Analysis of *CYP2C9*2*, *CYP2C19*2*, and *CYP2D6*4* polymorphisms in patients with type 2 diabetes mellitus

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Abstract

This is the first study performed in population from Bosnia & Herzegovina (BH), in which we analysed a significance of genetic variations in drug-metabolising enzyme, cytochrome P450 (CYP), in pathogenesis of Type 2 diabetes. We have determined allele frequencies for *CYP2C9*2*, *CYP2C19*2*, and *CYP2D6*4* in diabetic patients and nondiabetic controls. Genomic DNA was extracted from blood samples collected from 37 diabetic and 44 nondiabetic subjects. A real-time polymerase chain reaction was used for the detection of specific CYP polymorphisms, with the application of the specific TaqMan® SNP genotyping tests (*Applied Biosystems*). Interestingly, results from this study have demonstrated that frequencies of *CYP2C19*2* and *CYP2D6*4* variants were in line, while frequency of *CYP2C9*2* polymorphism seemed to be lower in this sample of BH population as compared to the Caucasians genotype data. Furthermore, no significant difference in allele frequencies for *CYP2C9*2*, CYP2C19*2, and CYP2D6*4 was demonstrated between diabetic and nondiabetic subjects. Thus, results form this study seem to indicate no relationship between *CYP2C9*, *CYP2C19*, and *CYP2D6* genotype and diabetes susceptibility in Bosnian population. This in part may reflect a limited study population included in our study and would require larger cohorts to reveal potential relationships between analysed CYP genetic variants and diabetes risk. In addition, it would be pertinent to further explore possible effects of CYP genetic variations on therapeutic and adverse outcomes of oral antidiabetics, which might be the key in optimising therapy for individual patient with Type 2 diabetes.

KEY WORDS: drug-metabolising enzymes, CYP2C9, CYP2C19, CYP2D6, diabetes, pharmacogenetics

Introduction

It has been estimated that around one billion people are obese worldwide, with around 250 million people suffering from Type 2 diabetes mellitus (T2DM) and projected rise to 380 million in next 15 years [1]. Patients with T2DM are often treated with more than one drug, including one or coprescription with additional oral antidiabetic (e.g. metformin, sulfonylureas, thiazoldinediones, repaglinide) and medicines used to treat diabetic complications, such as hypertension and dyslipidemia (e.g. statins). Effects of these drugs depend on the extent of drug absorption from the gut lumen, on metabolism of the drug in the liver, and on the extent of its transport back into the systemic circulation for extrahepatic effects [2]. Drug-metabolising enzymes (DME), which include phase I and II metabolising enzymes, play a central role in the intestinal absorption/permeability, metabolism, elimination, and detoxification of various drugs. Cytochrome P450 (CYP) enzymes are major phase I metabolising enzymes, which play particularly important role in disposition of various drugs used in treatment of Type 2 diabetes and its complications. The human CYP superfamily contains 57 functional genes and 58 pseudogenes, where *CYP2C8*, *CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5,* and *CYP1A2* metabolise around 90% of drugs [3,4]. Importantly, CYP-regulated drug metabolism is prone to genetic variability that can result in an enzyme with normal, low, or no activity. CYP polymorphisms are responsible for observed variations in drug response among patients with different ethnic origins [5,6], which could be combined with genetic variations in other DME, drug transporters, and drug receptors. Interestingly, polymorphisms of *CYP2D6*, *CYP2C19*, and *CYP2C9* genes account for the most frequent variations in phase I metabolism of drugs, since almost 80% of drugs in use today are metabolised by these enzymes. Approximately 5-14% of Caucasians, 0-5% Africans, and 0-1% of Asians lack *CYP2D6* activity, and these individuals are

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known as poor metabolizers. *CYP2C9* is another clinically significant enzyme that demonstrates multiple genetic variants with a potentially functional impact on the efficacy and adverse effects of drugs that are mainly eliminated by this enzyme. Diminished rates of drug clearance can increase the incidence of toxicity from many drugs, but may also enhance efficacy, as in the case of the proton-pump inhibitor omeprazole, that maintains therapeutic serum concentrations in individuals that carry null alleles for *CYP2C19*. Thus, analysis of pharmacogenetic variation in CYP enzymes is pertinent to enhance safety and efficacy of drug therapy and streamline this therapy for individual patients suffering from Type 2 diabetes and its complications.

TABLE 1. Locations and effects of *CYP2D6*4*, *CYP2C9*2* and *CYP2C19*2* polymorphisms (adopted from [7]).

Polymorphism	Nucleotide change	rs number*	Location, protein effect	Functional effect
*CYP2D6*4*	1846G>A	rs3892097	Splicing defect	Null allele
*CYP2C9*2*	3608C>T	rs1799853	R144C	Decreased activity
*CYP2C19*2*	19154G>A	rs4244285	Splicing defect	Null allele

*rs number - reference Single Nucleotide Polymorphism (SNP) ID assigned by the SNP database at National Center for Biotechnology Information (dbSNP).

In addition to environmental effects, ethnic and individual genetic variations contribute to both diabetes susceptibility and drug response variability. Interestingly, CYP polymorphisms, such as *CYP3A4* and *CYP2J2*, have been recently associated with the genetic risk for Type 2 diabetes in Japanese and Chinese population, respectively [8, 9]. However, a significance of genetic variations in other CYP isoforms in development of Type 2 diabetes is not completely understood, as the genes that contribute to the genetic susceptibility of this disorder remain to be identified. Furthermore, since the ethnic differences include lifestyle, environmental factors and genetic background, it would be pertinent to analyse gene polymorphisms related to Type 2 diabetes in different ethnic groups. To address these issues, in this study we have analyzed frequency of specific gene polymorphisms of *CYP2C9*, *CYP2C19*, and *CYP2D6* (Table 1) in diabetic patients from Bosnia and Herzegovina.

Materials and Methods

Patients

In this study we have analysed CYP polymorphisms in a group of 37 patients diagnosed with Type 2 diabetes mellitus and 44 nondiabetic participants. All human subjects involved in this study were patients of General hospital in Tesanj, BH. All research involving human subjects and material derived from human subjects in this study was done in accordance with the ethical recommendations and practices of the General Hospital in Tesanj and complied with ethical principles outlined in World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000). Subjects included in this study were free of evidence of acute infection and/or inflammation, active liver and kidney damage, and other metabolic or endocrine disorders. Characteristics of all subjects participating in this study are shown in Table 2. Individuals with Type 1 diabetes mellitus and patients taking drugs that cause secondary diabetes mellitus were also excluded from this study.

Clinical Diagnosis of Type 2 Diabetes Mellitus

Type 2 DM was clinically diagnosed by standardized clinical examination conducted by specialist of Internal Medicine. All subjects underwent medical history and clinical examination. Selection of patients included in this study was done following IDF criteria, so diabetes mellitus was diagnosed when fasting plasma glucose levels were higher than 7.0 mmol/L, postprandial serum glucose more than 11.1 mmol/L or a blood glycosylated hemoglobin (HbA1c) content of >6.5%, or were taking oral antidiabetic drugs. All patients had abdominal obesity - waist circumference more than 80 cm in women and 94 cm in men; atherogenic dyslipidemia-triglycerides > 1.7 mmol/L, HDL < 1.0 mmol/L and controlled blood pressure levels. Nondiabetic subjects were of approximately same age (30-60 years old, mean 45±1.5 years), but with normal glucose-tolerance test, and they were not on any drug treatment during the course of this study. In all subjects participating in this study, systolic and diastolic blood pressure, body mass index (BMI), and waist circumference were measured.

Sample Collection and Biochemical Analysis

Blood samples were obtained from all participants in fasting conditions from antecubital vein into siliconized tubes (BD Vacutainer Systems, Plymouth, UK). Large-scale phenotype data were collected, including fasting plasma glucose, HbA1c, insulin levels, triglycerides, total, LDL, and HDL cholesterol. Standard IFCC protocols were used for all analyses employing VITROS 350 Chemistry System analyser (*Ortho-Clinical Diagnostics*).

Genotyping Analysis

Genomic DNA was extracted from blood samples by the Miller salting-out procedure [10]. The total concentration of isolated genomic DNA was determined by UV/VIS spectrophotometer *NanoDrop ND-1000*. A real-time polymerase chain reaction was used for the detection of specific CYP polymorphisms, with the application of the specific TaqMan® SNP genotyping assays (*Applied Biosys-*

TABLE 2. Characteristics of the study participants.

	Diabetic patients (n=37)	Nondiabetics (n=44)	*p*
Age (years)	49.4 ± 1.2	45.1 ± 1.5	0.028
Male/Female	15/22	11/33	0.136
BMI (kg/m^2)	32.7 ± 0.9	30.3 ± 1.2	0.122
Waist circumference (cm)	112.3 ± 3.0	96.7 ± 3.3	0.001
Systolic BP (mm Hg)	151.8 ± 3.2	124.4 ± 5.0	<0.001
Diastolic BP (mm Hg)	94.8 ± 1.9	76.8 ± 3.0	<0.001
Fasting plasma glucose (mmol/L)	11.1 (8.3-15.3)	5.3 (4.9-5.6)	<0.001
Blood HbA1c (%)	6.8 (5.9-7.7)	4.4 (4.2-5.0)	<0.001
Total cholesterol (mmol/L)	5.6 ± 0.2	5.8 ± 0.2	0.559
LDL-cholesterol (mmol/L)	3.22 ± 0.18	3.05 ± 0.18	0.521
HDL-cholesterol (mmol/L)	1.09 ± 0.06	1.71 ± 0.05	<0.001
Triglycerides (mmol/L)	2.32 (1.70-3.20)	1.95 (1.46-2.96)	0.225

tems): C__27102431_D0 (*CYP2D6*4*), C__25625805_10 (*CYP2C9*2*) and C__25986767_70 (*CYP2C19*2*). Real-time PCR method was performed on the ABI PRISM™ 7000 Sequence Detection System (Applied Biosystems) for *CYP2D6*4* and on the LightCycler®480 Real-Time PCR System (Roche Diagnostics) for *CYP2C9*2* and *CYP2C19*2*.

Statistical Analysis

The differences between frequencies of specific genotypes between diabetic patients and nondiabetic controls were analysed by $\chi 2$ test and Fisher exact test (in the case where frequencies were less or equal to 5). Association of the analysed CYP polymorphisms and clinical and biochemical parameters were calculated by Student's t-test for data that followed normal distribution (including age, BMI, waist circumference, systolic and diastolic blood pressure, total, LDL, and HDL cholesterol) or Mann-Whitney test for data that did not follow normal distribution (such as fasting plasma glucose, HbA1c, and triglyceride levels).

TABLE 3. Variant allele frequencies for *CYP2D6*4*, *CYP2C9*2* and *CYP2C19*2* polymorphisms in a sample of BH population compared to other data in Caucasians.

Polymorphism	rs number	Mutated allele frequency	
		BH population study group	Caucasians*
*CYP2D6*4*	rs3892097	0.22	0.15-0.25
*CYP2C9*2*	rs1799853	0.09	0.13-0.17
*CYP2C19*2*	rs4244285	0.17	0.15

* Frequencies of the indicated variant alleles for Caucasians obtained from dbSNP at http://www.ncbi.nlm.nih.gov/SNP (adopted from [7]).

Statistical data analysis was done using *SPSS 17.0 for Windows*. Data are presented as mean ± SEM or median (lower-upper quartile). Statistical significance was set as $p<0.05$.

Results

The characteristics of subjects participating in this study are shown in Table 2. The mean age difference between diabetic and nondiabetic participants was 4 years and should not be considered as clinically relevant. Waist circumference, systolic and diastolic blood pressure, fasting plasma glucose levels, and blood HbA1c content were higher, while the serum concentration of high density lipoprotein (HDL)-cholesterol levels were significantly lower in patients with Type 2 diabetes than in nondiabetic subjects. As shown in Table 3, results from this study have demonstrated that frequencies of *CYP2C19*2* and *CYP2D6*4* polymorphisms in BH population were in line with the Caucasians genotype data reported earlier [7]. However, the frequency of *CYP2C9*2* polymorphism seems to be lower in this sample of BH population as compared to data from earlier studies [7]. The analysed CYP genotype frequencies were in line with Hardy-Weinberg equilibrium ($p>0.05$). However, as shown in Table 4, no significant difference in genotype frequencies for *CYP2C9*2*,

TABLE 4. Genotype and variant allele frequencies for *CYP2D6*4*, *CYP2C9*2*, and *CYP2C19*2* polymorphisms in diabetic and nondiabetic subjects.

Polymorphism	Genotype	Diabetic patients		Nondiabetics		p*
		Number of subjects (%)	Mutated allele frequency	Number of subjects (%)	Mutated allele frequency	
*CYP2D6*4*	wild-type (G/G)	22 (64.7%)	0.19	26 (60.5%)	0.24	0.53
	heterozygotes (G/A)	11 (32.4%)		13 (30.2%)		
	mutated homozygotes (A/A)	1(2.9%)		4 (9.3%)		
	Total	34		43		
*CYP2C9*2*	wild-type (C/C)	29 (82.9%)	0.09	35 (81.4%)	0.09	1.00
	heterozygotes (C/T)	6 (17.1%)		8 (18.6%)		
	Total	35		43		
*CYP2C19*2*	wild-type (G/G)	23 (67.6%)	0.18	30 (69.8%)	0.16	0.97
	heterozygotes (G/A)	10 (29.4%)		12 (27.9%)		
	mutated homozygotes (A/A)	1 (2.9%)		1 (2.3%)		
	Total	34		43		

*significance of χ^2 test for comparison of genotype frequencies between diabetic and nondiabetic subjects.

*CYP2C19*2*, and *CYP2D6*4* polymorphisms was demonstrated between diabetic and nondiabetic subjects. Table 4 Genotype and variant allele frequencies for *CYP2D6*4*, *CYP2C9*2*, and *CYP2C19*2* polymorphisms in diabetic and nondiabetic subjects. In this study we have also explored genotype-phenotype associations to reveal potential relationships between selected CYP polymorphisms and phenotype information related to metabolic control and anthropomorphic measures. However, no significant association was observed between specific *CYP* gene variants and any of analysed phenotypic parameters in nondiabetic subjects (data not shown). Similarly, in diabetics, no associations of *CYP2C9*2*, *CYP2C19*2*, and *CYP2D6*4* polymorphisms with patients' anthropomorphic measures were demonstrated.

Discussion

In general, the distribution of different CYP alleles varies among ethnic groups [11]. Furthermore, CYP polymorphisms, such as CYP3A4, have been associated with the genetic risk for Type 2 diabetes in Japanese population [8]. In addition, recent study has suggested the association between the CYP2J2 G-50T polymorphism and T2DM risk in Chinese population [9]. CYP2J2 is expressed in vascular endothelium and metabolizes arachidonic acid to biologically active epoxyeicosatrienoic acids, which are potent endogenous vasodilators and inhibitors of vascular inflammation. To the best of our knowledge, the association of other CYP isoforms variation with diabetes risk has not been studied yet. Here we have analyzed the frequency of specific CYP polymorphisms, including *CYP2D6*4*, *CYP2C9*2*, and *CYP2C19*2*, in subjects from Bosnia and Herzegovina, and their possible correlation with development of Type 2 diabetes. Our data have demonstrated that frequencies of *CYP2C19*2* and *CYP2D6*4* variants in Bosnian population were in line, while the frequency of *CYP2C9*2* polymorphism seemed to be lower in this sample of BH individuals as compared to the Caucasians genotype data reported earlier [7]. Furthermore, results form this study seem to indicate no relationship between *CYP2D6*, *CYP2C9*, and *CYP2C19* genotype and diabetes in BH population. This finding can be, at least partially, attributed to the ethnic characteristics including lifestyle, environmental factors and genetic background, in addition to the number of subjects participating in our study. Since this study included a limited study population, it would probably require larger patient cohorts to reveal potential relationships between analysed CYP variants and diabetes risk. It is also important to note that polymorphisms in the CYP family may have had the most impact on the fate of therapeutic drugs. Since *CYP2D6*, *CYP2C19*, and *CYP2C9* polymorphisms account for the most frequent variations in phase I metabolism of drugs, it would be also pertinent to explore CYP genetic variations in terms of effectiveness of oral antidiabetics and their adverse outcomes. Recently, polymorphisms in sulfonylurea drug target genes and diabetes risk genes have been implicated as important determinants of sulfonylurea pharmacodynamics in patients with Type 2 diabetes. Particularly, *CYP2C9* and *CYP2C19* have been associated with interindividual variation of sulfonylureas metabolism, while CYP2C8 seems to be regulating biotransformation of thiazoldinediones [12]. Interestingly, acute myopathy was reported in Type 2 diabetic patient treated with combination therapy with metformin, fibrates, and thiazolidinediones [13] and possible genetic risk factors for this adverse effect have not been described yet. Furthermore, CYP3A4 and CYP3A5 variants have been demonstrated to influence the pharmacokinetics, efficacy, and safety of statins [14], which are often coprescribed in diabetic patients to treat cardiovascular complications. Although individualized statin therapy is not a common practice yet, evidence has been accumulating to suggest an especial caution when initiating therapy in certain patients groups, such as older patients and patients that are cooadministered with drugs affecting the CYP-regulated metabolism [15]. Therefore, in addition to variations of CYP isoforms analysed in this study, it would be important to determine the frequency of other CYP polymorphisms, such as CYP3A4 and CYP3A5 variations, in BH population in terms of diabetes risk and treatment outcomes. Importantly, information gained from the further identification of the major enzymes and transporters participating in antidiabetic drug disposition, as well as the elucidation of the genetic basis for this variation, should be applied for the effective, safe treatment of diabetes and its cardiovascular complications, paving the way towards personalized health care.

Conclusion

In summary, this is the first study performed in population from Bosnia & Herzegovina (BH), in which a significance of genetic variations of cytochrome P450 (CYP) was investigated in pathogenesis of Type 2 diabetes. Our data have demonstrated that frequencies of *CYP2C19*2* and *CYP2D6*4* variants were in line, while the frequency of *CYP2C9*2* polymorphism seems to be lower in this sample of BH population as compared to the Caucasians genotype data. Furthermore, results from this study seem to indicate no relationship between *CYP2D6*, *CYP2C9*, and *CYP2C19* genotype and diabetes pathogenesis in Bosnian population. This in part may reflect a limited study population includ-

ed in our study and would require larger cohorts to reveal potential relationships between analysed CYP genetic variants and diabetes risk. Furthermore, it would be pertinent to explore specific CYP polymorphisms in terms of effectiveness of oral antidiabetics and their adverse outcomes.

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Declaration of interest

Authors have no conflict of interest to declare.

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