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CYP2D6 gene polymorphism in psychiatric patients

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Analysis of CYP2D6 alleles and genotypes frequencies in patients with different response to the standard dose of psychotropic drugs is presented.

Key words: CYP2D6 gene, psychotropic drugs, schizophrenia, depression

Introduction

Genetically determined polymorphism of drug oxidation was revealed independently by Eichelbaum et al. [7] in case of sparteine and Mahgouba et al. [17] in case of debrisoquine. The extent of this drug metabolism to respective derivatives is an inherited trait associated with two phenotypes: EM (extensive metabolizer) and PM (poor metabolizer). The rate of metabolism is clinically determined with drug loading test. This allows for evaluation of metabolic coefficient, which represents the relation of amount of drug urine excretion in unchanged form to the amount of its excreted hydroxy-derivatives. On this basis the metabolic phenotype is determined [3,13]. The load test requires, however, temporary withdrawal of the applied drugs for at least two weeks and the patient's sufficient cooperation, what may often be difficult in case of mental illness.

The frequency of PM phenotype exhibits some ethnic differences. It has been estimated for 5-10% in Caucasian population [1,18]. In Poland it has been reported as 8,8% in sparteine group [20] and 5,8% in case of debrisoquine determined phenotype [15]. The alternative for phenotype identification is genotyping with restrictive fragment length polymorphism analysis (RFLP) and polymerase chain reaction (PCR) method. Genotyping, originally described by Heim and Meyer in 1990 [12] corresponds with phenotyping in 92-99% [4,12]. The gene encoding CYP2D6 isoenzyme is located in chromosome 22. Several mutations associated with poor metabolism of psychotropic drugs have been described. Point CYP2D6(*4) mutation leading to disturbed mRNA synthesis, which is associated with replacement of nucleotides within gene 1934 loca-

tion, CYP2D6(*5) mutation caused by entire gene deletion and CYP2D6(*3) involving single nucleotide deletion in gene 2637 locus are the most common among Caucasian population [2,8,12,14]. EM phenotype corresponds to homozygous or heterozygous genotype with autosomal-dominant trait responsible for extensive metabolism (normal allele – CYP2D6*1). PM phenotype corresponds with homozygous genotype with two recessive genes responsible for poor metabolism (allele CYP2D6 *3, *4, *5).

A number of drugs often applied in psychosis therapy enters metabolic pathway of debrisoquine involving cytochrome P-450 CYP2D6 isoenzyme. These are, among others, tricyclic antidepressant drugs (amitriptyline, desipramine, imipramine, nortriptyline, clomipramine), selective serotonin reuptake inhibitors (fluoxetine, norfluoxetine, paroxetine), neuroleptics (chlorpromazine, levomepromazine, thioridazine, perphenazine, fluphenazine, clopentixol, haloperidol, remoxypirid) [3,10,18,23]. The different metabolic course of drugs may be responsible for different results of the therapy with the same drug in case of patients presenting similar clinical symptoms. Non-liner pharmacokinetics of tricyclic antidepressants resulting in the increase of drug concentration and its toxic accumulation is observed more often in patients with poor metabolizer characteristics [3,21,26]. The standard doses of antidepressant drugs administered to persons with PM phenotype may be linked with an increased risk of side effect occurrence, which might be further misinterpreted as enhanced depressive symptoms [19]. The studies on pharmacokinetics of neuroleptic drugs confirmed that the serum elimination half time of some of these drugs was significantly longer in PM than in EM, along with the higher serum levels [5,6,27]. Further, more side effects of extrapyramidal origin were observed in persons with poor metabolism. However, in case of persons with extensive phenotype EM the same doses could achieve lower serum levels than that of therapeutic significance. Thus, the knowledge of the metabolism rate in a patient prior to the treatment may be of therapeutic value, enabling appropriate dose choice and the clinical evaluation of its effects.

The aim of this study was to evaluate the frequency of gene CYP2D6 alleles and respective genotypes determining the metabolism rate in psychiatric patients. The study covered patients on standard doses of psychotropic drugs, whose clinical improvement was achieved as compared to patients manifesting side effects associated with the applied therapy, or unsuccessful results of treatment.

The patients

The study included 36 psychiatric patients admitted to 1st and 2nd Psychiatric Clinic of Łódź Medical University and Babinski Hospital in Łódź. The population under study, 22 persons (13 male and 9 female, aged 19-59, mean 44,6) enrolled into group I, included: 18 persons with diagnosed schizophrenia and 4 with depression in the course of recurring depressive disorders (according to ICD-10). No clinical improvement during treatment of these patients was noted despite standard dose administration during at least two consecutive courses of treatment, each at least 4 weeks long. Moreover, in three cases, serious side effects were recognised, which led to change of their treatment. The control group (group II) consisting of 14 persons (6

male and 8 female, aged 20-66, mean 43) included 6 patients with diagnosed depression in the course of recurring depressive disorders and 8 with schizophrenia (according to ICD-10). During the treatment of these persons with standard doses of psychotropic drugs a satisfactory improvement of their clinical state was achieved and no side effects were observed.

The patients from each group with diagnosed depression were treated with antidepressant drugs metabolised by isoenzyme CYP2D6, and patients diagnosed as schizophrenics underwent at least one course of neuroleptic therapy with a drug engaging the same pattern of metabolism. Basing on retrospective analysis of the therapy we studied the differences between therapeutic results of monotherapy with these drugs applied. As the current therapy cannot influence the genotype, we neglected the data on the treatment during our study.

Methods

The genotyping of polymorphic CYP2D6 gene forms (*3) and (*4) and the normal allele CYP2D6(*1) was performed by means of nested PCR with pairs of primers specific for respective alleles [12]. DNA was isolated from peripheral blood cells with Easy DNA PREP kit (A & A Biotechnology). In order to avoid false positive amplifications that detect similar types of mutation within adjacent pseudogenes, the fragments of CYP2D6 gene with the mutations (29*3 and 29*4) were amplified first, using pairs of primers: 1\ATTTCCCAGCTGGAATCC; 2\GAGACTCCTCGGTCTC for fragment A with mutation 29*3 and the pair 3\GCGGAGCGAGAGACCGAGGA; 4\CCGGCCCTGACACTCCTTCT for fragment B with mutation 29*4, respectively. Amplification was conducted in 0,2 μmol/l dNTP, 0,25μmol/l of primers, 200-400 ng genomic DNA, and 1,5 U Taq polymerase (Perkin Elmer) in the volume of 20 μl, under the following conditions: denaturation at 94°C for 90s, further 35 cycles for 60s at the temperature of 94°C, 90s at 52°C and 90s at 72°C along with termination period of 7 min at temperature of 72°C.

The PCR product was analysed on 2% agarose gel stained with ethidium bromide in UV illumination. One μl of amplification product from its first stage was further amplified with a pair of primers specific for respective mutations, i.e., product A with primers 1/ and 7/CGAAAGGGGCGTCC and 1/ and 8/CGAAAGGGGCGTCT; product B with primers 4/ and 5/GCTAACTGAGCACA and 4/ and 6/GCTAACTGAGCACG under the conditions allowing for amplification only in case of perfect match between primers and complementary sequences. Amplification was conducted under similar conditions as those during the first amplification, except that 1,0 U Taq polymerase and only 15 cycles of PCR 60s at 94°C, 60s at 50°C and 60s at 72°C were used. Then, the products of the second amplification were analyzed on agarose gel. The presence of the specific product of amplification with respective primer pairs allowed for mutation and normal gene identification.

CYP2D6(*5) allele associated with complete gene deletion was typed with Expand PCR kit (Boehringer Mannheim) [24] applying the pair of primers: 1/ACCGGGCACCCTGTACTCCTCA and 2/GCATGAGCTAAGGCACCCAGAC under protocol

conditions of Expand PCR. The product amplification, 3,5 kb size, was evaluated in 0,8% agarose gel. The detection of this product proved the presence of CYP2D6(*5) in the investigated DNA. The evaluation of homo- or heterozygous genotype was performed to prove the presence (heterozygous type) or the absence (homozygous type) of another allele (CYP2D6 *1, *3 or *4).

The allele frequency was compared with Fisher's exact test, assuming that differences were statistically significant at $p<0,05$. The extent of allele and genotype correlation with a given feature was estimated with odds ratio (OR) from 2x2 table within 95% confidence interval (95%CI) calculated with Woolf's approximation.

Results

The frequency of gene CYP2D6 alleles occurrence is presented in table 1. In the group of patients well responding to standard treatment, the occurrence of normal allele (*1) of CYP2D6 gene (OR=9,13; 95% CI=2,4-34,7; p=0,0004) was significantly more frequent as compared with patients with no clinical improvement after standard therapy. Among the patients with no response to therapy CYP2D6 (*3) and (*4) alleles were significantly more frequent (for allele (*3): OR=11,4; 95% CI=0,62-208,2; p=0,0004 and for allele (*4): OR=3,89; 95% CI=1,00-15,08; p=0,05, respectively) than in the group of patients well responding to treatment. No significant differences were found in allele CYP2D6 (*5) distribution in both studied groups.

The comparison of homozygous genotype distribution in both groups (table 2) revealed significantly higher frequency (OR=16,5; 95% CI=3,09-88,07; p=0,0005) of CYP2D6*1/*1 genotype among the patients with good response to treatment. The frequencies of other homozygous genotypes did not differ significantly in both groups. The occurrence of genotypes determining extensive and poor metabolism did not differ significantly in both groups (table 2).

Table 1

Number and frequencies of CYP2D6 alleles

Alleles	CYP2D6 *1		CYP2D6 *3		CYP2D6 *4		CYP2D6 *5		n	
	n	%	n	%	n	%	n	%	n	%
Group I	21	48	7♦	16	14♦	32	2	4	44	100
GroupII	25*	89	0	0	3	11	0	0	28	100

Group I – patients with no clinical improvement on standard doses of psychotropic drugs
Group II – patients with clinical improvement on standard doses of psychotropic drugs
n – number of chromosomes
p – Fisher exact test: * p = 0,0004 ; ♦p<0,05

Table 2

Genotype (patients) number in study groups

Genotype	Extensive metabolism			Poor metabolism			
CYP2D6	*1/*1	*1/*3	*1/*4	*3/*3	*3/*4	*4/*4	*5/*5
Group I	4	4	9	1	1	2	1
Group II	1*	0	3	0	0	0	0

Group I – patients with no clinical improvement on standard doses of psychotropic drugs
Group II – patients with clinical improvement on standard doses of psychotropic drugs
p – Fisher exact test * – p = 0,0005

Discussion

Two groups with increased risk of other than expected drug biotransformation pattern can be distinguished among the patients on treatment with drugs entering oxidative metabolism with CYP2D6 isoenzyme. The group with PM (poor metaboliser) phenotype (genotype) consists of patients who are endangered with a number of serious adverse events as applied doses may reach toxic serum levels. The group with EM (extensive metaboliser) phenotype (genotype) includes persons whose enhanced metabolism may decrease serum drug levels below that of therapeutic significance.

In our study, we found that the clinical improvement observed in patients on standard doses of psychotropic drugs correlated positively with both genotype of CYP2D6*1/*1 (OR=16,5; p=0,0005) and allele CYP2D6 *1 occurrence (OR=9,13; p=0,0004). Phenotype kind of extensive metabolism EM of debrisoquine type is determined by both CYP2D6 *1/*1 genotype and the occurrence of heterozygous genotype with one CYP2D6 *1 allele [9]. As EM phenotype is most abundant in general population (approximately 90%), it should be assumed that the standard drug doses would be characterised with remarkable efficiency, especially in patients with EM type metabolism.

The higher frequency of CYP2D6 *1 allele observed among our study patients with clinical improvement on such drug doses would confirm this hypothesis. However, the higher frequency of genotypes determining extensive metabolism (homozygous CYP2D6 *1 or heterozygous) in the group with successful therapeutic effect was not statistically significant if compared to the group with unsuccessful therapeutic effect (table 2). The significant increase of allele CYP2D6 *3 and *4 frequencies was found in the group with unsuccessful therapeutic effects on standard doses but the occurrence of poor metabolism genotypes did not differ significantly in both groups. This results most probably from the relatively small number of study patients and low frequency of PM genotype in general population (5-10%). Studies on a larger group would obviously clarify the nature of the relation.

There were 5 patients with poor metabolism genotype and unsuccessful therapeutic effects on standard doses. The therapy was modified only in one case due to adverse

events, probably associated with increased serum levels. However, the adverse events in the course of psychotropic therapy may often be misinterpreted as symptoms of original disease progression or as a toxic side effect of the applied drug. Also, quite common therapy aimed to neutralise or decrease side effects (e.g. pridinol, biperiden) should be considered during neuroleptic drug administration. The retrospective analysis, as in our study, does not provide all necessary data to identify the reasons for withdrawal from the therapy. The unsuccessful effects of standard therapy in heterozygous CYP2D6 genotype (one allele determining poor metabolism and another normal CYP2D6 *1 - EM phenotype) may be associated with the occurrence of intermediate (normal under standard conditions) type of metabolism. It seems that considerable effects of co-therapy should be expected in these patients.

The effect of tricyclic antidepressant metabolism inhibition by neuroleptic drugs is often cited [10,25,26]. Administration of several drugs of both types to patients with intermediate extent of metabolism may decrease tricyclic antidepressant metabolism, leading to serum level increase and side effect appearance with further therapy modification. Similarly, inhibitors of selective serotonin reuptake inhibitors, as well as many other, e.g. antiarhythmic drugs (propafenon), calcium channel blockers, or beta-adrenergic receptor blockers applied along with tricyclic antidepressants or neuroleptics may inhibit metabolism of psychotropic drugs and the increase of their serum levels [11,21,22]. The optimal serum concentration of antidepressant drugs is important for successful clinical effects, as neither lower nor higher levels exhibit therapeutic value [26].

The unsuccessful therapeutic effects in our study, associated with slow metabolism alleles, may be the result of altered pharmacokinetics of the applied drugs and the failure to achieve therapeutic levels. The frequencies of gene CYP2D6 alleles we observed might also be caused by linkage disequilibrium between the inherited genes, the phenomenon that exists in general population. Therefore, CYP2D6*1 alleles more frequently found in patients with successful therapeutic effects, may constitute a genetic marker of other, adjacent or close genes actually responsible for successful effects of the applied therapy.

Llerena et al. [16] described the occurrence of different personality profiles in persons with different phenotypes of debrisoquine metabolism. The authors suggested a direct influence of genetically determined variations in the rate of metabolism on the endogenous substance biotransformation or the existence of other associated genes responsible for changes in the central nervous system. Numerous research data indicate a substantial role of genetic factors in psychiatric disorders origin. Many loci at chromosome 4, 5, 11, 18, 21 and X are correlated with affective diseases. So far, no genetic markers on chromosome 22 have been found correlated with etiology or clinical course of affective diseases.

Determination of cytochrome CYP2D6 phenotype (genotype) in psychiatric patients before psychotropic pharmacotherapy may be of a remarkable value prior to drug and its dose choice and evaluation of treatment efficiency. As patient cooperation is limited in psychiatric disorders, phenotype determination with drug loading tests is often impossible. Molecular biology assays, as a relatively simple and fast method

of CYP2D6 genotype determination, may solve the problem. Knowledge of the type of genes engaged in drug metabolic course in a given patient will help with pharmacotherapy selection. It may also be useful in diagnosis and prognosis evaluation in psychiatric diseases.

Conclusions

Significantly higher frequencies of CYP2D6 *1/*1 genotype and CYP2D6*1 alleles determining normal debrisoquine type metabolism (EM) were found in the group of psychiatric patients with successful therapeutic effects on standard drug doses.

Alleles CYP2D6 (*3) and (*4), responsible for poor metabolism (PM), were significantly more frequent among the patients with no clinical improvement on standard pharmacotherapy.

CYP2D6 polymorphism genotyping may be of substantial utility in efficient pharmacotherapy of psychiatric diseases.

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