A genome-wide association study of late-onset Alzheimer's disease in a Japanese population

Atsushi Hirano[a,b], Tomoyuki Ohara[a,c,d], Atsushi Takahashi[a], Masayuki Aoki[a], Yuta Fuyuno[a,b], Kyota Ashikawa[a], Takashi Morihara[e], Masatoshi Takeda[e], Kouzin Kamino[f], Etsuko Oshima[g], Yuko Okahisa[g], Nobuto Shibata[h], Heii Arai[h], Hiroyasu Akatsu[i], Masashi Ikeda[j], Nakao Iwata[j], Toshiharu Ninomiya[d], Akira Monji[k], Takanari Kitazono[b], Yutaka Kiyohara[d], Michiaki Kubo[a] and Shigenobu Kanba[c]

Objective Although a number of genome-wide association studies (GWASs) of late-onset Alzheimer's disease (LOAD) have been carried out, there have been little GWAS data on East Asian populations.

Design To discover the novel susceptibility loci of LOAD, we carried out a GWAS using 816 LOAD cases and 7992 controls with a replication analysis using an independent panel of 1011 LOAD cases and 7212 controls in a Japanese population. In addition, we carried out a stratified analysis by *APOE*-ε4 status to eliminate the established effect of *APOE* region.

Results Our data indicated that 18p11.32 (rs1992269, $P = 9.77 \times 10^{-7}$), *CNTNAP2* (rs802571, $P = 1.26 \times 10^{-6}$), and 12q24.23 (rs11613092, $P = 6.85 \times 10^{-6}$) were suggestive loci for susceptibility to LOAD.

Conclusion We identified three suggestive loci for susceptibility to LOAD in a Japanese population. Among these, rs802571, located at intron 1 of *CNTNAP2*, was considered to be a plausible candidate locus from a functional perspective. *Psychiatr Genet* 25:139–146

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Keywords: *CNTNAP2*, genome-wide association study, Japanese, late-onset Alzheimer's disease

[a]Laboratory for Genotyping Development, Center for Integrative Medical Sciences, RIKEN Yokohama Institute, Yokohama, [b]Department of Medicine and Clinical Science, [c]Department of Neuropsychiatry, [d]Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, [e]Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, [f]National Hospital Organization Yamato Mental Medical Center, Nara, [g]Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, [h]Department of Psychiatry, Juntendo University School of Medicine, Tokyo, [i]Choju Medical Institute, Fukushimura Hospital, [j]Department of Psychiatry, Fujita Health University School of Medicine, Aichi and [k]Department of Psychiatry, Faculty of Medicine, Saga University, Saga, Japan

Correspondence to Atsushi Hirano, MD, PhD, Laboratory for Genotyping Development, Center for Integrative Medical Sciences, RIKEN Yokohama Institute, 1-7-22, Suehiro-cho, Tsurumi, Yokohama, Kanagawa 230 0045, Japan
Tel: + 81 45 503 9607; fax: + 81 45 503 9606;
e-mail: atsuhira@intmed2.med.kyushu-u.ac.jp

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, the cause and pathogenesis of which are still uncertain (Ballard *et al.*, 2011). AD is divided into two types according to the age of onset, namely, early-onset AD and late-onset Alzheimer's disease (LOAD). Early-onset AD is a rare and Mendelian form of the disease caused by mutations of three genes [*APP* (Goate *et al.*, 1991), *PSEN1* (Sherrington *et al.*, 1995), and *PSEN2* (Rogaev *et al.*, 1995)], and LOAD accounts for the vast majority of AD cases. LOAD is the most common form of dementia in the elderly (Lobo *et al.*, 2000; Sekita *et al.*, 2010). Given the lack of disease-modifying therapies for LOAD, further elucidation of the pathogenesis of LOAD is required to develop a treatment strategy. Although LOAD is considered to be a multifactorial disease with many genetic and environmental factors contributing toward its development, the heritability estimates of LOAD are high [$h^2 \approx 60$–80% (Gatz *et al.*, 2006), λ's≈4–5 (Pericak-Vance and Haines, 2009)]. Therefore, genomic studies are considered to provide useful clues to elucidate the pathogenesis of LOAD. Until a genome-wide association study (GWAS) became feasible, only apolipoprotein E (*APOE*) was recognized as a LOAD susceptibility gene (Corder *et al.*, 1993). Since 2007, GWASs have identified several additional susceptibility loci for LOAD, including *CLU* (Harold *et al.*, 2009; Lambert *et al.*, 2009), *PICALM* (Harold *et al.*, 2009), *CR1* (Lambert *et al.*, 2009), *BIN1* (Seshadri *et al.*, 2010), *ABCA7* (Hollingworth *et al.*, 2011), *MS4A4A*/*MS4A4E*, *EPHA1*, *CD33*, and *CD2AP* (Hollingworth *et al.*, 2011; Naj *et al.*, 2011). Moreover, 11 susceptibility loci (*HLA* region,

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PTK2B, *SORL1*, *SLC23A4/RIN3*, *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2*, *CASS4*) were identified through large-scale meta-analysis of GWAS (Lambert *et al.*, 2013). However, most LOAD GWASs have been carried out in western populations, and there are little GWAS data on East Asian populations (Miyashita *et al.*, 2013). Although an *APOE*-ε4 allele has been confirmed to be a robust risk factor in Japanese populations (Ohara *et al.*, 2012; Miyashita *et al.*, 2013), the remaining loci outside of the *APOE* region have been little investigated; thus, ethnic-specific LOAD susceptibility loci remain to be identified. In this study, to elucidate the genetic background of LOAD pathology in a Japanese population, we carried out a GWAS using 816 LOAD cases and 7992 controls with a replication analysis in an independent panel of 1011 LOAD cases and 7212 controls. Subsequently, as disproportionately large *APOE* effects might mask the relatively small effects of LOAD susceptibility loci outside of the *APOE* region, we also carried out a stratified analysis by *APOE*-ε4 carrier status. In addition, we evaluated the associations of previously reported loci.

Materials and methods

Participants

A total of 1838 individuals with LOAD and 15 204 control individuals were enrolled in this study. All individuals were of Japanese descent. LOAD was diagnosed using clinical information, including neuroimaging results, according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (McKhann *et al.*, 1984). All LOAD cases in this study fulfilled the criteria for probable AD. LOAD cases for the GWAS were recruited at Kyushu University and 21 affiliated hospitals and institutes [$n = 827$; same set as the previous study (Ohara *et al.*, 2012)]. The cases used in the replication study included a total of 1011 participants recruited at Osaka University ($n = 364$), Okayama University ($n = 320$), Juntendo University ($n = 187$), Choju Medical Institute ($n = 108$), and Fujita Health University ($n = 32$). The control participants for the GWAS included 7992 individuals from the BioBank Japan project ($n = 6063$), Osaka-Midosuji Rotary Club ($n = 1023$), and the Pharma SNP consortium (PSC) ($n = 906$). The BioBank Japan project (*http://biobankjp.org*) was started in 2003 to collect genomic DNA, serum, and clinical information for about 300 000 patients diagnosed with any of the 47 diseases by a collaborative network of 66 hospitals in Japan (Nakamura, 2007). The participants from the Osaka-Midosuji Rotary Club included 1023 healthy volunteers and the participants from the PSC included 906 Japanese healthy volunteers from whom immortalized B lymphoblast cell lines were established by the PSC. For replication study controls, we used a nonoverlapping set of samples from the BioBank Japan project that included 7212 individuals. DNA was extracted from peripheral blood leukocytes. This study was approved by the ethics committees of each participating medical center and the RIKEN Yokohama Institute. Written informed consent was obtained from all appropriate proxies for LOAD patients and control participants.

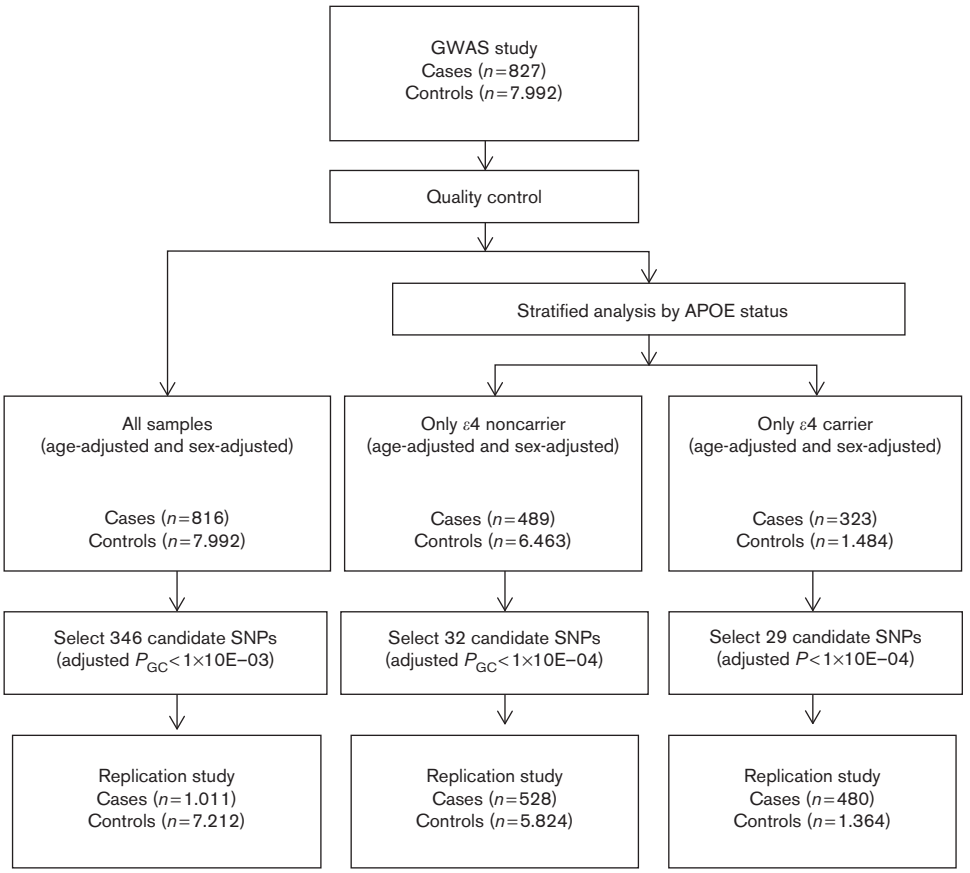
Single nucleotide polymorphism genotyping and quality controls

For the GWAS, we genotyped 827 LOAD cases and 7992 controls using the Illumina Human Omni Express BeadChip (Illumina, San Diego, California, USA). We excluded six LOAD cases with call rates lower than 0.98. After searching for close relatives using identity-by-descent as estimated by PLINK (Purcell *et al.*, 2007), we excluded five LOAD cases. We applied stringent quality control criteria to the single nucleotide polymorphism (SNP) data, with a genotype call rate 0.99 or more in both cases and controls, a Hardy–Weinberg equilibrium $P \geq 1 \times 10^{-6}$ in controls, and a minor allele frequency (MAF) over 0.01 in both the cases and the controls. After quality control filtering, we compared the frequencies of 561 143 autosomal SNPs among cases and controls. We carried out principal component analysis (PCA) on the genotype data from the participants along with data from European (CEU), African (YRI), and East Asian [Japanese (JPT) and Han Chinese (CHB)] individuals obtained from the phase 2 HapMap database using EIGENSTRAT (Price *et al.*, 2006), and identified no outliers. For the replication study, we genotyped 1011 LOAD cases using the multiplex PCR-based Invader assay (Hologic/Third Wave Technologies, Madison, Wisconsin, USA) (Ohnishi *et al.*, 2001). The control group included genome-wide data from 7992 participants who were genotyped using the Illumina Human Omni Express BeadChip. The *APOE*-ε4 status was classified either as ε4 carrier or as ε4 noncarrier according to the genotype of rs429358 (Belbin *et al.*, 2007). We genotyped rs429358 using the Invader assay.

Statistical analysis

In all stages, the age-adjusted and sex-adjusted associations and odds ratios (ORs) with their 95% confidence intervals (CIs) of each SNP were estimated using logistic regression analysis under an additive model. Departure from Hardy–Weinberg equilibrium was evaluated using the χ^2-test. Differences in basic characteristics were evaluated using Welch's *t*-test or the χ^2-test. The significance threshold of *P* value less than 5.0×10^{-8} was used for the GWAS (i.e. the genome-wide significance level). For the replication study, the association of *P* value less than 0.05 was considered 'nominally' significant, and if the *P* value surpassed the Bonferroni corrected threshold calculated as $\alpha = 0.05$, the association was considered significant. Combined analysis of the GWAS and the replication study was carried out using the inverse variance method. Heterogeneities among the

Fig. 1



The study design is shown. APOE, apolipoprotein E; GWAS, genome-wide association study; SNP, single nucleotide polymorphism.

studies were determined using Cochran's Q test. We initially carried out an analysis of all participants, and subsequently carried out a stratified analysis on the basis of *APOE*-ε4 status (Fig. 1). The GWAS and the replication data were calculated using R statistical environment (version 2.15.1) (*http://www.r-project.org/*) or PLINK (version 1.07) software. Haploview (version 4.2) software (Barrett *et al.*, 2005) was used to analyze linkage disequilibrium (LD) values. The association of previously reported loci was evaluated by VEGAS (Liu *et al.*, 2010) using the HapMap CHB + JPT population.

Results

Basic characteristics of the study participants

A total of 1827 LOAD cases and 15 204 control participants were included for the association study. The basic characteristics of the study population are summarized in Table 1. The number of men were lower among LOAD cases than the controls, whereas the mean age at sampling and the frequencies of APOE-ε4 carriers were higher among LOAD cases than the controls. With a MAF of 0.2, the statistical power of our samples was ~80% to detect a genotypic OR of 1.3 in all samples, 1.4 in the ε4 noncarrier subgroup, and 1.55 in the ε4 carrier subgroup at $\alpha = 5 \times 10^{-8}$.

A genome-wide association study and a replication study using all samples

We carried out a GWAS with 816 LOAD cases and 7992 control participants in a Japanese population. After applying stringent quality control criteria, we carried out an association analysis of 561 143 SNPs with MAF of 0.01 or more in both the cases and the controls. Although PCA showed no genetic heterogeneity among the LOAD cases and controls (Supplementary Fig. S1-A, Supplemental digital content 1, *http://links.lww.com/PG/A133*), the genomic inflation factor (λ_{GC}) was 1.087 (Supplementary Fig. S1-B, Supplemental digital content 1, *http://links.lww.com/PG/A133*), suggesting the possibility of the existence of a population substructure. To further examine the possibility of a population substructure and its influence on our GWAS results, we carried out PCA again using the HapMap JPT and CHB populations as references. Almost all participants fell into the two known main clusters of the Japanese population (Yamaguchi-Kabata *et al.*, 2008) (Supplementary Fig.

Table 1 Basic characteristics of the study participants

	Genome-wide association study			Replication study		
	Case	Control	*P* value	Case	Control	*P* value
Number of samples	816	7992		1011	7212	
Male [*n* (%)]	185 (22.7)	4606 (57.6)	< 0.001	360 (35.6)	3817 (52.9)	< 0.001
Age at sampling (mean ± SD) (years)	83.2 ± 6.5	58.6 ± 13.3	< 0.001	76.1 ± 8.6	44.9 ± 18.3	< 0.001
Status of APOE-ε4 allele [*n* (%)]						
Noncarrier	489 (60.2)	6463 (81.3)	< 0.001	528 (52.4)	5824 (81.0)	< 0.001
Carrier	323 (39.8)	1484 (18.7)		480 (47.6)	1364 (19.0)	
NA	4	45		3	24	

APOE, apolipoprotein E.

S1-C, Supplemental digital content 1, *http://links.lww.com/PG/A133*). When we evaluated the quantile–quantile plot using only the samples in the main (Hondo) cluster, the inflation factor did not improve ($\lambda_{GC} = 1.078$; Supplementary Fig. S1-D, Supplemental digital content 1, *http://links.lww.com/PG/A133*). Therefore, we considered that the population substructure might not be the cause of the difference in the inflation factor. To adjust for an unknown genetic heterogeneity in our results, we used a λ_{GC}-corrected *P* value (P_{GC}) for the GWAS results.

In the GWAS, six SNPs showed a genome-wide significance level of association with LOAD ($P_{GC} < 5 \times 10^{-8}$). All of these SNPs were located in the *APOE* region (Fig. 2a), and the top-associated SNP in the *APOE* region was rs769449 ($P_{GC} = 9.04 \times 10^{-22}$, OR = 4.01, 95% CI = 3.06–5.27). To identify new susceptibility loci for LOAD in the Japanese population, we carried out a replication study with 1011 independent LOAD cases and 7992 independent controls. Among 541 candidate SNPs with P_{GC} less than 1×10^{-3}, 346 SNPs that represent independent loci were selected after 195 SNPs with strong LD ($r^2 \geq 0.8$) were excluded. Among the 346 SNPs, 16 SNPs were excluded because genotype data from the controls were not available in the replication set. In the replication study, although 25 SNPs showed a nominal association with LOAD ($P < 0.05$) (Supplementary Table S4, Supplemental digital content 2, *http://links.lww.com/PG/A134*), no SNP remained significant after the Bonferroni correction ($P < 1.4 \times 10^{-4}$). When the results from the GWAS and the replication study were combined using an inverse variance weighted method, no SNP surpassed the genome-wide significance threshold. However, rs1992269 on 18p11.32 showed a suggestive association ($P_{combined} = 9.77 \times 10^{-7}$) (Table 2).

A stratified analysis by carrier status for the *APOE-ε4* allele

To eliminate the established effect of the *APOE* region, we stratified all GWAS participants and all replication participants, respectively, according to the presence or absence of the *APOE*-ε4 allele, and carried out association studies.

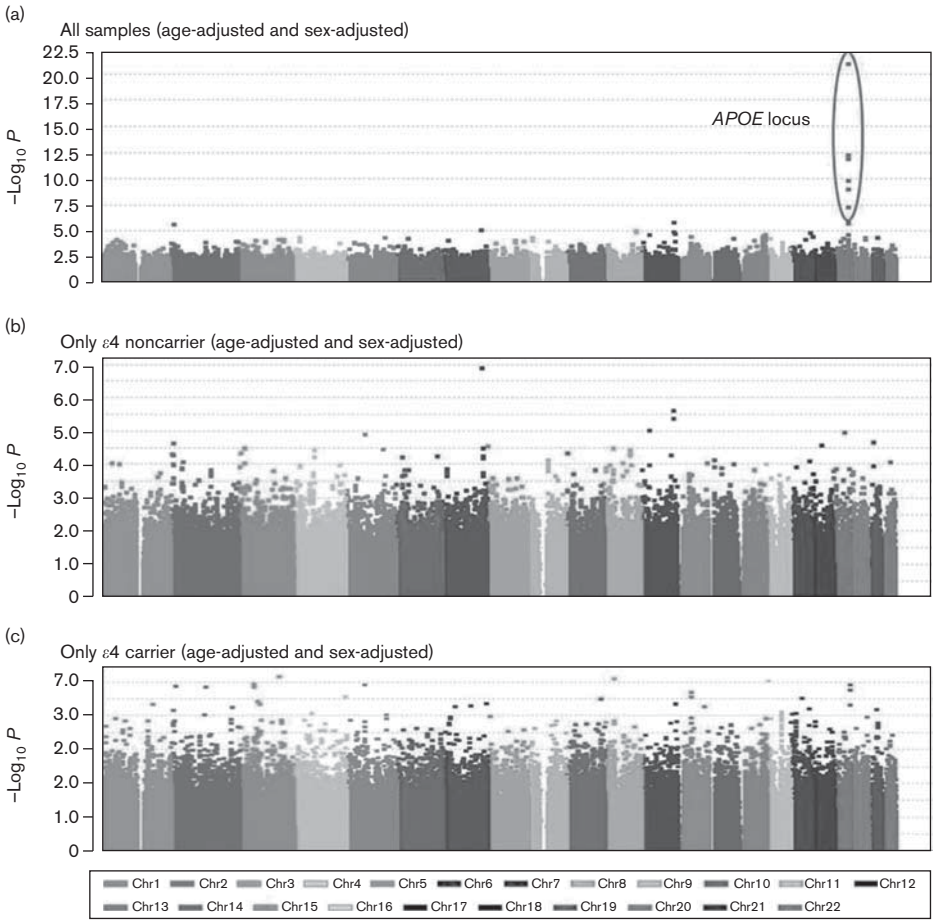
In the *APOE*-ε4 noncarrier subgroup, there were 489 LOAD cases and 6463 controls for the GWAS. As the λ_{GC} of GWAS was 1.068 (Supplementary Fig. S2-A, Supplemental digital content 3, *http://links.lww.com/PG/A135*), we used the λ_{GC}-corrected *P* value. No SNP showed a genome-wide significance level of association with LOAD in the GWAS (Fig. 2b). Subsequently, we carried out a replication study using 528 LOAD cases and 5824 controls. Out of 47 candidate SNPs with P_{GC} less than 1×10^{-4}, there were 32 SNPs that represent independent loci after 15 SNPs with strong LD ($r^2 \geq 0.8$) were excluded. In the replication study, four SNPs were nominally significant ($P < 0.05$) (Supplementary Table S5, Supplemental digital content 4, *http://links.lww.com/PG/A136*). Combining data from the GWAS and the replication study of the *APOE*-ε4 noncarrier subgroup, rs802571 in the contactin-associated protein-like 2 (*CNTNAP2*) on 7q35 ($P_{combined} = 1.26 \times 10^{-6}$) and rs11613092 on 12q24.23 ($P_{combined} = 6.85 \times 10^{-6}$) showed suggestive associations, although no SNP showed a genome-wide significance level of association with LOAD (Table 2).

In the *APOE*-ε4 carrier subgroup, there were 323 LOAD cases and 1484 controls for the GWAS. As the λ_{GC} was 1.031, indicating a low probability of false-positive associations resulting from genetic heterogeneity, we did not correct the *P* value (Supplementary S2-B, Supplemental digital content 3, *http://links.lww.com/PG/A135*). No SNP showed a genome-wide significance level of association with LOAD in the GWAS (Fig. 2c). As a consequence, we carried out a replication study with 480 LOAD cases and 1360 controls. Among 35 candidate SNPs with *P* value less than 1×10^{-4}, 29 SNPs that represent independent loci were selected after 15 SNPs with strong LD ($r^2 \geq 0.8$) were excluded. No SNP showed an association with LOAD in the replication study of the *APOE*-ε4 carrier subgroup.

Association study of previously reported loci

We examined the associations of previously reported loci in our GWAS set of all samples (816 LOAD cases and 7992 control participants) by VEGAS. Owing to the complex LD pattern of the *HLA* region, we tested all genes included in this region, although previously

Fig. 2



Manhattan plot showing the *P* value from the genome-wide association study of each group (age-adjusted and sex-adjusted logistic regression analysis). (a) Analysis in all samples. (b) Analysis in the *APOE*-ε4 noncarrier subgroup. (c) Analysis in the *APOE*-ε4 carrier subgroup. APOE, apolipoprotein E.

reported SNP exists near *HLA-DRB5/B1*. The result is shown in Table 3. Only *BIN1* and the *HLA* region surpassed the significance threshold ($P < 0.05$), and no significant association was observed in the other loci.

Discussion

We carried out a GWAS and a replication study with a total of 1827 LOAD cases and 15 204 controls in a Japanese population, and subsequently carried out a stratified analysis on the basis of *APOE*-ε4 carrier status. Although the strong association of the *APOE* region was reconfirmed, we could not identify any novel LOAD susceptibility loci surpassing the genome-wide significance threshold. Meanwhile, we found three suggestive loci for LOAD: rs1992269 was identified in an analysis of all samples, and rs802571 and rs11613092 were identified in an analysis of the APOE-ε4 noncarrier subgroup.

Among the three suggestive loci, rs1992269 was located in the gene desert on 18p11.32 (Supplementary Fig. S3-A, Supplemental digital content 5, *http://links.lww.com/PG/A137*). Rs11613092 was located ~37 kb downstream of suppressor of defective silencing 3 (*SUDS3*) and ~82 kb upstream of TAO kinase 3 (*TAOK3*). However, rs11613092 was not linked to these genes from the viewpoint of LD (Supplementary Fig. S3-B, Supplemental digital content 5, *http://links.lww.com/PG/A137*). Hence the association of these two loci with LOAD is also unclear from a functional perspective. Rs802571 is located in intron 1 of the *CNTNAP2* gene (Fig. 3). *CNTNAP2* encodes a member of the neurexin family, members of which function in the vertebrate nervous system as cell adhesion molecules and receptors. *CNTNAP2* is important for the clustering of Shaker-like K^+ channels and neural–glia interactions at the juxtaparanodal regions of myelinated axons of the central and peripheral nervous systems (Poliak *et al.*, 1999). As *CNTNAP2* is implicated in various neurodevelopmental disorders, including Gilles de la Tourette syndrome

Table 2 Summary of genetic loci showing a suggestive association for late-onset Alzheimer's disease in a Japanese population

SNPs	Allele	Minor allele	Chromosome	Chromosome location	Gene	Study	Number of samples		MAF		Age and sex adjusted			P_{het} [a]
							Case	Control	Case	Control	*P*	OR	95% CI	
All samples														
rs1992269	A/G	A	18	1 872 317	–	GWAS	816	7959	0.074	0.076	9.60E−04	1.80	1.32–2.72	0.42
						Replication	1006	7202	0.062	0.080	2.16E−04	1.57	1.24–2.00	
						Combined					9.77E−07	1.66	1.35–2.03	
Only ε4 noncarrier subgroup														
rs802571	T/C	C	7	145 962 186	CNTNAP2	GWAS	489	6459	0.065	0.084	3.14E−05	0.36	0.23–0.57	0.07
						Replication	524	5824	0.063	0.090	2.08E−03	0.61	0.45–0.84	
						Combined					1.26E−06	0.52	0.40–0.68	
rs11613092	A/G	A	12	118 893 248	–	GWAS	489	6455	0.876	0.907	2.31E−06	0.41	0.29–0.59	0.01
						Replication	526	5820	0.896	0.909	3.56E−02	0.75	0.57–0.98	
						Combined					6.85E−06	0.61	0.49–0.76	

The age-adjusted and sex-adjusted *P* values were calculated by logistic regression analysis under an additive model, and *P* values from GWAS were corrected by λ_{GC}.
The combined *P* values were calculated using the inverse variance method.
CI, confidence interval; MAF, minor allele frequency; GWAS, genome-wide association studies; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]The *P* values of heterogeneities (P_{het}) across the population were estimated using Cochran's *Q* test.

Table 3 Associations of previously reported loci evaluated by VEGAS

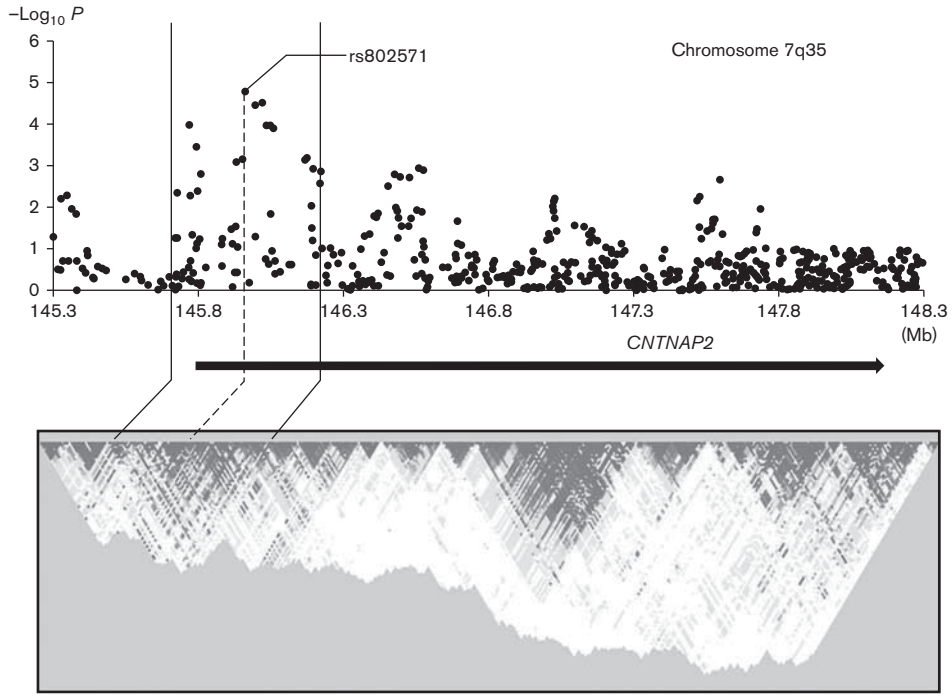
Genes	*P* value	Top-SNP *P* value
CR1	0.159	0.102
BIN1	0.010	0.003
CD2AP	0.079	0.139
EPHA1	0.052	0.064
CLU	0.236	0.113
MS4A4A	0.329	0.586
PICALM	0.632	0.810
ABCA7	0.070	0.425
CD33	0.276	0.420
HLA region		
HLA-DRB5	0.745	0.839
HLA-DRB1	0.108	0.177
HLA-DMA	4.30E−04	0.018
PTK2B	0.360	0.414
SORL1	0.846	0.665
SLC24A4/RIN3 region		
SLC24A4	0.438	0.439
RIN3	0.708	0.564
INPP5D	0.523	0.048
MEF2C	0.269	0.147
NME8	NA	NA
ZCWPW1	0.166	0.410
CELF1	0.510	0.742
FERMT2	0.107	0.160
CASS4	0.782	0.500

SNP, single nucleotide polymorphism.

(Verkerk *et al.*, 2003), schizophrenia (Friedman *et al.*, 2008), focal epilepsy (Strauss *et al.*, 2006), autism (Alarcón *et al.*, 2008; Arking *et al.*, 2008; Bakkaloglu *et al.*, 2008), and developmental language disorder (Vernes *et al.*, 2008), this gene is considered to play a crucial role in the central nervous system. Van Abel *et al.* (2012) showed that *CNTNAP2* is directly downregulated by STOX1A, which is a transcription factor binding to intron 1 of *CNTNAP2*, and CNTNAP2 expression is downregulated in the hippocampus of LOAD patients, where STOX1A expression has been shown to be upregulated. We speculate that a genomic variant linked to rs802571 might alter the binding affinity of the transcription factor and affect the development of LOAD. Although *CNTNAP2* has not shown an association with LOAD at the GWAS-significant level in the previous GWASs, rs10273775 located in intron 8 of *CNTNAP2* was reported to be a suggestive locus in the LOAD GWAS of African-Americans (Logue *et al.*, 2011). Together with this result and the reported function of *CNTNAP2*, *CNTNAP2* is one of the plausible candidate genes for LOAD susceptibility. Further genomic studies with larger sample sizes and functional studies are required to clarify whether *CNTNAP2* is a true susceptibility gene for LOAD or not.

We examined the associations of previously reported loci. Associations were shown in only two loci, whereas no significant association was found in the other loci. One reason for this may be our relatively small case–control samples. Another is that, because the LOAD cases and controls were all individuals of Japanese descent, genetic heterogeneity among different ethnicities could have weakened the associations.

Fig. 3



Case–control association plots, linkage disequilibrium (LD) map, and genomic structure of the regions on chromosome 7q35. The candidate region is shown between the two black lines. The black dots represent $-\log_{10} P$ values obtained from genome-wide association study (GWAS). We drew the LD map on the basis of D' values using the genotype data from the cases and controls in the GWAS samples. The black dashed line indicates the position of the landmark single nucleotide polymorphism (rs802571).

Some potential limitations of this study should be noted. None of the control individuals had undergone a cognitive examination, and therefore, it is unknown whether or not any cognitive impairments were present in these individuals. In addition, the control participants were significantly younger than the LOAD cases, which suggests that some of the controls will go on to develop LOAD as they age. As these limitations would cause our study to underestimate the impact of SNPs on the development of LOAD, the true associations may be stronger than those shown in this study.

In conclusion, we identified three suggestive loci for LOAD susceptibility in a Japanese population. Among these, *CNTNAP2* was considered to be a plausible candidate gene for LOAD. Further studies are required to clarify the role of the identified loci in the pathogenesis of LOAD.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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