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SHORT COMMUNICATION

Genetic association study between the detected risk variants based upon type II diabetes GWAS and psychotic disorders in the Japanese population

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Several epidemiological and genetic studies have suggested that the risk of type II diabetes (T2D) is likely to overlap with the susceptibility to psychotic disorders such as schizophrenia (SCZ) and bipolar disorder (BD). In this study, we aimed to examine the association of single-nucleotide polymorphisms (SNPs) detected in previous T2D genome-wide association studies (GWAS) with SCZ, BD and psychosis (SCZ plus BD). A total of 37 SNPs were selected from the literature. A two-stage analysis was conducted using a first set of screening samples (total $N = 3037$) and a second set of replication samples ($N = 4950$). None of the SNPs showed a significant association to the screening samples after correction for multiple testing. To avoid type II error, we genotyped the top three SNPs in *BCL11A*, *HMG20A* and *HNF4A* showing associations with any of the phenotypes ($P_{uncorrected} < 0.01$) using independent samples to replicate the nominal associations. However, we were unable to find any significant associations based on the screening results ($P_{uncorrected} > 0.05$). Our findings did not support the shared genetic risk between T2D and psychotic disorders in the Japanese population. However, further replication using a larger sample size is required.
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An increasing rate of prevalence of metabolic abnormalities, including type II diabetes (T2D), has been observed in subjects with major psychiatric disorders such as schizophrenia (SCZ) and bipolar disorder (BD).[1,2] The hypotheses concerning the comorbidity between SCZ/BD and T2D include three categories of risk factors:[3] (1) adverse effects of antipsychotic drugs (gene × drug interaction); (2) a high-risk lifestyle arising from psychiatric symptoms (for example, bulimia); and (3) common etiological factors including shared genetic risk. Interestingly, the prevalence of T2D is higher in young patients with SCZ and BD, who are expected to be minimally influenced by antipsychotics, compared with healthy individuals.[4] This finding strongly suggests a shared genetic risk between T2D and SCZ and/or BD.

Based on genetic association studies, a number of researchers have reported that loci associated with T2D risk overlap with SCZ risk genes.[5,6] To test the hypothesis that there is shared genetic risk between T2D and psychotic disorders, and to replicate the previous findings, we examined whether T2D risk SNPs selected on the basis of previous GWASs are associated with SCZ and BD in the Japanese population.

Two independent sets of samples were used in this study. In the screening analysis involving the first set, we examined 1032 SCZ and 1012 BD patients, and 993 controls. For the three SNPs that showed a nominal significant association signal in the screening analysis, we used an independent second set sample for replication analysis (1808 SCZ, 821 BD and 2321 controls). A detailed description of the general characterization and psychiatric assessments of our subjects has previously been reported[7] and listed in the Supplementary Information. Written informed consent was obtained from each subject. The ethics committees of Fujita Health University, RIKEN Brain Science Institute (BSI) and the institutes participating in the Collaborative Study of Mood Disorder (COSMO) approved this study.[7,8]

We selected 37 SNPs from T2D GWAS data published before September 2011 (see Supplementary Information). All of the SNPs were genotyped using the Sequenom iPLEX GOLD system (Sequenom, San Diego, CA, USA) with stringent quality control

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(Supplementary Information). We assessed the allelic associations (two-tailed) of the SNPs with three phenotypes: (1) SCZ vs control (referred to as a 'SCZ association'), (2) BD vs control (referred to as a 'BD association') and (3) 'psychosis' (SCZ + BD) vs control (referred to as a 'psychosis association') in the first and second sets of samples. A meta-analysis of the SNPs (nominally significant SNPs in the first set of samples and genotyped SNPs in the second set of replication samples) was conducted by combining the screening and the replication data sets. A fixed model (the I^2 heterogeneity index <50), or a random effect model ($I^2>50$), was applied in each analysis. All of the statistical procedures were calculated using PLINK 1.07 software.[9]

Following the quality control procedure, a total of 32 SNPs and 2675 samples in the first screening set remained eligible for association analysis (930 SCZ, 869 BD and 876 healthy controls). The results are listed in Table 1 and show the nominal association signals ($P_{uncorrected}<0.05$) found for all three phenotypes (SCZ, BD and psychosis). The full results can be found in Supplementary Table 2.

In the SCZ sample, only one SNP in *UBE2E2* (rs7612463) showed a nominal significant association ($P_{uncorrected}<0.05$). However, we observed that the direction of the effect of this SNP was opposite to the hypothesized direction (that is, that the risk for T2D shows the same direction as psychosis).

The BD associations exhibited nominal significance for SNPs in *BCL11A* (rs243021, $P_{uncorrected}=0.0031$) and *HMG20A* (rs7178572, $P_{uncorrected}=0.0043$), with the same direction of effect as T2D, and SNPs in *SLC30A8* (rs3802177, $P_{uncorrected}=0.042$) and *HNF4A* (rs4812829, $P_{uncorrected}=0.0082$), which showed the opposite direction of effect (Table 1).

When we corrected the multiple testing results using the Bonferroni method ($N=32$), none of the SNPs showed a significant association (Table 1). To avoid type II error, we examined the results of replication analysis of the top three SNPs with arbitrary and relaxed α-levels set at $P<0.01$ using an independent second set of replication samples. However, no significant association was revealed in these independent data sets (Table 1).

Finally, using a meta-analytical approach, we combined our two data sets (the first screening set and the second replication set) for the three SNPs (rs243021, rs7178572 and rs4812829) to maximize the sample size. Again, no association was observed (Table 2).

In this study, we did not find any significant association between risk SNPs for T2D with the SCZ, BD or psychosis phenotypes in the Japanese population. Our results therefore do not provide supportive evidence of shared genetic risk between T2D and SCZ/BD.[5,6] Interestingly, the results of the psychosis association analysis for the SNPs, which showed nominal associations only with SCZ or BD, did not enhance the significance, even when the sample size was doubled. Therefore, we speculate that the T2D SNPs examined in this study do

Table 2 Meta-analysis of the three SNPs detected in the first set of screening analysis

						Current study (screeing + replication)		
CHR	*SNP*	*GENE*	*A1*	*A2*	*Phenotype*	P-*value*	*OR*	I^2
2	rs243021	BCL11A	C	T	SCZ	0.63	0.97	60.6
					BD	0.30	0.90	78.9
					PSY	0.45	0.93	81.6
15	rs7178572	HMG20A	G	A	SCZ	0.95	1	58.3
					BD	0.17	0.89	68.8
					PSY	0.52	0.95	79.0
20	rs4812829	HNF4A	A	G	SCZ	0.97	1	38.3
					BD	0.22	0.91	68.1
					PSY	0.49	0.95	73.4

Abbreviations: A1, non-reference allele; A2, reference allele; BD, bipolar disorder; CHR, chromosome; SCZ, schizophrenia; SNP, single-nucleotide polymorphism; PSY, psychosis.

Table 1 Association analysis in the first set of screening samples (SNPs with nominal significant association)

							Screening							*Replication*					
CHR	*SNP (original SNP)*	*GENE*	*BP*[a]	*A1*[b]	*A2*[c]	*Pheno-type*	*F_A*[d]	*F_U*[e]	P-*value*	$P_{corrected}$[f]	*OR*[g]	*s.e.*	*Direc-tion*[h]	*F_A*	*F_U*	P-*value*	*OR*	*s.e.*	*Direc-tion*[h]
2	rs243021	BCL11A	60584819	C	T	SCZ	0.311	0.335	0.12	1	0.89	0.071	+	0.314	0.309	0.59	1.03	0.048	−
						BD	0.288		**0.0031**	0.100	0.81	0.073	+	0.307		0.92	0.99	0.063	+
						PSY	0.300		**0.0095**	0.30	0.85	0.062	+	0.312		0.71	1.02	0.044	−
3	rs7612463	UBE2E2	23336450	A	C	SCZ	0.184	0.155	0.021	0.68	1.23	0.089	−						
						BD	0.154		0.92	1	0.99	0.094	+						
						PSY	0.170		0.19	1	1.11	0.080	−						
8	rs3802177[i] (rs13266634)	SLC30A8	118185025	T	C	SCZ	0.424	0.423	0.95	1	1.00	0.068	−						
						BD	0.457		0.042	1	1.15	0.068	−						
						PSY	0.440		0.24	1	1.07	0.059	−						
15	rs7178572	HMG20A	77747190	G	A	SCZ	0.404	0.423	0.25	1	0.93	0.068	+	0.420	0.408	0.28	1.05	0.045	−
						BD	0.376		**0.0043**	0.14	0.82	0.069	+	0.400		0.56	0.97	0.059	+
						PSY	0.390		0.023	0.72	0.87	0.059	+	0.414		0.57	1.02	0.041	−
20	rs4812829	HNF4A	42989267	A	G	SCZ	0.459	0.477	0.281	1.0	0.93	0.067	−	0.463	0.455	0.49	1.03	0.045	+
						BD	0.432		**0.0082**	0.26	0.84	0.068	−	0.450		0.72	0.98	0.058	−
						PSY	0.446		0.034	1	0.88	0.059	−	0.458		0.72	1.02	0.041	+

Abbreviations: BD, bipolar disorder; CHR, chromosome; PSY, psychosis; SNP, single-nucleotide polymorphism; SCZ, schizophrenia.
[a]BP, base position based upon hg19.
[b]A1, minor allele based upon whole samples.
[c]A2, major allele.
[d]F_A: allele frequency of A1 in case.
[e]F_U: allele frequency of A1 in controls.
[f]$P_{corrected}$, corrected *P* with Boferroni correction ($N=32$).
[g]OR, odds ratio (A2 is reference).
[h]Direction: + same direction of the effect with the original study '−' opposite direction of the effect.
[i]This SNP is proxy SNP with the original one.
Bold numerals: A P-value < 0.01.

not exhibit a pleiotropic effect on psychotic disorders even though recent evidence has indicated that several identified SCZ risk SNPs are associated with BD and *vice versa*, suggesting the existence of shared genetic risk between SCZ and BD.[10,11]

The SNPs examined in this study were selected based on the results of T2D GWASs conducted in Caucasian and Asian populations, including individuals of Japanese ancestry. Therefore, a population difference cannot fully explain our results showing a lack of an association. However, owing to the modest sample size in this study (see Supplementary Information), this negative finding does not completely negate the hypothesis that shared genetic components contribute to both psychotic disorders and T2D. To increase the statistical power for our results in the first set of screening samples, we included two additional analyses, a polygenic component analysis[12] and a sign test to check the association of the cumulative effect of the T2D 'risk' SNPs. Again it showed lack of the significant enrichment between T2D SNPs (or 'risk' allele) and SCZ/BD (Supplementary Tables 3 and 4).

In summary, our association analysis did not support the shared genetic risk between T2D and psychotic disorders in the Japanese population. However, a larger sample size will be required to obtain conclusive results.

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