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CYP2D6 Phenotype Versus Genotype and the Potential Risk of Allergic Diseases

Porównanie wyników badań fenotypu i genotypu CYP2D6 i ich znaczenia jako czynników ryzyka w rozwoju chorób alergicznych

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Abstract

Background. Mediators of immunologic and allergic reactions, such as histamine, are catabolised mainly by processes of oxidative metabolism. The relationship between genetically determined polymorphic metabolism, its phenotypic expression and susceptibility to allergic diseases seemed to be very promising, but was not studied methodically yet.

Objective. Investigation of the distribution of the *CYP2D6* phenotypes versus genotypes in patients with allergic diseases, like atopic asthma and allergic rhinitis, and to compare results with the distribution among healthy persons from Polish population.

Material and Methods. Study completed 750 unrelated individuals from the southwestern region of Poland (Lower Silesia). The CYP2D6 phenotype was analyzed in 400 individuals (100 patients and 300 healthy persons), using sparteine as a model drug. The CYP2D6 genotype was analyzed in 400 individuals (100 patients and 300 healthy persons), by PCR-RFLP techniques for the CYP2D6*1, CYP2D6*3 and CYP2D6*4 alleles. Among patients, 50 persons were involved in both – the CYP2D6 genotyping and phenotyping part of the study in order to compare accordance between these methods.

Results. The results of the study revealed a statistically significant predominance of extensive metabolizer phenotype and genotype among patients with allergic diseases in comparison to healthy volunteers. Relative risk (odds ratio) of development of allergic diseases was 2.81 times higher ($p < 0.005$) for persons with extensive CYP2D6 phenotype and 1.85 times higher ($p < 0.05$) for persons with extensive CYP2D6 genotype. The comparison of phenotype and genotype in fifty randomly selected individuals showed a good concordance of the obtained PM_s results.

Conclusions. Obtained results represent some evidence for a possible relationship between extensive CYP2D6 genotype and especially phenotype and the higher susceptibility to development of allergic diseases. It also suggests that phenotyping methods may be as good as genotyping in predicting susceptibility to development of allergic diseases (Adv Clin Exp Med 2005, 14, 6, 1175–1180).

Key words: CYP2D6 phenotype, CYP2D6 genotype, allergic diseases.

Streszczenie

Wprowadzenie. Utlenianie jest jedną z głównych przemian metabolicznych wielu substancji, zarówno egzogennych, jak i endogennych, w tym także kluczowych mediatorów wczesnej i późnej reakcji alergicznej – histaminy, prostaglandyn i leukotrienów. Za procesy utleniania jest odpowiedzialny w głównej mierze układ enzymatyczny frakcji mikrosomalnej wątroby, którego głównym składnikiem jest cytochrom P-450. Aktywność kilku odmian – izozymów cytochromu P-450, zwłaszcza CYP2D6 (P-450 II D6), jest uwarunkowana genetycznie i charakteryzuje się osobniczą zmiennością.

Cel pracy. Ocena znaczenia fenotypu i genotypu CYP2D6 w etiologii wybranych chorób alergicznych na przykładzie astmy atopowej i alergicznego nieżytu nosa (pyłkowicy) oraz porównania efektywności obu metod badawczych.

Materiał i metody. Badania wykonano ogółem u 750 osób. Fenotyp oksydacji oznaczono u 400 osób, w tym u 100 osób z chorobami alergicznymi: astmą atopową i alergicznym nieżytem nosa (pyłkowicą). Grupę kontrolną stanowiło 300 zdrowych ochotników. Fenotyp oznaczano za pomocą testu ze sparteiną jako lekiem modelowym. Geno-

typ oksydacji oznaczono u 400 osób, w tym u 100 osób z chorobami alergicznymi i w grupie kontrolnej 300 zdrowych ochotników. Polimorfizm genu *CYP2D6* badano za pomocą reakcji łańcuchowej polimerazy i cięcia enzymami restrykcyjnymi (PCR/RFLP) w celu określenia występowania w obu grupach alleli *CYP2D6**1, *CYP2D6**3 and *CYP2D6**4. Spośród chorych wyodrębniono grupę 50 osób, u których wykonano zarówno badania fenotypu, jak i genotypu, w celu porównania efektywności obu metod badawczych.

Wyniki. Opisanie badania wykazały istnienie statystycznie istotnej przewagi ekstensywnych metabolizerów w grupie osób z chorobami allergicznymi (astmą atopową i allergicznym nieżytem nosa – pyłkowicą) w porównaniu z grupą zdrowych ochotników. Względne ryzyko zachorowania na choroby allergiczne jest 2,81 razy większe ($p < 0,005$) dla osób o ekstensywnym fenotypie *CYP2D6* i 1,85 razy większe ($p < 0,05$) dla osób o ekstensywnym genotypie *CYP2D6*.

Wnioski. Ekstensywny genotyp, a w jeszcze większym stopniu fenotyp utleniania może stanowić jeden z istotnych czynników, pozwalających przewidzieć zwiększone ryzyko wystąpienia chorób allergicznych, takich jak astma atopowa i allergiczny nieżyt nosa (pyłkowica). Metody badania fenotypu i genotypu utleniania mają podobną wartość naukową w odniesieniu do oceny ryzyka wystąpienia chorób allergicznych (*Adv Clin Exp Med* 2005, 14, 6, 1175–1180).

Słowa kluczowe: fenotyp *CYP2D6*, genotyp *CYP2D6*, choroby allergiczne.

The relationship between genetically determined polymorphic metabolism and susceptibility to numerous diseases has aroused much interest. All organisms are constantly exposed to foreign chemicals, or xenobiotics, which include chemicals such as drugs, industrial chemicals, pesticides, pollutants, pyrolysis products in cooked food, alkaloids, and toxins produced by plants, molds and animals. Enzymes belonging to the cytochrome P450 family, especially *CYP2D6*, which play crucial role in the metabolism of 20–25% clinically important drugs [1, 2], are also involved in the activation and deactivation of some endogenous substances and many environmental xenobiotics such as carcinogens and toxic substances [3–5]. The members of human cytochrome P450 family are subjects to genetic polymorphism. The activity of these enzymes varies broadly between individuals from absence to high activity and this variance can be responsible for adverse effects and toxicity of drugs and xenobiotics or plays a key role in the etiopathology of several malignancies. The activity of enzymes is dependent on hereditary polymorphism of genes, coding for these enzymes. *CYP2D6* polymorphism is relatively well known and more than 20 mutations of the *CYP2D6* gene have been described. *CYP2D6**3 (former A-mutation) and *CYP2D6**4 (former B-mutation) are most common non-functional *CYP2D6* alleles [6]. Therefore genotyping of these alleles allows prediction of the extensive (EM) and poor metabolizers phenotype (PM) with about 90–96% accuracy [7, 8].

Among other potential associations, relationship between genetically determined polymorphic metabolism and susceptibility to allergic diseases seemed to be very promising, but was not studied methodically yet. One could expect interesting result in this field, especially because histamine, very important mediator of immunologic and allergic reaction, such as early IgE-dependent

allergic reaction, is catabolised in over 75% by oxidative dealkylation, methylation and acetylation [9, 10].

The aim of the study was to evaluate whether patients with allergic diseases, like atopic asthma and allergic rhinitis differ from healthy persons in their *CYP2D6* phenotype and genotype and to compare the results of phenotyping and genotyping methods.

Material and Methods

Individuals

Persons with allergic diseases, suffering atopic asthma or allergic rhinitis or both, were in- and out-patients from Department and Clinic of Internal Medicine and Allergology, Wrocław Medical University.

Controls were healthy, non-pregnant unrelated volunteers with normal kidney and liver function. All were of native Polish origin, as indicated by family history over two generations. They were members of Medical University staff, students and out-patients with minor surgical disorders, living in the southwestern (Lower Silesia) region of Poland.

Among 100 patients with allergic diseases 52 subjects (52%) were male, 48 (48%) were female. Their ages ranged from 16 to 62 years with a mean (SD) 29.9 (10.8) years. Mean duration of allergic disease were 8.1 (SD) 6.9 years.

Three hundred volunteers were involved in the phenotyping part of the study. The ages ranged from 18 to 73 years with a mean (SD) 37.1 (15.5) years. One hundred forty-six subjects (49%) were male, one hundred fifty-four (51%) were female.

One hundred patients with allergic diseases were involved in the genotyping part of the study. Their ages ranged from 18 to 73 years with a mean

Table 1. Characteristic of phenotyped and genotyped patients with allergic diseases and healthy persons as a control group**Tabela 1.** Charakterystyka pacjentów z chorobami alergicznymi i osób zdrowych z grupy kontrolnej

Group (Grupa)	Age – years (Wiek – lata)		Gender – number of persons (Płeć – liczba osób)		Duration of disease – years (Czas trwania choroby – lata)	
	\bar{x}	\pm SD	female (kobiety)	male (mężczyźni)	\bar{x}	\pm SD
Phenotyped persons (Osoby fenotypowane)						
Allergic diseases (Choroby alergiczne) n = 100	29.9	10.8	48	52	8.13	6.91
Healthy persons (Osoby zdrowe) n = 300	37.1	15.5	154	146	–	–
Genotyped persons (Osoby genotypowane)						
Allergic diseases (Choroby alergiczne) n = 100	28.70	10.38	53	47	8.51	5.72
Healthy persons (Osoby zdrowe) n = 300	28.86	11.01	192	108	–	–

(SD) 28.3 (10.4) years. 53 subjects (53%) were male, 47 (47%) were female. Mean duration of disease were 8.5 (SD) 5.7 years.

The ages of 300 volunteers involved in the genotyping part of the study ranged from 19 to 88 years with a mean (SD) 28.9 (11.1) years. One hundred eight subjects (36%) were male, one hundred ninety-two (64%) were female.

Fifty patients, both phenotyped and genotyped (50% male, 50% female), were involved in the phenotyping/genotyping comparison part of the study. The ages of the subjects ranged from 17 to 62 years with a mean (SD) 31.3 (11.0) years.

The characteristic of allergic patients and controls is summarized in Table 1.

Informed consent was obtained in every case. The protocol for the study was approved by the Bioethics Committee of the Wrocław Medical University.

Methods

The subjects received 100 mg sparteine sulphate. All the urine excreted during 6 h was collected and stored at -20°C until analyzed. Sparteine (SP) and its metabolites 2- and 5-dehydrosparteine (DHS) were determined in urine by the gas chromatographic modified method of Eichelbaum [2]. The metabolic ratio (MR) was calculated as:

$$\text{Metabolic Ratio (MR)} = \frac{\% \text{ of dose excreted as sparteine}}{\% \text{ of dose excreted as 2-and 5-dehydrosparteine}}.$$

Individuals with a sparteine metabolic ratio lower than 2.5 were classified as extensive metabolizers (EM_s), with MR greater than 20 as poor metabolizers (PM_s) and with $2.5 < \text{MR} < 20$ as intermediate metabolizers of sparteine (IM_s).

DNA was arranged from leukocytes extracted from peripheral blood. Genotyping for the defective CYP2D6*3 and CYP2D6*4 alleles (a single base-pair deletion in exon 5 for CYP2D6*3 and a G1934 to A point mutation for CYP2D6*4) was performed by polymerase chain reaction amplification and restriction fragment length polymorphism (PCR-RFLP) modified techniques based on method described by Smith et al. [11]. Alleles carrying neither CYP2D6*3 nor CYP2D6*4 were classified, using this method, as CYP2D6*1 (wild-type) alleles.

Data Analysis

The statistical analysis of the results was performed by using χ^2 test, with or without Yates correction and Kolmogorov-Smirnoff test. Hardy-Weinberg law was applied to calculate expected genotype frequency. For the statistical analysis of the susceptibility to breast cancer the Relative Risk (odds ratio) was calculated.

Results

In the study the sparteine metabolic ratios varied widely among the subjects and ranged from 0.14 to 791. From phenotyped 100 patients and

Table 2. Extensive (EM_s), intermediate (IM_s) and poor (PM_s) CYP2D6 sparteine phenotype among patients with allergic diseases and healthy persons

Tabela 2. Ekstensywny (EM_s), pośredni (IM_s) i słaby (PM_s) fenotyp CYP2D6 u pacjentów z chorobami alergicznymi i u osób zdrowych

Group (Grupa)	EM _s		IM _s		PM _s	
	n	%	n	%	n	%
Allergic diseases (Choroby alergiczne)	89	89**	9	9	2	2*
Healthy persons (Osoby zdrowe)	231	77	44	14.7	25	8.3

Statistic significance of difference in comparison with healthy persons:

* $p < 0.05$, ** $p < 0.005$.

Istotność statystyczna różnicy w porównaniu z grupą osób zdrowych:

* $p < 0,05$, ** $p < 0,005$.

300 healthy participants of the study 2 patients (2%) and 25 controls (8.3%) were classified as poor metabolizers (PM_s), with MR > 20. Moreover, metabolic ratio distribution of individuals with MR < 20 shows extensive deviation from a normal distribution and therefore could be separated into two subgroups – EM_s and IM_s by cut-off point located between MR = 2.40 and 2.68 (anti-mode 2.5). Basing on these criteria 9 patients (9%) and 44 healthy persons (14.7%) were classified as intermediate metabolizers (IM_s). Eighty-nine patients (89%) and 231 healthy persons (77%)

Table 4. Predicted extensive (EM_{pred} wt/wt), intermediate (IM_{pred} wt/mut) and poor (PM_{pred} mut/mut) CYP2D6 genotype among patients with allergic diseases and healthy persons

Tabela 4. Przewidywany ekstensywny (EM_{pred} wt/wt), pośredni (IM_{pred} wt/mut) i słaby (PM_{pred} mut/mut) genotyp CYP2D6 u pacjentów z chorobami alergicznymi i u osób zdrowych

Group (Grupa)	EM _{pred} wt/wt		IM _{pred} wt/mut		PM _{pred} mut/mut	
	n	%	n	%	n	%
Allergic diseases (Choroby alergiczne)	73	73**	24	24	3	3*
Healthy persons (Osoby zdrowe)	178	59	98	33	24	8

Statistic significance of difference in comparison with healthy persons:

* $p < 0.1$, ** $p < 0.05$.

Istotność statystyczna różnicy w porównaniu z grupą osób zdrowych:

* $p < 0,1$, ** $p < 0,05$.

were classified as “normal” extensive metabolizers (EM_s) of sparteine (Table 2).

The CYP2D6 allele frequencies and genotypes in allergic patients and in Polish population are summarized in Table 3.

From genotyped 100 patients and 300 healthy participants of the study 3 patients (3%) and 24 controls (8%) were classified as predicted poor metabolizers (PM_{pred}), 34 patients (34%) and 140

Table 3. CYP2D6 genotype and allele frequencies among patients with allergic diseases and healthy persons as a control group

Tabela 3. Genotyp CYP2D6 i częstość występowania alleli CYP2D6 u pacjentów z chorobami alergicznymi i u osób zdrowych z grupy kontrolnej

	Patients with allergic diseases (Chorzy na choroby alergiczne)			Controls (Grupa kontrolna)				
CYP2D6 genotype (Genotyp CYP2D6)	number (liczba)	frequency (częstość) %	expected frequency (oczekiwana częstość) %	expected phenotype (oczekiwany genotyp)	number (liczba)	frequency (częstość) %	expected frequency (oczekiwana częstość) %	expected phenotype (oczekiwany genotyp)
*1/*1	73	73	72.3	EM	178	59.5	57.3	EM
*1/*3	1	1	0.9	IM	7	2.3	2.0	IM
*1/*4	23	23	24.6	IM	91	30.4	34.8	IM
*3/*4	0	0	0.1	PM	1	0.3	0.6	PM
*4/*4	3	3	2.1	PM	23	7.7	5.3	PM
Allele of CYP2D6 (Allele CYP2D6)								
CYP2D6*1	170	85.0			454	75.7		
CYP2D6*3	1	0.5			8	1.3		
CYP2D6*4	29	14.5			138	23.0		

EM – extensive metabolizers, IM – intermediate metabolizers, PM – poor metabolizers.

EM – ekstensywni metabolizerzy, IM – pośredni metabolizerzy, PM – słabi metabolizerzy.

controls (14.7%) as predicted intermediate metabolizers (IM_{pred}) and 64 patients (64%) and 135 controls (47%) as predicted extensive metabolizers (EM_{pred}) of sparteine.

The results of study revealed a statistically significant predominance of extensive metabolizer phenotype and genotype among patients with allergic diseases in comparison to healthy volunteers. Relative risk (odds ratio) of development of allergic diseases was 2.81 times higher ($p < 0.005$) for persons with extensive CYP2D6 phenotype and 1.85 times higher ($p < 0.05$)

The comparison of genotype and phenotype in 50 genotyped and phenotyped individuals showed a good concordance of the predicted PM_s phenotype and the obtained PM_s results. However, results revealed that heterozygous genotype (IM_{pred}) cannot be an indicator of IM_s phenotype and homozygous genotype (EM_{pred}) cannot be an indicator of EM_s phenotype. Among phenotyped 7 IM_s persons only 5 were heterozygous and among phenotyped 41 EM_s only 32 were homozygous.

The frequency of *CYP2D6**1, *CYP2D6**3 and *CYP2D6**4 alleles among genotyped three hundred persons was 75.7%, 1.3% and 23.0%, respectively. The group of twenty-four carriers of *CYP2D6* deficient genes (8.0% in Polish population) consisted of one heterozygous person with *CYP2D6**3/*4 genotype and twenty-three homozygous individuals carrying two nonfunctional alleles (*CYP2D6**4/*4 genotype). One hundred seventy-eight (59.5%) of three hundred volunteers were homozygous for the wild-type CYP2D6 allele (*CYP2D6**1/*1) and expected as extensive metabolizers (EM_s) phenotype. Among ninety-eight (32.7%) heterozygous persons, predicted as intermediate metabolizers (IM_s) phenotype, ninety-one (30.4%) individuals carried *CYP2D6* *1/*4 genotype (B-mutation), while seven (2.3%) carried the *CYP2D6**1/*3 genotype (A-mutation). The CYP2D6 genotypes in allergic patients and in Polish population are presented in Table 4.

The comparison of genotype and phenotype in sixty randomly selected individuals showed a good concordance of the predicted PM_s phenotype and the obtained PM_s results. However, results revealed that heterozygous genotype cannot be an indicator of IM_s phenotype and homozygous genotype cannot be an indicator of EM_s phenotype. Among phenotyped eight IM_s persons only six were heterozygous and among phenotyped forty-seven EM_s only thirty-two were homozygous.

Discussion

The study is the first that compares the distribution of CYP2D6 phenotypes versus genotypes within allergic diseases patients and within population from the southwestern region of Poland (Lower Silesia). The first aim of presented study was to evaluate whether patients with allergic diseases, like atopic asthma and allergic rhinitis differ from healthy persons in their CYP2D6 phenotype and genotype. In study the authors have observed a predominance of the percentage of extensive metabolizers genotype and especially phenotype among patients with allergic diseases in comparison to the percentage of extensive metabolizers in healthy controls. It may be caused by fact that histamine, very important mediator of immunologic and allergic reaction, such as early IgE-dependent allergic reaction, is catabolised also by oxidative dealkylation [9, 10]. The authors cannot compare their results with other studies, because relationship between genetically determined polymorphic metabolism and susceptibility to allergic diseases was not studied yet. But their findings are with agreement with results of studies showing general tendency to relationship between extensive CYP2D6 genotype and phenotype and the higher susceptibility to development of various diseases, probably caused by activating role of CYP2D6 in conversion of procarcinogens and non-toxic substances to proximate carcinogens and toxins [1, 3, 11].

The second aim of the study was to compare the efficacy of phenotyping and genotyping methods. Stronger correlation between oxidation phenotype than genotype and the risk of allergic diseases may be caused by the influence of environmental factors on genetic predisposition. It proved that older, well known and cheaper phenotyping method may be as good or even better than genotyping in predicting susceptibility to development of allergic diseases.

The results represent some evidence for a possible relationship between extensive CYP2D6 genotype and especially phenotype and the higher susceptibility to development of allergic diseases. Results also suggest that phenotyping methods may be as useful as genotyping methods in predicting susceptibility to development of diseases such as allergic diseases.

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