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ORIGINAL ARTICLE

Replication of a genome-wide association study of panic disorder in a Japanese population

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Panic disorder (PD) is an anxiety disorder characterized by recurrent and unexpected panic attacks, subsequent worry and phobic avoidance. Although a number of association and linkage studies have been conducted, no gene has been identified as a susceptibility locus. We previously conducted a genome-wide association analysis of PD in 200 Japanese patients and the same number of controls, using a 500 K single nucleotide polymorphisms (SNPs) chip. In this study, we report a replication analysis of PD using the DigTag2 assay. The second stage sample consisted of 558 Japanese patients and 566 controls. Thirty-two markers were tested in a replication sample. As a result, no significant association was found after correction for multiple testing. However, the difference was observed at the nominal allele *P*-value <0.05 for two SNPs (rs6733840 and rs132617). We also conducted haplotype analyses of SNPs in the *APOL3* and *CLU* genes. Our results failed to show any significant association with PD in these genes. Further studies on these variants with a larger sample size may be worth testing to confirm the results.
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INTRODUCTION

Panic disorder (PD) is an anxiety disorder characterized by recurrent and unexpected panic attacks, subsequent worry and phobic avoidance. Life prevalence of PD is 1–3% and twice as many women as men suffer from the disorder.[1] The disorder frequently takes a chronic course, with many remissions and relapses.[2] Genetic epidemiological studies including family and twin studies have shown that genetic as well as environmental factors have an important role in the pathogeneses of PD. First-degree relatives of proband with PD have an approximately fivefold increased risk of PD.[3,4] Twin studies show that about 40% of the liability towards PD consists of heritable factors[5–7] However, the etiology of PD is currently unknown.

Linkage analyses of PD have implicated several chromosomal regions including 1q,[8] 2q,[9] 7p,[10,11] 9q,[12] 12q,[13] 13q,[14,15] 15q[9] and 22q.[15] In association analyses, candidate genes including *HTR 1A*,[16] *2A*,[17,18] *CCK*,[19] *ADORA2A*,[20] *MAOA*[21] and *COMT*[22,23] have been investigated. Most of the association studies were, however, of small sample size ($N<200$) and the results were controversial. PD is considered to have polygenic- or oligogenic-multifactorial etiology, and most studies to date may be underpowered to detect the modest effect size of susceptibility loci.

We previously conducted a genome-wide association study of PD in the Japanese population using a 500 K single nucleotide polymorphism (SNP) chip.[24] In the first stage, 200 patients and 200 controls were studied using the 500 K SNP chip (the GeneChip Human Mapping 500 K Array Set). A portion of the results of the first stage was previously reported.[26] In the second stage, replication analysis of the SNPs, which were suggested in a previous study, was conducted in a Japanese second sample of PD (558 patients and 566 controls) using the DigiTag2 assay.

MATERIALS AND METHODS

Subjects

All patients and control subjects in the first and second stages were ethnically Japanese and were recruited in the vicinity of Tokyo and Nagoya, Japan. Subjects in the first stage consisted of 200 PD patients (64 males and 136 females; age=39.5 ± 9.4 years, mean ± s.d.) and 200 controls (64 males and 136 females; 38.2 ± 9.3 years). Details were described in a previous paper.[24] Subjects for the second sample comprised 558 unrelated PD patients (175 males and 383

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females; age=38.9 ± 11.1 years, 252 from Tokyo and 306 from Nagoya) and 566 controls (272 males and 294 females; age=35.7 ± 12.9 years), who were recruited around Tokyo (*n*=466) and the Nagoya-Tsu area (*n*=100). For the multimarker haplotype analyses in the most susceptible genes in replication, subjects comprised 563 PD patients (177 males and 386 females; age=38.5 ± 10.8 years; second sample and other five PD patients) and 506 controls (246 males and 260 females; age=35.2 ± 13.1 years; second sample). Most of the sample overlapped with the second sample. The diagnosis was confirmed according to the DSM-IV criteria,[25] using the MINI[26] and clinical records. The controls received a short interview by one of the authors and filled out questionnaires to exclude the history of major psychiatric illness. Objective of this study was clearly explained and written informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Graduate School of Medicine, University of Tokyo.

Genome-wide association analysis of PD

In the first stage, 200 Japanese PD cases and the same number of controls were genotyped using the GeneChip Human Mapping 500 K Array Set (Affymetrix, Santa Clara, CA, USA) below for the replication in the second stage.

SNP selection

In the first stage, we employed the following quality control criteria as in Miyagawa *et al.*[27] : call rate ⩾95% in patients or controls, Hardy–Weinberg equilibrium (HWE) *P*-value ⩾0.1% in controls and minor allele frequency ⩾5% in all samples. As a result, we observed 48 SNPs on autosomal chromosomes and one SNP on X-chromosome in female subjects at the level of *P*-value $< 10^{-4}$. For the second stage, 32 SNPs were selected out of 49 as follows. Among the 49 SNPs, 29 had a minor allele frequency of ⩾10% in all subjects. Out of the 29, rs12227600 was excluded because it was located in the same linkage disequilibrium (LD) block with another of the 28 SNPs. Rs16985077 on X-chromosome was also excluded because the sample size was relatively limited and the analysis by sex may be underpowered. Thus, 27 SNPs were selected for the replication. As the DigiTag2 system simultaneously genotypes 32 SNP on one plate, five others (rs3804375, rs12579350, rs941184, rs3816995 and rs1383290) with the lowest *P*-values in the rest of the 49 SNPs (minor allele frequency ⩾5%; *P*-value $< 10^{-4}$) were also selected and studied in the replication.

Genotyping

Genomic DNA was extracted from leukocytes by using the standard phenol–chloroform method. Genotyping was conducted at the SNP-Typing Center (University of Tokyo) using the DigTag2 assay (Olympus, Tokyo, Japan) following the manufacturer's protocol. A multiplex SNP-typing method designated DigTag2 is suitable for genotyping an intermediate number of SNPs (tens to hundreds of sites) with a high conversion rate (>90%), high accuracy and low cost.[28,29] The conversion rate is defined as the proportion of successfully genotyped SNPs among the total number of SNPs examined. The reproducibility of this assay was examined by duplicate experiments, resulting 100% identical between duplicate experiments.

Haplotype analyses

We conducted additional haplotype-based analyses to augment the signals of the most associated SNPs in known genes. We examined five SNPs in *CLU* (*APOJ*) and four in *APOL3* (Figure 1). Besides rs17466684 and rs132617 investigated in the second stage, four tag SNPs (rs2279591, rs9331949, rs2279590 and rs9331888) in *CLU* (*APOJ*) and three tag SNPs (rs3827346, rs1807672, rs132660) in *APOL3* were selected from the HapMap database (www.hapmap.org). These tag SNPs were selected to include at least one SNP within each LD block, which was determined with the criteria D′ ⩾0.75 using SNPbrowser software ver.3.5 (Applied Biosystems, Foster city, CA, USA). All SNPs were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems), according to the manufacturer's protocol (Applied Biosystems),

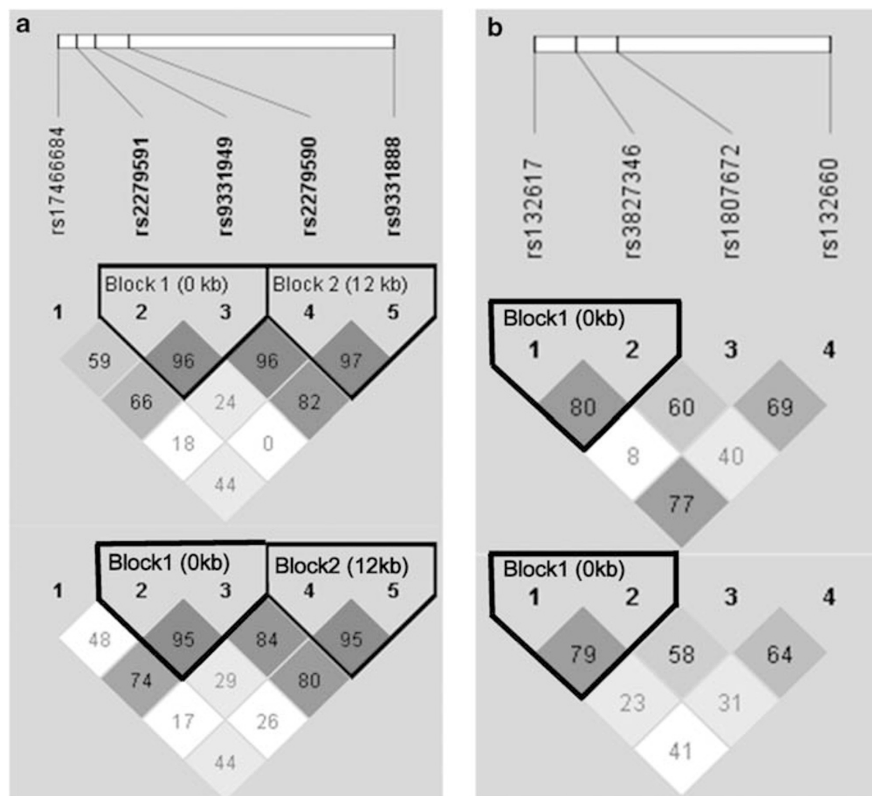

Figure 1 The strength of linkage disequilibrium (LD) between pairs of polymorphisms in patients and controls. The values of *D′* for patients are shown in the upper portion and those for controls in the lower portion. The heavy-line frames show suggested haplotype blocks. (**a**) *CLU (or APOJ)* (**b**) *APOL3*.

with ABI PRISM 7900 SDS2 Software (Applied Biosystems). To check for typing errors, we re-genotyped rs9331949 in duplicated samples. The concordance rates between the genotypes using the TaqMan were 100%.

Data analyses

Deviation from the HWE was tested using the χ^2-test. Allele and genotype frequencies of the patients and control subjects were compared using the χ^2-test. Significance for the result was set at $P<0.05$. Data analyses for replication and the TaqMan study were performed using the PLINK software package[30] (http://pngu.mgh.harvard.edu/purcell/plink/). LD and haplotype were analyzed in patients and controls who were studied using TaqMan method for all nine SNPs. D′ was used to analyze pairwise LD.[31] Haplotype block analysis was conducted using the Gabriel method.[32] The HaploView 4.1 program was used to conduct the LD and haplotype block analyses.[33] A power calculation was performed using the method as described elsewhere.[34,35]

RESULTS

Replication analysis

Three of 32 SNPs (SNP29, SNP30 and SNP31) were not successfully genotyped because of strong false-positive signals, which resulted from misligation in the encoding step. Therefore, 29 SNPs were investigated for the replication. Genotyping call rate was 99.57% in the 29 SNPs. Allele and genotype frequencies are summarized in Table 1. The genotype distributions of two SNPs were significantly deviated from the HWE (SNP16; $P=0.035$ in patients, SNP20; $P=0.024$ in controls, respectively). Other SNPs were within HWE ($P>0.05$) in both patients and controls. Significant differences were observed in the allele and genotype frequency of the SNP2 (rs860554; allele $P=0.015$, genotype $P=0.043$), SNP4 (rs6733840; allele $P=0.021$) and SNP32 (rs132617; allele $P=0.025$) between patients and controls (Tables 1 and 2), although the associations did not reach statistical significance after Bonferroni correction. Risk alleles of SNP4 and SNP32 were the same as the first sample whereas risk allele of SNP2 was different from the first sample. SNP32 (rs132617) is located within 3 kb of the *APOL3* gene and SNP4 (rs6733840) is located in an unknown gene. Four SNPs (SNP8, SNP12, SNP16 and SNP25) showed allele *P*-value between 0.05 and 0.1. Risk alleles of three SNPs (SNP12, SNP16 and SNP25) were the same as in the first sample, whereas risk allele of SNP8 was different from the first sample. SNP12 (rs17466684) is located within 1 kb of the *CLU* (or *APOJ*) gene and SNP25 (rs8013992) is located in the *STON2* gene, whereas SNP16 (rs12420498) is not located in or adjacent to any gene.

Haplotype analyses

Among seven SNPs at the allele *P*-value of <0.1 in the second sample, risk alleles of five SNPs were the same as in the first sample. Among five SNPs, three SNPs are located in known genes including *APOL3*,

Table 1 Allele and genotype frequencies of 29 SNPs successfully genotyped in the second stage between patients and controls

			Minor allele frequency			*Genotype frequency*[a]		
SNP	*dbSNP (Major/minor)*	*Chromosome*	*Patients*	*Controls*	P-value	*Patients*	*Controls*	P-*value*
1	rs2806561(A/G)	1p36	0.49	0.48	NS	0.25/0.51/0.24	0.25/0.54/0.21	NS
2	rs860554 (C/T)	1q32	0.14	0.17	0.015	0.75/0.24/0.01	0.69/0.28/0.03	0.043
3	rs6587428 (C/T)	1q44	0.41	0.43	NS	0.35/0.49/0.16	0.33/0.49/0.18	NS
4	rs6733840 (T/C)	2q14	0.18	0.22	0.021	0.67/0.30/0.03	0.60/0.35/0.04	0.059
5	rs751056 (A/G)	3p26	0.37	0.34	NS	0.40/0.46/0.14	0.44/0.45/0.12	NS
6	rs3749380 (C/T)	3p26	0.34	0.36	NS	0.40/0.46/0.14	0.41/0.46/0.13	NS
7	rs3804375 (G/A)	4q23	0.08	0.07	NS	0.85/0.15/0.01	0.87/0.13/0.00	NS
8	rs4702982 (C/T)	5q21	0.23	0.26	0.097	0.58/0.37/0.05	0.55/0.37/0.08	NS
9	rs2295767 (C/T)	6p25	0.15	0.15	NS	0.73/0.24/0.03	0.72/0.25/0.03	NS
10	rs2103868 (A/G)	6p21	0.21	0.20	NS	0.63/0.32/0.05	0.63/0.33/0.04	NS
11	rs9372078 (A/T)	6q25	0.28	0.29	NS	0.52/0.39/0.09	0.51/0.41/0.08	NS
12	rs17466684 (G/A)	8p21	0.20	0.17	0.061	0.65/0.30/0.05	0.69/0.28/0.03	NS
13	rs2016795 (G/A)	8q12	0.14	0.12	NS	0.74/0.24/0.02	0.77/0.22/0.01	NS
14	rs2118261(A/G)	8q12	0.14	0.12	NS	0.74/0.24/0.02	0.77/0.21/0.02	NS
15	rs7070456 (C/T)	10p12	0.30	0.32	NS	0.48/0.43/0.09	0.45/0.46/0.9	NS
16	rs12420498 (T/A)	11q23	0.44	0.40	0.074	0.29/0.54/0.17	0.35/0.49/0.16	0.080
17	rs12579350 (G/A)	12p13	0.13	0.12	NS	0.76/0.22/0.02	0.78/0.20/0.02	NS
18	rs11180828 (A/G)	12q12	0.18	0.18	NS	0.67/0.29/0.03	0.67/0.30/0.02	NS
19	rs2731006 (C/T)	12q12	0.34	0.36	NS	0.44/0.44/0.12	0.43/0.41/0.16	NS
20	rs2731000 (C/T)	12q12	0.34	0.36	NS	0.44/0.44/0.12	0.44/0.41/0.15	NS
21	rs941184 (C/T)	12q13	0.11	0.12	NS	0.79/0.20/0.01	0.77/0.22/0.01	NS
22	rs7299940 (C/G)	12q24	0.31	0.30	NS	0.46/0.46/0.08	0.48/0.43/0.08	NS
23	rs9302001 (C/T)	13q32	0.36	0.36	NS	0.42/0.43/0.14	0.42/0.43/0.15	NS
24	rs10872898 (G/C)	14q11	0.11	0.10	NS	0.80/0.19/0.01	0.81/0.18/0.01	NS
25	rs8013992 (G/A)	14q31	0.14	0.12	0.095	0.74/0.23/0.03	0.78/0.21/0.01	NS
26	rs3816995 (G/A)	17q25	0.16	0.15	NS	0.70/0.28/0.02	0.73/0.24/0.03	NS
27	rs1383290 (C/A)	18q12	0.11	0.11	NS	0.80/0.19/0.01	0.80/0.19/0.01	NS
28	rs11660890 (T/C)	18q23	0.47	0.48	NS	0.26/0.53/0.21	0.28/0.48/0.24	NS
32	rs132617 (C/T)	22q12.3	0.25	0.29	0.025	0.56/0.37/0.07	0.50/0.42/0.08	0.076

Abbreviation: NS, not significant
Three SNPs (SNP29, SNP30 and SNP31) were excluded for analyses because of conversion failure.
[a]Described as major homo/hetero/minor homo.

Table 2 Allele frequencies in the first and second samples of SNPs showing allele $P<0.001$ in the first sample and $P<0.1$ in the second sample

			First sample			*Second sample*			
			mAF			*mAF*			
SNP	*dbSNP (rs no.)*	*Chromosome*	*Patients*	Controls	P-*value*	Patients	*Controls*	P-*value*	*Gene*
2[a]	rs860554	1q32	0.17	0.05	4.68×10^{-8}	0.14	0.17	0.015	*PKP1*
4	rs6733840	2q14	0.13	0.24	6.02×10^{-5}	0.18	0.22	0.021	Unknown
8[a]	rs4702982	5q21	0.29	0.16	6.23×10^{-6}	0.23	0.26	0.097	—
12	rs17466684	8p21	0.21	0.09	6.82×10^{-7}	0.20	0.17	0.061	*CLU (APOJ)*
16	rs12420498	11q23	0.47	0.33	5.20×10^{-5}	0.44	0.40	0.074	—
25	rs8013992	14q31	0.18	0.06	1.45×10^{-5}	0.14	0.12	0.095	*STON2*
32	rs132617	22q12.3	0.21	0.34	7.16×10^{-5}	0.25	0.29	0.025	*APOL3*

Abbreviation: mAF, minor allele frequency.
[a]Risk alleles of SNP2 and SNP8 were different between the first and second samples.

Table 3 Allele and genotype frequencies of SNPs in the *CLU* and *APOL3* genes

		Minor allele frequency			*Genotype frequency*[a]		
Locus (Chr)	*dbSNP (Major/Minor)*	*Patients*	*Controls*	P-*value*	*Patients*	*Controls*	P-*value*
CLU	rs17466684 (G/A)	0.20	0.16	0.02	0.65/0.31/0.04	0.71/0.27/0.02	0.06
(8p21)	rs2279591 (T/C)	0.25	0.26	0.96	0.56/0.37/0.07	0.56/0.37/0.07	0.96
	rs9331949 (T/C)	0.21	0.21	0.82	0.63/0.33/0.05	0.60/0.37/0.03	0.21
	rs2279590 (C/T)	0.27	0.28	0.60	0.54/0.39/0.08	0.53/0.39/0.09	0.84
	rs9331888 (C/G)	0.43	0.40	0.18	0.33/0.47/0.20	0.36/0.48/0.16	0.32
APOL3	rs132617 (C/T)	0.25	0.29	0.03	0.56/0.38/0.06	0.50/0.41/0.09	0.10
(22p12.3)	rs3827346 (G/A)	0.34	0.37	0.19	0.42/0.47/0.11	0.39/0.48/0.13	0.42
	rs1807672 (G/T)	0.17	0.16	0.81	0.70/0.28/0.03	0.70/0.28/0.02	0.88
	rs132660 (T/G)	0.13	0.13	0.76	0.75/0.24/0.01	0.76/0.23/0.01	0.83

[a]Described as major homo/hetero/minor homo.

CLU (or *APOJ*) and *STON2*. We selected *APOL3* and *CLU* (or *APOJ*) in which SNPs showed significant or marginally significant allele *P*-values. Allele and genotype frequencies of SNPs in the *APOL3* and *CLU* genes between patients and controls were shown in Table 3. The genotype distributions of rs9331949 in controls were nominally deviated from the HWE (P=0.045). Other SNPs were within HWE ($P>0.05$) in both patients and controls. Significant differences were observed in allele frequencies of rs17466684 and rs132617 (P=0.019 and 0.033, respectively), although the statistical level became insignificant after correction for multiple testing. No significant difference was observed in allele frequencies of other SNPs or genotype frequencies of all SNPs between patients and controls.

The strength of LD denoted D′ between pairs of polymorphisms is shown in Figure 1. The LD pattern was broadly similar in the patient and control groups. In haplotype block analysis, two blocks consisting of SNP2–SNP3 (rs2279591-rs9331949) and SNP4–SNP5 (rs2279590-rs9331888) were suggested in *CLU*; and a block consisting of SNP1–SNP2 (rs132617-rs3827346) in *APOL3* was suggested to be in strong LD ($D'\geqslant0.75$). We then compared frequencies of the suggested three haplotypes between patients and controls in addition to two haplotypes consisting of all polymorphisms in each gene. A significant difference was observed in the frequency of the haplotype consisting of all polymorphisms between patients and controls in *CLU* (ATTCG: P=0.035; Table 4), whereas the statistical level became insignificant after correction for multiple testing. No significant difference was observed in frequencies of other haplotypes between both patients and controls (Table 4).

DISCUSSION

We conducted a two-stage association analysis of PD in the Japanese population. Genome-wide association analysis was conducted in the first stage and 32 candidate SNPs according to the first stage result were tested in the second stage using the DigiTag2 assay. Among 32 SNPs selected for replication, 29 SNPs were successfully genotyped. Although we did not find a significant association after Bonferroni correction, two SNPs (rs6733840 and rs132617) were associated with PD at 5% level with the same risk allele as in the first sample. We also conducted haplotype analyses of SNPs in the *APOL3* and *CLU* genes. Our results failed to show any significant association with PD in either a single SNP or haplotypes in these genes. However, rs132617 in the *APOL3* gene and rs17466684 in the *CLU* gene were associated with PD at 5% level. The haplotype ATTCG in the *CLU* gene was nominally associated with PD.

APOL proteins might be involved in lipid transport and metabolism. Six *APOL* genes (*APOL*1-6) are located near each other on the 22q12 locus. The cholesterol content of intracellular membranes of neurons might be involved in modulating gene transcription and signal transduction in the brain during neurodevelopment and in the

Table 4 Haplotype frequencies according to haplotypes in each blocks and all polymorphisms of the *CLU* and *APOL3* genes

	Haplotype	Frequencies[a] Patients	Controls	P-value
CLU				
SNP2-SNP3				
1	TT	0.54	0.53	0.77
2	CT	0.25	0.25	0.84
3	TC	0.21	0.21	0.88
SNP4-SNP5				
1	CG	0.43	0.40	0.14
2	CC	0.30	0.32	0.28
3	TC	0.27	0.28	0.63
SNP1-SNP2-SNP3-SNP4-SNP5				
1	GTTCG	0.20	0.22	0.26
3	GTCCC	0.19	0.19	0.89
3	ATTCG	0.13	0.10	0.04
4	GCTCC	0.12	0.13	0.29
5	GTTTC	0.11	0.12	0.64
6	GCTCG	0.09	0.07	0.13
7	GTTCC	0.07	0.08	0.41
APOL3				
SNP1-SNP2				
1	CG	0.63	0.59	0.12
2	TA	0.22	0.25	0.07
3	CA	0.12	0.11	0.62
SNP1-SNP2-SNP3-SNP4				
1	CGGT	0.58	0.55	0.13
2	TAGT	0.17	0.19	0.23
3	TATT	0.05	0.06	0.26

[a]Haplotypes whose frequencies were estimated >5% were described.

adult.[36] A postmortem study using the candidate gene cDNA array showed the upregulations of *APOL1*, *APOL2* and *APOL4* in the prefrontal cortex of schizophrenic patients,[37] whereas recent association study showed no association of this gene cluster with schizophrenia.[38] In this study, SNP rs132617 was nominally associated with PD. In haplotype analysis, we observed a trend of decrease in the frequency of the haplotype TA (s132617-rs3827346) in PD patients (P=0.07). The result suggested that this haplotype might be protective against PD.

Furthermore, Clusterin, which is a protein encoded by the *CLU* (or *APOJ*) gene located on chromosome 8p21, might function in membrane lipid recycling.[39] It may also be involved with ongoing synapse turnover.[40] *APOJ* might regulate the process of amyloid-β deposition in the brain of Alzheimer's disease.[41] Some studies suggested a correlation between cholesterol levels and the intensity and frequency of panic attacks reported by PD patients.[42,43] In our analyses, trends of increase in the frequencies of the allele A (rs17466684) and the haplotype ATTCG (rs17466684-rs9331888) in PD patients were observed. The results suggested that these, SNPs and haplotypes, might be a risk factor of PD. Previous findings from animal studies suggested that Clusterin increases post-ischemic damage and may exert a negative effect on the structural integrity and functionality of hippocampal neurons.[44]

Several limitations may be noted in this study. First, sample size (558 patients and 566 controls) might not be adequate. The second sample has statistical power of >0.80 (alpha=0.05) for the detection of the role of the polymorphism with minor allele frequency of 0.1, when the genotype relative risk is >1.52. A larger sample is requested for the detection of genes with a smaller effect than this. Second, proportions of the subjects from Tokyo and those from the Nagoya-Tsu area were not same between patients and controls. However, all subjects were ethnically Japanese and minor allele frequencies of significant SNPs were not different between the subjects from Tokyo and those from the Nagoya-Tsu area (data not shown).

In conclusion, we conducted a replication analysis of a genome-wide association study of PD in the Japanese population. As a result, no significant association with PD was found after correction for multiple testing. However, the difference was observed at the nominal allele P-value <0.05 for two SNPs (rs6733840 and rs132617). We also conducted haplotype analyses of SNPs in the *APOL3* and *CLU* genes. Our results failed to show any significant association with PD in these genes. Further studies on these variants with a larger sample size may be worth testing to confirm the results.

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1 Eaton, W. W., Kessler, R. C., Wittchen, H. U. & Magee, W. J. Panic and panic disorder in the United States. *Am. J. Psychiatry* **151,** 413–420 (1994).
2 Weissman, M. M., Bland, R. C., Canino, G. J., Faravelli, C., Greenwald, S., Hwu, H. G. *et al.* The cross-national epidemiology of panic disorder. *Arch. Gen. Psychiatry* **54,** 305–309 (1997).
3 Crowe, R. R., Noyes, R., Pauls, D. L. & Slymen, D. A family study of panic disorder. *Arch. Gen. Psychiatry* **40,** 1065–1069 (1983).
4 Goldstein, R. B., Wickramaratne, P. J., Horwath, E. & Weissman, M. M. Familial aggregation and phenomenology of 'early'-onset (at or before age 20 years) panic disorder. *Arch. Gen. Psychiatry* **54,** 271–278 (1997).
5 Hettema, J. M., Neale, M. C. & Kendler, K. S. A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am. J. Psychiatry* **158,** 1568–1578 (2001).
6 Kendler, K. S., Neale, M. C., Kessler, R. C., Heath, A. C. & Eaves, L. J. Major depression and generalized anxiety disorder. Same genes, (partly) different environments? *Arch. Gen. Psychiatry* **49,** 716–722 (1992).
7 Torgersen, S. Genetics of neurosis. The effects of sampling variation upon the twin concordance ratio. *Br. J. Psychiatry* **142,** 126–132 (1983).
8 Gelernter, J., Bonvicini, K., Page, G., Woods, S. W., Goddard, A. W., Kruger, S. *et al.* Linkage genome scan for loci predisposing to panic disorder or agoraphobia. *Am. J. Med. Genet.* **105,** 548–557 (2001).
9 Fyer, A. J., Hamilton, S. P., Durner, M., Haghighi, F., Heiman, G. A., Costa, R. *et al.* A third-pass genome scan in panic disorder: evidence for multiple susceptibility loci. *Biol. Psychiatry* **60,** 388–401 (2006).
10 Knowles, J. A., Fyer, A. J., Vieland, V. J., Weissman, M. M., Hodge, S. E., Heiman, G. A. *et al.* Results of a genome-wide genetic screen for panic disorder. *Am. J. Med. Genet.* **81,** 139–147 (1998).
11 Crowe, R. R., Goedken, R., Samuelson, S., Wilson, R., Nelson, J., Noyes, Jr. R. *et al.* Genomewide survey of panic disorder. *Am. J. Med. Genet.* **105,** 105–109 (2001).
12 Thorgeirsson, T. E., Oskarsson, H., Desnica, N., Kostic, J. P., Stefansson, J. G., Kolbeinsson, H. *et al.* Anxiety with panic disorder linked to chromosome 9q in Iceland. *Am. J. Hum. Genet.* **72,** 1221–1230 (2003).
13 Smoller, J. W., Acierno, J. S. Jr., Rosenbaum, J. F., Biederman, J., Pollack, M. H., Meminger, S. *et al.* Targeted genome screen of panic disorder and anxiety disorder proneness using homology to murine QTL regions. *Am. J. Med. Genet.* **105,** 195–206 (2001).
14 Weissman, M. M., Fyer, A. J., Haghighi, F., Heiman, G., Deng, Z., Hen, R. *et al.* Potential panic disorder syndrome: clinical and genetic linkage evidence. *Am. J. Med. Genet.* **96,** 24–35 (2000).

15 Hamilton, S. P., Fyer, A. J., Durner, M., Heiman, G. A., Baisre de Leon, A., Hodge, S. E. *et al*. Further genetic evidence for a panic disorder syndrome mapping to chromosome 13q. *Proc. Natl. Acad. Sci. USA* **100,** 2550–2555 (2003).
16 Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Mossner, R., Franke, P. *et al*. Association of a functional 1019C>G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. *Int. J. Neuropsychopharmacol.* **7,** 189–192 (2004).
17 Inada, Y., Yoneda, H., Koh, J., Sakai, J., Himei, A., Kinoshita, Y. *et al*. Positive association between panic disorder and polymorphism of the serotonin 2A receptor gene. *Psychiatry Res.* **118,** 25–31 (2003).
18 Rothe, C., Koszycki, D., Bradwejn, J., King, N., De Luca, V., Shaikh, S. *et al*. Association study of serotonin-2A receptor gene polymorphism and panic disorder in patients from Canada and Germany. *Neurosci. Lett.* **363,** 276–279 (2004).
19 Ebihara, M., Ohba, H., Hattori, E., Yamada, K. & Yoshikawa, T. Transcriptional activities of cholecystokinin promoter haplotypes and their relevance to panic disorder susceptibility. *Am. J. Med. Genet.* **118,** 32–35 (2003).
20 Deckert, J., Nothen, M. M., Franke, P., Delmo, C., Fritze, J., Knapp, M. *et al*. Systematic mutation screening and association study of the A1 and A2a adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. *Mol. Psychiatry* **3,** 81–85 (1998).
21 Deckert, J., Catalano, M., Syagailo, Y. V., Bosi, M., Okladnova, O., Di Bella, D. *et al*. Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. *Hum. Mol. Genet.* **8,** 621–624 (1999).
22 Hamilton, S. P., Slager, S. L., Heiman, G. A., Deng, Z., Haghighi, F., Klein, D. F. *et al*. Evidence for a susceptibility locus for panic disorder near the catechol-O-methyltransferase gene on chromosome 22. *Biol. Psychiatry* **51,** 591–601 (2002).
23 Woo, J. M., Yoon, K. S., Choi, Y. H., Oh, K. S., Lee, Y. S. & Yu, B. H. The association between panic disorder and the L/L genotype of catechol-O-methyltransferase. *J. Psychiatr. Res.* **38,** 365–370 (2004).
24 Otowa, T., Yoshida, E., Sugaya, N., Yasuda, S., Nishimura, Y., Inoue, K. *et al*. Genome-wide association study of panic disorder in the Japanese population. *J. Hum. Genet.* **54,** 122–126 (2009).
25 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* 4th ed. (American Psychiatric Association, Washington DC, 1994).
26 Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E. *et al*. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* **59** (Suppl 20), 22–33; Quiz 34–57 (1998).
27 Miyagawa, T., Nishida, N., Ohashi, J., Kimura, R., Fujimoto, A., Kawashima, M. *et al*. Appropriate data cleaning methods for genome-wide association study. *J. Hum. Genet.* **53,** 886–893 (2008).
28 Nishida, N., Tanabe, T., Hashido, K., Hirayasu, K., Takasu, M., Suyama, A. *et al*. DigiTag assay for multiplex single nucleotide polymorphism typing with high success rate. *Anal. Biochem.* **346,** 281–288 (2005).
29 Nishida, N., Tanabe, T., Takasu, M., Suyama, A. & Tokunaga, K. Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay. *Anal. Biochem.* **364,** 78–85 (2007).
30 Purcell, S., Neal, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559–575 (2007).
31 Lewontin, R. C. The Interaction of Selection and Linkage. I. General Considerations; Heterotic Models. *Genetics* **49,** 49–67 (1964).
32 Gabriel, S. B., Schaffner, S. F., Nguyen, H., Moore, J. M., Roy, J., Blumenstiel, B. *et al*. The structure of haplotype blocks in the human genome. *Science* **296,** 2225–2229 (2002).
33 Barret, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–265 (2005).
34 Ohashi, J., Yamamoto, S., Tsuchiya, N., Hatta, Y., Komata, T., Matsushita, M. *et al*. Comparison of statistical power between 2 * 2 allele frequency and allele positivity tables in case-control studies of complex disease genes. *Ann. Hum. Genet.* **65,** 197–206 (2001).
35 Ohashi, J. & Tokunaga, K. The expected power of genome-wide linkage disequilibrium testing using single nucleotide polymorphism markers for detecting a low-frequency disease variant. *Ann. Hum. Genet.* **66,** 297–306 (2002).
36 Vanhollebeke, B. & Pays, E. The function of apolipoproteins L. *Cell Mol. Life. Sci.* **63,** 1937–1944 (2006).
37 Mimmack, M. L., Ryan, M., Baba, H., Navarro-Ruiz, J., Iritani, S., Faull, R. L. *et al*. Gene expression analysis in schizophrenia: reproducible up-regulation of several members of the apolipoprotein L family located in a high-susceptibility locus for schizophrenia on chromosome 22. *Proc. Natl. Acad. Sci. USA.* **99,** 4680–4685 (2002).
38 McGhee, K. A., Morris, D. W., Schwaiger, S., Nangle, J. M., Donohoe, G., Clarke, S. *et al*. Investigation of the apolipoprotein-L (APOL) gene family and schizophrenia using a novel DNA pooling strategy for public database SNPs. *Schizophr. Res.* **76,** 231–238 (2005).
39 Jones, S. E. & Jomary, C. Clusterin. *Int. J. Biochem. Cell Biol.* **34,** 427–431 (2002).
40 Danik, M., Chabot, J. G., Hassan-Gonzalez, D., Suh, M. & Quirion, R. Localization of sulfated glycoprotein-2/clusterin mRNA in the rat brain by *in situ* hybridization. *J. Comp. Neurol.* **334,** 209–227 (1993).
41 DeMattos, R. B., Cirrito, J. R., Parsadanian, M., May, P. C., O'Dell, M. A., Taylor, J. W. *et al*. ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism *in vivo*. *Neuron* **41,** 193–202 (2004).
42 Hayward, C., Taylor, C. B., Roth, W. T., King, R. I & Agras, W. S. Plasma lipid levels in patients with panic disorder or agoraphobia. *Am. J. Psychiatry* **146,** 917–919 (1989).
43 Agargun, M. Y., Kara, H., Algun, E., Sekeroglu, R. & Trakcioglu, M. High cholesterol levels in patients with sleep panic. *Biol. Psychiatry.* **40,** 1064–1065 (1996).
44 Hakkoum, D., Imhof, A., Vallet, P. G., Boze, H., Moulin, G., Charnay, Y. *et al*. Clusterin increases post-ischemic damages in organotypic hippocampal slice cultures. *J. Neurochem.* **106,** 1791–1803 (2008).