ORIGINAL ARTICLE

Case–control association study of 59 candidate genes reveals the *DRD2* SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population

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The development of molecular psychiatry in the last few decades identified a number of candidate genes that could be associated with schizophrenia. A great number of studies often result with controversial and non-conclusive outputs. However, it was determined that each of the implicated candidates would independently have a minor effect on the susceptibility to that disease. Herein we report results from our replication study for association using 255 Bulgarian patients with schizophrenia and schizoaffective disorder and 556 Bulgarian healthy controls. We have selected from the literatures 202 single nucleotide polymorphisms (SNPs) in 59 candidate genes, which previously were implicated in disease susceptibility, and we have genotyped them. Of the 183 SNPs successfully genotyped, only 1 SNP, rs6277 (C957T) in the *DRD2* gene ($P=0.0010$, odds ratio=1.76), was considered to be significantly associated with schizophrenia after the replication study using independent sample sets. Our findings support one of the most widely considered hypotheses for schizophrenia etiology, the dopaminergic hypothesis.

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INTRODUCTION

Schizophrenia (F20, International Classification of Diseases—10th Revision (ICD X)) is a severe mental illness characterized by the alteration of higher functions, deterioration of behavior, cognition, emotions, motivation and perception, and marked by socio-occupational dysfunction. A wide variety of positive (auditory hallucinations, paranoid delusions), negative (affective flattening, anhedonia, alolia) and cognitive (declined attention, memory) symptoms comprise its diverse clinical heterogeneity, which, together with the lack of decisive laboratory tests, set obstacles for unanimous diagnosis.¹ Typically, the onset in male patients is in young adulthood, between 15 and 25 years, and in women it emerges about a decade later.² The lifetime incidence of schizophrenia is estimated to be about 1% worldwide, with untypical rates among certain groups of the society.² In DSM-IV-TR

(Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision, 2000), the criteria of schizoaffective disorder (F25, ICD X) include both characteristic schizophrenia symptoms (generally hallucinations or delusions) and affective symptoms (manic, depressive or mixed), which can be observed during the same episode of the illness, although during some periods the psychotic symptoms can be observed independently from prominent mood disorder features. Similar to schizophrenia, the onset of schizoaffective disorder is also usually in early adulthood, but it is more common in women than in men.³

The high heritability of schizophrenia was estimated to be about 80% and its complex (non-Mendelian) mode of transmission was suspected through the abundance of data from family, twin and adoption studies.^{1,4} It was also suggested that the disorder is a result

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of the complicated additive interaction between multiple genes, each having a small effect.^{2,5,6} The multifactorial polygenic threshold model, sustained by a number of linkage and association studies, proposes that both genetic background and environmental factors, including infections, stress and trauma, are necessary for triggering schizophrenia.^{4,5} The case-control association studies have been proven to be a more appropriate method for detection of genes with small effect, compared with linkage studies, as they allow recognition of relatively small differences if the sample size and the selection criteria are adequate.^{1,7}

Several hypotheses concerning the etiology of schizophrenia have been proposed, and most of them have focused on the alterations during neuronal development.⁸ According to the developmental model, various prenatal and perinatal noxae cause formation of inappropriate synaptic contacts, irregular cell signaling and migration during the fetal period in brain areas important for development of integration in the society. Such maldevelopment does not interfere with the basic brain functioning in the early years, but expresses itself when the subject is stressed by demands for integration in young adulthood.^{9,10}

The biochemical models are based on alterations in the neurotransmitter pathways supported by the effect of some of the antipsychotic medicaments that block certain receptors in the brain. The classical dopaminergic hypothesis asserts that the excessive dopaminergic signaling causes the psychotic symptoms, and is a result of increased sensitivity and density of dopamine D2 receptors in some brain regions.^{2,11} Similar are the serotonin, glutamate and other metabolic hypotheses, but none of them can sufficiently explain the complex symptoms of schizophrenia, and it is still unclear whether the affection of those circuits is a consequence of the treatment and years of persistency, rather than a cause for the disease.^{1,2}

Despite the incontestable significance of the genetic background of schizophrenia and great efforts in a large number of studies to clarify it, none of the candidates suspected to be involved in the pathogenesis was conclusively confirmed. To date, there are still no causative genes that are commonly accepted, even though some candidates were replicated in different studies and seemed to be promising.¹² Population heterogeneity, phenotypic heterogeneity, interweaving diagnostic criteria and misclassification, overestimated risk in the initial study, small sample size, influence of multiple loci (causal heterogeneity) and genetic-environmental interactions are among the indicated causes for the failure of replication of association studies.^{5,7,12,13} Some of the strongest causative candidates to date are considered to be *DISC1*, *DTNBP1*, *COMT*, *NRG1* and *RGS4*.

The aim of this study was to research the association between the common genetic variants in candidate genes, recently reported in the literature, and schizophrenia in an independent collective group from Bulgarian patients with schizophrenia or schizoaffective disorder, who differ in their genetic background from patients analyzed in other studies.

MATERIALS AND METHODS

Subjects

In this study, we have collected 811 unrelated Caucasians from Bulgaria, namely 198 patients with schizophrenia, 57 with schizoaffective disorder and 556 unrelated healthy control individuals. Samples from patients with schizophrenia (paranoid form) or schizoaffective disorder were recruited at four regional University psychiatric clinics—1st Psychiatric Dispensary of Sofia, Psychiatric Dispensary of Plovdiv, Psychiatric Dispensary of Blagoevgrad, and Psychiatric Dispensary of Radnevo. The patients were examined and diagnosed, according to the criteria of the DSM-IV, by two psychiatrists independently. The psychiatrists conducted interviews and provided written information to the participants. A written informed consent form was signed before the collection of blood samples. The identity of the patients was known only by the

interviewing psychiatrists and by the head of the corresponding psychiatric clinic. All samples and accompanying information (age, gender, severity and length of affection) were identified only by a unique code. All patients were on antipsychotic therapy. The mean age of the patients was 45.3 years (range: 23–81; s.d. $\pm$ 10.9) and the mean age of disease onset was 26.9 years (range: 13–52; s.d. $\pm$ 8.4). Of the 255 affected patients, 124 (49%) were males and 131 (51%) were females.

The control samples were collected from healthy volunteers in Bulgaria, who have provided written informed consents as well. This group included 289 (52%) males and 267 (48%) females, with an average age of 50.5 years (range: 18–86; s.d. $\pm$ 16.0). Each individual of the control group was interviewed by a physician so that any psychiatric disorder could be excluded, and data on their general medical history and information about their age, gender and ethnoses were obtained. Ethical approvals for conducting the study were obtained from the Ethical Committees in the SNP Research Center (present name: Center for Genomic Medicine), Yokohama Institute, The Institutes of Physical and Chemical Research (RIKEN, Yokohama, Japan) and the Local Medical Ethic Committees from each of the participating institutions.

DNA sampling

DNA was extracted from the peripheral venous blood (30 ml) and was stored in the DNA Bank at the Department of Medical Genetics (Medical Faculty, Medical University, Sofia, Bulgaria). Each tube was marked with a unique identification code. The DNAs were extracted by a standard phenol-chloroform procedure and stored at -20°C before their transportation to Japan, where the genotyping was performed.

Genotyping

A total of 64 single nucleotide polymorphisms (SNPs) from 25 genes were determined with commercially available pre-designed TaqMan probes and primers by means of the GeneAmp PCR System 9700 from Applied Biosystems (Foster City, CA, USA), according to the manufacturer's protocol. The genotypes were determined by an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The remaining 119 SNPs were genotyped by a combined Multiplex PCR and Invader assay, as described elsewhere.¹⁴

Gene and SNP selection

A total of 59 candidate genes from 18 chromosomes were selected if they met at least one of the following criteria: evidence for positive association with schizophrenia from previous case-control or family-based studies; positional candidates—located in some of the most promising candidate regions from genome-wide scans and large-scale studies of schizophrenia; or functional candidates—plausible involvement in the disease pathogenesis consistent with the neurodevelopment and biochemical (neurotransmitter and cell signaling) etiological models. Some candidate genes were excluded when most of their previously reported markers were already examined in Bulgarian trios in earlier studies.

We have selected 202 candidate SNPs from 59 candidate genes according to the public information in NCBI (Builds 35 and 36), the Schizophrenia Research Forum (<http://www.schizophreniaforum.org>) and the literatures. Most of the SNPs had minor allele frequencies of >0.05 , although exceptions were made for non-synonymous SNPs with an unknown frequency from genes of interest as well as from six SNPs with minor allele frequencies of <0.05 showing an association in Caucasian studies. For some genes (*DISC1*, *DTNBP1*, *RGS4*, *GABRB2*, *ERBB4* and *ERBB3*), we have selected additional non-synonymous SNPs, and polymorphisms at the 5' and 3' untranslated regions with presumptive effect over the transcriptional level. Detailed information about genotyped SNPs is illustrated in Table 1.

Table 1 Candidate genes and SNPs successfully genotyped in the first screening for association with schizophrenia

Locus	Gene	SNP ID	Allele 1	Allele 2	Position/effect	MAF		HWE (P-value)		P-value		
						Cases	Controls	Cases	Controls	1 vs 2	vs 11	vs 22
1p13	<i>NTNG1</i>	rs1373336	T	C	Intron 5	0.46	0.48	0.90	0.13	0.12	0.64	0.042
1p34	<i>GRIK3</i>	rs6691840	A	C	Ser 310 Ala	0.29	0.27	0.74	0.26	0.62	0.83	0.53
1p36	<i>MTHFR</i>	rs1801131	T	G	Glu 429 Ala	0.30	0.35	0.59	0.66	0.16	0.21	0.41
		rs1801133	G	A	Ala 222 Val	0.36	0.33	1.00	0.51	0.39	0.34	0.88
1q23	<i>RGS4</i>	rs10917670	T	C	5' UTR	0.45	0.39	0.77	0.98	0.085	0.13	0.22
		rs951439	C	T	5' UTR	0.46	0.40	0.76	0.97	0.14	0.32	0.18
		rs951436	A	C	5' flanking	0.44	0.48	0.12	0.45	0.032	0.039	0.19
		rs2661319	T	C	Intron 1	0.45	0.47	0.37	1.00	0.044	0.044	0.27
		rs10759	A	C	Exon 5 untr.	0.26	0.27	0.49	0.70	0.87	1.00	0.83
1q23	<i>UHMK1</i>	rs10494370	A	G	Intron 1	0.12	0.12	1.00	0.49	1.00	0.90	0.69
		rs7513662	A	G	Intron 4	0.34	0.26	0.46	0.81	0.027	0.027	0.24
		rs6427680	T	C	Intron 7	0.45	0.40	0.38	0.34	0.18	0.27	0.36
1q23	<i>CAPON</i>	rs348624	T	C	Exon 9 syn.	0.12	0.15	0.44	1.00	0.23	1.00	0.18
1q24	<i>MPZL1</i>	rs2051656	A	G	Intron 5	0.20	0.16	0.16	1.00	0.13	0.078	1.00
1q32	<i>CHI3L1</i>	rs4950928	G	C	5' UTR	0.19	0.22	0.94	1.00	0.36	0.59	0.38
1q32	<i>PLXNA2</i>	rs841865	A	G	Intron 14	0.14	0.14	1.00	0.67	0.83	0.69	1.00
		rs752016	A	G	Intron 12	0.19	0.17	0.34	0.71	0.50	0.57	0.60
		rs2498020	A	G	Intron 8	0.30	0.27	0.55	0.70	0.57	0.92	0.34
1q42	<i>GNPAT</i>	rs487047	A	G	Intron 1	0.49	0.49	1.00	0.28	0.71	0.46	1.00
		rs508908	A	T	Intron 1	0.49	0.50	1.00	0.22	0.71	0.39	0.90
		rs538643	T	C	Intron 4	0.48	0.50	0.84	0.74	0.77	0.90	0.81
		rs539699	T	C	Intron 10	0.48	0.50	1.00	0.39	0.50	0.90	0.39
1q42	<i>DISC1</i>	rs3738401	A	G	Arg 264 Gln	0.32	0.30	0.77	1.00	0.58	1.00	0.53
		rs2793092	G	A	Intron 3	0.19	0.19	0.70	0.97	1.00	0.79	0.83
		rs2812393	C	G	Intron 6	0.35	0.39	0.71	0.68	0.32	0.24	0.88
		rs1322784	A	G	Intron 6	0.30	0.26	0.37	0.53	0.25	0.60	0.083
		rs1322783	C	T	Intron 6	0.12	0.12	0.43	0.41	1.00	1.00	1.00
		rs2255340	C	T	Intron 8	0.29	0.23	0.45	0.66	0.075	0.24	0.065
		rs2738864	C	T	Intron 8	0.29	0.24	0.53	0.79	0.095	0.25	0.11
		rs6675281	C	T	Leu 607 Phe	0.13	0.12	1.00	0.55	0.74	0.62	0.72
		rs2295959	T	C	Intron 9	0.12	0.079	0.95	1.00	0.065	1.00	0.064
		rs999710	A	G	Intron 10	0.37	0.38	0.55	0.72	0.82	0.76	0.91
		rs11122359	G	A	Intron 10	0.35	0.31	0.41	0.99	0.31	0.25	0.86
		rs821597	A	G	Intron 10	0.36	0.36	0.77	0.88	1.00	1.00	1.00
		rs843979	C	G	Intron 10	0.34	0.33	0.79	0.84	0.82	0.83	1.00
		rs821616	A	T	Ser 704 Cys	0.28	0.33	1.00	0.34	0.68	0.53	0.84
		rs2806465	C	G	Intron 12	0.42	0.45	0.42	0.17	0.41	0.79	0.11
2q14	<i>IL1b</i>	rs16944	G	A	5' UTR	0.34	0.32	0.36	0.81	0.53	0.75	0.53
2q14	<i>IL1RN</i>	rs1794068	A	G	Intron 2	0.22	0.21	0.33	0.87	0.72	0.65	0.91
2q31	<i>GAD1</i>	rs3749034	A	G	Exon 1 utr.	0.23	0.24	0.65	0.31	0.86	0.68	1.00
		rs2241165	C	T	Intron 2	0.25	0.24	0.68	0.50	0.61	0.67	0.40
2q33	<i>ERBB4</i>	rs7598440	C	T	Intron 25	0.43	0.50	0.83	0.80	0.065	0.13	0.16
		rs839523	C	T	Intron 26	0.35	0.28	0.23	0.36	0.056	0.12	0.19
		rs707284	C	T	Intron 27	0.37	0.33	0.76	0.98	0.19	0.29	0.28
		rs1905139	A	G	Intron 28	0.48	0.45	0.41	0.71	0.46	1.00	0.26
3p25	<i>SYN2</i>	rs308963	C	G	Intron 2	0.22	0.20	0.53	1.00	0.47	1.00	0.39
		rs795009	T	G	Intron 8	0.22	0.20	0.59	1.00	0.59	0.52	1.00
3p26	<i>CHL1</i>	rs2272522	C	T	Leu 17 Phe	0.20	0.20	0.24	0.28	1.00	0.66	0.17
3q13	<i>DRD3</i>	rs2134655	T	C	Intron 5	0.26	0.26	0.090	0.48	0.93	0.62	1.00
		rs324030	G	C	Intron 2	0.32	0.31	0.31	0.66	0.75	0.59	0.40
		rs7625282	A	G	Intron 2	0.25	0.26	0.32	0.60	0.80	0.83	0.27

Table 1 Continued

Locus	Gene	SNP ID	Allele 1	Allele 2	Position/effect	MAF		HWE (P-value)		P-value		
						Cases	Controls	Cases	Controls	1 vs 2	vs 11	vs 22
		rs324029	A	G	Intron 2	0.32	0.31	0.34	0.66	0.75	0.59	0.40
		rs6280	C	T	Ser 9 Gly	0.38	0.35	0.96	0.82	0.36	0.65	0.40
		rs1800828	C	G	Intron 1	0.23	0.22	0.33	1.00	0.79	1.00	0.51
		rs2399504	T	C	5' UTR	0.18	0.19	1.00	0.31	0.70	0.41	1.00
		rs905568	C	G	5' UTR (ZNF 80)	0.44	0.42	0.65	0.58	0.60	0.79	0.66
4p16	<i>GPR78</i>	rs1282	C	T	5' flanking	0.21	0.25	0.66	0.80	0.22	0.39	0.23
4q22	<i>CLDN5</i>	rs10314	G	C	Exon 2 utr.	0.16	0.13	0.0040	1.00	0.21	0.63	0.053
5q12	<i>KIF2</i>	rs2289883	A	G	Intron 5	0.38	0.39	1.00	0.25	0.82	0.65	0.51
		rs464058	A	G	Intron 17	0.25	0.25	0.35	0.44	0.86	0.92	1.00
5q23	<i>Neurog1</i>	rs8192558	A	C	Exon 1 utr.	0.27	0.26	0.95	0.53	0.74	0.60	0.84
		rs2344484	A	G	5' UTR	0.36	0.34	0.57	0.93	0.70	0.83	0.64
5q31	<i>GRIA1</i>	rs707176	T	C	Exon 4 syn.	0.35	0.35	0.41	1.00	0.82	0.67	0.87
		rs2963944	A	G	Intron 5	0.35	0.35	0.41	0.91	1.00	0.83	0.75
5q34	<i>GABRB2</i>	rs2229944	A	G	Exon 10 syn.	0.10	0.12	0.99	0.39	0.40	0.45	0.51
		rs187269	A	G	Intron 2	0.36	0.31	0.45	1.00	0.16	0.35	0.15
		rs252944	C	G	Intron 2	0.14	0.12	1.00	0.52	0.38	0.47	0.62
		rs1816071	C	T	Intron 2	0.39	0.33	0.22	1.00	0.12	0.10	0.40
		rs3816596	C	T	Ser 179 Phe	0.34	0.42	1.00	0.25	0.027	0.14	0.031
6p21	<i>TNF/LTA</i>	rs2229094	C	T	Cys 13 Arg	0.30	0.25	0.88	0.25	0.19	0.20	0.35
6p21	<i>TNF</i>	rs1800629	A	G	5' UTR	0.089	0.13	0.42	0.75	0.078	0.25	0.10
6p21	<i>TNXB</i>	rs1009382	C	T	Gly 2578 Glu	0.16	0.15	1.00	1.00	0.92	1.00	1.00
		rs204887	A	G	Exon 22 syn.	0.17	0.17	0.88	0.89	1.00	0.75	0.91
6p21	<i>CREBL1</i>	rs8283	A	G	Exon 18 utr.	0.26	0.22	0.28	0.92	0.23	0.52	0.14
6p21	<i>NOTCH4</i>	rs204993	A	G	Intron 4 (PBX2)	0.17	0.17	0.087	0.38	1.00	0.50	0.14
		rs2071287	C	T	Intron 20	0.36	0.38	0.57	0.0080	0.82	0.33	0.40
		rs520692	T	C	Asp 272 Gly	0.49	0.43	0.15	0.20	0.044	0.64	0.0053
		rs367398	A	G	5' UTR	0.46	0.35	0.20	0.05	0.0039	0.00016	0.22
6p22	<i>DTNBP1</i>	rs1047631	A	G	Exon 10 utr.	0.12	0.14	1.00	0.83	0.50	0.62	0.45
		rs17470454	A	G	Ser 272 Pro	0.038	0.060	0.45	0.98	0.17	1.00	0.15
		rs742106	T	C	Intron 9	0.33	0.39	0.26	0.40	0.14	0.38	0.17
		rs760666	A	G	Intron 7	0.26	0.24	0.94	0.28	0.49	1.00	0.40
		rs3213207	A	G	Intron 4	0.10	0.14	0.036	0.018	0.11	0.15	0.41
		rs1011313	T	C	Intron 4	0.068	0.092	0.83	0.93	0.28	0.50	0.39
		rs760761	T	C	Intron 3	0.18	0.20	0.96	0.93	0.51	0.57	0.66
		rs2619522	A	C	Intron 1	0.18	0.20	0.92	0.74	0.63	0.82	0.57
		rs1018381	T	C	Intron 1	0.10	0.08	1.00	0.60	0.51	0.50	0.68
		rs1474605	A	G	Intron 1	0.18	0.20	0.76	0.93	0.58	0.74	0.41
		rs1997679	T	C	Intron 1	0.36	0.32	0.53	1.00	0.21	0.61	0.17
		rs909706	T	C	Intron 1	0.34	0.36	0.085	0.48	0.44	0.92	0.051
		rs2619538	T	A	5' UTR	0.45	0.41	0.93	0.65	0.23	0.18	0.59
6q23	<i>TAAR6</i>	rs4305745	G	A	3' flanking	0.43	0.45	0.41	0.88	0.60	0.35	1.00
		rs6903874	T	C	3' flanking	0.20	0.20	0.16	0.13	0.93	0.39	0.070
		rs6937506	G	A	3' flanking	0.20	0.20	0.065	0.22	0.93	0.51	0.046
7q21	<i>GRM3</i>	rs917071	C	T	Intron 1	0.30	0.30	0.84	1.00	0.94	1.00	0.86
		rs6465084	A	G	Intron 2	0.21	0.25	0.55	0.60	0.25	0.29	0.53
		rs1468412	A	T	Intron 3	0.29	0.31	0.65	0.43	0.63	0.75	0.73
		rs2228595	T	C	exon 3 syn.	0.043	0.079	1.00	1.00	0.064	1.00	0.076
		rs187993	T	G	3' UTR	0.34	0.31	0.67	0.91	0.48	0.46	0.73
8p21	<i>VMAT1</i>	rs2270637	C	G	Thr 98 Ser	0.19	0.16	0.19	0.084	0.21	0.65	0.011
8p21	<i>PPP3CC</i>	rs2272080	T	G	5' UTR	0.086	0.055	0.49	0.84	0.11	0.069	1.00

Table 1 Continued

Locus	Gene	SNP ID	Allele 1	Allele 2	Position/effect	MAF		HWE (P-value)			P-value	
						Cases	Controls	Cases	Controls	1 vs 2	vs 11	vs 22
8q23	<i>YWHAZ</i>	rs983583	T	C	Intron 1	0.42	0.40	0.69	0.61	0.46	0.68	0.52
9q34	<i>GRIN1</i>	rs11146020	G	C	Exon 1 utr.	0.11	0.11	0.33	0.74	0.91	0.90	0.37
10p12	<i>PIP5K2A</i>	rs1053454	A	C	3' UTR	0.32	0.30	0.17	0.58	0.75	0.62	1.00
		rs746203	T	C	Intron 8	0.34	0.32	0.43	0.73	0.58	0.75	0.63
10q21	<i>HTR7</i>	rs4933194	C	T	Intron 1	0.47	0.43	0.93	0.014	0.24	0.046	0.80
		rs12412496	A	G	Intron 1	0.24	0.25	0.24	0.07	0.86	0.055	0.53
11p13	<i>BDNF</i>	rs6265	C	T	Val 66 Met	0.20	0.24	0.84	0.73	0.16	0.20	0.37
11p15	<i>TPH1</i>	rs1799913	G	T	Intron 6	0.38	0.41	0.58	0.52	0.45	0.23	1.00
		rs684302	A	G	Intron 1	0.41	0.42	0.50	0.37	0.82	0.58	0.38
		rs4537731	T	C	5' near	0.40	0.39	0.30	1.00	0.76	0.51	0.77
11q22	<i>GRIK4</i>	rs4430518	T	G	Intron 6	0.20	0.20	0.20	0.93	1.00	0.75	0.38
		rs2156635	C	G	Intron 10	0.43	0.39	0.93	1.00	0.33	0.51	0.41
11q23	<i>DRD2</i>	rs1800497	A	G	Glu 713 Lys	0.20	0.20	0.46	0.40	0.93	0.41	0.59
		rs6277	A	G	exon 7 syn.	0.48	0.40	0.28	0.41	0.018	0.0050	0.43
		rs6275	A	G	exon 7 syn.	0.32	0.25	0.32	0.53	0.040	0.84	0.013
		rs1079597	T	C	Intron 1	0.17	0.15	0.81	0.90	0.42	0.50	0.57
11q24	<i>NRGN</i>	rs7113041	C	G	Intron 1	0.24	0.28	1.00	0.91	0.35	0.29	0.83
		rs1804829	A	G	3' UTR	0.042	0.027	1.00	1.00	0.31	1.00	0.31
		rs12541	T	C	3' UTR (ESAM)	0.16	0.19	0.51	0.75	0.49	0.51	0.72
12p12	<i>GRIN2B</i>	rs1805502	A	G	Exon 13 (3'utr.)	0.17	0.21	0.84	0.22	0.22	0.37	0.32
		rs1805476	T	G	Exon 13 (3'utr.)	0.46	0.42	0.83	1.00	0.37	0.69	0.36
		rs890	A	C	Exon 13 (3'utr.)	0.46	0.43	0.12	0.41	0.50	0.42	0.89
		rs1805246	A	G	Exon 13 syn.	0.030	0.033	1.00	1.00	0.84	1.00	0.83
		rs1805247	A	G	Exon 13 syn.	0.12	0.087	0.48	0.81	0.18	0.23	0.68
		rs1806191	A	G	Exon 13 syn.	0.42	0.48	1.00	0.14	0.14	0.59	0.065
		rs1806201	A	G	Exon 13 syn.	0.34	0.33	0.48	0.84	0.94	0.86	0.83
		rs1805482	A	G	Exon 8 syn.	0.32	0.28	0.86	0.18	0.23	0.17	0.47
		rs7301328	C	G	Exon 2 syn.	0.44	0.39	0.51	0.29	0.20	0.43	0.23
12q13	<i>ERBB3</i>	rs1019385	A	C	5' flanking	0.48	0.49	0.51	0.64	0.82	0.46	0.72
		rs3741499	C	T	Intron 1	0.24	0.27	0.66	0.46	0.35	0.35	0.65
		rs2271194	A	T	Intron 2	0.30	0.33	0.64	0.51	0.53	0.72	0.53
		rs877636	A	G	Intron 4	0.25	0.27	0.53	0.59	0.56	0.60	0.65
		rs705708	A	G	Intron 15	0.44	0.41	0.33	0.42	0.46	0.52	0.58
		rs2292238	A	C	Intron 25	0.39	0.36	0.15	0.65	0.45	1.00	0.14
		rs2271189	A	G	Exon 27 syn.	0.36	0.34	0.29	0.45	0.49	0.16	1.00
		rs773123	A	T	Ser 1119 Cys	0.10	0.11	1.00	0.63	0.72	0.60	1.00
12q24	<i>NOS1</i>	rs1047735	A	G	Exon 12 syn.	0.31	0.28	0.13	0.82	0.42	0.30	0.75
		rs3782219	C	T	Intron 1	0.19	0.20	0.70	1.00	0.93	0.83	1.00
		rs3782221	A	G	Intron 1	0.24	0.22	0.96	0.83	0.66	1.00	0.67
13q14	<i>KPNB3</i>	rs2761072	A	G	Intron 6	0.48	0.44	0.33	0.53	0.37	0.45	0.57
		rs626716	A	G	Exon 10 syn.	0.038	0.048	1.00	0.10	0.58	0.85	0.24
		rs624066	A	G	Intron 11	0.47	0.43	0.095	0.32	0.37	0.38	0.65
13q14	<i>KPNA3</i>	rs3736830	G	C	Intron 6	0.17	0.18	0.88	0.51	0.77	0.75	0.58
		rs2181185	T	C	Intron 2	0.13	0.12	1.00	0.42	0.91	1.00	0.37
13q14	<i>HTR2A</i>	rs659734	A	G	Intron 2	0.049	0.071	0.010	0.77	0.22	0.070	0.25
		rs6313	A	G	Exon 1 utr.	0.46	0.46	0.90	0.52	0.94	0.61	0.82
14q32	<i>CHGA</i>	rs9658635	T	C	5' flanking	0.21	0.25	0.42	0.0029	0.22	0.094	0.75
14q32	<i>AKT1</i>	rs2494732	T	C	Intron 11	0.46	0.47	1.00	0.25	0.88	0.73	0.45

Table 1 Continued

Locus	Gene	SNP ID	Allele 1	Allele 2	Position/effect	MAF		HWE (P-value)		P-value		
						Cases	Controls	Cases	Controls	1 vs 2	vs 11	vs 22
19q13	MAG	rs3730358	A	G	Intron 3	0.16	0.18	0.48	0.019	0.69	0.053	0.73
		rs10149779	A	G	Intron 2	0.27	0.30	0.88	0.14	0.33	0.84	0.15
		rs1130214	A	C	Intron 1	0.28	0.33	0.76	0.17	0.23	1.00	0.094
		rs3803300	T	C	5' flanking	0.17	0.10	0.21	0.59	0.013	0.22	0.028
		rs2301600	C	T	Exon 4 syn.	0.22	0.20	0.85	0.13	0.47	0.24	0.49
		rs720309	A	T	Intron 7	0.14	0.12	0.58	0.22	0.58	0.28	0.33
		rs720308	A	G	Intron 7	0.14	0.12	0.64	0.22	0.66	0.39	0.28
20p12	CHGB	rs236152	C	G	Ala 353 Gly	0.39	0.33	0.035	1.00	0.092	0.025	1.00
		rs236153	T	C	Exon 4 syn.	0.40	0.33	0.019	1.00	0.079	0.018	1.00
		rs236155	A	G	Intron 4	0.40	0.33	0.042	1.00	0.067	0.87	0.019
21q22	S100B	rs9722	G	A	Exon 3 utr.	0.10	0.083	0.47	0.19	0.52	0.48	1.00
		rs11542311	C	G	Exon 2 syn.	0.29	0.36	0.47	0.23	0.041	0.27	0.046
		rs2839364	T	C	5' UTR	0.14	0.16	0.24	0.33	0.46	0.62	0.48
		rs2839365	C	G	5' UTR	0.39	0.42	1.00	1.00	0.32	0.57	0.38
		rs9975039	A	G	5' UTR	0.071	0.068	1.00	1.00	0.89	0.88	1.00
21q22	OLIG2	rs6517135	T	C	5' UTR	0.15	0.16	0.88	1.00	0.92	0.82	1.00
		rs1005573	C	T	Intron 1	0.29	0.33	0.18	0.12	0.37	0.43	0.49
		rs1122807	A	T	Intron 1	0.0080	0.0030	1.00	1.00	0.62	0.62	1.00
		rs1059004	A	C	Exon 2 utr.	0.42	0.45	0.78	0.75	0.37	0.43	0.49
		rs9653711	G	C	3' UTR	0.42	0.45	0.60	0.78	0.41	0.49	0.57
		rs2073776	T	C	3' UTR	0.31	0.33	0.22	0.05	0.58	0.032	0.60
22q11	DGCR2	rs2072123	A	G	Ala 473 Val	0.37	0.41	0.73	0.22	0.37	0.91	0.12
		rs807759	A	G	5' flanking	0.43	0.49	0.10	0.036	0.14	0.82	0.0035
		rs713729	T	A	Intron 3	0.27	0.27	0.83	0.65	0.87	0.68	0.84
22q13	PICK1	rs3952	A	G	Intron 3	0.33	0.34	0.079	1.00	0.75	0.83	0.29
		rs2076369	T	G	Intron 4	0.38	0.36	0.32	0.59	0.59	0.87	0.52
22q13	SYNGR1	rs909685	A	T	Intron 1	0.34	0.29	0.73	0.90	0.11	0.16	0.25
		rs715505	G	C	Intron 1	0.25	0.22	0.96	0.41	0.29	0.53	0.23
22q13	MCHR1	rs133068	C	G	5' flanking	0.38	0.40	0.85	0.93	0.71	0.75	0.89
		rs133069	A	C	5' flanking	0.38	0.40	0.85	0.84	0.76	0.89	0.83
		rs133073	C	T	Exon 1 syn.	0.40	0.41	0.66	1.00	0.76	1.00	0.66
Total	59	180										

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism. The order of the genes and SNPs corresponds to the human genome reference sequence. P-values of SNPs estimated by Fisher's exact test.

Statistical analyses

Genotype and allele frequencies were evaluated by using the two-tailed Fisher's exact test in allelic, dominant- and recessive-inheritance models. Deviations from the Hardy–Weinberg equilibrium were evaluated by the HAPLOVIEW 4.0 program (Broad Institute of MIT and Harvard).

RESULTS

This study was performed in two subsequent screenings. In the first screening, all selected candidate SNPs were genotyped in 185 cases and 184 controls collected in three major psychiatric centers in Bulgaria. During the first screening, additional samples for verification were collected from the dispensary in Radnevo. All SNPs showing a

P-value of <0.05 in the two-tailed Fisher's exact test in the first screening proceeded to the second screening. Three SNPs (two from *DISC1* and one from *DTNBP1* genes) showed deviation from the Hardy–Weinberg equilibrium in the case group and were excluded from further analysis. Six SNPs (two from the *ERBB3* gene and four from the *ERBB4* gene) were found to be monomorphic in this population.

The genotyping in the initial set of samples identified 21 SNPs as possible candidates for susceptibility for schizophrenia/schizoaffective disorder with a P-value of <0.05 by Fisher's exact test. Subsequently, these 21 variants were genotyped in an additional 70 case samples from Bulgarian patients with schizophrenia and 372 Bulgarian control samples. Two polymorphisms—rs2255340 from *DISC1* gene and

rs909706 from *DTNBP1* gene—were included in the follow-up screening, because some trend for association was considered in the initial genotyping and because those two candidates were suggested to be important functional candidates in previous studies. Probably because of the small sample size of the additional independent case group, only one SNP (rs6277) from the *DRD2* gene revealed a positive association ($P=0.00067$, Table 2). The SNP remained significantly associated with schizophrenia after the adjustment for multiple testing, according to strict Bonferroni's correction. These data, together with the negligible discrepancies of the allele and genotype frequencies with previous studies in Caucasian populations: Northern European populations living in Australia, Spain, Finland and Russia,^{15–18} support the association of *DRD2* with schizophrenia.

DISCUSSION

We have performed a case–control association study for schizophrenia in the Bulgarian population using 183 SNPs present in 59 candidate genes, the implication of which in the disease etiology had been previously reported. We have successfully assessed 180 SNPs in a group of 185 cases and 184 controls, and selected 21 SNPs from 15 genes to be possible candidates for the association ($P<0.05$). Later, we managed to obtain additional sets and performed adjustments for multiple testing, according to strict Bonferroni's correction. Finally, we implied that the marker, rs6277 (*DRD2*), preserved statistical significance for association with schizophrenia/schizoaffective disorder. We could replicate only rs6277 (corrected P -value of 0.014). However, the previously reported markers were not replicated, either because of the limitations in the number of patients and controls (lack of the power) or because of false-positive results in papers reported earlier.

Neurotransmitters have been considered to be involved in the development of schizophrenia in the past. Dysfunction of the dopaminergic pathway in its pathogenesis has been extensively discussed in the literature for the last few decades. The classical dopaminergic hypothesis remains one of the most commonly acknowledged hypotheses, although it still remains unclear whether the observed alterations are causal or result from long-term treatment with antipsychotic medications. It postulates that psychotic symptoms are a consequence of dopamine excess in specific brain regions (particularly in the mesolimbic and nigrostriatal areas).² Postmortem and *in vivo* neuroimaging studies indicate increased dopamine D2 receptor density and binding affinity in the striatum of schizophrenic patients.^{11,19,20} Enhanced expression of *DRD2* in the caudate nucleus was implicated in the cognitive dysfunction of affected individuals.² Furthermore, common antipsychotic medications are capable of affecting the positive symptoms of the disease² as a result of antagonistic or partially agonistic interactions with the dopamine D2 receptor, whereas dopamine agonists, such as amphetamines, provoke schizophrenia-like psychosis.^{2,11,21}

The dopamine receptor genes comprise a large superfamily that encodes G-protein-coupled receptors important for regulation of some higher functions, such as locomotion, cognition and emotions.^{22–24} Five human dopamine receptors are described and classified into two major groups, dopamine 1-like (D1) and dopamine 2-like (D2), in accordance with their transcriptional and pharmacological properties.²⁵ The dopamine D2 receptors (*DRD2*) are abundant on the postsynaptic membranes in the mesocortical pathway,²⁶ implicated in the control of the feelings of reward and pleasure. At least two different isoforms of the protein exist as a result of alternative splicing of exon 6.^{23,27}

Several case–control association studies have been performed to evaluate the likely contribution of variants in the *DRD2* gene to

schizophrenia susceptibility. Positive results for the association between the A allele of the TaqI A polymorphism (rs1800497) and schizophrenia were discovered by case–control studies in few independent groups, where an overtransmission of certain haplotypes, including the TaqI A2 allele, was observed.^{28,29} After Itokawa *et al.*³⁰ focused on the implication of the Ser311Cys polymorphism, Arinami *et al.*³¹ were the first to describe a positive association between this non-synonymous SNP of the *Dopamine D2 receptor* gene and schizophrenia. However, over 20 subsequent studies attempted to elucidate this variant's contribution to the disorder and most of them failed to reveal that correlation. Comprehensive meta-analyses suggested a weak but positive effect of the Cys allele as the risk factor for susceptibility to schizophrenia ($P=0.007$).^{32,33} In another study, Arinami *et al.*³⁴ reported a positive association for another *DRD2* gene polymorphism, –141C (Ins/Del), which became one of the most commonly tested in the quest for susceptibility variants. In an attempt to replicate these data, several case–control studies and meta-analyses were conducted, with controversial results.^{2,35}

Our results in the Bulgarian sample group (Table 2) are consistent with the previous reports by other researchers on the positive association between the synonymous C957T (rs6277) polymorphism and schizophrenia, in different Caucasian populations.^{15–18} In our study, the G allele (allele 2), which corresponds to the C allele in the single nucleotide polymorphism database (dbSNP) and the literature, was identified as the risk allele. After the initial study by Lawford *et al.*,¹⁷ reporting association of rs6277 in the Australian (Caucasian) population, three subsequent studies confirmed significantly higher frequencies of the C allele and the C/C genotype in patients with schizophrenia who were of European descent (in the Finnish,¹⁵ Spanish¹⁶ and Russian¹⁸ populations). However, a case–control study using subjects from India failed to confirm the positive relation with schizophrenia, although some tendency for association was seen.³⁶ Furthermore, the results of two meta-analyses, including the data from all five studies¹⁸ or those from Caucasian populations,³⁷ found a nominally significant association of the C allele and the C/C genotype with schizophrenia with pooled allelic odds ratios (ORs) of 1.42 (95% confidence interval (CI): 1.26–1.61)¹⁸ and 1.45 (95% CI: 1.21–1.73),³⁷ and summary OR for the genotype of 1.60 (95% CI 1.32–1.95).¹⁸ As all positive associations were found in populations from European descent, ancestry-specific effect must be taken into consideration, and the genetic effect of this variant across populations with different ancestral descents must be evaluated.³⁷

Our findings have provided additional evidence for the importance of variants in the *DRD2* genes for the vulnerability to schizophrenia. Despite rs6277 being a synonymous polymorphism causing no amino acid substitution (Pro319Pro), it was reported to cause some effect on the gene expression in an *in vitro* assay, suggesting that the 957T variant significantly affects the stability and the expression level of the *DRD2* mRNA.^{15,17,23}

Recent *in vivo* study in healthy volunteers discovered a correlation between the C957T polymorphic variants and the intrasynaptic dopamine levels and D2 receptor-binding affinity in the striatum. In support of the dopaminergic hypothesis for the etiology of schizophrenia, the highest binding potential of the receptor was found in the C/C homozygotes, whereas the T/T homozygotes showed the lowest affinity.^{15,17,38} Furthermore, C957T showed a population attributable risk for schizophrenia of 24% and an attributable risk in patients with schizophrenia of 42%.¹⁷

In summary, our findings further suggest that *DRD2* may play an important, but most probably not independent, role in the pathogenesis of schizophrenia in the Bulgarian population. Previous genetic

Table 2 Genotyping results for 21 SNPs after the first and the second screening

Locus	Gene	SNP ID	Screening	Case			Control			Frequency, case			Frequency, control			Risk allele ^a	P-value		
				11	12	22	11	12	22	11	12	22	11	12	22		1 vs 2	vs 11	vs 22
1p13	NTNG1	rs1373336	1st	53	94	38	48	81	55	0.29	0.51	0.21	0.26	0.44	0.30	1	0.12	0.64	0.042
			2nd	16	36	17	98	185	85	0.23	0.52	0.25	0.27	0.50	0.23		0.64	0.65	0.76
			Total	69	130	55	146	266	140	0.27	0.51	0.22	0.26	0.48	0.25		0.42	0.86	0.29
1q23	RGS4	rs951436	1st	63	80	42	44	85	53	0.34	0.43	0.23	0.24	0.47	0.29	1	0.032	0.039	0.19
			2nd	24	33	13	95	192	83	0.34	0.47	0.19	0.26	0.52	0.22		0.20	0.14	0.53
			Total	87	113	55	139	277	136	0.34	0.44	0.22	0.25	0.50	0.25		0.028	0.009	0.37
1q23	RGS4	rs2661319	1st	56	80	39	41	91	51	0.32	0.46	0.22	0.22	0.50	0.28	1	0.044	0.044	0.27
			2nd	22	34	14	83	199	89	0.31	0.49	0.20	0.22	0.54	0.24		0.17	0.13	0.54
			Total	78	114	53	124	290	140	0.32	0.47	0.22	0.22	0.52	0.25		0.017	0.0049	0.28
1q23	UHMK1	rs7513662	1st	75	84	17	100	73	11	0.43	0.48	0.10	0.54	0.40	0.06	2	0.027	0.027	0.24
			2nd	29	35	6	169	166	37	0.41	0.50	0.09	0.45	0.45	0.10		0.77	0.60	0.83
			Total	104	119	23	269	239	48	0.42	0.48	0.09	0.48	0.43	0.09		0.18	0.12	0.79
1q42	DISC1	rs2255340	1st	93	70	18	103	67	8	0.51	0.39	0.10	0.58	0.38	0.04	2	0.075	0.24	0.065
			2nd	37	26	7	216	131	19	0.53	0.37	0.10	0.59	0.36	0.05		0.19	0.36	0.16
			Total	130	96	25	319	198	27	0.52	0.38	0.10	0.59	0.36	0.05		0.013	0.077	0.0093
5q34	GABRB2	rs3816596	1st	82	82	21	67	81	36	0.44	0.44	0.11	0.36	0.44	0.20	1	0.027	0.14	0.031
			2nd	30	34	6	144	184	43	0.43	0.49	0.09	0.39	0.50	0.12		0.44	0.59	0.54
			Total	112	116	27	211	265	79	0.44	0.45	0.11	0.38	0.48	0.14		0.067	0.12	0.18
6p21	NOTCH4	rs367398	1st	44	82	59	15	93	68	0.24	0.44	0.32	0.09	0.53	0.39	1	0.0031	0.00010	0.19
			2nd	14	32	24	56	174	142	0.20	0.46	0.34	0.15	0.47	0.38		0.35	0.37	0.59
			Total	58	114	83	71	267	210	0.23	0.45	0.33	0.13	0.49	0.38		0.0031	0.00050	0.12
6p21	NOTCH4	rs520692	1st	50	81	53	52	96	28	0.27	0.44	0.29	0.30	0.55	0.16	2	0.044	0.64	0.0037
			2nd	21	38	11	117	179	75	0.30	0.54	0.16	0.32	0.48	0.20		0.78	0.89	0.42
			Total	71	119	64	169	275	103	0.28	0.47	0.25	0.31	0.50	0.19		0.085	0.41	0.040
6p22	DTNBP1	rs909706	1st	76	94	15	77	80	27	0.41	0.51	0.08	0.42	0.43	0.15	1	0.44	0.92	0.051
			2nd	35	28	7	156	165	50	0.50	0.40	0.10	0.42	0.44	0.13		0.21	0.24	0.45
			Total	111	122	22	233	245	77	0.44	0.48	0.09	0.42	0.44	0.14		0.20	0.70	0.037
6q23	TAAR6	rs6937506	1st	114	68	3	111	49	10	0.62	0.37	0.02	0.65	0.29	0.06	1	0.93	0.51	0.046
			2nd	47	19	4	229	121	22	0.67	0.27	0.06	0.62	0.33	0.06		0.50	0.42	1.00
			Total	161	87	7	340	170	32	0.63	0.34	0.03	0.63	0.31	0.06		0.43	0.94	0.077
8p21	VMAT1	rs2270637	1st	124	51	10	128	55	1	0.67	0.28	0.05	0.70	0.30	0.01	2	0.21	0.65	0.011
			2nd	55	14	1	274	85	12	0.79	0.20	0.01	0.74	0.23	0.03		0.36	0.46	0.70
			Total	179	65	11	402	140	13	0.70	0.25	0.04	0.72	0.25	0.02		0.30	0.56	0.18
10q21	HTR7	rs4933194	1st	51	94	40	69	73	42	0.28	0.51	0.22	0.38	0.40	0.23	2	0.24	0.046	0.80
			2nd	24	29	17	119	183	66	0.34	0.41	0.24	0.32	0.50	0.18		0.64	0.78	0.24
			Total	75	123	57	188	256	108	0.29	0.48	0.22	0.34	0.46	0.20		0.18	0.20	0.40
11q23	DRD2	rs6277	1st	45	101	39	70	82	32	0.24	0.55	0.21	0.38	0.45	0.17	2	0.018	0.0050	0.43
			2nd	13	27	27	122	171	79	0.19	0.40	0.40	0.33	0.46	0.21		0.00067	0.031	0.0012
			Total	58	128	66	192	253	111	0.23	0.51	0.26	0.35	0.46	0.20		0.0010	0.0013	0.054
11q23	DRD2	rs6275	1st	15	87	83	13	64	106	0.08	0.47	0.45	0.07	0.35	0.58	1	0.040	0.84	0.013
			2nd	3	39	28	33	154	182	0.04	0.56	0.40	0.09	0.42	0.49		0.62	0.24	0.19
			Total	18	126	111	46	218	288	0.07	0.49	0.44	0.08	0.39	0.52		0.14	0.58	0.023
14q32	AKT1	rs3803300	1st	8	46	131	3	32	149	0.04	0.25	0.71	0.02	0.17	0.81	1	0.013	0.22	0.028
			2nd	5	20	45	8	99	261	0.07	0.29	0.64	0.02	0.27	0.71		0.11	0.041	0.32
			Total	13	66	176	11	131	410	0.05	0.26	0.69	0.02	0.24	0.74		0.031	0.024	0.13

Table 2 Continued

Locus	Gene	SNP ID	Screening	Case			Control			Frequency, case			Frequency, control			Risk allele ^a	P-value		
				11	12	22	11	12	22	11	12	22	11	12	22		1 vs 2	vs 11	vs 22
20p12	CHGB	rs236152	1st	61	103	21	82	82	20	0.33	0.56	0.11	0.45	0.45	0.11	2	0.092	0.025	1.00
			2nd	26	31	13	148	180	44	0.37	0.44	0.19	0.40	0.48	0.12		0.29	0.79	0.12
			Total	87	134	34	230	262	64	0.34	0.53	0.13	0.41	0.47	0.12		0.085	0.053	0.49
20p12	CHGB	rs236153	1st	59	105	21	81	83	20	0.32	0.57	0.11	0.44	0.45	0.11	2	0.079	0.018	1.00
			2nd	26	31	13	148	180	44	0.37	0.44	0.19	0.40	0.48	0.12		0.29	0.69	0.17
			Total	85	136	34	229	263	64	0.33	0.53	0.13	0.41	0.47	0.12		0.067	0.036	0.49
20p12	CHGB	rs236155	1st	22	103	60	20	82	82	0.12	0.56	0.32	0.11	0.45	0.45	1	0.067	0.87	0.019
			2nd	13	31	26	44	180	148	0.19	0.44	0.37	0.12	0.48	0.40		0.29	0.17	0.69
			Total	35	134	86	64	262	230	0.14	0.53	0.34	0.12	0.47	0.41		0.059	0.42	0.044
21q22	S100B	rs11542311	1st	13	81	89	20	94	70	0.07	0.44	0.49	0.11	0.51	0.38	2	0.041	0.27	0.046
			2nd	11	31	28	47	170	155	0.16	0.44	0.40	0.13	0.46	0.42		0.63	0.45	0.89
			Total	24	112	117	67	264	225	0.09	0.44	0.46	0.12	0.47	0.40		0.10	0.34	0.13
22q11	DGCR2	rs2073776	1st	14	88	83	27	69	88	0.08	0.48	0.45	0.15	0.38	0.48	2	0.58	0.032	0.60
			2nd	8	36	26	44	152	175	0.11	0.51	0.37	0.12	0.41	0.47		0.28	1.00	0.15
			Total	22	124	109	71	221	263	0.09	0.49	0.43	0.13	0.40	0.47		0.95	0.10	0.23
22q11	DGCR2	rs807759	1st	54	87	28	57	76	51	0.32	0.51	0.17	0.31	0.41	0.28	1	0.11	0.91	0.015
			2nd	20	40	10	122	168	79	0.29	0.57	0.14	0.33	0.46	0.21		0.78	0.49	0.20
			Total	74	143	38	178	245	130	0.29	0.56	0.15	0.32	0.44	0.24		0.33	0.37	0.0051

Abbreviations: SNP, single nucleotide polymorphism.

First screening in 185 case samples and 184 control samples.

Second screening in additional 70 case samples and 372 control samples.

Total in 255 case samples and 556 control samples.

and functional studies point out this gene as a possible candidate for implication in schizophrenia etiology. Although the association of *NOTCH4*, *DISC1*, *DTNBP1*, *RGS4* and other highly putative candidate genes was not confirmed after the statistical evaluation, we observed some tendency for association with schizophrenia in the Bulgarian population. Hence, these genes should be further evaluated using a larger set of samples.

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