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SHORT REPORT

GAB2 is not associated with late-onset Alzheimer's disease in Japanese

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The ε4 allele of the apolipoprotein E gene (*APOE*) is unequivocally recognized as a genetic risk factor for late-onset Alzheimer's disease (LOAD). Recently, single-nucleotide polymorphisms (SNPs) of the GRB2-associated binding protein 2 gene (*GAB2*) were shown to be associated with LOAD in Caucasians carrying the *APOE-ε4* allele through a genome-wide association study. Here, we attempted to replicate the finding by genotyping these SNPs in a large clinical cohort of Japanese. We observed no association of any of the SNPs with LOAD. *GAB2* may not be a disease susceptibility gene for LOAD in Japanese.
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Introduction

It is well known that the development of Alzheimer's disease (AD) is consequence of complex interactions between multiple genetic and environmental factors. To date, only the *ε4* allele of the apolipoprotein E gene (*APOE*) is universally recognized as a genetic risk factor for late-onset AD (LOAD) in a variety of populations,[1–3] but not in elderly Nigerians.[4] As the presence of risk genes other than *APOE* is speculated,[5] many studies have been performed to identify them.

Genome-wide association studies (GWAS) involving high-density single-nucleotide polymorphism (SNP) genotyping technologies have led to great success in the identification of risk genes for various common diseases.[6,7] With regard to LOAD, the GRB2-associated binding protein 2 gene (*GAB2*) on chromosome 11q was recently identified in Caucasians through GWAS: 10 SNPs of this gene have been shown to be associated with LOAD in *APOE-ε4* carriers.[8] It is noteworthy that the most significant SNP, rs2373115, exhibits an odds ratio (OR) of 4.1 (95% confidence intervals (Cis), 2.8–14.7), which is almost equal to the strong risk effect exerted by the *APOE-ε4* allele (*ε3 vs ε4*, OR = 3.2–4.1).[2] Furthermore, the following

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findings with relation to AD neuropathology have been made:[8] in LOAD brains GAB2 is detected in highly dystrophic neurons, including neurofibrillary tangle (NFT)-bearing neurons, and interference with *GAB2* expression increases TAU phosphorylation, which leads to NFT formation. With this genetic and biological evidence, *GAB2* is considered to be a promising candidate for LOAD, although a recent replication study revealed a lack of association of this gene with LOAD in Caucasians.[9] Therefore, we here assessed whether or not the genetic association of *GAB2* with LOAD can be reproduced in Japanese.

Subjects and methods

Subjects

Blood samples were collected by the Japanese Genetic Study Consortium for AD (JGSCAD): the members are listed in our recent publications.[10,11] The LOAD patients were clinically validated, and satisfied 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.[12] Non-demented controls living in an unassisted manner in the local community were recruited from among elderly subjects. The Mini-mental State Examination (MMSE) and Clinical Dementia Rating and/or the Function Assessment Staging were used to assess severity of the cognitive impairment. Basic information on the sample sets used is presented in Table 1. The total sample size is 1656 LOAD patients (female, 71.6%) and 1656 controls (female, 58.7%), which is large enough to detect risk alleles assuming OR > 1.3 (range of risk allele frequency = 0.1–0.9, $\alpha = 0.05$, power = 80%). This subject group is referred to as overall sample set *All* in this study (Table 1). A large proportion (79.4%) of the subjects are the same as in our previous overall sample set.[10,11] To construct two subsample sets, the *All* set was stratified as to the *APOE-ε4* carrier status: *Negative-ε4* (LOAD, 790; control, 1378) and *Positive-ε4* (LOAD, 866; control, 278) (Table 1).

This study was approved by the Institutional Review Board of Niigata University, and by all participating institutes. Informed consent was obtained from all controls and appropriate proxies for patients, and all subjects were anonymously subjected to SNP genotyping.

Genotyping

Genomic DNA preparation and genotyping were described previously.[10.] We did not genotype additional SNPs to the 10 ones reported by Reiman *et al*,[8] as strong LD ($|D'| > 0.8$) was observed across the *GAB2* (see Supplementary Figure 1).

Statistical analysis

We carried out a Hardy–Weinberg equilibrium (HWE) test based on an exact test, single SNP and haplotype-based case–control studies, haplotype inference, and computation of LD measures (D′). As an estimate of the relative risk of disease, OR with 95% CIs of each marker or haplotype was calculated from a 2×2 contingency table. For all statistical analyses mentioned above, we used SNPAlyze® software version 6.0.1 (DYNACOM): the analytical methods were described in detail elsewhere.[11] For evaluation of the LD block structure in and around *GAB2*, Haploview software version 3.32 was used. We considered $P < 0.05$ statistically significant.

Results

To determine whether the *GAB2* association can be replicated in Japanese or not, we analyzed the 10 SNPs using a total of 3312 clinical subjects for genotyping (see Supplementary Figure 2). These SNPs are encompassed by *GAB2* (Table 2), which consists of 10 exons and spans about 202.4 kb on chromosome 11. To examine population

Table 1 Information on sample sets

		AAO/AAE		MMSE		APOE Genotype						APOE Allele		
Sample set	*No. of subjects*	*Mean (SD)*	*Range*	*Mean (SD)*	*Range*	*2*2*	*2*3*	*2*4*	*3*3*	*3*4*	*4*4*	*ε2*	*ε3*	*ε4*
Overall set														
All														
LOAD	1656	73.1 (6.2)	60–85	16.5 (6.7)	0–30	1	53	21	736	686	159	76	2211	1025
Control	1656	75.3 (6.1)	64–96	28.3 (1.7)	24–30	4	126	17	1248	248	13	151	2870	291
Subsets														
Negative-ε4														
LOAD	790	73.7 (6.4)	60–85	16.2 (7.2)	0–30	1	53	0	736	0	0	55	1525	0
Control	1378	75.4 (6.2)	64–96	28.3 (1.7)	24–30	4	126	0	1248	0	0	134	2622	0
Positive-ε4														
LOAD	866	72.5 (5.9)	60–85	16.9 (6.3)	0–30	0	0	21	0	686	159	21	686	1025
Control	278	75.1 (5.8)	64–95	28.3 (1.7)	24–30	0	0	17	0	248	13	17	248	291

AAO, age at onset; AAE, age at examination.

differences in the allele frequencies of the SNPs, we first assessed the HapMap genotype data (http://www.hapmap.org/index.html) for four populations: Japanese in Tokyo (JPT), US Utah residents with northern and western European ancestry (CEU), Han Chinese in Beijing (CHB) and Yoruba in Ibadan, Nigeria. These 10 SNPs for JPT exhibited similar allelic frequencies to CHB, but not to CEU: for example, the frequencies for allele G of SNP rs2373115 were 0.47 for JPT and 0.89 for CEU (see Supplementary Figure 3). HWE exact tests were performed to detect genotyping errors. SNP rs7101429 slightly deviated from the HWE in the *All* ($P=0.0497$) and *Negative-ε4* ($P=0.0416$) sample sets in LOAD. Remaining nine SNPs were in HWE ($P\geq0.05$) (Table 2).

A single SNP case–control study (χ^2 test) was then carried out. We did not observe any significant association of the SNPs with LOAD in not only the *All* set but also the two subsets (*Negative-ε4* and *Positive-ε4*) (Table 3). Multiple logistic regression analysis, with adjustment for the carrier status of the *APOE-ε4* allele, age and gender as covariates, did not reveal any significant evidence of association (data not shown).

Pairwise LD measures, D′, of the SNPs are given in Supplementary Table 1. We found a strong correlation ($|D'|>0.93$) between the 10 SNPs in each of the three sample sets. No difference in the LD block structure was observed between LOAD and control subjects. Using the HapMap genotype data for JPT and CEU, we further performed *in silico* LD mapping of a genomic region spanning about 500 kb. It was found that *GAB2* was completely encompassed by a highly structured single LD block in both JPT and CEU (see Supplementary Figure 1). However, there was an evident difference in the LD block boundary in the 5′ region of *GAB2*: in JPT, we observed a definitive break point in the block, but not in CEU (see Supplementary Figure 1).

In the LD block, including the whole *GAB2*, three common haplotypes (frequency >1%), H1, H2 and H3, were inferred in all sample sets (see Supplementary Table 2). Haplotype H2 consisted of all major alleles of the 10 SNPs. In every sample set, no haplotypes exhibited significant differences between LOAD and controls (see Supplementary Table 2).

Discussion

Recently, it was shown that *GAB2*, encoding a scaffolding adaptor protein involved in several signal-transduction pathways, is associated with LOAD in Caucasians.[8] At SNP rs2373115 located within this gene, a noticeable significance in allelic association ($P_{\text{allele}}=9.7\times10^{-11}$) has been observed.[8] Interestingly, the disease risk of this gene is increased by the *APOE-ε4* allele: maximum OR of 24.6 (95% CIs, 7.4–116.8) was computed in carriers with both the

Table 2 SNP information on 10 *GAB2* SNPs

						All				*Negative-ε4*				*Positive-ε4*			
			Allele			*Allele frequency*		*HWE*		*Allele frequency*		*HWE*		*Allele frequency*		*HWE*	
dbSNP	*Physical position (bp)*	*SNP position*	*Maj*	*Min*	*GSR (%)*	*Maj*	*Min*	*LOAD*	*Control*	*GSR (%)*	*Maj*	*Min*	*LOAD*	*Control*	*GSR (%)*	*Maj*	*Min*	*LOAD*	*Control*
rs901104	77 608 147	intron 9	G	A	99.1	0.58	0.42	0.1417	0.5789	99.3	0.57	0.43	0.1894	0.8252	98.6	0.58	0.42	0.4388	0.3866
rs1385600	77 613 814	exon 5	A	G	99.7	0.56	0.44	0.0792	0.6180	99.7	0.56	0.44	0.0962	0.7433	99.7	0.57	0.43	0.4042	0.6249
rs1007837	77 618 724	intron 3	T	C	98.4	0.56	0.44	0.0964	0.4818	98.3	0.56	0.44	0.1102	0.6206	98.7	0.57	0.43	0.4421	0.5383
rs2510038	77 643 682	intron 2	G	A	99.0	0.57	0.43	0.0695	0.3665	99.0	0.56	0.44	0.0944	0.5456	99.0	0.58	0.42	0.3633	0.3910
rs4945261	77 667 908	intron 2	G	A	98.5	0.57	0.43	0.0768	0.4817	98.6	0.56	0.44	0.0582	0.6203	98.2	0.58	0.42	0.5278	0.6205
rs7101429	77 670 615	intron 1	A	G	99.0	0.56	0.44	**0.0497**	0.5479	99.0	0.56	0.44	**0.0416**	0.7019	98.9	0.57	0.43	0.4857	0.5344
rs10793294	77 674 051	intron 1	C	A	98.0	0.80	0.20	0.3389	0.2567	98.3	0.80	0.20	0.7184	0.0780	97.5	0.79	0.21	0.0905	0.2930
rs4291702	77 678 896	intron 1	G	A	98.4	0.57	0.43	0.0681	0.5456	98.4	0.56	0.44	0.0794	0.6593	98.3	0.58	0.42	0.3992	0.7089
rs7115850	77 722 719	intron 1	G	C	99.7	0.57	0.43	0.1194	0.4842	99.8	0.56	0.44	0.0960	0.7017	99.4	0.58	0.42	0.5772	0.4600
rs2373115	77 768 798	intron 1	C	A	98.8	0.56	0.44	0.0770	0.5824	99.0	0.56	0.44	0.0930	0.7846	98.3	0.57	0.43	0.4019	0.4606

The genomic position of each SNP is according to NCBI build 36.2. The allele frequency for each sample set was calculated by combining LOAD and control subjects. Maj, major; Min, minor; GSR, genotyping success rate; HWE, *P*-value of Hardy–Weinberg equilibrium exact test (bold value indicates statistical significance at $P<0.05$).

Table 3 Genotypic and allelic associations

		Genotype							*Allele*					
		LOAD			*Control*				*LOAD*		*Control*			
Sample set	*dbSNP*	*Maj*	*Ht*	*Min*	*Maj*	*Ht*	*Min*	*P-value (d.f. = 2)*	*Maj*	*Min*	*Maj*	*Min*	*P-value (d.f. = 1)*	*OR (95% CIs)*
Overall set														
All	rs901104	559	768	306	554	793	301	0.8206	1886	1380	1901	1395	0.9539	1.00 (0.91–1.11)
	rs1385600	550	775	326	518	804	328	0.473	1875	1427	1840	1460	0.4007	1.04 (0.95–1.15)
	rs1007837	541	769	324	514	788	324	0.6366	1851	1417	1816	1436	0.5163	1.03 (0.94–1.14)
	rs2510038	550	766	321	531	789	321	0.7156	1866	1408	1851	1431	0.6263	1.02 (0.93–1.13)
	rs4945261	549	763	318	523	789	319	0.5864	1861	1399	1835	1427	0.4978	1.03 (0.94–1.14)
	rs7101429	541	764	329	524	797	323	0.6084	1846	1422	1845	1443	0.7602	1.02 (0.92–1.12)
	rs10793294	1044	518	54	1035	519	77	0.1347	2606	626	2589	673	0.2034	1.08 (0.96–1.22)
	rs4291702	555	760	314	525	789	315	0.5021	1870	1388	1839	1419	0.438	1.04 (0.94–1.15)
	rs7115850	551	777	321	530	799	323	0.6982	1879	1419	1859	1445	0.5612	1.03 (0.93–1.13)
	rs2373115	543	763	321	519	800	326	0.5071	1849	1405	1838	1452	0.4355	1.04 (0.94–1.15)
Subsets														
Negative-ε4	rs901104	265	365	152	456	666	249	0.6473	895	669	1578	1164	0.8361	1.01 (0.89–1.15)
	rs1385600	262	365	162	425	672	275	0.4462	889	689	1522	1222	0.579	0.97 (0.85–1.09)
	rs1007837	255	362	162	421	659	272	0.5943	872	686	1501	1203	0.7715	0.98 (0.87–1.11)
	rs2510038	260	361	160	436	661	268	0.617	881	681	1533	1197	0.8747	0.99 (0.87–1.12)
	rs4945261	264	357	159	429	660	269	0.4285	885	675	1518	1198	0.5942	0.97 (0.85–1.10)
	rs7101429	258	355	165	427	669	273	0.3526	871	685	1523	1215	0.8232	0.99 (0.87–1.12)
	rs10793294	519	228	27	873	418	67	0.2019	1266	282	2164	552	0.0953	0.87 (0.74–1.02)
	rs4291702	264	357	156	432	660	265	0.4674	885	669	1524	1190	0.6136	0.97 (0.85–1.10)
	rs7115850	263	365	162	433	670	271	0.5133	891	689	1536	1212	0.751	0.98 (0.87–1.11)
	rs2373115	259	358	159	425	672	274	0.4068	876	676	1522	1220	0.5528	0.96 (0.85–1.09)
Positive-ε4	rs901104	294	403	154	98	127	52	0.9071	991	711	323	231	0.9743	1.00 (0.82–1.21)
	rs1385600	288	410	164	93	132	53	0.9997	986	738	318	238	0.9994	1.00 (0.82–1.21)
	rs1007837	286	407	162	93	129	52	0.9866	979	731	315	233	0.9244	0.99 (0.82–1.20)
	rs2510038	290	405	161	95	128	53	0.9637	985	727	318	234	0.9757	1.00 (0.82–1.21)
	rs4945261	285	406	159	94	129	50	0.9616	976	724	317	229	0.7902	0.97 (0.80–1.18)
	rs7101429	283	409	164	97	128	50	0.7886	975	737	322	228	0.5107	0.94 (0.77–1.14)
	rs10793294	525	290	27	162	101	10	0.6649	1340	344	425	121	0.3862	1.11 (0.88–1.40)
	rs4291702	291	403	158	93	129	50	0.9981	985	719	315	229	0.9674	1.00 (0.82–1.21)
	rs7115850	288	412	159	97	129	52	0.8926	988	730	323	233	0.8083	0.98 (0.80–1.19)
	rs2373115	284	405	162	94	128	52	0.9571	973	729	316	232	0.8382	0.98 (0.81–1.19)

Maj, major; Ht, heterozygous; Min, minor; d.f., degree of freedom.

APOE-ε4 and *GAB2* SNP rs2373115 risk (G) alleles,[8] suggesting a genetic interaction between these two genes. On the basis of these findings, we attempted here to replicate the genetic association of *GAB2* with LOAD in Japanese. In Reiman *et al*'s study,[8] neuropathologically well-characterized brains of Caucasians were largely used (LOAD, 643; control, 404), whereas we utilized only clinically confirmed subjects (LOAD, 1656; control, 1656). However, no evidence of association of this gene was obtained in the *All*, *Negative-ε4* and *Positive-ε4* sets (Table 3). *GAB2* may not be a disease susceptibility gene for LOAD in Japanese.

As a possible explanation for the discrepancy between our results and the initial study,[8] we consider an ethnic difference (Japanese *vs* Caucasian), genotyping technology (TaqMan® *vs* GeneChip® genotyping) and subject selection (clinically *vs* neuropathologically verified subjects) described above. With regard to the ethnic difference, Wright's F_{ST} statistic has been proposed for clarifying the level of between-population differentiation.[13] F_{ST} is 0.145 (estimated from 3845 SNPs) among Asian (Japanese and Chinese), African-American and European-American, and 0.013 (estimated from 8801 SNPs) between Japanese and Chinese.[14] Across the 10 *GAB2* SNPs, we calculated F_{ST} using HapMap genotype data of JPT, CHB and CEU. The mean F_{ST} of these SNPs was 0.012 (standard deviation (SD), 0.006; range, 0.000–0.025) between JPT and CHB, and 0.219 (SD, 0.077; range, 0.164–0.435) between JPT and CEU. These data indicate that a higher level of genetic differentiation exists between JPT and CEU for *GAB2*. Recently, Chapuis *et al*[9] could not replicate the initial finding[8] even in European-Caucasian subjects ($N > 3000$), suggesting that *GAB2* is at best a minor disease susceptibility gene for LOAD. A meta-analysis is needed to confirm the association of *GAB2* with LOAD.

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