

Pharmacogenetics of sulfonylurea: Presence of CYP2C9*2, CYP2C9*3 and a novel allele, CYP2C9*61, in Type 2 diabetes patients under sulfonylurea therapy

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Abstract: *CYP2C9* is an important member of the cytochrome P450 gene family involved in the metabolism of 15% of the drugs including an oral antidiabetic agent sulfonylurea. This study aims to investigate the frequency of CYP2C9*2 and CYP2C9*3 alleles of the gene in the sulfonylurea treated diabetic subjects in Pakistan. Briefly, total 105 patients were included in the study and segregated as control (24) and test (81) based on the clinical manifestations after taking sulfonylurea. Genomic DNA was extracted from blood of the subjects and amplified using CYP2C9 specific primers for exon 3 and exon 7 and then subjected to DNA sequencing. Alignment of the sequences with the reference sequence shows presence of CYP2C9*3/*3, CYP2C9*1/*3 and CYP2C9*1/*2 genotypes in the test cases but only the latter two were found in the control cases. In addition a novel allele, CYP2C9*61 in the heterozygous state, was also identified frequently in the test cases. Molecular structure comparison also showed variations in the structural features of protein encoded by the allelic variants. To the best of our knowledge, the present data is the first report for CYP2C9 allelic variations in the indigenous diabetic subjects and also report the existence of novel allelic variant of CYP2C9, CYP2C9*61.

Keywords: Diabetes mellitus, *CYP2C9*, pharmacogenetics, sulfonylurea.

INTRODUCTION

Diabetes mellitus (DM) is a polygenic and multifactorial disease characterized by the persistent hyperglycemia. According to international federation of diabetes (2019), approximately 463 million adults (20-79 years) were living with diabetes. Type 2 Diabetes is “non-insulin-dependent diabetes” previously known as “adult onset diabetes” and it accounts for 90–95% of all diabetes cases. Around, 79% of adults with diabetes were living in low- and middle-income countries and the prevalence of T2DM in Pakistan is 13.7%, a lot higher than had been anticipated (Adnan and Aasim, 2020).

Use of modern medicine and life style modification in regard to dietary habits and physical exercise play a fundamental role in the management of T2DM. However, once individual become diabetic, with nearly no exception patient requires pharmacological management. Pharmacological interventions of diabetes are broadly classified as injectable and oral intervention. Amongst the several oral antidiabetic agents, sulfonylurea holds profound importance. Sulfonylurea (SUs) is an oldest oral anti-diabetic drug class used as second line or as add on therapy in T2DM patients (Qian *et al.*, 2018). Drugs included in sulfonylurea class are divided in to four

generations. First generation include acetohexamide, chlorpropamide, tolbutamide and tolazamide. Glyburide, glipizide, gliclazide, gliclazide MR are the members of second generation whereas glimepiride is an example of third generation SUs. Compared to first and second generation, SUs drugs of third generation have improved safety and tolerability profile (Cordiner and Pearson, 2018). JB253, which is under clinical trials, potentially represent fourth generation sulfonylurea (Broichhagen *et al.*, 2014). First-generation SUs are still in use but comprises only 3% of all oral anti hyperglycemic drug prescriptions. However, second- and third-generation SUs are more widely used, accounting for 20% to 30% of all anti diabetic drug consumption (Rados *et al.*, 2014). Mechanistically, after oral absorption, SUs bind to the receptor SUR subunit present on plasma membrane of β -cell. This binding blocks ATP sensitive potassium channels of β -cells thus inhibiting K⁺ efflux and cause depolarization of membrane. This in turn stimulates influx of Ca²⁺ ions resulting in the exocytosis of preformed insulin (Sola *et al.*, 2015; Lv *et al.*, 2020). SUs are metabolized in the liver by cytochrome P450C9 (*CYP2C9*) enzyme and mutations in the enzymes may result in the impaired metabolism and unwanted retention and reduced clearance of the drug. This consequently leads to the development of adverse side effects of SUs which is mainly hypoglycemia (Loganadan *et al.*, 2016).

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In total 60 allelic variants of *CYP2C9* have been reported with some holding clinical and pharmacological importance (Cavallari *et al.*, 2019). Two non-synonymous allelic variants, CYP2C9*2 (R144C; rs1799853) and CYP2C9*3 (I359L; rs1057910), located at exon 3 and exon 7, respectively, are considered to impair the metabolism of SUs (Loganadan *et al.*, 2016). It has been previously reported that these variants exist in different frequency in populations with different demographic and/or genetic background. In Pakistan some studies have been conducted in relation to the frequency of CYP2C9*2 and CYP2C9*3 alleles amongst subjects with warfarin therapy (Siddiqi *et al.*, 2010; Yasmeen *et al.*, 2015; Qayyum *et al.*, 2017; 2019). However, no investigation of similar nature has been carried out for the type 2 diabetic patients exhibiting sulfonylurea toxicity. This work not only entails a novelty with reference to the identification of the frequency of these important allelic variants of CYP2C9 in the T2DM indigenous population with SUs therapy but also leads to the identification of novel allelic variant of CYP2C9 gene.

MATERIALS AND METHODS

Subject recruitment

Total 101 subjects with diabetic history of less than 5 years and age range of 25-70 years were recruited from National Institute of Diabetes and Endocrinology unit, Dow University of Health Sciences (NIDE-DUHS). Patients were segregated primarily into two groups: control (n=24), those exhibiting no toxicity upon administration of sulfonylurea and test (n=81) include diabetic patients that show signs and symptoms (mainly hypoglycemia) upon taking sulfonylurea. Patients with renal and liver impairments and taking any medication that inhibit and/or interfere in the normal functionality of CYP2C9 such as amiodarone, fluconazole, metronidazole and sulphaphenazole, cefoxib, phenytoin and warfarin were excluded. Prior ethical approval was granted by Institutional Review Board of Dow University of Health Sciences (IRB-672/DUHS/Approval/2015/132). For sample size calculation following formula was considered and justified as discussed by Billingham *et al* (2013).

$$n = \frac{z^2 \times p(1-p)}{\epsilon^2}$$

Where, n, z, p and ϵ refer to sample size of infinite population, z score, population proportion and margin of error, respectively.

Genotyping of CYP2C9 Alleles

Genotyping of CYP2C9 alleles was done by DNA sequencing (Qayyum *et al.*, 2019). Venous blood (2-3ml) was collected from each recruited subject after taking informed consent. Genomic DNA was extracted from whole blood by using QIAGEN DNA extraction kit.

Exon3 and Exon7 of the *CYP2C9* gene were amplified using primers:

Exon3 Forward: 5'-TACAAATACAATGAAAATA TCATG-3',

Exon 3 Reverse: 5'-CTAACAACCAGACTCATAATG-3',

Exon 7 Forward: 5'-CTGAATTGCTACAACAAA TGTG-3'

Exon 7 Reverse: 5'-GATACTATGAATTTGGGACTTC-3'

PCR amplification was performed at the final volume of 35 μ L. Each reaction mixture includes 10-20ng of sample DNA, 2 μ M dNTP, 1 μ M each of forward primer and reverse primer, 4.2 μ M magnesium chloride (MgCl₂), 7 units of Taq DNA polymerase, 7ul to ammonium sulphate PCR buffer (5x) to attain the final concentration 1x in the total reaction volume. Finally, appropriate amount of autoclaved sterile water was added to make up the final volume of 35 μ L. PCR reaction was carried out in 24 channel Thermal cycler (Thermo Hybaid) set for initial denaturation at 94°C for 10 minutes followed by 35 cycles of 94°C for 30 seconds, 61°C for 5 minutes and 72°C for 3 minutes. Final extension was performed at 72°C for 10 minutes. PCR products were stored at 4°C till sent to Macrogen, South Korea, for DNA sequencing. Electropherograms of DNA sequences were analyzed using Chromas to monitor quality of sequencing and identification of homozygous and/or heterozygous variants. Nucleotide sequences of all samples were aligned along with the respective reference *CYP2C9* exon 3 and exon 7 sequence (NM_000771.4 and NP_000762.2) using ClustalW (Thompson *et al.*, 1994) and visualized using CLC sequence viewer.

Molecular Modelling and Structure Analyses of CYP2C9 Alleles

Molecular models of all identified alleles were developed by Swiss Model (Waterhouse *et al.*, 2018) using atomic coordinates of resolved structure of CYP2C9, PDBid:1R9O (Wester *et al.*, 2004). The models after structural and thermodynamic refinement were superimposed over 1R9O and structural variations were observed in C α backbone in Å. The ligand binding cavity dimensions of all structures were estimated using DoG Site Scorer (Volkamer *et al.*, 2012).

STATISTICAL ANALYSIS

All statistical analyses were conducted using Graph Pad Prism v5.01 and in all cases p-value of <0.05 was considered significant. Distribution of all the data variables was estimated by Kolmogorov-Smirnov test. Chi-square test was employed to statistically compare the frequencies of alleles and genotypes with other studies and projected values.

RESULTS

Sequencing of the subjects revealed presence of both clinically important alleles of CYP2C9, namely CYP2C9*2 and CYP2C9*3 in both control and cases, with noticeable variations in the frequency. In the present study, no subject was found homozygous for CYP2C9*2/*2 genotype, however, compared to 8.33% in control, 11.11% patients were detected to bear heterozygous CYP2C9*1/*2 genotype (fig.1A, table 1). Out of the three homozygous states of genotypes, CYP2C9*1/*1 (wild type), CYP2C9*2/*2, and CYP2C9*3/*3, only CYP2C9*1/*1 was found in both control and test subjects. Importantly, CYP2C9*3/*3 was exclusively identified in test subjects (fig.1B, table 1), suggesting the association of the CYP2C9*3/*3 genotype with the sulfonylurea induced toxicity in T2DM patients. Consistently, the heterozygous form of the allele genotype, CYP2C9*1/*3 is also more frequently found in the test cases (17.28%) compared to control cases (8.82%) (fig.1C, table 1). This again indicates the link between CYP2C9*3 allele with the impaired metabolism of sulfonylurea in the T2DM patients. CYP2C9*2 is another clinical important allele reported for its association with sulfonylurea induced hypoglycemia in the T2DM patients. Interestingly, compared to the 4.17% of control cases, 8.64% of subjects in the test cases bears a sequence variant (G>A) in the exon7 of CYP2C9 in heterozygous state. Previously, information in relation to this allelic variant is not known in the literature and dedicated databases and we dubbed this novel variant as CYP2C9*61 (fig.1D, table 1).

Principles of Hardy-Weinberg Law were employed on the observed allele frequencies on total population to explore the underlying selection pressure. Comparison of frequencies of genotypes of CYP2C9*3 between observed and expected values were not found to be significantly different in statistical terms having p value (0.50605) (fig.1E). This not only ousted the possibility the effect of selection pressure on CYP2C9*1 variant in relation to CYP2C9*3 genotype, but also shows that observed genotypic differences is not due to the errors/bias in the sample collection or genotyping. Genotypes of CYP2C9*2 and CYP2C9*61 could not be explored in similar fashion because of the absence of any homozygous form of the allele among the recruited subjects.

Compared to the wild type allele, CYP2C9*1, all three allelic variants of CYP2C9 found in the present study, CYP2C9*2, CYP2C9*3 and CYP2C9*61, correspond to the non-synonymous mutations, resulting in R144C, I359L and E328K amino acid substitutions, respectively. Comparison of the molecular structures of these variants with the wild type structure did not reveal any noticeable change in the backbone architecture of the protein, where

superimposition of C α backbone of R144C, I359L and E328K variants over wild type CYP2C9 structures were found as 0.11Å, 0.23Å and 0.23Å, respectively (fig.2A,B). However, noticeable changes were observed in the dimensions of ligand binding cleft (volume and area) of the variants compared to the wild type protein structure. Increase in the volume and surface area was observed in case of protein encoded by CYP2C9*3 and CYP2C9*61, whereas the volume and surface area of CYP2C9*2 encoded protein drops to some extent compared to the wild type protein (fig. C, table 2).

DISCUSSION

Sulfonylurea is a drug of choice for many T2DM patients; however, due to the presence of faulty variants of drug metabolizing enzyme, CYP2C9, it induces hypoglycemia in the T2DM patients (Loganadan *et al.*, 2016). Since this is the first study to investigate the sequence polymorphism in CYP2C9 gene in sulfonylurea induced toxicity in T2DM in Pakistan. We compared our finding with similar studies conducted elsewhere in world. Interestingly, CYP2C9*3 alleles was found more prevalent in indigenous population compared to studies conducted in other parts of world (Adithan *et al.*, 2003; Hasmehi-Soteh *et al.*, 2012; Saberi *et al.*, 2020). Similarly, the frequency of the CYP2C9*3/*3 genotype is more pronounced in our test group and less in the control group compared to the similar studies conducted in Indian (Bhatt *et al.*, 2014), Greece (Ragia *et al.*, 2009) and Caucasian (Holstein *et al.*, 2011) populations. However, CYP2C9*2 alleles and its corresponding genotypes (CYP2C9*1/*2 and CYP2C9*2/*2) are less frequent in the indigenous population of T2DM patients compared to similar population sets of Greece (Ragia *et al.*, 2009), Egyptian (Salam *et al.*, 2014) and Caucasian (Holstein *et al.*, 2011). An argument could be made because of the sample size difference, however, except for the study of Holstein *et al.* (2011), where the total sample size is 203, other studies have used comparable sample sizes as 92 (Ragia *et al.*, 2009), 100 (Salam *et al.*, 2014) and 109 (Bhatt *et al.*, 2014) compared to present study (n=101). Secondly, difference in the sample size of control test groups could also be considered as a limitation. Since, association of CYP2C9*2 and CYP2C9*3 alleles are already established in relation to the impaired metabolism of sulfonylurea (Loganadan *et al.*, 2016), therefore, sample size of control group is more or less become irrelevant. Consistently, in some studies of similar nature in different population sets, control groups are simply not included in the investigations (Salam *et al.*, 2014; Bhatt *et al.*, 2014). Since in this study and Siddiqui *et al.* (2010) mostly individuals with sulfonylurea and warfarin (respectively) induced toxicity are recruited, they might not be the best representative of CYP2C9 allele and genotype distribution in the healthy population of Pakistan. Therefore, a separate study covering only

Table 1: Frequency of CYP2C9 Genotypes

Gene Variants	Control (n=24)		Test (n=81)		Total n=105 (%)
	Number	%	Number	%	
CYP2C9*1/*1	20	83.33%	50	61.7%	70(68.3)
CYP2C9*1/*2	2	8.33%	9	11.11%	7(16.83)
CYP2C9*1/*3	3	8.82%	14	17.28%	17(3.96)
CYP2C9*1/*61	1	4.17%	7	8.64%	8()
CYP2C9*2/*2	0	0%	0	0%	0(0)
CYP2C9*3/*3	0	0%	4	4.94%	4(0)
CYP2C9*61/*61	0	0%	0	0%	0(0)

Table 2: Dimensions of main ligand binding cavity of CYP2C9 variants

Protein	Volume (Å ³)	Surface Area (Å ²)	Drug Score
Wild type (1R9O)	1317.56	1703.30	0.81
CYP2C9*2 (R144C)	1209.58	1753.98	0.82
CYP2C9*3 (I359L)	1515.24	1848.86	0.82
CYP2C9*61 (E328K)	2174.64	2494.98	0.82

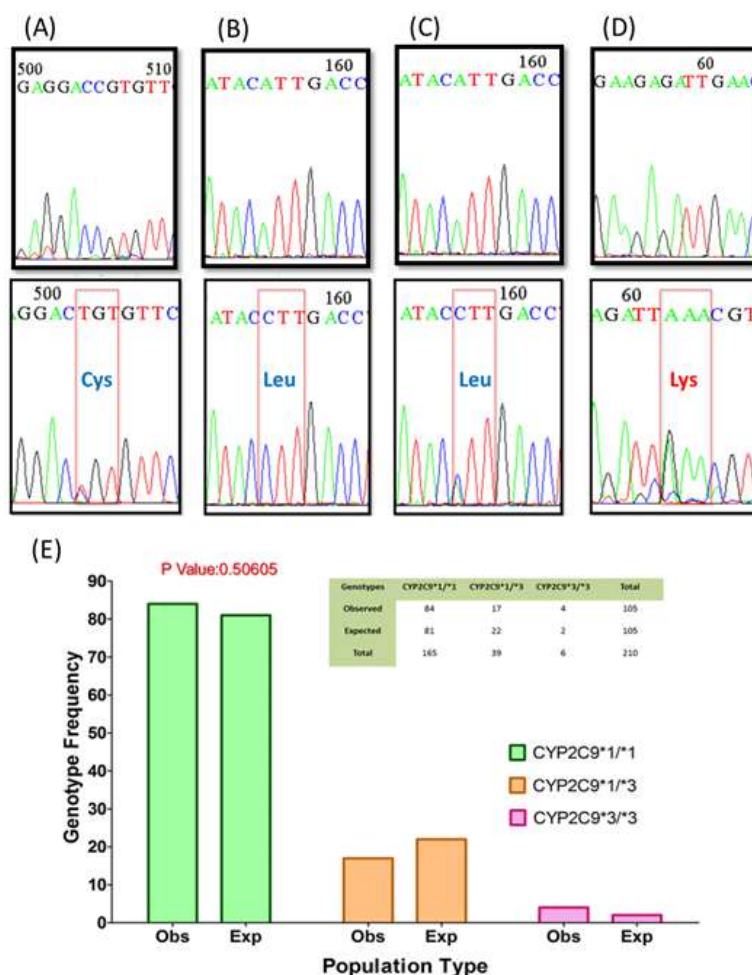


Fig. 1: Electropherograms of CYP2C9 sequence. Representative regional images of electropherograms of (A) wild type CYP2C9*1/*1 (first row) and CYP2C9*1/*2 (second row) (B) wild type CYP2C9*1/*1 (first row) and CYP2C9*3/*3 (second row) (C) wild type CYP2C9*1/*1 (first row) and CYP2C9*1/*3 (second row) (D) wild type CYP2C9*1/*1 (first row) and CYP2C9*1/*61 (second row). (E) Histogram showing differences between observed and expected values of CYP2C9*3 allele genotypes for the Hardy-Weinberg Equilibrium Analysis.

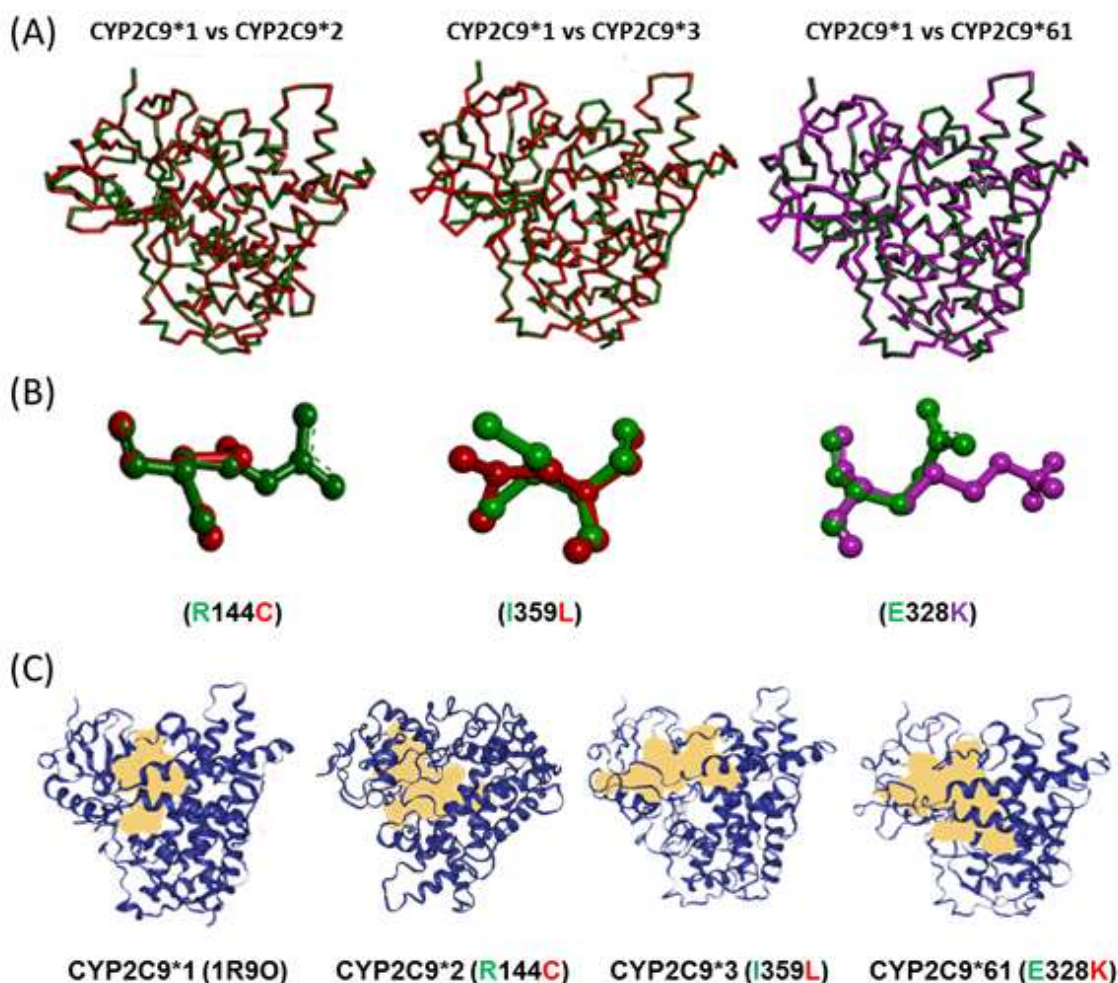


Fig. 2: Structural Analysis of *CYP2C9* Alleles. (A) Superimposition of C α backbone of different *CYP2C9* alleles as labeled over the resolved structure of the wild type protein (B) Spatial orientation of substituted residues compared to the wild type residues as labeled are shown in ball and stick (C) Span of main ligand binding cavity (yellow) of different *CYP2C9* alleles are represented.

healthy population could be carried out to investigate the unbiased allelic and genotypic frequency of *CYP2C9* distribution in Pakistan. Nevertheless, in this study *CYP2C9*3* allele and *CYP2C9*3/*3* genotype are more frequently found compared to Siddiqui *et al.* 2010 where the subjects are recruited on the basis of warfarin induced toxicity. This potentially points to different degree of selection pressure incurred by SUs (present study) and warfarin. Nevertheless, no significant difference was noticed between observed and expected populations employing principles of Hardy Weinberg equilibrium. This denotes that the selected/recruited population is under neutral natural selection with reference to *CYP2C9* as also observed by Qayyum *et al.*, 2017.

Although sequencing might take a considerable amount of the time to genotype an individual, certain other methodologies such as capillary electrophoresis and RFLP could be considered for rapid identification of clinically relevant genotypes. However, DNA sequencing

raises a possibility of identification of novel mutational variant in a gene. Indeed, in the present study, a non-synonymous mutation in the *CYP2C9*, *CYP2C9*61*, has been found. Non-synonymous mutation in protein encoding genes like *CYP2C9* may affect the structure and function of the encoded protein. To explore this possibility, *in silico* molecular models of *CYP2C9*2* (R144C), *CYP2C9*3* (I359L) and *CYP2C9*61* (E328K) were constructed by homology modelling. In comparison to crystal structure *CYP2C9*1* (1R90), *in silico* models of *CYP2C9*2*, *CYP2C9*3* and *CYP2C9*61* shows no major structural change with reference to overall topology and number and span of β sheets, helices and coils in the structures (Wester *et al.*, 2004). Compared to the wild type protein, all three variants have shown variations in the dimensions of the binding cavity suggesting potential variation in the drug binding capacity that may lead to potential alteration in the metabolism of the SUs. Of these, *CYP2C9*3* variant is more influential in relation to SU induced toxicity compared to *CYP2C9*2* allele. As in

comparison to homozygous wild type CYP2C9*1/*1, only 12% and <5% enzymatic activity have been reported in case of homozygous CYP2C9*2/*2 and homozygous CYP2C9*3/*3 variants, respectively (Hosseinkhani *et al.*, 2018). Since the novel CYP2C9 allele, CYP2C9*61 identified in this study also shows increase in the volume and surface area of ligand binding cavity and more frequently present in the test cohort, it certainly raises an interesting possibility of the presence of new allele of pharmacogenetic importance at least in the Pakistani population. However, to identify the true clinical significance of the allele, a separate study with much large sample size is warranted.

CONCLUSION

In summary, the findings of the present investigation shows that CYP2C9 allele, CYP2C9*3/*3, is more frequently present in the indigenous T2DM patients with sulfonylurea related toxicity. In addition, another novel allele of CYP2C9, CYP2C9*61 could also be considered as a potential genetic reason that underpin sulfonylurea induced toxicity in T2DM patients.

REFERENCES

- Adithan C, Gerard N, Vasu S, Balakrishnan R, Shashindran CH and Krishnamoorthy R (2003). Allele and genotype frequency of CYP2C9 in Tamilnadu population. *Eur. J. Clin. Pharmacol.*, **59**(8-9):707-709.
- Adnan M and Aasim M (2020). Prevalence of Type 2 Diabetes Mellitus in Adult Population of Pakistan: A Meta-Analysis of Prospective Cross-Sectional Surveys. *Ann. Glob. Health*, **86**: 1.
- Bhatt D, Chauhan N, Sharma A, Dhawan D, Bhatt RV, Phatak S and Padh H (2014). Investigating the role of plasma glucose concentration as a phenotypic marker for CYP2C9 genetic variants, in the diabetic population of Gujarat. *Indian J. Pharma. Sci.*, **76**: 72.
- Billingham SA, Whitehead AL and Julious SA (2013). An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med. Res. Methodol.*, **13**: 104.
- Broichhagen J, Schönberger M, Cork SC, Frank JA, Marchetti P, Bugliani M, Shapiro AJ, Trapp S, Rutter GA, Hodson DJ and Trauner D (2014). Optical control of insulin release using a photoswitchable sulfonylurea. *Nat. Commun.*, **5**: 1-1.
- Cavallari LH and Momary KM (2019). Pharmacogenetics in Cardiovascular Diseases. In *Pharmacogenomics Academic Press, USA*, pp.133-179.
- Cordiner RL and Pearson ER (2019). Reflections on the sulphonylurea story: A drug class at risk of extinction or a drug class worth reviving? *Diabetes Obes. Metab.*, **21**(4): 761-771.
- Hashemi-Soteh SM, Shahabi-Majd N, Gholizadeh AR and Shiran MR (2012). Allele and genotype frequencies of CYP2C9 within an Iranian population (Mazandaran). *Genet. Test Mol. Bioma.*, **16**(7):817-821.
- Holstein A, Hahn M, Patzer O, Seeringer A, Kovacs P and Stingl J (2011). Impact of clinical factors and CYP2C9 variants for the risk of severe sulfonylurea-induced hypoglycemia. *Eur. J. Clin. Pharmacol.*, **67**(5): 471-476.
- Hosseinkhani Z, Sadeghalvad M, Norooznezhad F, Khodarahmi R, Fazilati M, Mahnam A, Fattahi A and Mansouri K (2018). The effect of CYP2C9* 2, CYP2C9* 3, and VKORC1-1639 G> A polymorphism in patients under warfarin therapy in city of Kermanshah. *Res. Pharm. Sci.*, **13**(4): 377.
- Loganadan NK, Huri HZ, Vethakkan SR and Hussein Z (2016). Genetic markers predicting sulphonylurea treatment outcomes in type 2 diabetes patients: current evidence and challenges for clinical implementation. *Pharmacogenomics J.*, **16**(3): 209-219.
- Lv W, Wang X, Xu Q and Lu W (2020). Mechanisms and characteristics of sulfonylureas and glinides. *Curr. Top. Med. Chem.*, **20**: 37-56.
- Qayyum A, Najmi MH, Mansoor Q, Farooqi ZU, Naveed AK, Hanif A, Kazmi SA and Ismail M (2017). Frequency of common CYP2C9 polymorphisms and their impact on warfarin dose requirement in Pakistani population. *Clin Appl Thromb-Hem.*, **23**(7): 800-6.
- Qayyum A, Najmi MH, Raza SI, Rajput TA and Naveed AK (2019). Relationship of S/R warfarin ratio with CYP2C9 genotypes in Pakistani population. *Pak. J. Pharm. Sci.*, **32**(4): 1581-1588.
- Qian D, Zhang T, Tan X, Zheng P, Liang Z, Xie J, Jiang J and Situ B (2018). Comparison of antidiabetic drugs added to sulfonylurea monotherapy in patients with type 2 diabetes mellitus: A network meta-analysis. *PLoS One.*, **13**(8): e0202563.
- Rados DV, Pinto LC, Remonti LR, Leitao CB and Gross JL (2016). The association between sulfonylurea use and all-cause and cardiovascular mortality: a meta-analysis with trial sequential analysis of randomized clinical trials. *PLoS Med.*, **13**(4): e1001992.
- Ragia G, Petridis I, Tavridou A, Christakidis D and Manolopoulos VG (2009). Presence of CYP2C9* 3 allele increases risk for hypoglycemia in Type 2 diabetic patients treated with sulfonylureas. *Pharmacogenomics*, **10**(11): 1781-1787.
- Saberi M, Ramazani Z, Rashidi H and Saberi A (2020). The Effect of CYP2C9 genotype variants in Type 2 diabetes on the pharmacological effectiveness of sulfonylureas, diabetic retinopathy, and nephropathy. *Vasc. Health Risk Manag.*, **16**: 241.
- Salam RF, Zeyada R and Osman NA (2014). Effect of CYP2C9 gene polymorphisms on response to treatment with sulfonylureas in a cohort of Egyptian type 2 diabetes mellitus patients. *Comp. Clin. Path.*, **23**: 341-346.

- Siddiqi AI, Khan DA, Khan FA, Naveed AK (2010). Impact of CYP2C9 genetic polymorphism on warfarin dose requirements in Pakistani population. *Pak. J. Pharm. Sci.*, **23**(2): 417-422.
- Sola D, Rossi L, Schianca GP, Maffioli P, Bigliocca M, Mella R, Corliano F, Fra GP, Bartoli E and Derosa G (2015). Sulfonylureas and their use in clinical practice. *Arch. Med. Sci.*, **11**(4): 840.
- Thompson JD, Higgins DG and Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**(22): 4673-4680.
- Volkamer A, Kuhn D, Rippmann F and Rarey M (2012). DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment. *Bioinformatics.*, **28**(15): 2074-2075.
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TA, Rempfer C, Bordoli L and Lepore R (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.*, **46**: W296-303.
- Wester MR, Yano JK, Schoch GA, Yang C, Griffin KJ, Stout CD and Johnson EF (2004). The structure of human cytochrome P450 2C9 complexed with flurbiprofen at 2.0-Å resolution. *J. Biol. Chem.*, **279**(34): 35630-35637.
- Yasmeen F, Ghafoor MB, Khalid AW, Latif W, Mohsin S and Khaliq S (2015). Analysis of CYP2C9 polymorphisms (*2 and *3) in warfarin therapy patients in Pakistan. Association of CYP2C9 polymorphisms (*2 and *3) with warfarin dose, age, PT and INR. *J. Throm. Thrombolys.*, **40**(2): 218-224.