Short Communication

Lack of Genetic Association Between TREM2 and Late-Onset Alzheimer’s Disease in a Japanese Population


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Abstract. Rare non-synonymous variants of TREM2 have recently been shown to be associated with Alzheimer’s disease (AD) in Caucasians. We here conducted a replication study using a well-characterized Japanese sample set, comprising 2,190 late-onset AD (LOAD) cases and 2,498 controls. We genotyped 10 non-synonymous variants (Q33X, Y38C, R47H, T66M, N68K, D87N, T96K, R98W, H157Y, and L211P) of TREM2 reported by Guerreiro et al. (2013) by means of the TaqMan and dideoxy sequencing methods. Only three variants, R47H, H157Y, and L211P, were polymorphic (range of minor allele frequency [MAF], 0.0002–0.0059); however, no significant association with LOAD was observed in these variants. Considering low MAF of variants examined and our study sample size, further genetic analysis with a larger sample set is needed to firmly evaluate whether or not TREM2 is associated with LOAD in Japanese.

Keywords: Alzheimer’s disease, Japanese, rare variants, SNP, TREM2

INTRODUCTION

Alzheimer’s disease (AD) is the main cause of dementia in the elderly. AD is thought to be caused by complex interactions between genetic and environmental factors. A twin study demonstrated that the heritability of late-onset AD (LOAD) is approximately 60–80% [1]. It is also assumed that multiple genes/loci contribute to LOAD development [2]. Rare non-synonymous mutations of APP, PSEN1, and PSEN2 are well known to cause familial cases of early-onset AD (EOAD) [3], which accounts for several percent of AD. Concerning LOAD, genome-wide association studies with large numbers of subjects have been conducted, based on the common diseases-common variants hypothesis. As a result, over a dozen genes other than APOE have been to be associated with the susceptibility to LOAD [4–10].

TREM2 was recently identified as a novel susceptibility gene for LOAD in Caucasians by two independent study groups [11, 12], both studies being performed on the basis of the common diseases-rare variants hypothesis. A noteworthy fact is that the most significant non-synonymous variant, R47H
(rs75932628: CGC→CAC; and minor allele frequency [MAF] < about 1%), located within exon 2 of TREM2, shows an odds ratio (OR) range of 2.0–5.0 [11, 12], which is almost equal to the risk magnitude for the APOE ε4 allele [13, 14]. The association of this variant with LOAD [15–19] as well as EOAD [20] has been reproducibly confirmed in multiple Caucasian populations. As to Asians, at present there has only been one genetic association study on TREM2 variants and LOAD, a northern Han Chinese population being involved [21]. In that study, it was demonstrated that no TREM2 variants, including R47H, examined show significant association with LOAD [21]. It is assumed that TREM2 may be a Caucasian-specific susceptibility gene for AD. Therefore, in this study we attempted to replicate the association of TREM2 with LOAD utilizing a Japanese sample set, comprising 4,688 subjects in total.

SUBJECTS AND METHODS

Subjects

This study was approved by the Institutional Review Board of Niigata University and by all participating institutes. All subjects were Japanese and anonymously genotyped. We prepared a Japanese sample set, comprising 2,190 LOAD cases (clinically-verified, n = 1,977; and neuropathologically-characterized, n = 213) and 2,498 controls (clinically-verified, n = 2,128; and neuropathologically-characterized, n = 370) (Table 1). From power analysis on the basis of Guerreiro et al.’s study with Caucasians [11], this sample set was estimated to be large enough to detect risk alleles with an OR of 1.1–2.5 (range of risk allele frequency = 0.01–0.99, α = 0.05, power = 80%) [29]. A large proportion of the clinically-verified subjects were the same (74.8%) as those in the overall sample set used in our previous genetic study on GAB2 [22]. The LOAD patients met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association for a diagnosis of probable AD [23]. Non-dementia controls were recruited from among elderly people living in an unassisted manner in the local community. Mini-Mental State Examination [24], Clinical Dementia Rating [25], and/or Function Assessment Staging [26] were applied to assess the severity of the cognitive impairment. All neuropathologically-characterized subjects were utilized in our recent genetic study on SORL1 [27].

Extraction and quantification of genomic DNA, and APOE genotyping are described elsewhere [27, 28]. The APOE alleles exhibited strong association with LOAD, as expected: pallele = 6.71E-171 with χ² test (χ² value = 783.7, degree of freedom = 2), and ORε4/ε3 (95% confidence interval [CI]) = 4.81 (4.26–5.42) and ORε2/ε3 (95% CI) = 0.59 (0.46–0.76).

TREM2 variants and genotyping

To determine whether or not TREM2 is associated with LOAD in Japanese, we focused on 12 non-synonymous variants of this gene, which were examined in Guerreiro et al.’s study with Caucasians [11]: Q33X (rs104894002), Y38C (rs ID, not available), R47H (rs75932628), R62H (rs143332484), T66M (rs201258663), N68K (rs ID, not available), D87N (rs142232675), T96K (rs2234253), R98W (rs147564421), R136Q (rs149622783), H157Y (rs2234255), and L211P (rs2234256). However, two variants, R62H and R136Q, were excluded since one (R62H) did not satisfy the design criteria for the TaqMan® genotyping assay and the other (R136Q) did not work well on TaqMan® genotyping. Consequently, we determined the genotypes of the remaining ten TREM2 variants using the TaqMan® method (Table 2, Supplementary Table 1). Heterozygotes were further evaluated by means of dideoxy DNA sequencing. Information on sequencing primers is available on request.

Statistical analysis

To detect genotyping errors, a Hardy-Weinberg equilibrium (HWE) test based on Fisher’s exact test was conducted. From a 2 × 2 contingency table (case-control status and genotype [MM and Mm]), we computed genotypic p (pgenotype) based on Fisher’s exact test and OR with 95% CI as the relative risk of disease for each polymorphic variant. We further performed multiple variant analysis as one of gene-based case-control association studies: distribution of minor-allele carriers (Mm) and non-carriers (MM) as to three polymorphic variants, R47H, H157Y and L211P, was compared between cases and controls on the basis of χ² test from a 2 × 2 contingency table. Subjects with undetermined genotype data in these variants were omitted for this analysis, with 4,582 subjects remaining. We used SNPAlyze software (DYNACOM, Japan; http://www.dynacom.co.jp/) for these statistical analyses, as described in detail elsewhere [35]. The statistical significance was set at p < 0.05.
RESULTS AND DISCUSSION

We attempted to replicate the association of \textit{TREM2} with LOAD in a Japanese sample set, comprising 4,688 subjects in total: cases, \( n = 2,190 \); and controls, \( n = 2,498 \) (Table 1). Three variants, R47H, 157Y, and L211P, were found to be polymorphic; however, the remaining seven, Q33X, Y38C, T66M, N68K, D87N, T96K, and R98W, did not show polymorphisms (Table 2, Supplementary Table 1). The MAF of the variants, R47H, 157Y, and L211P, were less than 0.01 (Supplementary Table 1). Concerning variant R47H \cite{11, 12}, three heterozygous subjects were observed: one clinically-verified case (female, age at onset of 76 years old, and \textit{APOE-\(e^3\)*3}) and two neuropathologically-characterized controls (one female, age at death of 99 years old, and \textit{APOE-\(e^3\)*3}; and one male, age at death of 79 years old, and \textit{APOE-\(e^3\)*3}). Variant L211P exhibited the highest MAF among them: 0.0041 in cases and 0.0059 in controls (Supplementary Table 1). Variants R47H, 157Y, and L211P were all in HWE (Supplementary Table 1). In both single and multiple variant analyses, we observed no significant association of \textit{TREM2} with LOAD (Table 2).

\textit{TREM2} is mainly expressed in microglia in the brain \cite{30}. This protein directly interacts with a type I transmembrane adapter protein, DAP12 \cite{30}. Recent whole transcriptome analysis of microglia, purified from mouse brains by means of flow cytometry, revealed that \textit{TREM2} belongs to a DAP12-centered protein network, in which multiple microglial marker proteins such as Cd68 are included \cite{31}. A \textit{TREM2}-DAP12 signaling pathway is involved in innate immune responses as well as the differentiation of myeloid progenitor cells into mature microglia \cite{30, 32}. Microglia play an important role in the clearance of amyloid-\(\beta\) protein in the brain \cite{33}. Thus, it is likely that genomic variants of not only \textit{TREM2} but also other genes involved in the \textit{TREM2}-DAP12 signaling pathway may accelerate amyloid plaque deposition through microglial dysfunction \cite{34}. Although none of the rare non-synonymous \textit{TREM2} variants investigated here exhibited association with LOAD in our sample sets (Table 2), we could not rule out the possibility that \textit{TREM2} is one of the crucial proteins for AD from the point of view of biological functions of this protein.

In conclusion, we were not able to detect the significant association of \textit{TREM2} variants examined with LOAD in Japanese, which is consistent with a recent study involving Chinese \cite{21}. On the other hand, \textit{TREM2} has been reproducibly shown to be strongly associated with both LOAD \cite{15–19} and EOAD \cite{20} in multiple Caucasian sample sets. Given these data, \textit{TREM2} may contribute to the susceptibility of LOAD only in Caucasians, i.e., not or only weakly in Asians. However, considering the very low MAF of variants investigated (Table 2, Supplementary Table 1) and our study sample size (Table 1), a large-scale meta-analysis is further needed to comprehensively evaluate whether or not \textit{TREM2} is associated with LOAD in Asians.

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Table 2

Genotypic distribution of three polymorphic variants, R47H, H157Y, and L211P, on TREM2 in Japanese

<table>
<thead>
<tr>
<th>Single variant analysis</th>
<th>Allele</th>
<th>Cases (frequency)</th>
<th>Controls (frequency)</th>
<th>(P_{\text{genotype}})^a</th>
<th>ORMm (95% CI)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant</td>
<td>dbSNP</td>
<td>M</td>
<td>m</td>
<td>MM</td>
<td>Mm</td>
</tr>
<tr>
<td>R47H</td>
<td>rs75932628</td>
<td>G</td>
<td>a</td>
<td>2.171 (0.9995)</td>
<td>1 (0.0005)</td>
</tr>
<tr>
<td>H157Y</td>
<td>rs2234255</td>
<td>C</td>
<td>t</td>
<td>2.147 (0.9972)</td>
<td>6 (0.0028)</td>
</tr>
<tr>
<td>L211P</td>
<td>rs2234256</td>
<td>T</td>
<td>c</td>
<td>2.161 (0.9917)</td>
<td>18 (0.0083)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Multiple variant analysis</th>
<th>Combind genotype</th>
<th>Cases (frequency)</th>
<th>Controls (frequency)</th>
<th>(P_{\text{genotype}})^c</th>
<th>ORCG-2 (95% CI)^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant</td>
<td>dbSNP</td>
<td>Combind</td>
<td>Allele</td>
<td>MM</td>
<td>Mm</td>
</tr>
<tr>
<td>R47H</td>
<td>rs75932628</td>
<td>Ga-CC-TT</td>
<td>2.104 (0.9883)</td>
<td>25 (0.0117)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>H157Y</td>
<td>rs2234255</td>
<td>GG-CC-TT</td>
<td>2.104 (0.9883)</td>
<td>25 (0.0117)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>L211P</td>
<td>rs2234256</td>
<td>GG-CC-Tc</td>
<td>2.104 (0.9883)</td>
<td>25 (0.0117)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

In single variant analysis, only three variants, L211P, H157Y, and R47H, are shown here since heterozygotes (Mm) were observed. M, major allele; m, minor allele; MM, major genotype; Mm, heterozygous genotype; mm, minor genotype; CG, combined genotype. ^aFisher’s exact test; ^bORMm (95% CI) for the heterozygote (Mm); ^cchi-squared test (degree of freedom = 1); ^dORCG-2 (95% CI) for CG-2 (Ga-CC-TT, GG-CT-TT, and GG-CC-Tc).
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SUPPLEMENTARY MATERIAL

The supplementary table is available in the electronic version of this article: http://dx.doi.org/10.3233/SUPPLEMENTARY-MATERIAL.

REFERENCES


