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Association of Tagging Single Nucleotide Polymorphisms on 8 Candidate Genes in Dopaminergic Pathway with Schizophrenia in Croatian Population

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Aim To perform a comprehensive evaluation of association of common genetic variants in candidate genes in the dopaminergic pathway with schizophrenia in a sample from Croatian population.

Methods A case-control association study was performed on 104 unrelated patients with schizophrenia recruited from a psychiatric hospital in Zagreb and 131 phenotypically normal Croatian subjects. Forty-nine tagging single nucleotide polymorphisms (tagSNPs) in 8 candidate genes in the dopaminergic pathway were identified from the HapMap database and tested for association. Genotyping was performed using the SNPlex platform. Statistical analysis was conducted to assess allelic and genotypic associations between cases and controls using a goodness of fit χ^2 test and trend test, respectively; adjustment for multiple testing was done by permutation based analysis.

Results Significant allele frequency differences between schizophrenia cases and controls were observed at 4 tagSNPs located in the genes *DRD5, HTR1B1*, *DBH,* and *TH1* ($P < 0.005$). A trend test also confirmed the genotypic association ($P < 0.001$) of these 4 tagSNPs. Additionally, moderate association ($P < 0.05$) was observed with 8 tagSNPs on *SLC6A3*, *DBH*, *DRD4*, *SLC6A4*, and *COMT*.

Conclusions Common genetic variants in genes involved in the dopaminergic pathway are associated with schizophrenia in the populations of Caucasian descent.

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Schizophrenia is a chronic, severe, and disabling brain disease affecting about 1% of the global population (1). There is substantial evidence that genetic factors are involved in the etiology of the disease (2). High heritability (~80%) and higher concordance in monozygotic (~50%) than in dizygotic (~17%) twins are strong indicators for an inherited basis of schizophrenia (3-5). During the past decade, numerous loci and plausible candidate genes have been identified by linkage and association studies. However, the findings have remained inconclusive (2,6). Like other complex diseases, a complex genetic etiology compounded by involvement of other non-genetic factors has hindered the precise identification of schizophrenia gene variants. Second, a major limitation in most association studies has been testing of a few variants within a gene of interest rather than a thorough assessment of the entire gene region. With the availability of the sequence of the genome and large body of data on human genetic variation from the HapMap project (7), it is now possible to undertake more comprehensive association studies.

Genes involved in the dopamine pathway are biologically plausible candidates in schizophrenia susceptibility. In this study, we report on the association of single nucleotide polymorphisms (SNPs) in 8 dopaminergic genes (*DRD4*, *DRD5*, *SLC6A3*, *SLC6A4*, *HTR1B*, *DBH*, *TH*, and *COMT*) with schizophrenia in a Caucasian sample from Croatia. We performed a comprehensive association study using tagging SNPs (tagSNPs). Overall, 49 tagSNPs were identified from the HapMap database (7), 4 of which showed strong evidence of association with schizophrenia susceptibility.

MATERIALS AND METHODS

Subjects

Cases, consisting of 104 unrelated individuals (61 men and 43 women) of Croatian ancestry were recruited from the Jankomir hospital in Zagreb, Croatia. Diagnoses, which were coded according to ICD-10 (8), included general schizophrenia and 4 subtypes of schizophrenia that all belong to a wider group of schizophrenia disorders (ICD-10 codes F20-F20.9). Of the 104 participants (Table 1), general schizophrenia (ICD-10 code F20) was assigned to 3 individuals (excluding acute schizophrenia-like psychotic disorder [F23.2], undifferentiated schizophrenia [F20.3], cyclic schizophrenia [F25.2], and schizotypal disorder [F21]). Altogether 59 subjects suffered from paranoid schizophrenia (ICD-10 code F20.0), 36 from residual schizophrenia (ICD-10 code F20.5), 3 from hebephrenic schizophrenia (ICD-10 code F20.1), and 3 from simple schizophrenia (ICD-10 code F20.6). Mean age of the subjects were 43.6 ± 10.6 years, with a range of 23-74 years. Age at the onset of the disease was not available.

TABLE 1. Summary of cases and their diagnosis

Diagnosis* (ICD-10 code)	Male	Female	Total
General schizophrenia (F20)	3	0	3
Paranoid schizophrenia (F20.0)	32	27	59
Hebephrenic schizophrenia (F20.1)	3	0	3
Residual schizophrenia (F20.5)	23	13	36
Simple schizophrenia (F20.6)	0	3	3
Total	61	43	104

*According to ICD-10 (8).

For controls, blood samples were obtained from 131 phenotypically healthy unrelated Croatian individuals, who were recruited as volunteers in anthropological field surveys. Control individuals were sampled from Zagreb and 6 other cities representing 6 geographically dispersed regions of mainland Croatia. Mean age of the controls was 50.3 ± 7.5, with a range of 30-74 years.

DNA analysis

Cases' DNA was isolated from whole blood (7 mL) by either Nucleon® Genomic DNA Extraction Kit (Tepnel Life Sciences PLC, Manchester, UK) standard protocols or by protocol of salting-out method given by Miller et al (9). DNA samples of control group were extracted from whole blood samples using the chloroform-phenol extraction method, previously described by Ponz et al (10). DNA was suspended in TE-buffer for use in genotyping.

The candidate genes, their chromosomal map positions, and the number of tagSNPs are listed in Table 2. TagSNPs were selected using the tagging approach of Carlson et al (11) implemented in the SNPbrowser Software, version 3.5 (Applied Biosystems, Foster City, CA, USA). As the study population is of European descent, we used the Caucasian HapMap database (7) based on pairwise r^2 (≥0.8) among all common SNPs with minor allele frequency (MAF)≥0.05 for selection of the tagSNPs. Altogether 49 SNPs tagged the 8 candidate genes.

Genotyping was performed on the SNPlex platform. The SNPlex Genotyping System is based on multiple oligonucleotide ligation/PCR assay with a universal ZipChute probe detection for high-throughput multiplexed SNP genotyping. Fluorescently labeled ZipChute probes were

TABLE 2. Candidate genes, map locations, and tagging single nucleotide polymorphisms (SNP)

Gene symbol	Gene name	Map position	No. of tagSNPs
DRD5	dopamine receptor D5	4p16.1	3
SLC6A3	solute carrier family 6 member 3	5p15.3	8
HTR1B	5 hydroxytryptamine (serotonin) receptor 1B	6q13	5
DBH	dopamine beta-hydroxylase	9q34.2	8
DRD4	dopamine receptor D4	11p15.5	4
TH	tyrosine hydroxylase isoform a	11p15.5	5
SLC6A4	solute carrier family 6 member 4	17q11.1-q12	4
COMT	catechol-O-methyltransferase isoform MB-COMT	22q11.21	12

hybridized to complementary ZipCode sequences that were part of genotype-specific amplicons. These ZipChute probes were eluted and detected by electrophoretic separation on Applied Biosystems 3130 DNA Analyzer. The GeneMapper® software version 3.7 was used for automated allele calling for all SNPs.

Statistical analysis

Allele frequencies were estimated by gene counting. Conformity of genotype proportions to Hardy-Weinberg expectations (HWE) was performed by the exact test (12). Allelic association between cases and controls was performed using a goodness of fit χ^2 test and adjustment for multiple testing was performed by permuting the association results 10 000 times to define the smallest empirical significance level (13). We used the PLINK whole genome analysis toolset (version 1.0.6) for allelic and genotypic association (trend test) and for inferring age-adjusted odds ratios (OR) (14).

RESULTS

The results of allelic and genotypic associations are summarized in Table 3. This table presents the genomic base positions of the tagSNPs, MAFs in cases and controls, HWE *P*-values and the permuted *P*-values for allelic association with schizophrenia susceptibility, and trend test *P*-values for primary comparisons of genotypes. There was no deviation from the expectations of HWE at any SNP. We found a significant association of 4 tagSNPs located one each in *DRD5* (*rs1850744, P*=0.002), *HTR1B1* (*rs2143823, P*=0.005), *DBH (rs2007153, P*<.001), and *TH (rs4320932, P*<.001). MAFs at these 4 SNPs differed by >10% between schizophrenia cases and controls. Additionally, modest allele frequency differences and significant association (allele frequency difference <10%) was observed with 8 tagSNPs on *SLC6A3* (*rs464049, P*=0.045), *DBH* (*rs2283123, P*=0.039), *DRD4* (*rs11246226, P*=0.014; and *rs4331145, P*=0.038), *SLC6A4* (*rs140700, P*=0.037; and *rs1050565, P*=0.034), and *COMT* (*rs2020917, P*=0.037; and *rs165815, P*=0.021). We then performed logistic regression to infer the age- and sex-adjusted OR and their respective 95% confidence intervals for the 12 significant tagSNPs that showed association at allelic level (Table 4). All of the 4 significantly associated tagSNPs showed significant associations of genotypes under one or more of the inherited models (additive, dominant, recessive, and log-additive), providing additional confirmation of the findings. Four of the 8 tagSNPs with modest associations (*rs464049* in *SLC6A3*, *rs11246226 and rs4331145* in *DRD4*, and *rs2020917* in *COMT*) also showed associations under one or more of the genetic models.

As case-control design can result in spurious association due to population stratification (15), we performed a structure analysis (16) using a set of 86 SNPs distributed over 11 chromosomes. We did not find evidence for substructure in our case-control population (data not shown).

DISCUSSION

This study reports the association of tagSNPs in 8 candidate genes involved in the dopaminergic pathway with schizophrenia in a Caucasian sample from Croatia. The analysis revealed significant allele and genotype frequency differences between the schizophrenia cases and controls at 4 tagSNPs located in *DRD5*, *HTR1B*, *DBH*, and *TH*. In addition, moderate levels of association were observed with 8 tagSNPs in *SLC6A3*, *DBH*, *DRD4*, *SLC6A4*, and *COMT*. These results reaffirm that common sequence variants in dopaminergic genes are associated with susceptibility to schizophrenia in populations of Caucasian descent.

TABLE 3. Allelic and genotypic associations on 8 genes with schizophrenia susceptibility*

Genes (map position)	TagSNP rs number	Alleles	NCBI SNP position	HWE *P*	Minor allele frequency controls (n = 131)	Minor allele frequency cases (n = 103)	χ^2	$P_{\chi 2}$	P_{Trend}
DRD5 (4p16.1)	*rs10033951*	C/T	9455849	0.151	0.278	0.223	1.296	0.255	0.316
	rs2867383	A/G	9464204	1	0.018	0.005	1.439	0.230	0.364
	rs1850744	A/G	9466981	0.466	0.179	0.071	9.713	0.002	0.001
SLC6A3 (5p15.3)	*rs40184*	A/G	1448077	0.01	0.452	0.39	1.138	0.286	0.438
	rs11133767	A/G	1454580	0.149	0.239	0.196	0.787	0.375	0.473
	rs6869645	C/T	1457548	0.643	0.098	0.064	1.283	0.257	0.398
	rs37022	A/T	1468629	0.532	0.25	0.275	0.244	0.622	0.851
	rs464049	C/T	1476905	0.725	0.355	0.465	4.015	0.045	0.005
	rs460000	A/C	1485825	0.112	0.283	0.256	0.169	0.681	0.565
	rs403636	G/T	1491354	1	0.05	0.02	2.547	0.111	0.255
	rs3756450	C/T	1501148	0.969	0.212	0.195	0.151	0.698	0.805
HTR1B (6q13)	*rs2143823*	C/T	78219236	0.05	0.41	0.244	7.985	0.005	0.001
	rs9359271	A/C	78222839	0.139	0.349	0.368	0.110	0.740	0.714
	rs2000292	A/G	78223664	0.815	0.272	0.309	0.534	0.465	0.924
	rs6297	A/G	78228660	1	0.159	0.1	2.759	0.097	0.182
	rs1213371	C/T	78236764	0.03	0.358	0.371	0.041	0.839	0.745
DBH (9q34.2)	*rs1076150*	A/G	133528315	0.325	0.367	0.429	1.377	0.241	0.066
	rs1611115	C/T	133530069	1	0.733	0.774	0.751	0.386	0.643
	rs2007153	A/G	133533373	1	0.470	0.658	11.135	0.001	0.0004
	rs3025399	A/C	133538528	0.971	0.113	0.119	0.019	0.889	0.774
	rs1541333	C/G	133540939	0.837	0.442	0.480	0.459	0.498	0.716
	rs2283123	C/T	133544851	0.422	0.063	0.020	4.274	0.039	0.050
	rs77905	C/T	133547651	0.454	0.475	0.475	0.000	0.999	0.763
	rs732833	A/G	133550216	0.118	0.416	0.335	2.268	0.132	0.221
DRD4 (11p15.5)	*rs3758653*	C/T	626399	0.019	0.395	0.450	0.421	0.517	0.526
	rs11246226	A/C	631191	0.736	0.183	0.078	6.100	0.014	0.016
	rs936465	C/G	633568	0.417	0.395	0.352	0.267	0.606	0.697
	rs4331145	A/G	633683	0.039	0.327	0.202	4.298	0.038	0.031
TH (11p15.5)	*rs4320932*	A/G	2128177	0.555	0.236	0.065	12.305	0.001	0.0003
	rs7924316	G/T	2130023	0.702	0.455	0.456	0.000	0.985	0.550
	rs3842748	C/G	2137971	0.001	0.442	0.468	0.199	0.655	0.774
	rs2070762	C/T	2142911	1	0.389	0.446	1.036	0.309	0.142
	rs7396243	G/T	2162468	0.208	0.355	0.41	1.042	0.307	0.218
SLC6A4 (17q11.1-q12)	*rs3794808*	A/G	25555919	1	0.204	0.257	1.754	0.185	0.055
	rs140700	A/G	25567515	0.049	0.143	0.078	4.349	0.037	0.062
	rs2066713	C/T	25575791	0.778	0.421	0.417	0.007	0.936	0.805
	rs1050565	A/G	25600202	0.641	0.323	0.434	4.496	0.034	0.038
COMT (22q11.21)	*rs2020917*	C/T	18303438	0.917	0.460	0.581	4.376	0.037	0.122
	rs933271	C/T	18305961	0.382	0.407	0.479	1.514	0.219	0.084
	rs1544325	A/G	18306222	0.06	0.452	0.454	0.001	0.972	0.925
	rs5992500	C/T	18316501	1	0.008	0.006	0.077	0.782	0.125
	rs740603	A/G	18319731	0.085	0.352	0.272	1.943	0.163	0.177
	rs165656	C/G	18323417	0.06	0.434	0.357	1.607	0.205	0.193
	rs4646316	C/T	18326686	0.935	0.156	0.097	1.889	0.169	0.087
	rs165774	A/G	18327115	0.279	0.433	0.454	0.106	0.744	0.982
	rs174696	C/T	18327730	0.506	0.412	0.375	0.209	0.648	0.5
	rs174697	A/G	18328386	1	0.075	0.081	0.040	0.842	0.685
	rs165728	C/T	18331577	0.07	0.125	0.062	3.210	0.073	0.465
	rs16581)5	C/T	18334027	0.244	0.298	0.449	5.357	0.021	0.142

*Abbreviations: SNPs – single nucleotide polymorphism; NCBI – National Center for Biotechnology Information; $P_{\chi 2}$ – *P*-value from χ^2 test for allelic frequency differences; P_{Trend} – *P*-value from genotypic trend test; HWE – Hardy Weinberg expectations.

TABLE 4. Genotypic associations of 12 significant tag single nucleotide polymorphisms (tagSNP) with schizophrenia susceptibility

SNP	Genotype model	Genotypes	Frequency		Odds ratio (95% confidence interval)	*P*
			control	case		
rs1850744 (DRD4)	additive	G/G	0.67	0.86	1	<0.001
		A/G	0.27	0.14	0.37 (0.17-0.82)	
		A/A	0.06	0.00		
	dominant	G/G	0.67	0.86	1	0.002
		A/G-A/A	0.33	0.14	0.30 (0.14-0.66)	
	recessive	G/G-A/G	0.94	1.00	1	0.005
		A/A	0.06	0.00		
	log-additive				0.30 (0.15-0.62)	<0.001
rs464049 (SLC6A3)	additive	C/C	0.52	0.28	1	<0.001
		C/T	0.33	0.56	3.62 (1.87-7.02)	
		T/T	0.14	0.17	2.37 (0.99-5.68)	
	dominant	C/C	0.52	0.28	1	<0.001
		C/T-T/T	0.48	0.72	3.23 (1.74-5.97)	
	recessive	C/C-C/T	0.86	0.83	1	0.641
		T/T	0.14	0.17	1.21 (0.55-2.67)	
	log-additive				1.84 (1.21-2.82)	0.004
rs2143823 (HTR1B)	additive	T/T	0.42	0.61	1	0.003
		C/T	0.27	0.31	0.79 (0.34-1.85)	
		C/C	0.31	0.09	0.17 (0.06-0.51)	
	dominant	T/T	0.42	0.61	1	0.046
		C/T-C/C	0.58	0.39	0.47 (0.23-0.99)	
	recessive	T/T-C/T	0.69	0.92	1	<0.001
		C/C	0.31	0.09	0.18 (0.06-0.53)	
	log-additive				0.47 (0.28-0.78)	0.003
rs2007153 (DBH)	additive	G/G	0.21	0.42	1	0.004
		A/G	0.49	0.47	0.48 (0.23-1.04)	
		A/A	0.30	0.11	0.20 (0.07-0.54)	
	dominant	G/G	0.21	0.42	1	0.007
		A/G-A/A	0.80	0.58	0.38 (0.18-0.78)	
	recessive	G/G-A/G	0.70	0.89	1	0.006
		A/A	0.30	0.11	0.31 (0.13-0.74)	
	log-additive				0.45 (0.28-0.73)	<0.001
rs2283123 (DBH)	additive	C/C	0.89	0.96	1	0.140
		C/T	0.10	0.04	0.36 (0.10-1.32)	
		T/T	0.01	0.00		
	dominant	C/C	0.89	0.96	1	0.07
		C/T-T/T	0.11	0.04	0.32 (0.09-1.15)	
	recessive	C/C-C/T	0.99	1.00	1	0.25
		T/T	0.01	0.00		
	log-additive				0.33 (0.10-1.12)	0.056
rs11246226 (DRD4)	additive	A/A	0.74	0.89	1	0.09
		A/C	0.24	0.11	0.40 (0.17-0.95)	
		C/C	0.01	0.00		
	dominant	A/A	0.74	0.89	1	0.031
		A/C-C/C	0.26	0.11	0.39 (0.16-0.94)	
	recessive	A/A-A/C	0.99	1.00	1	0.560
		C/C	0.01	0.00		
	log-additive				0.39 (0.16-0.94)	0.029
rs4331145 (DRD4)	additive	A/A	0.36	0.64	1	0.046
		A/G	0.62	0.33	0.36 (0.16-0.83)	
		G/G	0.02	0.04	1.20 (0.09-15.39)	
	dominant	A/A	0.36	0.64	1	0.022
		A/G-G/G	0.64	0.37	0.39 (0.17-0.88)	
	recessive	A/A-A/G	0.98	0.96	1	0.590
		G/G	0.02	0.04	1.98 (0.16-25.08)	
	log-additive				0.49 (0.23-1.03)	0.055

TABLE 4. Genotypic associations of 12 significant tag single nucleotide polymorphisms (tagSNP) with schizophrenia susceptibility – continued

SNP	Genotype model	Genotypes	Frequency		Odds ratio (95% confidence interval)	*P*
			control	case		
rs4320932 (TH)	Additive	G/G	0.56	0.87	1	
		A/G	0.43	0.13	0.19 (0.07-0.51)	0.001
		A/A	0.02	0.00		
	Dominant	G/G	0.56	0.87	1	<0.001
		A/G-A/A	0.44	0.13	0.18 (0.06-0.49)	
	Recessive	G/G-A/G	0.98	1.00	1	0.25
		A/A	0.02	0.00		
	Log-additive				0.18 (0.07-0.49)	<0.001
rs140700 (SLC6A4)	Additive	G/G	0.77	0.85	1	
		A/G	0.18	0.14	0.68 (0.31-1.49)	0.290
		A/A	0.05	0.01	0.27 (0.03-2.45)	
	Dominant	G/G	0.77	0.85	1	0.190
		A/G-A/A	0.23	0.15	0.61 (0.29-1.28)	
	Recessive	G/G-A/G	0.95	0.99	1	0.220
		A/A	0.05	0.01	0.28 (0.03-2.60)	
	Log-additive				0.62 (0.33-1.17)	0.130
rs1050565 (SLC6A4)	Additive	A/A	0.48	0.32	1	
		A/G	0.40	0.50	1.74 (0.88-3.42)	0.160
		G/G	0.13	0.19	2.11 (0.82-5.40)	
	Dominant	A/A	0.48	0.32	1	0.063
		A/G-G/G	0.53	0.68	1.82 (0.96-3.45)	
	Recessive	A/A-A/G	0.88	0.81	1	0.300
		G/G	0.13	0.19	1.57 (0.66-3.75)	
	Log-additive				1.51 (0.97-2.37)	0.068
rs2020917 (COMT)	Additive	T/T	0.33	0.33	1	
		C/T	0.38	0.55	1.32 (0.63-2.75)	0.041
		C/C	0.29	0.13	0.44 (0.17-1.12)	
	Dominant	T/T	0.33	0.33	1	0.850
		C/T-C/C	0.67	0.68	0.93 (0.47-1.85)	
	Recessive	T/T-C/T	0.71	0.87	1	0.016
		C/C	0.29	0.13	0.37 (0.16-0.86)	
	Log-additive				0.72 (0.46-1.12)	0.140
rs165815 (COMT)	Additive	T/T	0.50	0.31	1	
		C/T	0.30	0.49	2.27 (0.94-5.47)	0.175
		C/C	0.20	0.20	1.75 (0.61-5.03)	
	Dominant	T/T	0.50	0.31	1	0.076
		C/T-C/C	0.50	0.69	2.08 (0.93-4.61)	
	Recessive	T/T-C/T	0.80	0.80	1	0.747
		C/C	0.20	0.20	1.18 (0.45-3.06)	
	Log-additive				1.42 (0.85-2.36)	0.180

Dysregulation of dopaminergic neurotransmission has been implicated in several neuropsychiatric diseases including schizophrenia, bipolar disease, and attention deficit disorder. Therefore, genes involved in the metabolic pathway of dopamine are biologically important candidates in the susceptibility of these disorders. Although a large body of data exists in the literature on association between polymorphisms in dopaminergic genes and schizophrenia, the results are inconsistent (17-21). While the inconsistent results could be attributed to phenotypic and genetic heterogeneity of schizophrenia, many studies considered a few polymorphisms within a gene based on past literature reports.

We pursued a more comprehensive approach of tagging the entire gene regions taking the advantage of the HapMap database. TagSNPs were selected on 8 genes that include 2 dopamine receptors, *DRD5* and *DRD4*; 2 genes involved in dopamine synthesis, *TH* and *DBH*; 2 neurotransmitter transporters, *SLC6A3* and *SLC6A4*; a serotonin recep-

tor, *HTR1B*; and 1 gene involved in dopamine degradation, *COMT*. Forty-nine tagSNPs in these genes were tested for association with schizophrenia risk in a Croatian population. Our study shows significant allelic association of 12 tagSNPs with schizophrenia susceptibility in the Croatian sample. Eleven of these tagSNPs also show significant genotypic associations. Four of the associated SNPs located in *DRD5 (rs1850744), HTR1B (rs2143823), DBH (rs2007153), and TH (rs4320932)* showed significant allelic and genotypic associations. The remaining 8 SNPs that showed moderate levels of association are located in *SLC6A3 (rs464049), DBH (rs2283123), DRD4 (rs11246226, rs4331145), SLC6A4 (rs140700, rs1050565),* and *COMT (rs2020917, rs165815)*. We further evaluated these 12 tagSNPs under 4 genetic models (additive, dominant, recessive, and log-additive) to identify at-risk genotypes. Interestingly, all of 4 most significantly associated SNPs showed significant ORs in at least 2 of the genetic models reaffirming the signals of associations. While tagSNPs provide a comprehensive assessment of genetic association, haplotype analysis based on tagSNPs is not likely to provide consequential information. Note that we used tagSNPs based on a pair-wise r^2 (≥0.8) among the common SNPs within the tagged region that would likely render statistical independence among the tagSNPs with the possibility of haplotypes being unstable.

A limitation of our study is a comparatively small sample size and the results should be considered preliminary. However, the observed associations are biologically relevant. Animal model and expression studies have implicated these genes in the metabolism of catecolamines (22-25). *DRD5* encodes the D5 subtype of the dopamine receptor, which is expressed in neuron in the limbic regions of the brain. *HTR1B*, located on 6q13-q26, is identified as one of the schizophrenia susceptibility gene and is linked with many neuropsychiatric diseases (26,27). The protein encoded by dopamine beta-hydroxylase (*DBH*) converts dopamine to norepinephrine. Variants in *DBH* were associated with modulation in psychotic symptoms in schizophrenia (28). *TH* is the rate-limiting step in catecholamine biosynthesis (29). Seeman et al (30) suggested involvement of dopamine supersensitivity and elevated activity of *TH*, *DBH*, and *DRD4* genes in rat striatal tissue along with elevation of other dopamine pathway genes. One of the more well studied genes in neuropsychiatric disorders is the catechol-O-methyltransferase (*COMT*) gene, which catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. A large case-control study of Ashkenazi Jews showed highly significant association between a *COMT* haplotype and schizophrenia (17). However, the findings have been inconclusive, with several studies, including a meta-analysis, showing no association (31). We found 2 *COMT* SNPs (*rs2020917* and *rs165815*) that were associated with risk of schizophrenia. A recent study reported haplotype-based association of the serotonin transporter 5-HTT (*SLC6A4*) with schizophrenia, but failed to find association at single marker level (32). It is notable that we found moderate association at 2 SNPs (*rs140700* and *rs1050565*) in this gene.

The present study is based on a candidate gene approach. It should be noted that, as with other complex diseases, genome wide association studies have been initiated to identify genetic risk variants associated with psychiatric disorders (33). While genome wide associations will potentially uncover the risk variants following an unbiased approach, candidate gene association studies will be important in pursuing the roles of known genes with functional implications in the pathophysiology of the disease. However, a comprehensive assessment of the genetic variation within the genes of interest will be important. The tagSNPs are not likely to be causal variants, but are rather in statistical associations with putative functional variants. Therefore, assessment of biological relevance of these indirectly associated sequence variants is not imperative; however, they provide the basis for further investigation leading to the discovery of sequences directly implicated in the disease pathophysiology.

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