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SHORT COMMUNICATION

A case–control association analysis of *CABIN1* with schizophrenia in a Japanese population

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Calcineurin (CN) is a calcium/calmodulin-dependent serine/threonine protein phosphatase and regulates neuronal structure, neurotransmission and activity-dependent gene expression. Several studies have indicated that CN signaling is likely to be involved in the pathogenesis of schizophrenia. The gene encoding CN-binding protein 1 (CABIN1) is located on 22q11.23, one of the common susceptibility loci for schizophrenia. Therefore, *CABIN1* is a promising functional and positional candidate gene for schizophrenia. To assess whether *CABIN1* is implicated in vulnerability to schizophrenia, we conducted a case–control association study between *CABIN1* and schizophrenia. The results showed no evidence of an association between *CABIN1* and schizophrenia using 11 tagging single nucleotide polymorphisms in 1193 Japanese subjects. Our results suggest that *CABIN1* may not confer increased susceptibility for schizophrenia in the Japanese population.
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Schizophrenia is a complex genetic disorder that affects approximately 1% of the global population. The pathogenesis of schizophrenia is currently unclear, but there is cumulative evidence that calcineurin (CN) may be implicated in its pathophysiology. CN, which consists of a catalytic subunit (CNA) and a regulatory subunit (CNB), is a calcium/calmodulin-dependent serine/threonine protein phosphatase and regulates neuronal structure, neurotransmission and activity-dependent gene expression. Forebrain-specific *Cnb1* knockout mice displayed several schizophrenia-like behavioral abnormalities.[1] A microarray analysis showed a significant increase in the levels of *CNA* mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia.[2] Hippocampal *CNA* mRNA expression levels, however, were decreased in patients with schizophrenia, as evaluated using reverse transcriptase PCR.[3] The results of earlier studies of CNA protein levels in the hippocampus of patients with schizophrenia have been inconsistent.[3,4] An association between schizophrenia and CN-related genes including *PPP3CC*,[5–8] *EGR3*[8] and *NRGN*[9] has been shown, but negative findings for *PPP3CC*[10–13] and *NRGN*[14] have also been reported. These findings suggest that CN signaling is likely to have an important function in the pathogenesis of schizophrenia.

CN-binding protein 1 (CABIN1) binds specifically to the activated form of CN and inhibits CN-mediated signal transduction. The gene encoding CABIN1 is located on 22q11.23, one of the common susceptibility loci for schizophrenia.[15,16] *CABIN1* is therefore a promising functional and positional candidate gene for schizophrenia. *CABIN1* has been tested for an association with schizophrenia by only one study. Fallin *et al.*[10] examined seven polymorphisms in *CABIN1* with an average density of one marker per 20.9 kb and failed to find any association with schizophrenia. Detailed studies in which all common variations within a candidate gene are considered jointly are required to ascertain whether *CABIN1* contributes to vulnerability to schizophrenia. Here, we aimed to increase statistical power by testing more markers, taking into account linkage disequilibrium structure. We conducted a case–control association study between *CABIN1* and schizophrenia using 11 tagging single nucleotide polymorphisms (SNPs) from the HapMap database in 1193 Japanese subjects.

This study was approved by the Ethics Committee on Genetics of the Niigata University School of Medicine. Written informed consent was obtained from all participants. All participants were unrelated Japanese living in the Niigata Prefecture or Fukushima Prefecture. The study population consisted of 595 patients with schizophrenia (313 men and 282 women; mean age, 40.2 (s.d. 14.1) years) and 598 control subjects (311 men and 287 women; mean age, 38.1 (s.d. 10.5) years). Case and control groups were matched for sex (P=0.836). Although the mean age of the patients was significantly higher than that of the control subjects (P=0.004), the absolute difference in mean age between the groups was relatively small (2.1 years). We conducted a psychiatric assessment of every participant, as described earlier.[17] In brief, the patients were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* criteria by at least two experienced psychiatrists. The control subjects were mentally healthy subjects with no self-reported history of psychiatric disorders.

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Table 1 Genotype and allele frequencies of 11 tagging SNPs for *CABIN1*

			Patients					Controls					P	
SNP #	*dbSNP ID*	*Allele*[a]	n	*1/1*[b]	*1/2*[b]	*2/2*[b]	*MAF*	n	*1/1*[b]	*1/2*[b]	*2/2*[b]	*MAF*	*Genotype*	*Allele*
1	rs422674	C/A	592	268	268	56	0.321	593	287	247	59	0.308	0.453	0.489
2	rs9624386	C/G	592	434	139	19	0.150	596	436	146	14	0.146	0.631	0.809
3	rs6004041	G/A	586	348	201	37	0.235	590	344	217	29	0.233	0.451	0.927
4	rs873833	A/G	582	148	280	154	0.505	588	148	308	132	0.486	0.224	0.364
5	rs5760189	C/T	595	358	216	21	0.217	596	348	223	25	0.229	0.741	0.474
6	rs2073396	A/G	593	376	186	31	0.209	596	374	195	27	0.209	0.784	0.990
7	rs2282476	C/A	592	320	238	34	0.258	594	322	229	43	0.265	0.541	0.710
8	rs6004052	C/T	594	432	144	18	0.152	595	431	150	14	0.150	0.732	0.895
9	rs3788367	C/T	586	334	213	39	0.248	596	358	205	33	0.227	0.496	0.232
10	rs5760220	C/T	593	490	96	7	0.093	593	490	99	4	0.090	0.703[c]	0.831
11	rs2267067	C/T	594	223	274	97	0.394	598	234	282	82	0.373	0.444	0.291

Abbreviations: CABIN1, calcineurin-binding protein 1; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[a]Major/minor alleles.
[b]Genotypes, major and minor alleles are denoted by 1 and 2, respectively.
[c]Calculated using Fisher's exact test.

Tagging SNPs for *CABIN1* (chr22:22736066.22905061) were selected from the HapMap database (release#24, population: Japanese in Tokyo; minor allele frequency: more than 0.05). We applied the criterion of an r^2 threshold greater than 0.8 in the 'pairwise tagging only' mode using the 'Tagger' program, as implemented in Haploview v4.1.[18] Eleven SNPs were selected as tagging SNPs for *CABIN1*. However, a probe for rs2267068 could not be designed. When the other 10 SNPs were forced to be selected as tagging SNPs, rs873833 was selected instead of rs2267068 as a tagging SNP. All SNPs were genotyped using the TaqMan 5′-exonuclease assay, as described earlier.[17]

Deviation from the Hardy–Weinberg equilibrium was tested using a χ^2 test for goodness-of-fit. The allele and genotype frequencies of the patients and control subjects were compared using a χ^2 test or Fisher's exact test. Linkage disequilibrium blocks defined in accordance with Gabriel's criteria[19] were determined using Haploview v4.1. The haplotype association test was performed using Haploview v4.1, which obtains counts by summing the fractional likelihoods of each individual for each haplotype estimated using an accelerated expectation maximization algorithm. A power calculation was performed using Genetic Power Calculator.[20] Power was estimated with an α of 0.05, assuming a disease prevalence of 0.01.

We genotyped 11 tagging SNPs for *CABIN1* (Table 1). None of the SNP genotype distributions deviated significantly from the Hardy–Weinberg equilibrium in both groups. None of the genotype or allele frequencies of the SNPs examined differed significantly between patients and control subjects. Nine SNPs between rs9624386 (SNP #2) and rs5760220 (SNP #10) constituted a linkage disequilibrium block spanning 135 kb of *CABIN1*. There were no significant associations between haplotypes of this linkage disequilibrium block and schizophrenia (Table 2).

Table 2 Haplotype analyses of *CABIN1*

Haplotype[a]	*Patients*	*Controls*	P
CGACAACCC	0.260	0.261	0.949
CGGCACCTC	0.247	0.225	0.199
CGATACCCC	0.215	0.225	0.566
GAGCGCTCC	0.143	0.146	0.823
CAGCACCCT	0.047	0.046	0.945
CAGCGCCCT	0.043	0.043	0.955
CGACACCCC	0.022	0.025	0.550
CGGCGCCCC	0.013	0.018	0.336

Abbreviations: CABIN1, calcineurin-binding protein 1; SNP, single nucleotide polymorphism.
[a]SNP #2-#10 (Global *P*-value=0.899).

In this study, we found no evidence for an association between *CABIN1* and schizophrenia using 11 tagging SNPs in 1193 Japanese subjects. Our study is in line with the negative findings from 274 Ashkenazi case–parent trios.[10] These results suggest that *CABIN1* may not confer increased susceptibility to schizophrenia. However, it remains possible that the sample sizes of these two studies may not provide sufficient power to detect associations between schizophrenia and SNPs with low-risk allele frequencies and small effects. Indeed, our sample size (595 cases and 589 controls) had statistical power of only 0.29, assuming a risk allele frequency of 0.10 and a genotypic relative risk for homozygous risk allele carriers of 1.44 under the multiplicative model of inheritance. Large sample sizes ($\sim$2400 cases and 2400 controls or 2400 trios) would be required to detect an association between the risk allele with frequency of 0.10 and schizophrenia with a power of 0.80. To draw a definitive conclusion, therefore, further studies using larger sample sizes and sufficient markers should be conducted in multiple ethnic populations.

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