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SHORT COMMUNICATION

A two-stage case–control association study of the dihydropyrimidinase-like 2 gene (*DPYSL2*) with schizophrenia in Japanese subjects

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We examined the association of schizophrenia (SCZ) and dihydropyrimidinase-like 2 (*DPYSL2*), also known as collapsin response mediator protein 2, which regulates axonal growth and branching. We genotyped 20 tag single nucleotide polymorphisms (SNPs) in 1464 patients and 1310 controls. There were two potential associations in a screening population of 384 patients and 384 controls (rs2585458: *P*=0.046, rs4733048: *P*=0.014). However, we could not replicate these associations in a confirmatory population of 1080 patients and 926 controls (rs2585458: *P*=0.39, rs4733048: *P*=0.70) or a joint analysis (rs2585458: *P*=0.72, rs4733048: *P*=0.10). We conclude that *DPYSL2* does not have a major function in SCZ in Japanese subjects.
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Keywords: case–control study; *DPYSL2*; imputation; Japanese subjects; neuronal polarity; schizophrenia

INTRODUCTION

Schizophrenia (SCZ) is a severe debilitating neuropsychiatric disorder that affects ~1% of the general population. Family, twin and adoption studies support a substantial genetic contribution to SCZ, but its etiology remains unclear.[1] Moreover, irregularities consistent with abnormal brain development, including faulty neuronal migration and altered spatial neuronal arrangement have been reported in SCZ. Together with behavioral, neuromotor and other functional abnormalities that occur in childhood and predict SCZ, such as low IQ, poor motor skills and poor development of language and social skills, these morphological findings indicate a developmental origin for SCZ.[2,3]

Dihydropyrimidinase-like 2 (DPYSL2), also known as collapsin response mediator protein 2, is involved in the regulation of axon formation during neuronal polarization.[4,5] Overexpression of *Dpysl2* induces the formation of multiple axons and can alter an established dendrite to become an axon, indicating that overexpressed *Dpysl2* confers axonal identity not only on immature neurites but also on established dendrites. These observations indicate that DPYSL2 has a crucial function in axon formation of hippocampal neurons, thereby establishing and maintaining neuronal polarity. DPYSL2 interacts with tubulin heterodimers and promotes microtubule assembly *in vitro*. Thus, DPYSL2 seems to promote neurite elongation and axon specification by regulating microtubule assembly, endocytosis of adhesion molecules and reorganization of actin filaments.[6]

DPYSL2 is located on chromosome 8p21.2. This region has been reported as positive in meta-analysis of genome-wide linkage studies of SCZ.[7] One study showed that chromosomal 8p is a potential hub for developmental neuropsychiatric disorders and contains 21 genes (ADRA1A, ARHGEF10, CHRNA2, CHRNA6, CHRNB3, DKK4, DPYSL2, EGR3, FGF17, FGF20, FGFR1, FZD3, LDL, NAT2, NEF3, NRG1, PCM1, PLAT, PPP3CC, SFRP1 and VMAT1/SLC18A1) that are likely to contribute to the developmental neuropsychiatric disorders (that is SCZ, autism, bipolar disorder and depression) and neurodegenerative disorders (Parkinson's and Alzheimer's disease).[8] *DPYSL2* is involved in neuropsychiatric disorders's biology and clearly associated with SCZ and probably with bipolar disorder. The expression of *DPYSL2* in the hippocampus is increased in SCZ patients. Expression of the dihydropyrimidinase-related protein 2 in Down's syndrome and Alzheimer's disease brain is downregulated at the mRNA and dysregulated at the protein level. Several clinical studies have described a variety of neurodevelopmental abnormalities in subjects with defects of *DPYSL2*. *DPYSL2* is a marker for escitalopram resistance in stress model of depression. *DPYSL2* was reported to be a SCZ susceptibility gene in Japanese subjects.[9] However, the results of replication studies using smaller sets of markers have been inconsistent.[10,11] Therefore, to assess whether *DPYSL2* has a function in vulnerability to SCZ, we conducted a two-stage case–control association study in a Japanese population.

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Table 1 Allele frequencies of the 20 SNPs of *DPYSL2* in the screening population

			First set						
			Single marker (allele-wise)						
dbSNP	*Physical position*	*Minor allele*	*Cases*[a]	*Controls*[a]	*P*[b]	*OR*	*L95*[c]	*U95*[c]	*pHWE*[d]
rs327233	26500769	G	0.27	0.31	0.09	0.83	0.66	1.03	0.72
rs327232	26511632	T	0.20	0.24	0.09	0.81	0.64	1.03	0.48
rs13249543	26519261	G	0.40	0.39	0.81	1.03	0.84	1.26	0.59
rs2585458	26523749	A	0.09	0.13	**0.046**	0.72	0.52	1.00	0.24
rs182748	26527132	C	0.50	0.49	0.68	1.04	0.85	1.27	0.04
rs17321828	26527954	A	0.15	0.12	0.11	1.28	0.95	1.72	1.00
rs4733013	26528572	A	0.34	0.37	0.20	0.87	0.71	1.08	0.08
rs371255	26530311	A	0.25	0.25	0.86	0.98	0.78	1.23	0.04
rs403185	26532638	A	0.39	0.36	0.28	1.12	0.91	1.38	0.05
rs7829347	26536534	C	0.09	0.07	0.17	1.29	0.89	1.86	1.00
rs327221	26536952	A	0.10	0.12	0.12	0.77	0.56	1.07	0.48
rs4733033	26545142	C	0.38	0.41	0.20	0.88	0.71	1.07	0.20
rs327217	26546233	C	0.10	0.12	0.20	0.81	0.58	1.12	0.45
rs1972921	26551657	T	0.21	0.25	0.10	0.82	0.64	1.04	0.49
rs1442337	26552304	A	0.15	0.16	0.87	0.98	0.74	1.29	1.00
rs4733048	26555006	C	0.17	0.22	**0.014**	0.73	0.56	0.94	0.36
rs708621	26566709	C	0.33	0.31	0.39	1.10	0.89	1.37	0.81
rs11863	26570233	T	0.28	0.26	0.41	1.10	0.88	1.38	0.79
rs920633	26570696	T	0.46	0.44	0.50	1.07	0.88	1.31	0.25
rs17666	26571375	C	0.15	0.14	0.92	1.02	0.76	1.35	0.67

Abbreviations: OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Minor allele frequency.
[b]Fisher exact test.
[c]Lower (L) and upper (U) 95% confidence intervals.
[d]Hardy–Weinberg equilibrium *P*-value in controls.
Bold values indicate that they are lower than *P*-value 0.05.

MATERIALS AND METHODS

Participants

This study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from each participant. Patients were included in the study if they (1) met DSM-IV criteria for SCZ, (2) were physically healthy and (3) had no mood disorders, substance abuse, neurodevelopmental disorders, epilepsy or known mental retardation. Consensus diagnoses were made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of unstructured interviews with patients and families and review of medical records. The rate of the samples excluded due to the loss of the consensus was <5%. Controls were selected from the general population who had no history of mental disorders based on self-administered questionnaire during sample inclusion step, and based on unstructured diagnostic interview done by a experienced psychiatrist during the blood collection step. All subjects were unrelated to each other, living in the central area of the main land of Japan and self-identified as Japanese population.

Participants consisted of 1464 unrelated Japanese patients with SCZ (age 44.8 ± 15.0 years (mean ± s.d.), male 52.6%) and 1310 unrelated healthy controls (age 36.0 ± 13.7 years (mean ± s.d.), male 51.0%). In a screening population, participants consisted of 384 unrelated Japanese patients with SCZ (age 50.5 ± 15.1 years (mean ± s.d.), male 52.6%) and 384 unrelated healthy controls (age 38.1 ± 14.2 years (mean ± s.d.), male 60.3%). In a confirmatory population, participants consisted of 1080 unrelated Japanese patients with SCZ (age 42.7 ± 14.4 years (mean ± s.d.), male 49.8%) and 926 unrelated healthy controls (age 35.1 ± 13.36 years (mean ± s.d.), male 55.4%). Characterization of general samples and sampling procedures are available elsewhere.[12]

Genotyping and data analysis

In the screening population of 384 patients and 384 controls, we examined 20 single nucleotide polymorphisms (SNPs) including the positive SNP (rs17666) in an earlier study of a Japanese population.[9] The 20 SNPs were selected by Haploview ver4.1 program[13] according to the HapMap database (release no. 24, population: Japanese in Tokyo, minor allele frequency >10%). SNP tagging criteria were based on minor allele frequency (>10%) and correlation coefficient (r^2) between loci (>0.8) as reported in the HapMap database. We genotyped all SNPs by TaqMan assay (Applied Biosystems Japan, Tokyo, Japan). For quality control, we checked the deviation from the Hardy–Weinberg equilibrium, the sample-wise call rate >95% and the SNP-wise call rate >95%. A power calculation was done by Power Calculator for Genetics Studies.[14] We did statistical calculations using PLINK ver2.05.[15] The Fisher exact test was used to compare allele frequencies between patients and controls. Significance level was set at $P<0.05$. As tagging SNPs were selected based on r^2,

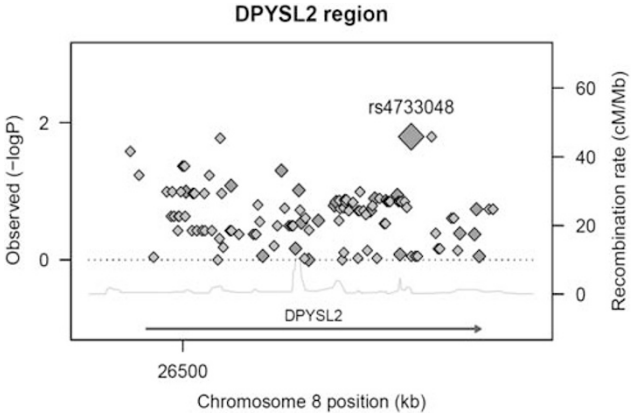

Figure 1 Allele *P*-values of the typed and the imputed SNPs. Red represents typed SNPs and gray represents imputed SNPs. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 2 Allele frequencies of the two SNPs of *DPYSL2*

			Second set							*Joint analysis*				
			Single marker (allele-wise)							*Single marker (allele-wise)*				
dbSNP	*Physical position*	*Minor allele*	*Cases*[a]	*Controls*[a]	*P*[b]	*OR*	*L95*[c]	*U95*[c]	*pHWE*[d]	*P*[e]	*OR*	*L95*[c]	*U95*[c]	*P_BD*[f]
rs2585458	26523749	A	0.12	0.11	0.39	1.09	0.89	1.34	0.86	0.72	0.97	0.82	1.15	0.03
rs4733048	26555006	C	0.18	0.19	0.70	0.97	0.82	1.14	0.91	0.10	0.89	0.77	1.02	0.06

Abbreviations: OR, odds ratio; SNP, single nucleotide polymorphism.
[a]Minor allele frequency.
[b]Fisher exact test.
[c]95% confidence intervals.
[d]Hardy–Weinberg equilibrium *P*-value in controls.
[e]Cochran–Mantel–Haenszel test.
[f]Breslow-day test.

we included imputation as an exploratory method to compute genotypes of SNPs that were not genotyped.[16] The starting point of imputation methods is a reference data set such as the HapMap, in which a large set of SNPs is being genotyped. The underlying assumption is that the reference samples, the cases and the controls are all sampled from the same population. Under this assumption, the three populations share the same linkage disequilibrium structure and the same haplotype distribution for every set of SNPs. Thus, the structure of the linkage disequilibrium in the reference population, in conjunction with the structure of the linkage disequilibrium of the observed SNPs within both the cases and the controls, can be used to impute the alleles of a hidden SNP. Imputed SNPs can then be tested for association using an appropriate statistical test. The advantage of imputing untyped SNPs is that the coverage of common variants within the locus of interest can be enhanced, boosting the statistical power and reducing type 2 errors in the screening population. The MACH program was used to calculate the genotypic prediction of 96 untyped SNPs using directly typed information from the 20 SNPs in the screening scan and the HapMap database (recombination map and haplotype data for the Japanese/Chinese population, release 24; phase II).[17] The MACH program was recently reported to have similar imputation accuracy rates to IMPUTE and to outperform fastPHASE, PLINK and Beagle.[18] The targeted region of imputation was limited to the *DPYSL2* locus.

RESULTS

We identified two association signals by PLINK ver2.05 between two SNPs and SCZ before multiple comparisons in the screening population (rs2585458: P=0.046, rs4733048: P=0.014) (Table 1). These two SNPs are located in intron. We imputed the genotype of 96 untyped SNPs using the MACH program and calculated the allelic *P*-value of each imputed SNP by Haploview ver4.1 (Figure 1). The lowest *P*-value in imputation was 0.016 at both typed rs4733048 and imputed rs4076071. To confirm the potential associations of these two SNPs and SCZ, we genotyped the two SNPs in a confirmatory population. However, we could not replicate these associations in the confirmatory population of 1080 patients and 926 controls (rs2585458: P=0.39, rs4733048: P=0.70) or in a joint analysis (rs2585458: P=0.72, rs4733048: P=0.10) (Table 2).

DISCUSSION

In this study, we investigated the association between 20 SNPs of *DPYSL2* and SCZ in a Japanese population. We did not observe significant associations between *DPYSL2* and SCZ. The positive association observed in the screening population might be due to a type 1 error. After Bonferroni correction, all SNPs in the screening population were negative. Sample size of this study is larger than earlier studies and we used an imputation method, boosting statistic power, so a type 2 error seems unlikely. However, other SNP might be associated with SCZ in the screening sample. Furthermore, we have conducted meta-analysis of data reported previously for rs17666,[9–11] but we could not detect association (fixed model OR_{ci95}=0.877–1.175, P=0.843). However, this study may not have sufficient power to detect associations between SNPs with smaller effects and SCZ. It should be noted that a larger sample is required for the detection of a smaller effect. The present sample has statistical power >0.8 for the detection of the role of the polymorphism with a minor allele frequency of 0.1, when the genotype relative risk is 1.35. As all participants in this study were of Japanese descent and recruited from the main island of Japan, the likelihood of population stratification is low.[19]

There are several limitations in our study. The first limitation in our association study is that cases and controls were not matched in terms of age. In other words, the controls may develop SCZ at some point in life, as they were significantly younger than cases. This might affect the statistic power. The second limitation in our study is that our study design was based on the common disease common variant hypothesis, so we applied minor allele frequency $>10\%$ when we selected the 20 tagSNPs. It is difficult to evaluate the association between rare variants and SCZ in our study. In conclusion, this study did not show evidence for the association of *DPYSL2* with SCZ in the Japanese population. *DPYSL2* may not have a major function in genetic susceptibility to SCZ.

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