Positive association of the pericentrin (*PCNT*) gene with major depressive disorder in the Japanese population

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Background: Pericentrin (*PCNT*) interacts with disruption-in-schizophrenia 1 (*DISC1*), a known genetic risk factor for schizophrenia, bipolar disorder and major depressive disorder (MDD). We sought to determine whether the *PCNT* gene is implicated in MDD. **Methods:** We performed case–control association analyses in the Japanese population. We analyzed 9 single nucleotide polymorphisms (SNPs) in 173 patients with MDD and 348 healthy controls. **Results:** We found a significant allelic association between 3 SNPs (rs3788265, rs2073376 and rs2073380) of the *PCNT* gene and MDD ($p = 0.006$, 0.005 and 0.021, respectively). After correction for multiple testing, 2 SNPs (rs3788265 and rs2073376) retained significant allelic associations with MDD. In addition, we found a significant association between the 2 marker haplotypes (r3788265 and rs2073376) and MDD (permutation $p = 0.011$). **Limitations:** Our sample was small and comprised only Japanese participants. In addition, owing to the late onset of MDD, it is possible that the disorder will develop in at least some participants in our control group. Finally, we did not show how SNPs of the *PCNT* gene alter its function. **Conclusion:** Our results suggest that genetic variations in the *PCNT* gene may play a significant role in the etiology of MDD in the Japanese population.

Contexte : La péricentrine interagit avec un facteur reconnu de risque génétique de schizophrénie, de trouble bipolaire et de trouble dépressif majeur, le *DISC1* (pour disruption-in-schizophrenia 1). Nous avons voulu vérifier si le gène de la péricentrine est associé au trouble dépressif majeur. **Méthodes :** Nous avons effectué des analyses d'association cas–témoins dans la population japonaise. Nous avons analysé 9 polymorphismes de nucléotides simples (PNS) chez 173 patients atteints de trouble dépressif majeur et chez 348 témoins en bonne santé. **Résultats :** Nous avons découvert une association allélique significative entre 3 PNS (le rs3788265, le rs2073376 et le rs2073380) du gène de la péricentrine et le trouble dépressif majeur ($p = 0{,}006$, 0,005 et 0,021, respectivement). Après correction pour tests d'hypothèses multiples, 2 PNS (le rs3788265 et le rs2073376) maintenaient des liens alléliques significatifs avec le trouble dépressif majeur. De plus, nous avons observé un lien entre les 2 haplotypes marqueurs (le r3788265 et le rs2073376) et le trouble dépressif majeur (permutation $p = 0{,}011$). **Limites :** Notre échantillon était de petite taille et les participants étaient tous japonais. En outre, compte tenu du déclenchement tardif du trouble dépressif majeur, il est possible que la maladie frappe éventuellement au moins quelques-uns des participants de notre groupe témoin. Finalement, nous n'avons pas démontré de quelle façon les PNS du gène de la péricentrine en modifient la fonction. **Conclusion :** Selon nos résultats, les variations génétiques du gène de la péricentrine pourraient jouer un rôle important dans l'étiologie du trouble dépressif majeur chez la population japonaise.

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Introduction

The lifetime population prevalence for major depressive disorder (MDD) is 5%–10%. Heritability based on twin studies is 40%–50%, and adoption studies provide some support for a role for genetic factors in MDD.[1] The pericentrin (*PCNT*) gene, also called the kendrin gene, is located at 21q22.3, a region of susceptibility for mood disorders.[2,3]

Disrupted-in-schizophrenia 1 (*DISC1*) is an important genetic risk factor for mental disorders such as schizophrenia,[4,5] bipolar disorder[6] and MDD.[7] It localizes to the centrosome by binding to *PCNT*, and *PCNT* anchors the γ-tubulin complex to the centrosome, providing microtubule nucleation sites. Thus *DISC1–PCNT* interaction might be involved in the pathophysiology of mental disorders owing to their putative effect on centrosomal function.[8]

Recently, Anitha and colleagues[9] reported that significantly higher expression of *PCNT* was observed in brain samples of patients with bipolar disorder compared with controls and that the mRNA expression of the *PCNT* gene in peripheral blood lymphocytes was significantly higher in patients with bipolar disorder and MDD than in controls.

Taken together, these findings suggest that the *PCNT* gene may be a candidate gene in MDD. We sought to determine whether the *PCNT* gene is implicated in MDD in the Japanese population.

Methods

Participants

We recruited biologically unrelated Japanese patients from Tokushima University and the Ehime University Hospital in Japan. At least 2 experienced psychiatrists (S.N., J.I., M.N., S.U., and T.O.) diagnosed MDD according to DSM-IV criteria[10] on the basis of extensive clinical interviews and a review of medical records. All patients underwent medical, neurologic, psychological and laboratory evaluation before participating in this study. We excluded patients with organic disorders or with other comorbid psychiatric disturbances (e.g., alcohol or substance abuse). We recruited volunteers for the control group among hospital staff, students and company employees. We included those who were genetically unrelated residents living in Japan with no personal or family history (among first-degree relatives) of mental disorders. For the genetic studies, we obtained genomic DNA samples from the participants. All participants provided written informed consent, and the institutional ethics committees approved our genetic association studies.

Genetic analysis

We performed the genotyping using commercially available TaqMan probes for the *PCNT* gene with the 7500 Fast Real-Time PCR System, according to the protocol recommended by the manufacturer (Applied Biosystems). We selected 8 tagging single nucleotide polymorphisms (SNPs) using SNP Browser 3.5 (Applied Biosystems; pair-wise $r^2 > 85\%$, minor allele frequency > 20%, Japanese population): rs11702684, rs2249057, rs11701058, rs2839226, rs2839231, rs3788265, rs2073376 and rs1010111. We additionally selected rs2073380, because the 8 tagging SNPs did not cover the third block of the *PNCT2* gene from HapMap data. We determined haplotype block structure using the Haploview program.[11] Blocks were defined according to the criteria set by Gabriel and colleagues.[12]

Statistical analysis

We compared the allelic and genotypic frequencies of patients and controls using the Fisher exact test. In single-marker analyses, we used the program SNPSpD,[13] which is able to reflect the correlation of markers (linkage-disequilibrium) on corrected p values, to control inflation of the type I error rate. We used SNPAlyze 3.2Pro software (Dynacom) to estimate haplotype frequencies, linkage-disequilibrium, permutation p values (10 000 replications) and deviation from Hardy–Weinberg distribution of alleles. We calculated pair-wise linkage-disequilibrium indices (D´ and r^2) for the control group. We performed power calculations for our sample size using the G*Power program.[14] We set statistical significance at $p < 0.05$ for all tests.

Results

Participants

We included 173 unrelated Japanese patients with MDD (74 men, mean age 45.1, standard deviation [SD] 12.4 yr; 99 women, mean age 45.0, SD 15.3 yr). The characteristics of patients with MDD are shown in Table 1. We included 348 healthy controls (148 men, mean age 45.1, SD 12.4 yr; 200 women, mean age 44.9, SD 13.0 yr) recruited from hospital staff, students and company employees.

We genotyped 9 SNPs in the *PCNT* gene (Table 2). Genotypic distributions of these 9 SNPs did not deviate significantly from Hardy–Weinberg equilibrium in the control group. The values of absolute D´ and r^2 for the healthy controls are presented in Figure 1. There were 3 linkage-disequilibrium blocks[12] in the *PCNT* gene, with rs2249057, rs11701058, rs2839226 and rs2839231 residing in block 1, rs3788265 and rs2073376 residing in block 2 and rs2073380 and rs1010111 residing in block 3 (Fig. 1). We observed significant differences in allelic frequencies between patients with MDD and controls for 3 SNPs (rs3788265, rs2073376 and

Table 1: Demographic data and clinical characteristics of patients with major depressive disorder and controls

Characteristic	MDD	Control
Age, mean (SD) [range], yr	45.1 (14.1) [21–79]	45.0 (12.7) [18–71]
Sex, male:female	74:99	148:200
No. of episodes, single:recurrent	92:81	—
Age at onset, mean (SD), yr	41.0 (13.7)	—
Family history, yes:no	52:121	0:348

MDD = major depressive disorder; SD = standard deviation.

rs2073380) in intron29, exon38 and exon45 (p = 0.006, 0.005 and 0.021, respectively). The T allele of rs3788265, the G allele of rs2073376 and the C allele of rs2073380 occurred more frequently in the MDD group than in the control group. After applying the SNPSpD software to correct for multiple testing, 2 SNPs (rs3788265 and rs2073376) retained significant allelic associations with MDD. The control minor allele frequency of rs2073376 in our study was almost the same as that in the study by Anitha and colleagues[9] involving Japanese participants (33% in our study v. 31.7% that of Anitha and colleagues[9]). Next, we performed haplotype analyses. We found a significant association between the 2 marker haplotypes (r3788265 and rs2073376) and MDD (permutation p = 0.011) (Table 3). In power calculations using the G*Power program, our sample size had a post-hoc power of 0.99 to detect an effect size of 0.5 (moderate) at the 0.05 significance level (2-tailed).

Table 2: Allele frequencies of 9 single nucleotide polymorphisms in the *PCNT* gene in patients with major depressive disorder and controls

SNP; group	Allele, no.		*p* value*	Frequency†
rs11702684 Intron 9	C	T		
MDD	195	151	0.12	0.44
Control	427	267		0.39
rs2249057 Exon 10	C	A		
MDD	179	159	0.18	0.47
Control	397	295		0.43
rs11701058 Intron 12	C	T		
MDD	185	161	0.10	0.54
Control	333	363		0.48
rs2839226 Intron 14	C	T		
MDD	78	268	0.81	0.23
Control	152	542		0.22
rs2839231 Intron 15	A	G		
MDD	83	263	0.07	0.24
Control	204	488		0.29
rs3788265 Intron 29	G	T		
MDD	164	182	0.006	0.53
Control	393	301		0.43
rs2073376 Exon 38	A	G		
MDD	85	261	0.005	0.25
Control	230	466		0.33
Rs2073380 Exon 45	C	A		
MDD	183	163	0.021	0.53
Control	313	381		0.45
Rs1010111 Intron 47	A	G		
MDD	269	77	0.99	0.22
Control	539	155		0.22

MDD = major depressive disorder; SNP = single nucleotide polymorphism.
*Fisher exact test.
†Percentage of minor allele frequency.

Discussion

To our knowledge, ours is the first study to examine an association between the *PCNT* gene and MDD. We observed significant allelic association with rs3788265 and rs2073376 after

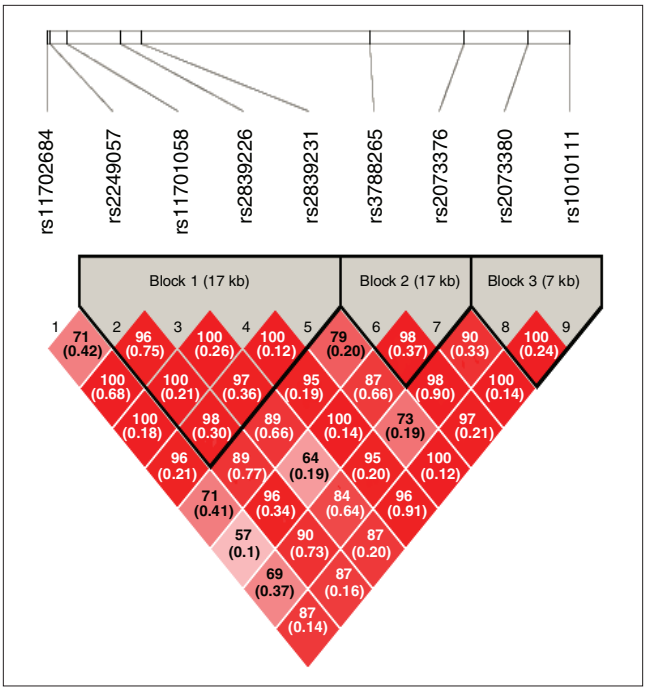

Fig. 1. Haplotype block structure of the *PCNT* gene. We determined the structure using the Haploview program.[11] We defined blocks according to the criteria of Gabriel and colleagues.[12] There were 3 linkage-disequilibrium blocks in the *PCNT* gene: rs2249057, rs11701058, rs2839226 and rs2839231 reside in block 1, rs3788265 and rs2073376 reside in block 2 and rs2073380 and rs1010111 reside in block 3. Each box represents D´ (r^2) values corresponding to each pair-wise single nucleotide polymorphism.

Table 3: Haplotype analysis among patients with major depressive disorder and controls*

Haplotype	Overall, %	MDD	Control	χ^2	*p* value	Permutation *p* value
GG	23.4	22.8	23.7	0.091	0.76	0.78
TG	46.3	52.6	43.2	8.234	0.004	0.005
GA	30.2	24.6	33	7.711	0.005	0.007
Select locus rs3788265 and rs2073376†				10.5	0.015	0.011

MDD = major depressive disorder.
*Haplotypes were omitted from analysis if the estimated haplotype probabilities were less than 5%.
†The 2 marker haplotypes of block 2 (rs3788265 and rs2073376) were associated with MDD (permutation p = 0.011). Replications = 10000.

applying multiple test correction. Furthermore, 2 marker haplotypes of block 2 (rs3788265 and rs2073376) were significantly associated with MDD. In block2, the most common haplotype (TG) was present in 53% of patients with MDD and 43% of controls. Therefore, this haplotype might be a risk factor for MDD. The second most common haplotype (GA) was present in 25% of patients with MDD and 33% of controls, suggesting that this haplotype might be protective against MDD.

Anitha and colleagues[9] reported that expression of the *PCNT* gene in peripheral blood lymphocytes was significantly higher in medication-naive patients with MDD than in healthy controls. A nonsynonymous exonic SNP (rs2073376, Gln2792Arg) that was significantly associated with MDD in our study may contribute to altered expression of this gene in peripheral lymphocytes of MDD.

Hashimoto and colleagues[7] reported an association between a genetic variation of *DISC1* (Ser704Cys) and MDD and brain morphology in the Japanese population. In our study, we provided the evidence that *PCNT*, an interacting partner of *DISC1*, is a candidate gene for MDD. Thus the *DISC1–PCNT* pathway may play an important role in the pathophysiology of MDD owing to abnormalities in centrosomal function.

Limitations

Our study has several limitations. First, our sample size was relatively small; however, our study had a power of 0.99 to detect an effect size of 0.5 (moderate) at the 0.05 significance level (2-tailed). Larger studies with participants of different races and meta-analyses are needed to confirm these associations. Second, owing to the late onset of MDD, it is possible that the disorder will develop in at least some of the participants in our control group. This potential misclassification in the control group would bias and weaken any association between the *PCNT* gene and MDD. On the other hand, selected controls who were recruited from employees without a personal or family history of mental disorders may enhance the likelihood of a positive association. Third, we did not reveal how SNPs of the *PCNT* gene, which were significantly associated with MDD in our study, alter its function. Recently it has been reported that Seckel syndrome, an autosomal recessive disorder of markedly reduced brain and body size, is associated with mutations in the *PCNT* gene.[15] The mutations have defects in DNA signalling. Further investigations, including intermediate phenotype approaches, are needed to reveal how genetic variations in the *PCNT* gene are involved in the etiology of MDD.

Conclusion

Our findings indicate that *PCNT* is a candidate gene for MDD in the Japanese population. Larger studies are needed to confirm these associations.

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Competing interests: None declared.

Contributors: Drs. Numata and Iga designed the study and acquired the data. Drs. Nakataki, Itakura, Ueno and Ohmori also acquired the data, which Drs. Numata, Nakataki, Tayoshi, Tanahashi, Ueno and Ohmori analyzed. Dr. Numata wrote the article, which all authors reviewed and approved for publication.

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