Original Article

# Distribution of Genetic Polymorphisms in Drug Metabolizing Gene *Cytochrome P450 (CYP2C8*3 and CYP2C9*2)* in a North Indian Type 2 Diabetes Population

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## Abstract

***Background and objective(s):*** Diabetes is growing as an epidemic, with around 250 million diabetics estimated globally and which is expected to rise up to 380 million in the next 15 years. Differences in the efficacy and toxicity of various antidiabetic drugs have been linked to polymorphisms in various drug metabolizing enzymes. In this study we have investigated the frequency of occurrence of two single nucleotide polymorphisms in the *CYP* gene *(CYP2C8*3* and *CYP2C9*2)* in type 2 diabetes mellitus (T2DM) patients from North India. ***Methods:*** This study included 360 T2DM patients from North India. Real-time polymerase chain reaction was carried out for the evaluation of specific *CYP450* gene polymorphisms via the specific TaqMan® SNP genotyping assays (Applied Biosystems Inc.) for detection of *CYP2C8*3 (rs10509681)* and *CYP2C9*2 (rs1799853)* polymorphisms in the *CYP450* gene. ***Results:*** For the *CYP2C8*3* polymorphism, the genotype frequencies detected were 0%, 92.78% and 7.22% for CC, TT and CT genotypes while the frequency of the C allele was 3.61% and that of the T allele was 96.39%. For the *CYP2C9*2 (rs1799853)* polymorphism, the frequencies were 3.1%, 12.5% and 84.44% for CC, AA and CA genotypes. The frequency of occurrence of A and C alleles were 54.72% and 45.28% respectively. ***Conclusions:*** Frequency of occurrence of the T and A alleles of *CYP2C8*3 (rs10509681)* and *CYP2C9*2 (rs1799853)* polymorphisms was higher in T2DM patients from North India.

## Introduction

Type 2 diabetes mellitus (T2DM), also known as adult-onset or noninsulin-dependent diabetes, is emerging as a topic of major global concern related to the body's ability to metabolize glucose. Pre-diabetics can maintain their blood glucose levels by making a few modifications to their diet and exercise routine, but individuals suffering from full-blown T2DM rely on medications or, most of the time, on insulin therapy. Effectiveness of these drugs is determined by various factors, including extent of drug absorption, its metabolism in liver and transport to the blood to exert its antidiabetic effects.[1] It has been shown that 20% to 95% of variability in the drug response is due to variability in inter-individual genetic composition.[2] These differences in the efficacy and toxicity of various antidiabetic drugs was found to be linked to polymorphisms in genes encoding various drug metabolizing enzymes, drug transporters and receptors.[3] Drug metabolizing enzymes are of two types, Phase I and Phase II metabolizing enzymes which aid in absorption, metabolism, elimination and detoxification of drugs. Among the various drug metabolizing enzymes, the cytochrome P450 (CYP450) enzymes, which are phase I metabolizing enzymes, play an important role in the disposition of variety of antidiabetic drugs. They are named CYP450 because they remain membrane-bound inside the cell (cyto) and harbor a heme pigment (chrome and P) that absorbs light of wavelength 450 nm after being exposed to carbon monoxide.[4] These enzymes are predominantly expressed in the liver, but they are also found in the small intestine, lungs, placenta and kidneys.[5] The CYP450 family includes more than 50 enzymes, among which CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 metabolize more than 90% of the drugs.[5,6] CYP-regulated drug metabolism exhibits genetic variability that affects enzyme activity and, thus, variations in drug response.[7–9] Polymorphisms in the *CYP450* gene occur when a mutant/variant allele replaces a wild-type allele; the individuals carrying the variant allele show reduced or no enzyme activity. Thus, persons with single or double variant alleles metabolize drugs poorly, as compared to individuals carrying two copies of wild-type functional allele.[6]

*CYP2C8* and *CYP2C9* are clinically relevant enzymes that display genetic variability, thereby affecting the efficacy of drugs metabolized by these enzymes.[10] In humans, the *CYP2C8* gene is localized within the long (q24.1) arm of chromosome 10, and consists of 9 exons and spans about 31 kilo bases.[11] To date, more than 20 polymorphisms in this gene have been reported in different populations.[12] Most of the *CYP2C8* polymorphisms, including *CYP2C8*3* in exons 3 and 8 (416G>A/1196A>G, R139K/K399R), lead to reduced enzyme activity. On the other hand, the *CYP2C9* gene, located on chr10q24.2 is the most abundant of all the *CYP2C* isoforms, constituting about 20% of the total CYP450 hepatic content.[13] Out of the 41 different variant alleles in the

**Keywords:** Cytochrome p450; Drug metabolism; Genetic polymorphism; Type 2 diabetes mellitus.

**Abbreviations:** CYP450, Cytochrome P450; T2DM, Type 2 Diabetes Mellitus; BMI, Body Mass Index; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; HbA1C, Acetylated Hemoglobin; EDTA, Ethylenediaminetetraacetic Acid; DNA, Deoxyribonucleic Acid; SD, Standard Deviation; RT-PCR, Real Time Polymerase Chain Reaction; FAM, 6-carboxyfluorescein; PRD, Passive Reference Dye; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBS, Fasting Blood Sugar; RBS, Random Blood Sugar; VLDL, Very Low-Density Lipoprotein; PPBS, Post Prandal Blood Sugar; IQR, Interquartile Range.

*Received: 03 June 2016; Revised: 23 July 2016; Accepted: 10 August 2016*

☆DOI: 10.14218/ERHM.2016.00004

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**Table 1. Biochemical and clinical characteristics of the study subjects**

| No. | Variable | T2DM Cases, *n*=360 |
|---|---|---|
| 1 | Age, years | 34 to 59 years |
| 2 | BMI, kg/m$^2$ | 27.2±2.3 |
| 3 | SBP, mmHg | 132±2.7 |
| 4 | DBP, mmHg | 88±1.83 |
| 5 | FBS, mmol/L | 7.6±1.8 |
| 6 | RBS, mmol/L | 12.51±5.6 |
| 7 | PPBS, mmol/L | 13.94±4.65 |
| 8 | HbA1c, % | 7.01±1.92 |
| 9 | Serum creatinine, mmol/L | 1258±520 |
| 10 | Serum cholesterol, mmol/L | 11.1±1.5 |
| 11 | Triglyceride, mmol/L | 1.8 (1.4–2.45) |
| 12 | HDL, mmol/L | 3.09±0.9 |
| 13 | LDL, mmol/L | 5.54±1.95 |
| 14 | VLDL, mmol/L | 3.1±1.2 |

Values are presented as mean±SD (standard deviation), except for HbA1c which is presented as *n* (%) and Triglyceride which is presented as median (IQR). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; RBS, random blood sugar; PPBS, post-prandial blood sugar; HbA1c, acetylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

*CYP2C9* gene, *CYP2C9*2* (430C>T, Arg144Cys), which is located in exon 3, and *CYP2C9*3* (1075A>C, Ile359Leu), which is located in exon 7, are the most studied alleles that lead to reduction in CYP2C9 enzyme activity.[11] Thus, it seems important to analyze the distribution of these genetic variants in the *CYP* gene, as the findings from such studies might help in optimizing therapy for individual patients with T2DM through enhancing safety and efficacy of antidiabetic drugs by means of a personalized medicine approach. In the present study, we investigated the frequency of occurrence of various genetic polymorphisms in the *CYP gene (CYP2C8*3* and *CYP2C9*2)* in T2DM patients from North India.

## Materials and methods

### *Study design*

This hospital lab-based research study was carried out as a collaborative effort between the Department of Biochemistry and Department of Medicine in Era's Lucknow Medical College & Hospital (Lucknow, India). This study was approved by the Institutional Ethical Committee, and the study strictly followed good clinical practice guidelines and the Helsinki declaration.

### *Patient selection*

This study involved 360 T2DM patients of North Indian ethnicity aged over 35 years. T2DM patients, diagnosed according to the International Diabetes Federation criteria, were consecutively recruited from the Department of Medicine and provided informed consent prior to study participation.[14] We excluded patients suffering from acute infection or inflammation, showing liver and/or kidney damage and having other metabolic or endocrine disorders. Patients with type 1 diabetes mellitus and those taking drugs that cause secondary diabetes mellitus were also excluded.

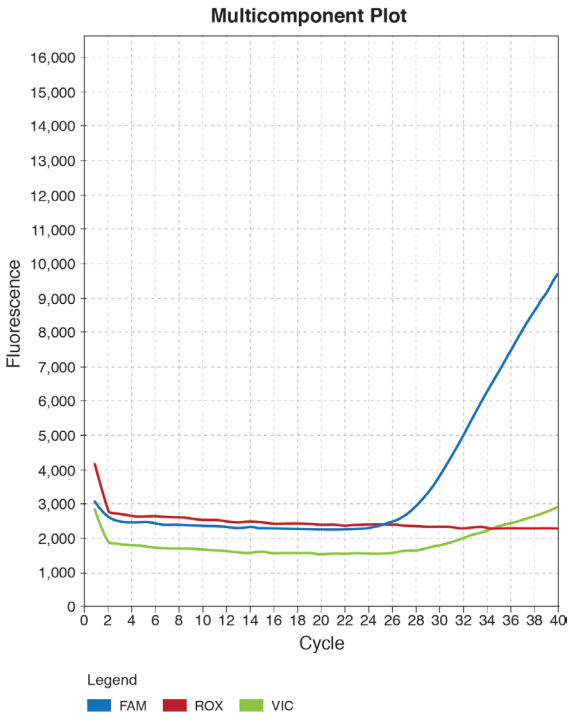


**Fig. 1. Multicomponent plot of the *CYP2C8*3* polymorphism generated through RT-PCR (StepOne Plus; Applied Biosystems Inc.) and showing the TT genotype. Allele C was amplified separately from the alternative allele T by using region-specific forward and reverse primers and two allele-specific TaqMan® probes (VIC® specific for allele C, and 6-carboxyfluorescein [FAM™] specific for allele T) that were designed to target the polymorphism. ROX dye was used as a passive internal reference.**

Data collection for each patient was performed to collect clinical variables including age, alcohol consumption, body mass index (BMI), height, weight, cigarette smoking and family history, etc. Blood samples were collected for biochemical and molecular assays. BMI was calculated by the Quetelet equation ([weight in kilograms/height in meter square]). Fasting plasma glucose (via the glucose oxidase-peroxidase method), serum cholesterol (via the cholesterol oxidase-peroxidase method), serum triglyceride (via the glycerol phosphate oxidase-peroxidase amidopyrine method), high-density lipoprotein (HDL) and cholesterol (both via immunoinhibition assay) were assessed by the XL-300 Fully-Automated Analyzer (Transasia, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald formula.[15] HbA1C was measured using the Transasia Semi-Auto-analyzer. All the assays were performed according to the manufacturer's protocols. Characteristics of all subjects participating in this study are shown in Table 1.

### *Analysis of CYP2C8*3 (rs10509681) and CYP2C9*2 (rs1799853) polymorphisms*

Venous blood (4 mL) was collected from all the subjects in 0.5 M vacutainers with EDTA as anticoagulant for DNA extraction and biochemical analysis. Genomic DNA was isolated from the whole blood samples using a DNA extraction kit (MACHEREY-NAGEL,

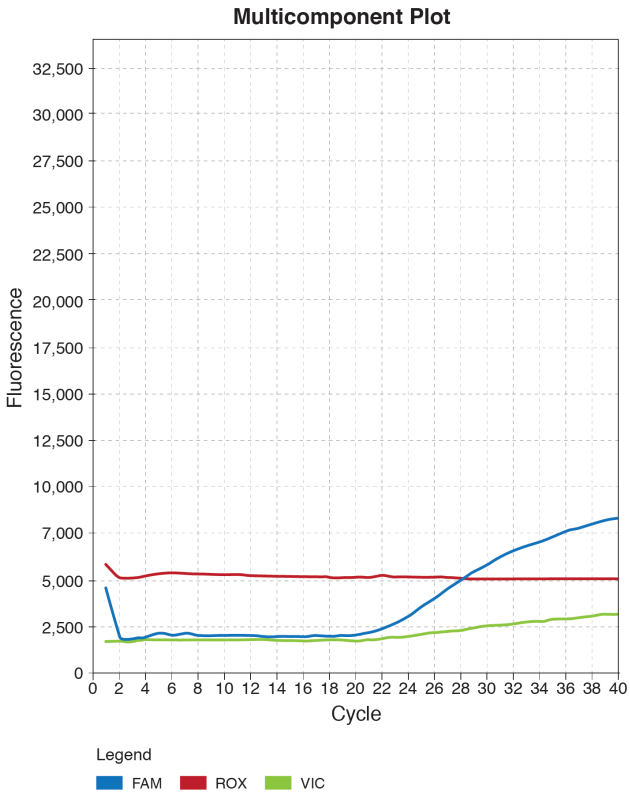


**Fig. 2. Multicomponent plot of the *CYP2C9*2* polymorphism generated through RT-PCR (StepOne Plus; Applied Biosystems Inc.) and showing the AA genotype. Allele C was amplified separately from the alternative allele A by using region-specific forward and reverse primers and two allele-specific TaqMan® probes (VIC® specific for allele C, and 6-carboxyfluorescein [FAM™] specific for allele A) that were designed to target the polymorphism. ROX dye was used as a passive internal reference.**

Duren, Germany) and following the manufacturer's protocol. The DNA concentration was determined by a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and samples were stored at −20°C.

Pre-validated allelic discrimination using TaqMan Real-time PCR Assays (Assay IDs: C_25625782 and C_25625805_10; Applied Biosystems Inc., Foster City, CA, USA) was carried out for detection of *SNPs rs10509681and rs1799853* in the *CYP450* gene respectively (multicomponent graphs for both polymorphisms are shown in Fig. 1 and 2).

### *Statistical analysis*

Clinical data are expressed as mean±SD. Data were compiled according to the genotype and allele frequencies estimated from the observed numbers of each specific allele. The chi-squared test was used to determine if the data were in agreement with Hardy-Weinberg equilibrium.

## Results

Our study included 360 T2DM patients from North India. The ages of subjects recruited in our study ranged from 34 years-old to 59 years-old. The clinical and biochemical parameters of studied subjects are shown in Table 1.

### *Hardy-Weinberg equilibrium test*

The genotype distributions of *CYP2C8*3 (rs10509681)* and *CYP2C9*2 (rs1799853)* polymorphisms in all subjects were in line with Hardy-Weinberg equilibrium ($p$=0.96 and $p$=0.55 respectively).

### *Distribution of genotype frequencies*

*CYP2C8*3 (rs10509681)* polymorphism analysis

Frequency of the CC genotype was 0% since no T2DM patient was found to carry the genotype. On the other hand, most of the studied subjects carried the TT genotype and its frequency was 92.78%. Frequency of occurrence of the heterozygous genotype CT was low, at 7.22%. Frequency of the C allele was 3.61%, while it was 96.39% for the T allele.

*CYP2C9*2 (rs1799853)* polymorphism analysis

Frequency of the CC genotype was 3.1%, while that of the AA genotype was a little higher, at 12.5%. Most of the studied subjects carried the heterozygous genotype and its frequency was 84.44% in T2DM patients from North India. In context to the frequency of occurrence of wild-type and mutant alleles, it was observed that A and C alleles were 54.72% and 45.28% respectively.

All of the above mentioned data are shown in Table 2.

## Discussion

Diabetes is growing as an epidemic, with around 250 million current diabetics estimated globally; moreover, this number is expected to increase to 380 million in the next 15 years.[16] Thousands of genes and their variants are associated with risk of T2DM. Pharmacogenetics is an important field emerging in context to diabetes research, primarily due to genetic polymorphisms that have been found in drug metabolizing genes and which affect absorption, metabolism and excretion of almost all antidiabetic drugs.[3] CYP450 enzymes play a major role in drug metabolism, and mutations in the genes encoding the CYP450 enzymes have been linked to inter-individual differences in efficacy and toxicology of a number of medications.[3] It has been reported that a standard drug dose may not lower blood glucose levels or is capable of leading to other complications if the drug is not metabolized efficiently in a diabetic patient due to mutations in *CYP450* gene.[17] Thus, it is important to use pharmacogenetic information in drug dosing and selection in order to enhance the efficacy of therapeutic treatment for T2DM.

The CYP2C8 enzymes play significant roles in metabolizing antidiabetes drugs (*e.g.* troglitazone, pioglitazone, rosiglitazone and repaglinide), apart from other anticancer and antihypertension drugs. Mutations in the *CYP2C8* gene have only recently been described, and most of them appear to occur at low frequencies. *CYP2C8*1* is the wild-type allele of the *CYP2C8* polymorphisms and four variant alleles (*CYP2C8*2, CYP2C8*3, CYP2C8*4* and *CYP2C8*5*) have been reported to date. The *CYP2C8*3* allelic variant encodes an enzyme containing two amino acid changes,

**Table 2. Frequency distribution of various genotypes of *CYP2C8*3* and *CYP2C9*2* genetic polymorphisms in North Indian T2DM patients**

| Gene Polymorphism | Genotype/Allele | Number of Patients, *n*=360 | Frequency of Occurrence, % |
|---|---|---|---|
| *CYP2C8*3 (rs10509681)* | CC | 0 | 0.00 |
| | CT | 26 | 7.22 |
| | TT | 334 | 92.78 |
| | C | 26 | 3.61 |
| | T | 694 | 96.39 |
| *CYP2C9*2 (rs1799853)* | CC | 11 | 3.10 |
| | CA | 304 | 84.44 |
| | AA | 45 | 12.50 |
| | C | 326 | 45.28 |
| | A | 349 | 54.72 |

namely R139K and K399R.[18] The variant alleles code for enzymes with hampered activity that lead to impaired drug metabolism. Individuals homozygous for the variant allele (**2/*2* or **3/*3*) were found to have lower intrinsic clearance of *CYP2C8* substrates (drugs) than those who are heterozygous (**1/*2* or **1/*3*).[11] The T allele frequency was found to be 96.39% in our diabetic North Indian population, which was remarkably higher than the frequency reported previously in a healthy Japanese population (0%) and an African Americans (2%) population.[18,19] Two other studies on white populations also reported low frequency for the T allele (13% and 15% respectively).[18,20] Apart from this, other studies on healthy subjects of Finnish, Swedish, British, French and Spanish Caucasian ethnicities have found much lower *CYP2C8*3* (K399R) allele frequencies (12%, 9%, 15.5%, 13% and 17% respectively); however, this frequency was higher than the frequency of the C allele (3.61%) that was observed in our North Indian diabetic subjects.[18,20–23]

The *CYP2C9* gene is the most abundant of all the *CYP2C* isoforms and the enzymes encoded by the *CYP2C9* gene basically metabolize the anti-inflammatory drugs including diclofenac, ibuprofen and naproxen, as well as various antidiabetic drugs such as glimepiride and glipizide through an oxidative process. Out of the 41 known variants of the *CYP2C9* gene, *CYP2C9*2* (430C>T, Arg144Cys) in exon 3 and *CYP2C9*3* (1075A>C, Ile359Leu) in exon 7 lead to decreased enzyme activity and have been found to cause adverse drug reactions, including hypoglycemia. Frequencies of the C and A alleles of the *CYP2C9*2* polymorphism in our diabetic population were 45.2% and 54.72%, which is higher than the frequencies reported for populations of Lebanese (11.305%), Egyptian (12%), American (8%), British (12.5%), Swedish (10.7%), Turkish (10.6%), Japanese (0%), Korean (0%), Chinese-Taiwanese (0%) and African American (0%).[24–32]

Finally, the main limitation of this study is the limited sample size. Further studies are needed to more accurately determine the incidence of the *CYP2C* polymorphic genotypes in North Indians. In addition, further development in this field is needed, along with more clinical trials, to determine whether treatment following a personalized medicine approach would improve clinical outcome by preventing adverse drug effects while being cost effective.

## Conclusions

This is the first study on the genetic polymorphisms of *CYP2C8*3* and *CYP2C9*2* in a North Indian diabetic population. A major difference was observed in the distribution of allele frequencies of the *CYP2C8*3* and *CYP2C9*2* polymorphisms in the North Indian population as compared to other global populations, indicating that these polymorphisms might also be associated with T2DM susceptibility since its frequency of occurrence in T2DM patients was found to be much higher than those of healthy subjects of different ethnicities. These data will be useful for future assessments of the role(s) of *CYP2C* polymorphisms in the diabetic population in India. The results from such studies might help in enhancing the efficacy of drugs by contributing to the decision-making process for choosing drug dosage and type for each and every individual, separately, through a personalized medicine approach.

## Acknowledgements

We are thankful for the financial support given by an intramural grant from Era's Lucknow Medical College and Hospital in Lucknow, Uttar Pradesh, India.

## Conflict of interest

The authors have no conflict of interests related to this publication.

## Author contributions

Conceiving & designing and revising the manuscript (STR, RK, FM), analysis and interpretation of data (SR), manuscript writing (SR), molecular genetic studies (SA), reading and approving the final manuscript (FM, STR, SR, SA, RK).

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