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SHORT COMMUNICATIONS

Genome-Wide Analysis of the Risk Association for the Development of Paranoid Schizophrenia in Russians: Search for Genetic Markers in the 1q43 Chromosomal Region

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Abstract—Schizophrenia is a highly hereditary disorder. Genetic risk is associated with a large number of alleles, including common alleles with little effect that can be found in genome-wide association studies. The aim of this study was to study genetic risk factors for the development of schizophrenia in a genome-wide association analysis (GWAS) in Russians from the Republic of Bashkortostan. The studied sample consisted of 320 patients with paranoid schizophrenia and 402 healthy individuals. GWAS genotyping of DNA samples was carried out on the PsychChip biochip, which included 610000 single nucleotide polymorphic variants (SNPs).

Keywords: genetics, schizophrenia, genome-wide association analysis, ethnicity, ethnospecific markers, Republic of Bashkortostan, Psychiatric Genomics Consortium (PGC)

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Schizophrenia is a complex multifactorial disease. Even after many years of scientific research, the pathogenesis of this heterogeneous disease remains unclear. This disease is known to involve disruption of brain function, likely caused by the interactions of multiple genes that are influenced by environmental factors leading to aberrant neurodevelopment and/or neurodegeneration [1]. Genome-wide association studies (GWAS) make it possible to simultaneously genotype several hundred thousand polymorphic gene loci and find every gene in the genome, and, like linkage analyses, the method is hypothesis-free and thus is able to identify genes, revealing as yet unknown pathogenetic mechanisms that may play an important role in the development of schizophrenia.

In order to identify ethnospecific genetic risk factors for the development of paranoid schizophrenia, we conducted a genome-wide association analysis in Russians from the Republic of Bashkortostan (Fig. 1).

The object of study is 320 patients (173 men, 147 women) of Russian ethnicity with a diagnosis of paranoid schizophrenia (PS) F20.0 according to the international classification of diseases, tenth revision (ICD-10), being treated at the Republican Clinical Psychiatric Hospital No. 1 of the Ministry of Health of the Republic of Bashkortostan. The average age of the patients was 24.9 ± 8.9 years. The average age of onset of the disease was 22.4 ± 7.3 years. Information on ethnicity up to the third generation was obtained by questionnaire. The control group consisted of 402 healthy individuals of the same ethnicity and age group, who were not registered with a psychiatrist or narcologist and denied a family history of mental illness. The average age of healthy donors was 32.4 ± 12.4 years.

Whole-genome genotyping of DNA samples was carried out on an Illumina Human 610-Quad PsychChip biochip, which included 610000 single nucleotide polymorphic variants (SNPs). Genome-wide analysis of the association of single-nucleotide polymorphic loci was performed using the PLINK 2.0 software package [2]. A detailed description of genome-wide association analysis was published previously [3].

To reduce the type 1 error, the FDR-BH correction (False Discovery Rate Bengamini–Hochberg) was applied to the number of multiple comparisons [4]. A genome-wide association analysis performed in individuals of Russian ethnicity revealed the most pronounced differences between patients with PS and the control group in polymorphic loci located in the 1q43 region (Fig. 1). The highest level of association of PS was found with SNP rs946936 (p = 1.42E-05) (Table 1). According to the 1000 Genomes Project, the frequency of allele *rs946936*A* in world populations varies from 24.3% in the Indian (GIH) to

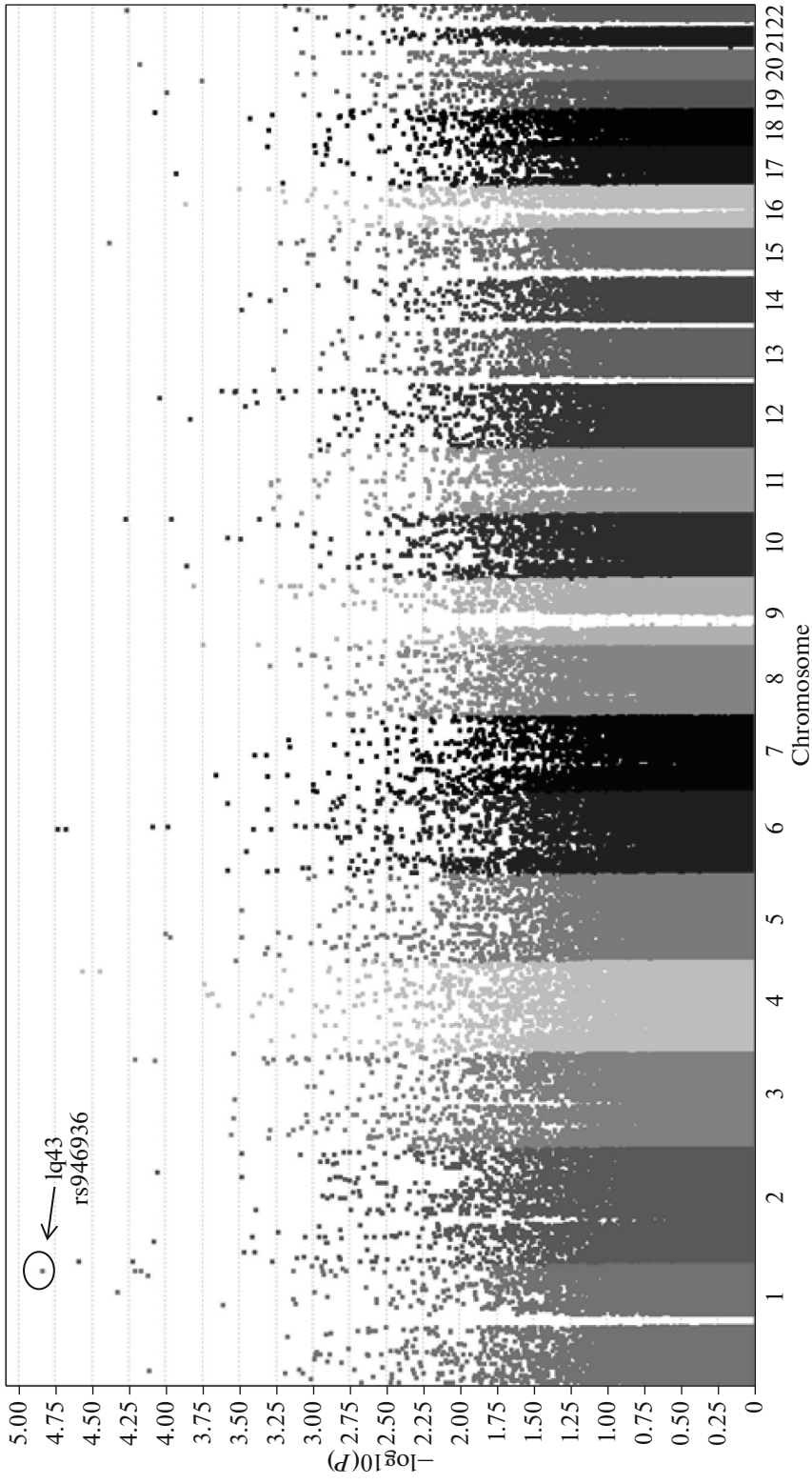

Fig. 1. Graphic representation of the results of a genome-wide analysis of the association of 395 832 SNPs with paranoid schizophrenia in Russians (Manhattan plot). The *x* axis shows the chromosomal localization of SNPs, and the *y* axis shows the values of the negative decimal logarithm of the *p*-value significance level.

Table 1. Single nucleotide polymorphic variants localized in the 1q43 region and associated with paranoid schizophrenia in Russians

Gene	rs no.	SNP	Allele 1	Frequency of allele 1, patients, %	Frequency of allele 1, control, %	Allele 2	p	p_{fdr}	OR
–	rs946936	g.237093381A>C	*A*	0.3516	0.245	*C*	1.42E-05	0.251	1.669
MTR	rs2853522	237061056A>C	*A*	0.3703	0.2706	*C*	6.11E-05	0.616	1.591
–	rs4351629	g.237077480G>T	*G*	0.3359	0.2388	*T*	6.67E-05	0.627	1.602
–	rs10802577	g.237126155C>T	*C*	0.2641	0.1903	*T*	0.001134	0.999	1.506
–	rs6428977	g.237083719A>G	*A*	0.3766	0.296	*G*	0.001301	0.999	1.442
MTR	rs10925257	g.92580A>G	*G*	0.2078	0.255	*A*	0.03421	0.999	0.762
MTR	rs1805087	g.94920A>G	*G*	0.2078	0.2537	*A*	0.03918	0.999	0.7673
–	rs1417303	g.237126385G>T	*G*	0.4078	0.3545	*T*	0.03929	0.999	1.252

51.6% in the Chinese population (CDX) (http://www.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=1:236929581-236930581;v=rs946936;vdb=variation;vf=713358).

The closest coding gene, located at a distance of about 30 kb from the given polymorphic locus is the gene *MTR* (1q43), encoding one of the key enzymes involved in the metabolism of homocysteine (HC), metabolizing the remethylation of HC into methionine-methionine synthase (MS). Vitamin B12 takes part as a cofactor in this reaction. The high level of activity of *MTR* leads to a decrease in plasma homocysteine [1]. Gene *MTR* consists of 33 exons spanning about 123 kb of genomic DNA (https://www.genecards.org/cgi-bin/carddisp.pl?gene=MTR). A number of studies demonstrate changes in the level of homocysteine in the plasma of patients with schizophrenia and other mental illnesses [1, 5, 6].

High levels of homocysteine can increase intracellular concentrations of free radicals, which can damage neuronal membranes and further impair brain function. As a member of the B vitamin family, folic acid is involved in the metabolism of HC, and it exhibits potent antioxidant activity. Moreover, folic acid deficiency and excess HC levels can increase intracellular calcium. Accumulated calcium can promote the production of free radicals and exacerbate nerve cell damage [6]. Antipsychotics can significantly increase folic acid and vitamin B12 levels, thereby causing a decrease in serum HC levels, which ultimately leads to a reduction in symptoms of schizophrenia [7]. The integrated actions of the folate and methionine cycles required for HC metabolism also support methylation and nucleotide synthesis, which are vital for supporting embryonic growth, proliferation, and development. Hyperhomocysteinemia has been associated with negative effects on embryonic development, including neural tube defects [8].

In patients with paranoid schizophrenia, the frequency of genotype *rs946936*A/A* was significantly higher than in the control group of individuals (11.88 and 6.47%, respectively) (p = 0.011, OR = 1.95, CI95% 1.16–3.29). Heterozygous genotype *rs946936*A/C* was also detected in patients with a higher frequency (46.56%) than in the control group (36.07%) (p = 4.4E-03, OR = 1.54, CI95% 1.14–2.08). Genotype *rs946936*C/C* in patients was rare, in 41.56% of cases, and in the controls, it was detected more often—in 57.46% of cases (p = 2.2E-05, OR = 0.53, CI95% 0.39–0.71). However, after introducing a correction for multiple comparisons to estimate the proportion of false positive results using the FDR-BH method, the differences in these genotypes turned out to be statistically insignificant (*rs946936*A/A* p_{fdr} = 0.999, *rs946936*A/C* p_{fdr} = 0.999, *rs946936*C/C* p_{fdr} = 0.999) (Table 2). Analysis of the allele frequency distribution of this polymorphic locus showed that the frequency of allele *rs946936*A* in patients the PS was higher (35.16%) than in the controls (24.5%) (p = 1.42E-05, p_{fdr} = 0.999, OR = 1.67, CI95% 1.33–2.1). The odds ratio for allele *rs946936*C* was 0.6 (CI95% 0.48–0.75) (Table 2).

A pronounced association with the development of paranoid schizophrenia was also established with single-nucleotide polymorphic loci rs2853522 and rs4351629, located in the chromosomal region 1q43 (Table 1). In patients with paranoid schizophrenia, the frequency of the homozygous genotype *rs2853522*A/A* (12.81%) was significantly higher than that in the control group (7.73%) (p = 0.024, OR = 1.75, CI95% 1.04–2.97). The frequency of heterozygous genotype *rs2853522*A/C* (48.44%) in patients PS was also higher than in healthy individuals (38.65%) (p = 8.4E-03, OR = 1.49, CI95% 1.11–2.01). Genotype *rs2853522*C/C* was more common in the control group of individuals (53.62%) than in patients with PS (38.75%) (p = 7.1E-05, OR = 0.55, CI95% 0.40–0.75). When introducing the FDR-BH correction, the significance level turned out to be statistically: insignificant (*rs2853522*A/A* p_{fdr} = 0.999,

Table 2. Distribution of frequencies of genotypes and alleles of polymorphic variants in samples of patients with paranoid schizophrenia and in control groups among Russians

Genotype, allele	Patients		Control		p	p_{fdr}	OR (CI95%)
	n_i	$p_i \pm S_p$ CI, %	n_i	$p_i \pm S_p$ CI, %			
rs946936							
A/A	38	11.88 ± 1.81 8.54–15.93	26	6.47 ± 1.23 4.27–9.33	0.011	0.999	OR = 1.95 (1.16–3.29)
A/C	149	46.56 ± 2.79 41–52.19	145	36.07 ± 2.4 31.37–40.98	4.4E-03	0.999	OR = 1.54 (1.14–2.08)
C/C	133	41.56 ± 2.75 36.11–47.18	231	57.46 ± 2.47 52.47–62.35	2.2E-05	0.999	OR = 0.53 (0.39–0.71)
A	225	35.16 ± 1.89 31.45–39	197	24.5 ± 1.52 21.57–27.63	1.42E-05	0.999	OR = 1.67 (1.33–2.1)
C	415	64.84 ± 1.89 61–68.55	607	75.5 ± 1.52 72.37–78.43	1.42E-05	0.999	OR = 0.6 (0.48–0.75)
rs2853522							
A/A	41	12.81 ± 1.87 9.35–16.98	31	7.73 ± 1.33 5.31–10.79	0.024	0.999	OR = 1.75 (1.07–2.86)
A/C	155	48.44 ± 2.79 42.84–54.06	155	38.65 ± 2.43 33.86–43.61	8.4E-03	0.999	OR = 1.49 (1.11–2.01)
C/C	124	38.75 ± 2.72 33.38–44.33	215	53.62 ± 2.49 48.6–58.58	7.1E-05	0.999	OR = 0.55 (0.41–0.74)
A	237	37.03 ± 1.91 33.28–40.9	217	27.06 ± 1.57 24.01–30.27	6.11E-05	0.999	OR = 1.59 (1.27–1.99)
C	403	62.97 ± 1.91 59.1–66.72	585	72.94 ± 1.57 69.73–75.99	6.11E-05	0.999	OR = 0.63 (0.5–0.79)
rs4351629							
G/G	35	10.94 ± 1.74 7.74–14.88	26	6.47 ± 1.23 4.27–9.33	0.032	0.999	OR = 1.78 (1.05–3.02)
G/T	145	45.31 ± 2.78 39.77–50.94	140	34.82 ± 2.38 30.17–39.71	4.2E-03	0.999	OR = 1.55 (1.15–2.09)
T/T	140	43.75 ± 2.77 38.24–49.38	236	58.71 ± 2.46 53.72–63.56	6.4E-05	0.999	OR = 0.55 (0.41–0.74)
G	215	33.59 ± 1.87 29.94–37.4	192	23.88 ± 1.5 20.97–26.98	6.67E-05	0.999	OR = 1.61 (1.28–2.03)
T	425	66.41 ± 1.87 62.6–70.06	612	76.12 ± 1.5 73.02–79.03	6.67E-05	0.999	OR = 0.62 (0.49–0.78)

*rs2853522*A/C* p_{fdr} = 0.999, *rs2853522*C/C* p_{fdr} = 0.999) (Table 2).

The frequency of allele *rs2853522*A* in patients with PS (37.03%) exceeded its frequency in the control group, where it was 27.06% (p = 6.11E-05, p_{fdr} = 0.999, OR = 1.59, CI95% 1.27–1.99). The frequency of allele *rs2853522*C* in the group of healthy individuals was significantly higher (72.94%) than in patients with PS (62.97%) (p_{fdr} = 6.11E-05, OR = 0.63, CI95% 0.5–0.79) (Table 2).

Analysis of the frequency distribution of genotypes and alleles of the polymorphic locus rs4351629 showed that genotypes *rs4351629*G/G* and *rs4351629*G/T* in patients with PS are more common (10.94 and 45.31%) than in healthy individuals (6.47 and 34.83%): for genotype *rs4351629*G/G*, p = 0.032, OR = 1.78, CI95% 1.05–3.02, for genotype *rs4351629*G/T*, p = 4.2E-03, OR = 1.15, CI95% 1.13–2.12. Genotype *rs4351629*T/T* in patients was determined with a frequency of 43.75%, and in the control group, it was determined with a frequency of

58.71% (p = 6.4E-05, OR = 0.55, CI95% 0.40–0.74). With the introduction of the FDR-BH correction, the significance level became statistically insignificant (*rs4351629*G/G* p_{fdr} = 0.999, *rs4351629*G/T* p_{fdr} = 0.999, *rs4351629*T/T* p_{fdr}= 0.999).

Alleles *rs4351629*G* and *rs4351629*T* in patients occur in 33.59 and 66.41% of cases, respectively, compared to 23.88 and 76.12% in healthy individuals. The odds ratio for allele *rs4351629*G* was 1.61 (CI95% 1.28–2.03), p = 6.67E-05; for allele *rs4351629*T*, it was 0.62 (CI95% 0.49–0.78). Correction for multiple comparisons showed no statistically significant differences (*rs4351629*G* p_{fdr} = 0.999, *rs4351629*T* p_{fdr}= 0.999) (Table 2).

Some studies have reported that the polymorphic variant rs1805087 (A2756G) of gene *MTR* led to an increase in the concentration of HC in carriers of allele *MTR*A*. Thus, an association was established for the functional polymorphic locus rs1805087 (A2756G) of gene *MTR* with schizophrenia [9, 10], with depressive disorders in Indians [11], and with Down syndrome in the East Indian population [12]. In the course of this study, no association of these SNPs with schizophrenia was found in Russians (Table 2).

As a result of a number of studies, an association of SNP genes localized in the chromosomal region 1q43 with schizophrenia and other mental and neurodegenerative diseases has been established [14–17]. In a study conducted in Norwegians and Icelanders, none of the SNPs reached the genome-wide level of significance; however, a number of polymorphic loci with a fairly high level of significance were identified, including the association of SNP rs6679053 of the phospholipase D5 gene—*PLD5* (1q43)—with the development of schizophrenia [13]. The GWAS study by M. Hamshere et al. Using the CLOZUK sample [14], consisting of patients with schizophrenia taking clozapine, an association of three polymorphic loci with the development of schizophrenia was established, one of which is rs6703335 of the gene for serologically detectable colon cancer antigen 8—*SDCCAG8* 1q43. It is known that the protein encoded by this gene may be involved in the organization of the centrosome during interphase and mitosis. Mutations in this gene are associated with nephroretinal syndrome (p = 4.22E-08) [14]. Subsequent GWAS also revealed an association of chromosomal region 1q43 (rs6703335 *SDCCAG8*) with the development of schizophrenia in European populations [15]. A genome-wide analysis of the association with the risk of developing schizophrenia in 2111 patients and 2535 individuals of Swedish ethnicity revealed an association with the gene that regulates G-protein signaling activity *RGS7* 1q43 (rs984402, p = 3.43E-07, OR = 0.79) with a genome-wide significance level [16]. In addition, according to a number of genome-wide studies, chromosomal region 1q43 is linked to multiple sclerosis, a neurodegenerative disease characterized by impaired myelination processes in the central nervous system [17].

Thus, the genome-wide analysis carried out in this study showed the absence of an association of paranoid schizophrenia in Russians with SNP rs946936, located in the 1q43 region, in close proximity to the gene *MTR*, while many published data have shown an association of chromosomal region 1q43 with the development of schizophrenia in various populations. The results of this work can be explained both by the insufficient sample size for this type of research and by interpopulation differences in the formation of hereditary predisposition to paranoid schizophrenia.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research ethics committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed voluntary consent was obtained from each of the participants included in the study. The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics-Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences, protocol No. 4 dated March 27, 2009. All participants were adults.

CONFLICT OF INTEREST

The author of this work declares that she has no conflicts of interest.

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