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Badanie asocjacji pomiędzy polimorfizmem genów 5-HTT, MAOA i DAT a samobójstwem u mężczyzn z populacji polskiej

Search for association between suicide and 5-HTT, MAOA and DAT polymorphism in Polish males

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Znalezienie markerów genetycznych umożliwiających ocenę ryzyka popełnienia samobójstwa miałyby istotne znaczenie w praktyce klinicznej. Celem przeprowadzonych badań było określenie czy istnieje asocjacja pomiędzy polimorfizmami w genach 5-HTT, MAOA i DAT a samobójstwem, a także określenie czy współwystępowanie wariantów alleli tych genów może predysponować do samobójstwa. Uzyskane wyniki wykazały brak statystycznie istotnych różnic w częstości alleli i genotypów w genach 5-HTT, MAOA i DAT pomiędzy grupą kontrolną a badaną. Analiza genotypów we wszystkich 4 loci wykazała różnice w częstości pomiędzy grupą kontrolną a samobójcami dla genotypu (3;12-12;S-S;9-10). Genotyp ten występował tylko w grupie kontrolnej z częstością 8% ($p=0,03$).

A better understanding of genetic determinants of suicidal behavior might be very useful in clinical practice. The objectives of the present study were to answer the question whether there is an association between functional polymorphic forms of 5-HTT, MAOA or DAT and suicidality, and to examine whether the combination of functional alleles in 5-HTT, MAOA and DAT genes would predict a predisposition to suicidal behavior. Functional polymorphisms in

5-HTT, MAOA and DAT genes were investigated in 66 male suicide completers and 51 male control subjects from the Polish population. There were no significant differences in the allele and genotype frequencies between the case and control group. In the individual genotype tests, examination of the distribution differences of each genotype showed that genotype (3;12-12;S-S;9-10) differed between the suicide victims and control subjects. This genotype existed only in the control sample and appeared with the frequency of 8% ($p=0.03$).

Key words: suicide, 5-HTT, MAOA, DAT

BACKGROUND

In the last decade, a growing number of molecular genetic studies have been carried out to identify candidate genes that may be involved in pathophysiological mechanisms of suicidal behavior. Post-mortem studies revealed interesting data on the serotonergic, noradrenergic and dopaminergic neurotransmitter systems of suicide victims. However, most of the attention is focused on serotonergic abnormalities, which are additionally related to a variety of psycho-

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pathological dimensions such as anxiety, depressed mood, impulsivity and aggression [1].

The crucial role in the regulation of serotonergic transmission by determining the magnitude and duration of 5-HT synaptic signal is played by the serotonin transporter (5-HTT) [2]. The human serotonin transporter is encoded by a single copy gene located on chromosome 17q11.1-q12. [3]. Two polymorphisms of the 5-HTT gene, which differently modulate transcription, have been identified: a 44-bp insertion-deletion in the promoter region (5-HTTLPR), and a variable number of tandem repeats in the second intron of the gene (VNTR) [4].

Monoamine oxidase-A (MAOA) is a mitochondrial enzyme, encoded by a gene located on chromosome Xp11.23-Xp11.4, which catalyzes oxidative deamination of biogenic amines such as noradrenaline, dopamine and serotonin [5]. Sabol et al. identified a common polymorphism of a variable number of tandem repeats (VNTR) in the promoter region of the MAOA gene, which was shown to be associated with MAOA transcriptional activity. This polymorphism is located 1.2 kb upstream of the MAOA coding sequences and consists of a 30-bp repeated sequence present in 3, 3.5, 4, or 5 copies [6].

The dopamine transporter (DAT) is a plasma membrane transport protein, encoded by a gene located on chromosome 5p15.3, which mediates an uptake of dopamine into presynaptic neurons. The 3' untranslated region of the dopamine transporter gene contains a 40-bp variable number of tandem repeat (VNTR), with two common alleles of 9 and 10 repeat elements [7]. A number of studies was devoted to investigation of the functional role played by DAT VNTR polymorphism, although the results remain inconclusive.

Over the past few years, several groups have investigated the possible association between suicidal behavior and the above-mentioned polymorphisms, but usually applied to only one of them. The aim of our study was to answer the question whether there is an association between functional polymorphic forms of 5-HTT, MAOA or DAT and suicidality, as well as to examine whether the combination of functional alleles in 5-HTT, MAOA and DAT genes would predict a predisposition to suicidal behavior.

MATERIAL AND METHODS

The case sample consisted of 66 male suicide completers (mean age 42.9 ± 17.9 years),

who were autopsied at the Institute of Forensic Medicine of Collegium Medicum in Bydgoszcz. The methods of committing suicide included hanging ($n=62$), jumping from heights ($n=2$), use of firearms ($n=1$) and jumping under a train ($n=1$) and were classified as violent. Buccal swabs were obtained from 51 randomly selected unrelated male individuals (mean age 35.3 ± 12.9 years) from the general population in the Pomerania-Kujawy region of Poland, who served as controls. We selected both suicide victims and controls of male gender because of the gender-specific association with suicidality [3].

The study protocol was approved by the Ethical Committee of Collegium Medicum in Bydgoszcz. Human genomic DNA was extracted from blood or saliva according to the standard procedures. Quantification of DNA was performed spectrophotometrically. The PCR procedures for the examined gene polymorphisms were described elsewhere: serotonin transporter and monoamine oxidase-A [8], dopamine transporter [9]. The PCR products for MAOA and DAT were separated by 2.5% agarose gel electrophoresis followed by ethidium bromide staining and visualized under UV light. Various alleles were determined using Gene Ruler 50 bp DNA ladder (Fermentas). The PCR products for 5-HTT labeled with different fluorescent dyes (5-HTTLPR –labeled with FAM and 5-HTTVNTR –labeled with HEX) were separated and detected by capillary electrophoresis on ABI PRISM 3130xl (Applied Biosystems).

A simultaneous determination of antidepressant drugs (amitriptyline, chlordiazepoxide, carbamazepine, chlorpromazine, citalopram, paroxetine, clomipramine, doxepin, fluoxetine, levomepromazine, maprotiline, paroxetine, perazine, mianserine, promazine, sertraline, thioridazine) in blood samples, hair and nails was performed using high-performance liquid chromatography with mass spectrometry (LC/MS).

The statistical significance of differences between the case and control group distribution for alleles and genotypes was determined using the chi-squared tests. The association analysis was performed using logistic regression analysis. The Fisher exact test was performed to compare distributions of the obtained genotypes between the case and control groups. The statistical analyses were performed using Statistica software (version 8). The Arlequin program was employed to determine departure from Hardy-Weinberg equilibrium and linkage disequilibrium between two loci. The significance level for all

statistical tests was 0.05. We applied Bonferroni correction for multiple tests (the level of significance was set to $\alpha=0.01$).

RESULTS

The sample of 66 suicide victims and 51 control subjects was genotyped for 5-HTTLPR and intron 2 polymorphism of 5HT transporter gene and two polymorphisms of a variable number tandem repeat: one in the promoter region of the MAOA gene and the other in 3' untranslated region of the dopamine transporter gene (DAT). Distribution of genotype frequencies in all loci was in accord with Hardy-Weinberg equilibrium in both groups. The allele frequen-

cies in suicide victims were not significantly different from those in the control group, for either 5-HTTLPR ($\chi^2=0.24$, $df=1$, $p=0.62$), or 5-HTTVNTR ($\chi^2=1.66$, $df=2$, $p=0.44$). We detected no significant linkage disequilibrium between the 5-HTTLPR and the 5-HTTVNTR polymorphism in suicide victims ($\chi^2=2.48$, $p=0.29$) and in control subjects ($\chi^2=2.15$, $p=0.34$). There were no significant differences between the controls and suicide victims in allele frequencies of the MAOA gene polymorphism ($\chi^2=3.72$, $df=2$, $p=0.16$) and DAT gene polymorphism ($\chi^2=0.91$, $df=1$, $p=0.34$). The result of association analysis obtained by logistic regression analysis showed no statistical significant association with suicidality.

Table 1. Polymorphism of 5-HTT, MAOA and DAT gene in suicide victims and control population.

Tabela 1. Polimorfizm genów 5-HTT, MAOA i DAT w grupie badanej i w grupie kontrolnej.

| Locus | Allel | Grupa badana (n=66) | Grupa kontrolna (n=51) | p |
|---------|-------|---------------------|------------------------|------|
| HTTLPR | L | 78 (59,1) | 57 (55,9) | 0,62 |
| | S | 54 (40,9) | 45 (44,1) | |
| HTTVNTR | 9 | 2 (1,5) | 4 (3,9) | 0,44 |
| | 10 | 51 (38,6) | 35 (34,3) | |
| | 12 | 70 (59,8) | 63 (61,8) | |
| MAOA | 3 | 22 (33,3) | 21 (41,2) | 0,16 |
| | 4 | 44 (66,7) | 28 (54,9) | |
| | 5 | 0 (0,0) | 2 (3,9) | |
| hDAT | 9 | 30 (22,7) | 18 (17,6) | 0,34 |
| | 10 | 102 (77,3) | 84 (82,4) | |

We obtained 41 genotypes from both the suicide victims and control subjects. We observed sixteen genotypes (genotypes with frequencies > 3% in the suicide group), which accounted for 75% and 56% of all the observed genotypes combinations in the suicide victims and the control sample, respectively. In the individual genotype tests, examination of the distribution differences of each genotype showed that only one genotype, existing only in the control sample with the frequency of 8% (tab. 2), differed significantly the between suicide victims and control subjects ($p=0.03$), but the significance was lost when applying Bonferroni correction.

Table 2. The genotype observed in the control sample only.

Tabela 2. Genotyp występujący tylko w grupie kontrolnej.

| Genotyp | | | | p |
|---------|-----------|----------|------|------|
| MAOA | 5-HTTVNTR | 5-HTTLPR | DAT | |
| 3 | 12,12 | S,S | 9,10 | 0,03 |

All the suicide victims underwent a toxicological screening of blood, hair and nails. Antidepressant drugs were found in 25 case subjects (38%). Positive screening results included SSRI ($n=10$) and other antidepressant drugs ($n=15$). A total of 22 individuals (33%) were under the influence of antidepressant drugs at the moment of death. In 15 of the cases, the traces of drugs were also found in hair and nails, which means that they were taking drugs for a prolonged period. Three individuals took antidepressants in the past but were not under their influence at the time of death (one had promazine in nails, the second had fluoxetine in nails and the third had amitryptiline in both hair and nails). Alcohol was detected in blood of 27 suicide victims (41%); 22 individuals had blood alcohol level above 1‰.

DISCUSSION

In the last decade, several studies concerning the genetics of suicidal behaviour were carried out. Since there is convincing evidence that the

serotonergic system is involved in susceptibility to suicide, it is reasonable that molecular genetic studies focused primarily on the genes of the serotonin pathway. Since the serotonin transporter acts as a key regulator of the 5-HT transmission, polymorphisms of the 5-HTT gene became an attractive target for association studies in suicide and were investigated extensively; in particular polymorphism in the promoter region of the gene. However, the results of these studies were inconsistent: some reported an association between the S-allele and suicidality [10, 11], while other found that the L-allele was more frequent in suicide victims [12]. In contrast, Fitch et al. (2001) and Mann et al. (2000) could not find any association between the 5-HTTLPR genotype and suicidality [13,14]. In addition, no association was found between suicide and VNTR polymorphism in the intron 2 of the 5-HTT gene. However, a combined analysis of the 5-HTTLPR and 5-HTTVNTR showed a tendency toward an increase of the 5-HTTLPR allele L and 5-HTTVNTR allele 10 in the suicide victims [15].

The main cause of these conflicting observations can be either a high genetic heterogeneity of European populations, inadequate sample sizes or differences in sample compositions. Different diagnostic groups, as well as different diagnostic distributions between samples, could lead to different results if the 5-HTT polymorphisms are not associated with suicidal behavior but rather with one of the psychiatric diagnoses which are prevalent among suicides. In addition, studies on different ethnic groups could lead to different outcomes because allele frequencies of both promoter and intron polymorphisms vary among the subjects of different ethnicities and races [16]. The role of DAT VNTR polymorphism in the etiology of neuropsychiatric disorders also seems unresolved. A number of studies investigated the possible association between this polymorphism and bipolar disorder, schizophrenia, alcoholism, also with mixed result [17, 18]. No association has been found between the MAOA gene VNTR polymorphism in the promoter region and vulnerability to a suicidal act. However, the MAOA gene variants may influence the methods used in suicide attempts [19].

The presence of the above-mentioned differences shows that the question how the genetic factors contribute to suicide is still open. We assume that it is unlikely that the few genes alone are conferring risk of suicidal behavior. Thus, in our study, the 5-HTT, MAOA and DAT data were analyzed together to find out whether

the simultaneous coexistence of functional polymorphic forms of the 5-HTT gene, MAOA gene and DAT gene may predispose to suicide. The study was conducted on a group of 117 males from Poland. The case sample consisted of 66 male subjects who committed suicide classified as violent. Because of the gender-specific association with suicidality we studied only males [3]. Despite the previous reports [10, 11], we were unable to show any association between the 5-HTT gene polymorphism and suicidality. In our study, there are no significant differences in allele and genotype frequencies between the case and control group. Therefore, we may argue that none of these genes alone predisposes to suicide. We checked the influence of all of the functional polymorphic forms on the occurrence of suicide by the logistic regression analysis. The result of this analysis did not show any association between the investigated polymorphic forms and suicidality. We determined all of the genotypes in 4 loci, but the individual genotype tests for all 4 loci did not show any associations with suicidality. However, one genotype (3;12-12;S-S;9-10) differed significantly between the suicide victims and control subjects. This genotype existed only in the control sample and appeared with frequency of 8%. Probably, this genotype includes variants of genes that may provide some protection against suicidal behavior. The "protective" genotype contains 9 and 10 allele in the DAT gene and low-activity allele in the MAOA gene. However, the high-activity alleles in the MAOA gene have been reported to show a significantly higher frequency in men who had attempted suicide by violent means [19]. The "protective" genotype also includes variants SS in 5-HTTLPR and 1212 in 5-HTTVNTR. In agreement with our results, an increase of the 5-HTTLPR allele L and the 5-HTTVNTR allele 10 was observed in the suicide victim group [15]. However, it is worth noting that after Bonferroni correction for multiple testing, the observed difference in frequency is no longer significant.

The results of our study should be considered with caution, for two reasons. First, the study sample was relatively small, and a larger sample would be more appropriate to detect genetic effect in association study of suicide. Second, in our study we had no detailed information about psychiatric diagnoses either in case subjects or control subjects. After toxicological screening of case subjects we only found that 38% of them had taken antidepressants in the past and 41% had alcohol in blood. This allows for suggest-

ing that a portion of the subjects might suffer from psychiatric disorders leading ultimately to suicide.

CONCLUSIONS

In conclusion, we did not find any association between the polymorphisms in the 5-HTT, MAOA and DAT genes and completed suicide in Polish population. Probably, the risk of suicide is a result of an action of a greater number of genes, each contributing a small part to the overall risk, while numerous non-genetic factors might also influence this genetic base of the susceptibility to suicide. A better understanding of the genetic determination of suicide needs further investigation into the interaction of genes involved in synthesis, release, uptake and receptor function for a variety of neurotransmitters.

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