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# Genetic association study on in and around the *APOE* in late-onset Alzheimer disease in Japanese

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ABSTRACT

The ε*4* allele of *APOE* is a well-characterized genetic risk factor for late-onset Alzheimer disease (LOAD). Nevertheless, using high-density single nucleotide polymorphisms (SNPs), there have only been a few studies involving genetic association and linkage disequilibrium (LD) analyses of in and around the *APOE*. Here, we report fine mapping of a genomic region (about 200 kb) including the *APOE* in Japanese using 260 SNPs (mean intermaker distance, 0.77 kb). A case-control study demonstrated that 36 of these SNPs exhibited significance after adjustment for multiple testing. These SNPs are located in a genomic region including four genes, *PVRL2*, *TOMM40*, *APOE* and *APOC1*. Recombination rate estimation revealed that the associated region is firmly sandwiched between two recombination hotspots. Strong LD between these SNPs was observed (mean $|D'| = 0.914$). These data suggest that the three genes other than *APOE*, i.e. *PVRL2*, *TOMM40* and *APOC1*, could also yield a predisposition to LOAD.



## Introduction

Alzheimer disease (AD) is the main cause of dementia in the elderly. Its main neuropathological features are extracellular deposition of amyloid β-protein (Aβ) and intracellular formation of neurofibrillary tangles. Late-onset AD (LOAD), accounting for ~95% of AD, is thought to be a multi-factorial disease, probably caused by complicated interactions between genetic and environmental factors.

To date, only the apolipoprotein E gene (*APOE*) on chromosome 19q is universally recognized as a major disease susceptibility gene for LOAD [1–3]. *APOE* has three common alleles, *APOE-ε2*, *APOE-ε3* and *APOE-ε4*. These three alleles are defined by two non-synonymous single nucleotide polymorphisms (SNPs), rs429358 (TGC→CGC, Cys112Arg) and rs7412 (CGC→TGC, Arg158Cys): *APOE-ε2*, T-T (Cys-Cys); *APOE-ε3*, T-C (Cys-Arg); and *APOE-ε4*, C-C (Arg-Arg). Among these alleles, the *APOE-ε3* one is the most frequent (0.49–0.91), the *APOE-ε2* one being the rarest (0.0–0.15), in all populations thus far investigated [4]. We previously reported that in Japanese normal controls (≥60 y.o.) the frequencies of the *APOE-ε2*, *APOE-ε3* and

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**Table 1**
Subject information

| | LOAD | Control |
|---|---|---|
| No. of subjects | 547 | 715 |
| Frequency of female | 0.717 | 0.544 |
| AAO/AAE (mean ± SD) | 73.0 ± 6.9 | 73.0 ± 6.8 |
| *APOE* genotype | | |
| 2*2 | 0 | 4 |
| 2*3 | 19 | 57 |
| 2*4 | 6 | 7 |
| 3*3 | 279 | 529 |
| 3*4 | 196 | 117 |
| 4*4 | 47 | 1 |
| *APOE-ε4* allele frequency | 0.269 | 0.087 |

*APOE-ε4* alleles are 0.05, 0.86 and 0.09, respectively [5,6]. Compared to the *APOE-ε3* allele, the *APOE-ε4* allele is a strong risk factor with an odds ratio (OR) of 2.0–4.0, the *APOE-ε2* allele being protective as to LOAD [7,8].

Recent genome-wide association studies (GWAS) reconfirmed that an *APOE* linkage disequilibrium (LD) locus is strongly associated with LOAD [9–11]. Through these GWAS, it appears to be finally demonstrated that reproducible, strong association signals are only observed in and around the *APOE*. Up to now, it has been implicitly assumed that the genetic association in and around the *APOE* in LOAD thoroughly reflects the *APOE-ε4* association itself, owing to strong LD with the two *APOE* SNPs rs429358 and rs7412 [11]. However, it was recently suggested that multiple (*cis*-regulatory) SNPs in and around the *APOE* may contribute to disease susceptibility via alteration of gene expression. Belbin et al. [12] found that the rate of the cognitive decline in AD patients is affected by a SNP (rs440446) lying in a regulatory element of intron 1 of the *APOE*. Interestingly, the effect of this SNP on the cognitive decline seems to be independent of the *APOE-ε4* allele. Quantitative trait analysis also indicated that three cis-acting SNPs (rs449647, rs17684509 and rs7247551) of the *APOE* and three SNPs (rs11556505, n17664883 and rs157584) within the translocase of the outer mitochondrial membrane 40 homolog (yeast) gene (*TOMM40*), proximally located upstream of the *APOE*, are associated with the level of cerebrospinal fluid APOE in healthy non-demented subjects [13]. These data raise the possibility that other SNPs which affect a variety of quantitative traits involved in the LOAD pathogenesis might be hidden in and around the *APOE*. Therefore, it would be very important to conduct a genetic association study using a sufficient number of SNPs to entirely survey the genomic region comprising the *APOE*.

Here, we carried out a high-density SNP-based case-control association study ($N = 1262$ [LOAD, 547; control, 715]) using 260 SNPs for an approximately 200 kb genomic region comprising 8 genes in addition to the *APOE*: the Cas-Br-M (murine) ecotropic retroviral transforming sequence c gene (*CBLC*), the basal cell adhesion molecule (Lutheran blood group) gene (*BCAM*), the poliovirus receptor-related 2 (herpesvirus entry mediator B) gene (*PVRL2*),

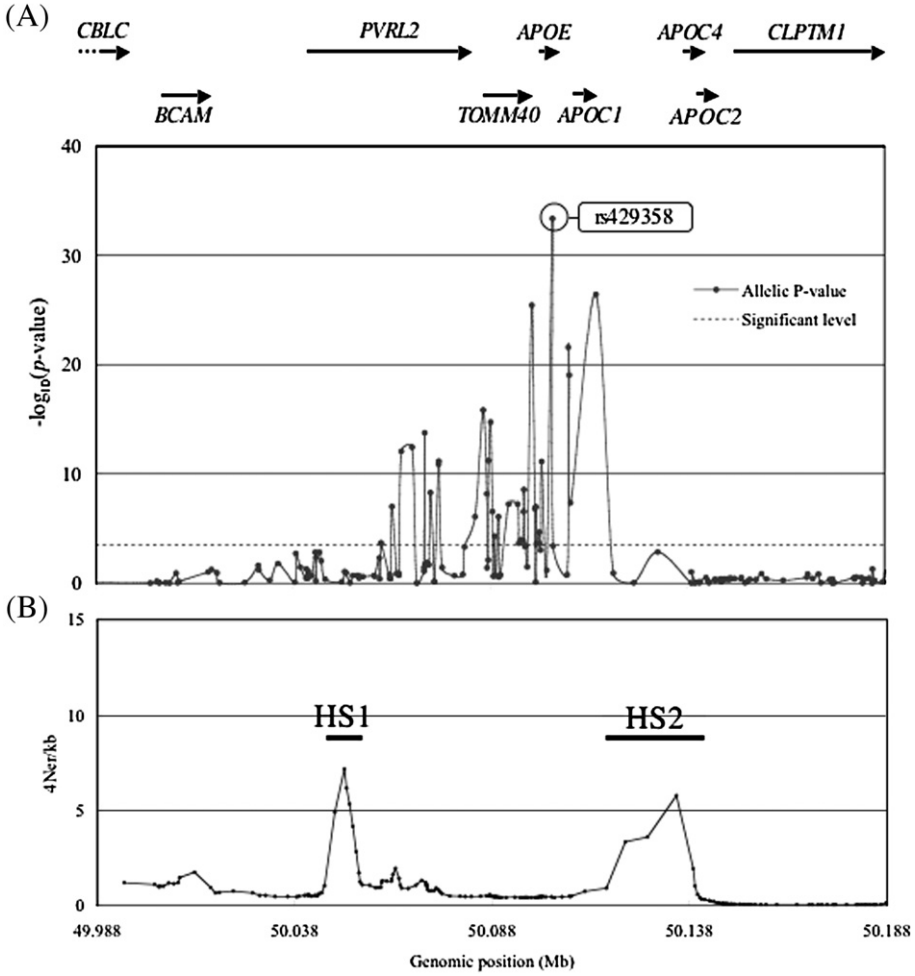


**Fig. 1.** Genomic region associated with LOAD. (A) Plot of $-\log_{10} P_{\text{unadj-allele}}$-values of 171 SNPs. Horizontal arrows indicate the transcriptional orientations of individual genes. The dotted line indicates the significant threshold of the $P_{\text{unadj-allele}}$-value = 0.0003 (Bonferroni correction). The most significant SNP, rs429358 ($P_{\text{unadj-allele}}$-value = 4.32E−34), is labeled. (B) Recombination hotspots, HS1 and HS2. Genomic positions are according to NCBI build 36.