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SHORT COMMUNICATION

An association analysis of *Per2* with panic disorder in the Japanese population

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Panic disorder (PD) is a severe and chronic psychiatric disorder, with genetic components underlying in its etiology. The *PERIOD2* (*Per2*) gene has been reported to be associated with familial advanced sleep phase syndrome. Considering the high frequency of sleep disturbance in PD, *Per2* may be a candidate gene for PD. Therefore, we conducted a two-stage case–control association study in the Japanese population. In the first screening sample of 203 patients and 409 controls, we investigated three single-nucleotide polymorphisms in *Per2*. We found a potential association in the screening sample (rs2304672, genotype *P*=0.046, uncorrected), whereas we could not replicate the association in the second sample of 460 patients and 460 controls. Our results suggest that *Per2* may not have a major role in the pathogenesis of PD in the Japanese population.
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Panic disorder (PD) is an anxiety disorder characterized by panic attacks and anticipatory anxiety, with a lifetime prevalence of 1–3% and a female:male ratio of 2:1.[1] PD frequently takes a chronic course, with many remissions and relapses, occasionally complicated by comorbidity with other psychiatric disorders, such as agoraphobia and major depression.[2] Twin and family studies have provided evidence for both genetic and environmental factors, contributing to susceptibility to PD.[3,4] Heritability of PD has been estimated about 40% on the basis of the meta-analyses of twin studies.[5] Because of the phenotypic and genetic heterogeneity of PD and the small effect size of individual variants, only a few candidate genes have been identified so far.[6]

Sleep disturbance is frequently present among psychiatric patients and has been incorporated in the diagnostic criteria for major depression, post-traumatic stress disorder and generalized anxiety disorder. Several studies demonstrated that individuals with PD report significant sleep disturbances.[7,8] The sleep–wake cycle, as well as other rhythmic variations in physiological and metabolic processes (hormone production, feeding behavior and brain activity), is generated by an endogenous pacemaker. In mammals, the principal circadian clock, which coordinates peripheral clocks in the body, is located in the suprachiasmatic nucleus and produces a nearly 24-h cycle through interacting positive/negative feedback loops. It is comprised of the positive regulators (CLOCK/ARNTL complexes) and the negative regulators (PER1, PER2, PER3, CRY1 and CRY2).[9]

A polymorphism of *PERIOD2* (*Per2*) has been reported to be associated with familial advanced sleep phase syndrome.[10] Carpen *et al.*[11] founded that the 111G allele of *Per2* (rs2304672) was associated with extreme morning preference. Lee *et al.*[12] reported that the G3853A single-nucleotide polymorphism (SNP) of *Per2* (rs934945) was associated with diurnal preference in Korean subjects. *Per2* was nominally associated with bipolar disorder,[13] whereas no associations of *Per2* with mood disorders and anxiety disorders have also been observed.[14,15] To find the possible involvement of *Per2* in PD, we conducted a two-stage case–control association study in the Japanese population.

The study was approved by the Ethical Committee of the Graduate School of Medicine, University of Tokyo. The objective of the present study was clearly explained and written informed consent was obtained from all subjects. All subjects were ethnically Japanese and were recruited in Tokyo and Nagoya, which are located in the main island of Japan. The first screening sample comprised of 203 unrelated PD patients (75 men and 128 women; age=38.8 ± 9.3 years (mean ± s.d.)) recruited from clinics for anxiety, and 409 unrelated healthy volunteers (165 men and 244 women; age=34.8 ± 10.7 years) served as controls. The replication sample consisted of 460 PD patients

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Table 1 Genotype and allele frequencies of the three SNPs in the *Per2* gene in the first screening sample

						Genotype distribution			
db SNP ID (M/m)	*Chromosome position (bp)*	*Phenotype*	n	*MAF*	P-*value*	M/M	*M/m*	*m/m*	P-*value*
rs934945	238819792	Patients	203	0.32	0.49	88	102	13	0.22
(G/A)		Controls	409	0.34		178	188	43	
rs2304674	238846642	Patients	203	0.27	0.84	105	85	13	0.55
(T/C)		Controls	409	0.28		216	158	35	
rs2304672	238851327	Patients	203	0.067	0.48	179	21	3	**0.046**
(C/G)		Controls	409	0.056		363	46	0	

Abbreviations: M, major allele; m, minor allele; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.
P-value in bold indicates $P<0.05$.

(143 men and 317 women; age=39.0 ± 11.1 years) and 460 controls (184 men and 276 controls; age=39.9 ± 10.9 years). The diagnosis of PD was confirmed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria and by using the Mini-International Neuropsychiatric Interview[16] and clinical records. The controls received a short interview by one of the authors and filled out questionnaires to exclude history of major psychiatric illness.

Genomic DNA was extracted from leukocytes by using the standard phenol–chloroform method. We selected two SNPs rs934945 and rs2304672 investigated in the previous studies.[11,14] We further added a tagging SNP (rs2304674) with minor allele frequency of 5% more in the Japanese population (HapMap database: http://www.hapmap.org), using HaploView 4.2 program.[17] Genotyping was performed using the ABI PRISM 7900HT Sequence Detection System according to the protocol of the manufacturer (Applied Biosystems, Foster City, CA, USA).

Hardy–Weinberg equilibrium was checked using χ^2-test. The allele and genotype frequencies of the patients and controls were compared using χ^2-test. Genotype data were analyzed using HaploView 4.2 program.[17] The expectation-maximization algorithm was used to estimate haplotype frequencies. The linkage disequilibrium block was defined by the Gabriel method.[18] D' was used to analyze pairwise linkage disequilibrium. Power calculation was performed using Genetic Power Calculator.[19] Significance for the result was set at $P<0.05$.

The genotype and allele frequencies of three SNPs in the *Per2* gene in the first screening sample are summarized in Table 1. Genotype frequencies of the SNPs rs934945 and rs2304672 in patients deviate from Hardy–Weinberg equilibrium (P=0.023 and 0.045, respectively). Genotype frequencies of rs2304674 in patients and all SNPs in controls were within Hardy–Weinberg equilibrium. Significant difference was found in the genotype frequencies of rs2304672 between patients and controls (P=0.046). No significant difference between patients and controls was observed in the genotype or allele frequency of the other SNPs. When the data were analyzed by sex, no significant difference was found between cases and controls (data not shown). Pairwise linkage disequilibrium (D') between the markers was between 0.98 and 1.0. The three SNPs were suggested to be in strong linkage disequilibrium. In the three-SNP haplotype analysis, no significant association was found between patients and controls (Table 2). To confirm the potential association between rs2304672 and PD, we examined the SNP in the second replication sample. The genotype distributions were within Hardy–Weinberg equilibrium in both patients and controls. The association between rs2304672 and PD was not replicated in the replication sample or in a combined sample (Table 3).

Table 2 Haplotype analyses of the *Per2* gene

	Frequency		
Haplotype	*Patients*	*Controls*	P-*value*
GTC	0.41	0.39	0.42
ATC	0.32	0.33	0.52
GCC	0.21	0.22	0.57
GCG	0.067	0.056	0.48

Haplotypes whose frequencies were estimated >5% were described.

Table 3 Genotype and allele frequencies of rs2304672 in the second replication and combined samples

					Genotype distribution			
Sample	*Phenotype*	n	*MAF*	P-*value*	*C/C*	*C/G*	*G/G*	P-*value*
Replication	Patients	460	0.064	0.63	402	57	1	0.88
	Controls	460	0.059		407	52	1	
Combined	Patients	663	0.065	0.40	581	78	4	0.24
	Controls	869	0.058		770	98	1	

Abbreviation: MAF, minor allele frequency.

In this study, we found nominally significant association between *Per2* and PD in the screening sample, whereas it was not significant after Bonferroni correction. In addition, the association was not replicated in the replication sample. Our results are consistent with previous studies that found no association between *Per2* and anxiety disorders, including PD in a Finnish population[15] and PD in our previous genome-wide association study[20] (rs2304672, allele P=0.44 and genotype P=0.71). One possible reason for this is the small magnitude of effect size, which is likely to be typical of single gene effects on complex phenotypes. The statistical power of the screening sample size was calculated as 0.63 at the level of α=0.05 (minor allele frequency=0.1, odds ratio=1.5). Hence, we could not exclude the possibility that other SNPs might be associated with PD in the screening sample. To detect an association between a risk allele with smaller effect and PD, studies with larger sample size would be needed. Because haplotype has a higher level of heterozygosity than individual single SNPs, the association study based on the haplotype has an increased power for detecting disease association compared with SNP-based analysis. However, we could not find the association between the three-SNP haplotype and PD in the screening sample.

In conclusion, this study suggest that *Per2* may not have a major role in susceptibility to PD in the Japanese population.

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