |
| Male           | 412 (51.3)    | 407 (51.8)     | 0.433                 |
| MMSE           | 28.67 (2.74)  | 15.89 (6.06)   | 0.000**               |
| Any ApoE ε4    | 134 (16.7)    | 355 (45.2)     | 0.000**               |

ApoE, apolipoprotein E; LOAD, late-onset Alzheimer’s disease; MMSE, Mini-Mental State Examination; NC, normal controls.

**Correlation is significant at the 0.01 level (two tailed).
variant rs75932628-T in the Han Chinese population was only 0.007%. Moreover, this rare variant was not detected in AD patients, indicating that the minor allele frequency expected for rs75932628-T was lower than 0.02% in the Han Chinese AD individuals.

TREM2 is a transmembrane glycoprotein that is expressed primarily on microglia, the resident histiocytes of the central nervous system. This protein interacts directly with a type I transmembrane adapter protein, DAP12 (Colonna, 2003). With respect to the function, TREM2 has been found to suppress inflammatory responses by repression of microglia-mediated cytokine production and secretion, as well as promote phagocytosis of Aβ and damaged neurons (Colonna, 2003). Several recent studies showed that the levels of TREM2 increased in both plasma and brain in patients with AD (Giraldo et al., 2013; Guerreiro et al., 2013; Hu et al., 2014).

In the current study, we evaluated the rs75932628 polymorphism in the Chinese Han population. However, we did not detect any rs75932628-T in our cohort, indicating that the single nucleotide polymorphism of TREM2 may not be a genetic marker to assess the risk of LOAD in the Chinese Han population.

Our result was consistent with that reported previously in Asia, including Japan (Miyashita et al., 2014) and China (Ma et al., 2014; Yu et al., 2014). However, these results are not in agreement with recent data from Whites (Benitez et al., 2013; Guerreiro et al., 2013; Jonsson et al., 2013; Ruiz et al., 2014), indicating that TREM2 may contribute toward the susceptibility of LOAD only in Whites. One possible reason could be the population-specific genetic causes related to AD. Therefore, the information on ancestry will be critical to evaluate the role of TREM2 polymorphism rs75932628 in AD and other neurodegenerative disorders.

Because of the vast population in China, our sample size is insufficient to illustrate that there is a lack of association between TREM2 polymorphism rs75932628 and LOAD in the Chinese population. Further study with a large population is highly warranted.

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Conflicts of interest
There are no conflicts of interest.

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