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ASSOCIATION STUDY OF DNA POLYMORPHIC LOCI OF SEROTONINERGIC NEUROTRANSMITTER SYSTEM GENES WITH SUICIDAL ATTEMPTS

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Several lines of evidence suggest that serotoninergic system genes are involved in the predisposition to suicidal behaviour. We examined whether the Msp1- restricted polymorphic marker of the serotonin 2A receptor gene (HTR2A), VNTR- and 5-HTTLPR polymorphic markers of the serotonin transporter gene (SLC6A4) were associated with one form of suicidal behaviour – suicidal attempts. 145 suicide attempters and 300 healthy volunteers were included in the study. All subjects were typed for the above-mentioned gene variants using polymerase chain reaction (PCR) technique. We found that allele A of the HTR2A gene (OR = 1.35) and genotype 12/10 of the SLC6A4 (OR = 1.57) were associated with suicidal behaviour. But our data showed that the 5-HTTLPR polymorphism of the SLC6A4 gene was unlikely to be involved in the biological susceptibility for suicide.

Introduction

Suicidal behaviour is a notion, which includes suicidal ideas and intention, suicide attempts, completed suicide. Suicide is an important public health problem, ranking among the top 10 causes of death for individuals all over the world [10]. The rate of suicide in Russia is 37,8 per 100 000 inhabitants per year. Suicidal attempt, less critical form of suicidal behaviour, have more wide distribution among the population.

Studies in twins and in adopted subjects have shown, that genetic risk factors accounted for approximately 55% of the variance in suicidal behaviour [21].

Resent molecular-genetical investigations of suicidal behaviour have been focused on a role of serotonergic system [7, 10]. The serotonin is one of the key neurotransmitters within both central and peripheric nervous systems. 7 classes and 14 subclasses of serotonin receptors are identified now [8]. The special attention is given to the serotonin receptor 2A gene (HTR2A). The human serotonin transporter (5-HTT) is believed to be essential for regulating the magnitude and duration of serotonergic responses [9]. Both genes are characterized by some polymorphisms, which are considered as genetic markers linked with behavioural disorders.

The results on the molecular-genetical investigations of suicidal behaviour are still separate and are discordant. One of possible reason results from a heterogeneity samples of subjects with suicide attempt by such criteria as a sex, age, mental status, mean of suicide, and also ethnic origin. Ethnic differences in allele frequencies are known for majority of polymorphisms of neutotransmitter systems genes [15, 21].

The aim of the present study was to analyse polymorphisms of the serotonin 2A receptor gene (HTR2A) and serotonin transpoter gene (SLC6A4) in suicidal behaviour.

Materials and methods

Genomic DNA was extracted from peripheral blood leukocytes using a standard phenol-chloroform method. Both polymorphisms were genotyped using amplification by polymerase chain reaction (PCR) and subsequent enzyme digestion. Polymerase chain reaction was carried out in a total volume of 12.5 (.iL con-taining 100 ng of genomic DNA; 0.25 μM of the specific primers; 0.25 mM dNTPs; 0.5 units of Taq DNA polymerase (Silex, Moscow, Russia) and 1.25 μL of 10 xbuffer (67 mM Tris-HCl, pH 8.8, 6.7 mM MgCI2, 16.6 mM $(NH_4)_2$ SO_4, 0.01% Tween-20).

The PCR products of the HTR2A gene were digested with 5 units of MspI enzime, The PCR products of all markers were electrophoresed on a 7% acrylamide gel (Table 1).

The genotype and allele frequency distribution of these polymorphisms were com- pared between suicidal and control groups using a chi-square test with Yets's correction; $p<0.05$ was considered statistically significant. Odds ratios(OR) with 95% confident intervals (CI) were calculated [17,19].

Table 1

Candidate genes of suicidal behaviour and their polymorphisms

Candidate genes, their localization	Polymorphism, localization	Primers	Alleles
HTR2A 13 q14-q21	(Msp1 - RFLP) promotor	5'-AAGCTGCAAGGTAGCAACAGC-3' 5'-AACCAACTTATTTCCTACCAC-3' [12].	A (468 bp) G (224, 244 bp)
SC6A4 17 q11.1 -q 12	(5-HTTLPR) promotor	5′-CTTGTTGGGGATTCTCCCGCCTGGCGTT-3′ 5′-CGAGGCTGAGCGTCTAGAGGGACTGAGCT G-3′ [19].	S (480 bp) L (520 bp)
	(VNTR) Intron 2	5′-GTCAGTATCAACAGGCTGCGAG-3′ 5′-TGTTCCTAGTCTTACGCCAGTG-3′ [5].	9 (250 bp) 10 (267 bp) 12 (301 bp)

Results and discussion

The HTR2A is a prime candidate gene of the suicidal behaviour. An alteration of the density of the serotonin 2A receptor has been demonstrated in cortical and subcortical regions in post-mortem brains of suicide victims. [8]. We investigated the Msp1 – RFLP (-1438G/A) in the HTR2A gene (table 1). This polymorphism or any other unknown polymorphisms close to the 1438G/A region are supposed to influence expression of the HTR2A gene. In both investigated groups genotype distribution was in Hardy-Weinberg equilibrium (Table 2). A/G genotype was the most frequent in both groups; its frequency was 0.55 in control group, and 0.61 – in suicide attempters group. G/G genotype had frequencies 0.31 (in control group) and 0.20 (in suicidal group); tA/A genotype was observed with almost equal frequency (0.19) in suicidal group, and its frequency was much lower (0.14) in control group. The differences in genotype frequencies of the HTR2A between these groups were not significant (χ^2= 5.52, p = 0.066).

The significant differences were revealed in allele frequencies between control and suicidal groups (χ^2 = 3.92, p = 0.048, OR = 1.35). The higher frequency of A allele was observed in suicidal group – 0.49 versus 0.42 in controls.

Thus, our data suggest the association of the HTR2A gene with suicidal behaviour. The subjects with suicide attempts had significantly higher frequency of A allele.

The data of some authors confirm association of suicidal behaviour with the Msp1-polymorphism of the HTR2A. For example, the associations of G/G genotype with suicide attempts in males from China [2], and of G allele with suicidal ideas in depression [7] were reported. There are also data about the association of this polymorphism with bipolar disorders in Korean population [3]. At the same time, Japanese researchers reported a lack of association of this polymorphism with completed suicide [15]. The allele frequencies of the A-1438G polymorphism seem to be ethic-specific. In studies of Japanese subjects, the frequencies of A allele were generally increased over G allele. In contrast, in studies of Caucasian subjects, the opposite frequencies were obtained: the frequency of G allele was higher than that of A allele.

Our data in healthy controls are in line with these reports: the frequency of G allele is higher than that of A allele.

Table 2

Genotype and allele frequency distribution of the MspI polymorphism of the serotonin 2A receptor gene in the suicidal and control groups

•Groups	**Value**	**Genotypes**			**Alleles**		χ^2 **(P)**
		A/G	G/G	A/A	G	A	
Control	277	152 (0.55)	85 (0.31)	40 (0.14)	322 (0.58)	232 (0.42)	2,28 (0.29)
Suicidal	143	87 (0.60)	29 (0.21)	27 (0.19)	145 (0.51)	141 (0.49)	3,39 (0.18)
• χ^2 (P) ~ conformity to Hardy-Weinberg equilibrium							

Таблица 3

Genotype and allele frequency distribution of the VNTR polymorphism of the serotonin transporter gene in the suicidal and control groups

•Groups	N	Genotypes					Alleles			χ^2 (P)
		12/10	12/12	10/10	10/9	12/9	12	10	9	
Control	301	117 (0.39)	133 (0.44)	42 (0.13)	4 (0.01)	5 (0.01)	388 (0.64)	205 (0.34)	9 (0.02)	2,04 (0.75)
Suicidal	140	70 (0.50)	47 (0.34)	18 (0.13)	2 (0.01)	3 (0.02)	167 (0.60)	108 (0.39)	5 (0.02)	0,43 (0.97)
N - value, • χ^2 (P) ~ conformity to Hardy-Weinberg equilibrium										

The serotonin transporter gene (SLC6A4) has been investigated in relation to behaviour. Many studies suggest that there is less 5-HTT binding in the thrombocytes of depressive subjects and in prefrontal cortex of suicide victims [8, 10]. We investigated 2 polymorphisms in the SLC6A4 gene – VNTR and 5-HTTLPR. Genotype distributions of both markers were in Hardy-Weinberg equilibrium.

VNTR polymorphism is located in intron 2 (9, 10 or 12 repeats) of the SCL6A4 (Tab. 1). The VNTR-polymorphism is suggested to influence gene expression on transcriptional level, or is in linkage disequilibrium with another functional polymorphism in the SLC6A4 gene. Allele with 9 repeats is likely to lead to the increased expression of this gene.

We revealed 5 genotypes of VNTR-polymorphic locus (tab. 3), from which 12/12 genotype was the most frequent in control group (0.44), and 12/10 genotype – in suicidal group (0.50). Genotypes with 9 allele (10/9, 12/9) had the lowest frequencies (< 5%) in the both groups. The genotype 9/9 was not revealed. Differences in genotype frequencies between the control and suicidal groups were not significant (χ^2 = 5.42, P = 0.24).

However, in suicidal group the significant increase of 12/10 genotype (χ^2 = 4.40, P = 0.036, OR= 1.57) and decrease of 12/12 genotype (χ^2 = 4.03, p = 0.045, OR = 0.64) were revealed.

Among three alleles of this marker, allele 12 was the most frequent – 0.60 and 0.64 (in group with suicidal behaviour and control group, respectively). Rare allele 9 in both groups had very low frequency (no more 0.02). There were no significant differences in allele frequencies between these groups (χ^2= 1.69, p = 0.44).

The obtained data suggest a possible significance of the SLC6A4 VNTR, namely, 12/10 genotype (OR = 1.57) in development of suicidal behaviour. The associations of 9 allele with depression [14], of genotype 12/12 and allele 12 with affective disorders have previoulsy been reported [22].

The 5-HTTLPR polymorphism consists of a 44 base pair insertion (long form, l) or deletion (short form, s) in the 5k regulatory region of the SLC6A4 gene. In studies of human lymphoblast cell lines expressing the different genotypes belonging to the l/s polymorphism, l/l lymphoblasts expressed more serotonin transporters than the l/s or s/s cells [13].

Table 4

Genotype and allele frequency distribution of the VNTR polymorphism of the serotonin transporter gene in the suicidal and control groups

•Groups	N	Genotypes			Alleles		χ^2 (P)
		S/L	L/L	S/S	L	S	
Control	268	127 (0,47)	76 (0,28)	65 (0,24)	279 (0.52)	257 (0,48)	0,37 (0,83)
Suicidal	143	58 (0.41)	48 (0.34)	37 (0.26)	154 (0.54)	132 (0.46)	2,11 (0.37)
χ^2 (P) ~ conformity to Hardy-Weinberg equilibrium							

Among three genotypes of the 5-HTTLPR (tab. 4), the genotype L/S was the most frequent: 0.47 - in control group, 0.44 - in group with attempts of suicide. The genotype L/L had frequencies 0.28 in control group and 0.33 in the group with attempts of suicide. The frequency of the S/S genotype in both groups was the lowest (0.24 and 0.23 - in control and suicidal group, respectively). In both groups, a long allele had a slightly higher frequency than a short allele : 0.55 versus 0.45 (in group with suicide attempts) and 0.52 versus 0.48 (in control group). There was no significant difference in frequencies of genotypes (χ^2 = 0.97, P = 0.60) and alleles (χ^2 = 0.62, P = 0.44) of the 5-HTTLPR in suicidal group and controls.

Our data do not confirm the association of this polymorphism with suicide; that is in line with results of some authors [4, 18]. However, there are multiple studies reported the association of the 5-HTTLPR with suicidal behaviour; the association was revealed for both short [1, 6, 16], and long allele [7]. Some researchers reported the possible association of this polymorphic marker with violent traits [20, 23].

Thus, our data allow to make conclusion that possible genetic markers of suicidal behaviour (attempts of suicide) are allele A of the HTR2A gene (OR = 1.35) and genotype 12/10 of the SLC6A4 gene (OR = 1.57). However, our data do not confirm the association of the 5-HTTLPR with vulnerability for suicide.

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