Association of functional genes polymorphisms of key enzymes in the metabolism of biogenic amines with paranoid schizophrenia susceptibility and the influence of these polymorphisms on PANSS results in antipsychotic treatment

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Summary
Introduction: The genetic components of schizophrenia susceptibility are calculated as being 50%.
Aim: We evaluated the frequency of alleles and genotypes of COMT and MAO-A genes polymorphisms in patients with schizophrenia and in the healthy population. We searched for associations between the genotypes and PANSS results among patients in a three month-long antipsychotic therapy.
Subjects and methods: The study comprised 72 unrelated patients who met ICD–10 criteria for schizophrenia, and 187 unrelated healthy controls. The analysis of COMT and MAO-A genes polymorphisms were performed using the polymerase chain reaction technique (RFLP-restriction fragments length polymorphism and VNTR-variable number tandem repeats). The severity of psychopathological symptoms was measured using the PANSS (Positive and Negative Schizophrenia Scale).
Results: We did not find any association between the genotype of COMT and MAO-A genes polymorphisms and schizophrenia. We found a statistically significant different allele distribution in MAO gene polymorphism: alleles with three tandem repeats in the promoter region were more frequent among females with schizophrenia. We did not find any association between the genotype of COMT and MAO-A genes polymorphisms and PANSS results in any time periods. Due to a small number of patients in this study the obtained results should be regarded as preliminary.

INTRODUCTION

Schizophrenia is a psychiatric disease which affects 1% of the world’s population. The chronic character of the illness and the damage it causes in patients’ cognitive skills, emotions and social functioning provide an impetus for research on the causes of the disease to predict its course and establish possibly effective treatment with few side effects.

Previous theories accounting for environmental, genetic, neurodevelopmental, biochemical and immunological or infectious factors seemed to explain, to some degree, the origins of schizophrenia. Despite the fact that the risk of developing schizophrenia in general population is similar, the disease was found to be diagnosed definitely more frequently within families (approx. 10% more often in the first degree relatives),
and the occurrence of the disease in monozygotic twins reaches 40–50% [1]. This data led to the conclusion that what plays an important part in schizophrenia morbidity is the genetic factor. However, the fact that schizophrenia morbidity among monozygotic twins was not found in 100% cases indicated that genetic predisposition was not the only background factor contributing to schizophrenia [2]. Further twin and adoption studies showed that a simple, Mendelian pattern of schizophrenia inheritance was invalid [3, 4, 5]. Some researchers believed that inheritance of susceptibility to schizophrenia was caused by epistatic activity of even tens of genes [6].

Nowadays, there are two widely accepted research strategies in psychiatric genetics: genetic linkage studies, where the whole genome is studied in search for sites responsible for schizophrenia, and association studies, which consist in comparing the distribution of alleles of the same locus in unrelated ill and healthy individuals from the general population [7]. Similar frequency of a particular allele in patients with schizophrenia may indicate that there are associations between the studied polymorphism and schizophrenia morbidity. Association studies are particularly useful in case of diseases of polygenic background, and therefore they are widely used in psychiatry [6].

So far, researchers have not determined any genome sites which could be related to schizophrenia morbidity. However, some results suggest that research is proceeding in the right direction [2, 8, 9]. Some studies have demonstrated differences in COMT activity resulting from the polymorphism of the gene which encodes this enzyme. The polymorphism consists in the G → A transition in exon 4 of the COMT gene. The functional effect of that transition is a 3 to 4-fold decrease in the activity of the COMT enzyme [10, 11]. The enzyme is most active in individuals with two valine alleles, and least active – in individuals with metionine homozygotes. Heterozygotes show an average level of the COMT enzyme activity.

There are two genes (MAO-A and MAO-B) located on chromosome X (Xp11.4–11.23) which are responsible for the MAO synthesis [12, 13]. In 1998, Sabol et al. [13] described the MAO gene polymorphism in the promotor region. This polymorphism consists in a different number of tandem repeats of 30 bp. The allelic forms contain 3, 3.5, 4 or 5 repeats. Alleles with 3.5 and 4 repeats feature a more productive transcription (2–10 times higher). It results in a lower activity of the form with 3 repeats as compared with the other alleles including those with 5 motives [13]. Approximately 97% of the general population has alleles with 3 or 4 repeats and only these were taken into consideration in our study. It was impossible to determine the genotype in terms of the VNTR polymorphism of the MAO-A gene in 6 men, which accounts for 8.33% of the studied population. Therefore, it is possible that the group also included individuals with other tandem repeats than 3 or 4.

**AIM OF THE STUDY**

1. Whether the polymorphisms of genes determining COMT and MAO-A synthesis affect paranoid schizophrenia morbidity rate.
2. How the above polymorphisms affect the PANSS results during antipsychotic treatment in the study periods.

**SUBJECTS AND METHODS**

**Subjects**

The study included 72 unrelated patients of Polish descent – 39 men and 33 women – diagnosed with paranoid schizophrenia. Their average age was as follows: men – 27.1 years (SD = 6.7), women – 31.4 years (SD = 9.4). The average age of onset of schizophrenia in the whole group under study was 24.1 years (SD = 6.64). The average age of onset in women was 26.45 years (SD = 7.58) whereas men developed the disease earlier, their average age of onset being 22.1 years (SD = 4.99). The average duration of the disease was 4.99 years in women and 5.0 years in men.

In order to make a diagnosis which would meet the ICD–10 criteria [14], the Polish version of CIDI (Composite International Diagnostic Interview) was used [15]. The assessment of mental condition was carried out by a psychiatrist or a physician specializing in psychiatry. Exclusion criteria included serious neurological disorders, major somatic disorders impairing cog-
nitive functions and diagnosed mental impairment.

The control group included 187 healthy individuals of Polish descent, unrelated to each other and to the individuals in the group under study. The control group consisted of 69 men and 118 women and their average age was 34.7 years (SD = 14.4).

All the participants were informed about the aim and course of the study and confidentiality guarantee. The examined individuals expressed their written consent to the examinations, including collection of blood necessary for genetic analysis. The intensity of psychopathological symptoms was examined using the PANSS scale [16], with which the positive, negative and general psychopathological symptoms present in schizophrenia can be evaluated. The examination was performed prior to starting the therapy and then after the 14th, 42nd and 84th day of treatment. Most of the patients were treated with the classic neuroleptic drug – pernazine and/or an atypical neuroleptic – olanzapine. The applied doses of the antipsychotic drugs complied with the standards for paranoid schizophrenia treatment and with the recommendations of the drug manufacturers. Due to the fact that the studied group was small, associations between the genotype on the efficacy of the applied drug dose were not analyzed.

The study protocol was accepted by the Commission of Ethics of the Pomeranian Medical University of Szczecin, Poland.

Methods

Genetic analysis

The blood used for genetic analysis was collected in the amount of 10 ml from the antecubital vein. The blood was collected from the patients after their informed consent, following the subsidence of psychotic symptoms of the disease. Genomic DNA was extracted from leucocytes using the salting method [17].

Val–158-met polymorphism of the COMT gene was analyzed using the PCR-RFLP technique [11]. Polymorphism in the promoter region gene of MAO-A was analyzed using the PCR-VNTR technique [13].

Statistical analysis

Statistical analysis was performed using the SPSS program, and specifically Pearson’s chi-square test. Associations between the treatment progress and the genotype were studied by analysis of variance (ANOVA) [8].

RESULTS

In the study, frequencies of particular val–158-met genotypes of the COMT gene were analyzed in patients with schizophrenia and in healthy individuals from the control group. The frequencies of metionine and valine alleles in the studied groups were also defined (Tab. 1). The control group was found to be in genetic equilibrium according to the Hardy-Weinberg law. Subsequently, the PANSS results were analyzed with regard to the functional aspect of the studied polymorphism. To evaluate associations between the genotype and the PANSS results, an improvement index was determined by multiplying the difference between the PANSS results at the beginning of the therapy and at the time of study by the PANSS results obtained at the beginning of the therapy.

Analogous analysis was applied to the MAO-A gene. It was studied whether there were any associations between a particular VNTR genotype and the occurrence of paranoid schizophrenia. The frequencies of particular alleles were determined in the studied groups of men and women (Tab. 2). The PANSS results were analyzed with respect to the genetic profile and the time of study.

No association was found between the genotypic distribution of the COMT and MAO-A genes and schizophrenia occurrence.

An analysis of the distribution of the VNTR alleles of the MAO-A gene showed that alleles with 3 tandem repeats within the promoter region were significantly more frequent in women suffering from paranoid schizophrenia than in a healthy population. No differences in the allelic distribution of the COMT gene polymorphism were found between the studied and the control groups.

No association was found between individual val–158-met genotypes of the COMT gene and
the influence of antipsychotic treatment on the PANSS results at any time of the study (Tab. 3).

A similar analysis was performed to determine the influence of VNTR polymorphism of the MAO-A gene on the PANSS results in the course of the applied treatment. No associations were found between the studied gene polymorphism and the PANSS score at different times of the study. In the analysis of the MAO-A gene polymorphism located on chromosome X, it was necessary to analyze the groups according to sex. Consequently, smaller groups were examined, which might have affected the results of the statistical analysis (due to the limited scope of this work, an analogous analysis of associations between the genotype and the improvement index was omitted). To obtain reliable results it is necessary to undertake further research in larger groups of patients.

Laboratory tests, particularly genetic analyses, are subject to the risk of a laboratory error (e.g. low quality of isolated DNA may result in problems with obtaining results for particular polymorphisms). The tables showing the study results contain the number of alleles and genotypes in the studied individuals (it was impossible to genotype 6 men for MAO-A gene polymorphism).

Due to the small number of studied individuals, the kind of applied neuroleptic drug was not taken into consideration.

**DISCUSSION**

Val-158-met polymorphism in the COMT gene became the focus of researchers’ attention in the 1990’s thanks to Carlsson’s theory, studies on the

| Table 1. Distribution of the val-158-met polymorphism of the COMT gene |
|---|---|---|---|---|---|---|---|
| Group | N | Distribution of genotypes | $\chi^2$ test (df=2) | Distribution of alleles | $\chi^2$ test (df=2) |
| | | val/val | met/val | met/met | | met | Val | |
| Patients | 67 | 16 | 36 | 15 | 0.239 | 0.537 | 0.224 | 0.798 | 0.671 | 68 | 66 | 0.507 | 0.493 | 0.077 | 0.782 |
| Control group | 187 | 53 | 89 | 45 | 0.283 | 0.476 | 0.241 | 0.521 | 0.479 |

* 5 individuals were not genotyped for COMT gene polymorphism

| Table 2. Distribution of the VNTR 30bp polymorphism of the MAO-A gene |
|---|---|---|---|---|---|---|---|
| Group | N | Distribution of genotypes | $\chi^2$ test (df=2) | Distribution of alleles | $\chi^2$ test (df=2) |
| | | 4VNTR | 3/4VNTR | 3VNTR | | 3VNTR | 4VNTR | |
| Female | 33 | 13 | 15 | 5 | 5.046 | 0.080 | 25 | 41 |
| Control group | 117 | 70 | 39 | 8 | 0.394 | 0.454 | 0.152 | 0.379 | 0.621 | 5.439 | 0.020 |
| Male | 33* | 21 | 21 | 12 | 0.636 | 0.364 | 0.364 | 0.636 |
| Control group | 67 | 49 | 18 | 18 | 0.731 | 0.269 | 0.269 | 0.731 |

* It was impossible to genotype 6 men for MAO-A gene polymorphism
psychiatric disorders in the VCFS syndrome [8] and the linkage studies of chromosome 22q (at the COMT gene locus). The genetic studies conducted so far, as well as biochemical studies on the COMT activity level in erythrocytes, have not produced any unequivocal results, which might suggest that there are no associations between the studied polymorphism and susceptibility to schizophrenia. It should be noted, however, that the studies used different methodologies and diagnostic criteria, and the distribution of the studied alleles in healthy populations differed considerably depending on the geographical zone [19]. Some researchers found no association between the COMT gene polymorphism and schizophrenia [20, 21] but admitted that such association might exist as the studied groups were small in size or suspected that the effect of the studied polymorphism was minimal, which corresponded to the polygenic model for inheritance of susceptibility to schizophrenia. Other researchers proved, however, that the COMT gene locus might be linked to schizophrenia [22, 23, 24]. This association is not with any particular genotype but with the allelic distribution which is not so obvious and in some studies shows an association with the disease. In their family studies, Li et al. [22] and Kunugi et al. [23] observed transmission disequilibrium for the valine allele, which was found to be transmitted more frequently by parents to affected children. However, these results were not confirmed in a similar study by Wei and Hemnings [25]. On the other hand, Ohmori et al. [24] reported that the metionine allele was significantly more frequent in schizophrenic patients. It should also be noted that in a healthy Japanese population studied by them, the metionine allele was less frequent than in Caucasian population [19]. With such contradictory results it is essential to use meta-analysis results for comparison in large groups of patients. Jin et al. [26] analyzed the COMT gene polymorphism in a group of 862 patients. Meta-analysis showed that there were no differences in genotype and allele frequencies among the patients compared with an equally large control group. Similarly, a meta-analysis of study results obtained worldwide in 1996–2003 did not show statistically higher frequency of any particular genotype or allele in patients with schizophrenia [27].

Low COMT activity resulting from a lower expression of mRNA which is responsible for the production of this enzyme [28] may be of significance to the prognosis of the disease and the efficacy of antipsychotic treatment. Herken and Erdal [29] studied possible associations between the COMT gene polymorphism and severity of schizophrenia symptoms. They reported that homozygous met/met individuals scored higher in the BPRS scale and patients from this group were hospitalized more frequently than others. Kirchheiner et al. [30] reported that response to treatment was worse in patients with a lower COMT enzyme activity. Such association was not found in the present study while analyzing the improvement index based on the PANSS scale. The reports on associations be-

<table>
<thead>
<tr>
<th>Geno-types</th>
<th>Total squares</th>
<th>df</th>
<th>Average square</th>
<th>F</th>
<th>Significance</th>
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<td>Positive symptoms</td>
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<tr>
<td>v/v 14</td>
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<td>2</td>
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<tr>
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<td>2</td>
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</tbody>
</table>
between VNTR polymorphism in the promotor region of the monoaminooxidase gene and the risk of developing schizophrenia are more consistent. Researchers admit that some slight influence is possible due to gene epistasis, however the studies referred to in this work show no statistically significant differences between patients with schizophrenia and a healthy population [31, 32, 33, 34, 35]. Similarly, in this study, no particular genotype was found to be more frequent in the affected individuals. The allelic distribution indicates that alleles with three tandem repeats are more frequent in affected women but no valid conclusion can be made before a larger group of patients is studied.

No associations were found between the VNTR polymorphism in the monoaminooxidase gene and the PANSS results at any stage of the treatment, which corresponded to the results of the COMT gene polymorphism analysis. Patients with low levels of COMT and MAO-A activity were not analyzed. If such an analysis had been carried out, the results might have been as interesting as those obtained by Kirchheiner et al. [30], who found that the risk of therapy failure was six times higher in individuals with a low level of activity of the analyzed enzymes. This confirms that there is an association between the synergetic effect of genes and therapy efficacy.

At this stage of research, it is necessary to collect more genetic material for further analysis. Considering the small size of the studied group the results obtained in this study should be regarded as preliminary.

CONCLUSIONS

1. No association was found between the genotype distribution in COMT and MAO-A gene polymorphisms and schizophrenia.

2. No differences were found in the allelic distribution in COMT gene polymorphism between the studied group and the control group.

3. An analysis of allele distribution in VNTR polymorphism of the MAO-A gene showed that an allele with three tandem repeats within the promotor region of this gene was significantly more frequent in women with paranoid schizophrenia compared with the healthy population.

4. No association was found between any particular genotype of the val–158-met polymorphism in the COMT gene nor the VNTR polymorphism in the MAO-A and the effect of antipsychotic treatment on the PANSS results in any of the study periods.

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