Review Article

Pharmacogenomics of Type 2 Diabetes Mellitus

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ABSTRACT
Diabetes mellitus is a complex metabolic disorder with multiple factors associated with its causation, outcome and response to treatment. Several researchers are working on identifying the genetic and non-genetic factors that are implicated in disease causation as well as response to therapy. It has been found that, some of the genetic factors such as ABCC8 and KCNJ11 polymorphisms are associated with disease causation as well as outcome of therapy with specific drugs. Presence of multiple associative factors have led to the realization of a much complex network of pathways that are involved in diabetes mellitus. In this review we discuss the genetic factors involved in type 2 diabetes mellitus and in its response to treatment with different classes of drugs. The genetic factors include single nucleotide polymorphisms in genes coding for drug metabolizing enzymes (e.g., CYP2C9), drug transporters (e.g., OCT1, OCT2, MATE1, MATE2), receptors and channels such as ABCC8, KCNJ11. The net effect of all genetic variations in each individual patient determines the outcome of therapy. To translate pharmacogenetics in to clinical practice, it is needed to have evidence on the net effect of genetic polymorphisms.

INTRODUCTION
Diabetes mellitus (DM) is a heterogeneous metabolic disorder that is now considered as a major health crisis in the past few decades. Type 2 diabetes mellitus commonly starts in adulthood and is often associated with obesity and accelerated atherosclerosis and hence becomes an important cardiovascular risk factor. The morbidities related to diabetes are numerous and involves multiple organs and their functions.\textsuperscript{1} The 7\textsuperscript{th} edition of the International Diabetes Federation IDF Diabetes Atlas reveals the worldwide prevalence of the disease to be 415 million people, and the estimated prevalence for the year 2040, is 642 million. Every 6 seconds a person dies from diabetes and its complications and it is also one among the top ten causes of disability worldwide.\textsuperscript{2} All these data refers to the ‘tip of the iceberg’, since many remain undiagnosed and unaware of the implications of diabetes mellitus.

The management of type 2 diabetes mellitus begins with lifestyle interventions like diet and exercise, oral hypoglycemic drugs belonging to various groups such as sulfonylureas, biguanides, meglitinides, thiazolidinediones, dipeptidyl peptidase 4 (DPP4) inhibitors, incretin analogues, α glucosidase inhibitors and sodium glucose co-transporter 2 (SGLT2) inhibitors.\textsuperscript{3,4} Delay in achieving optimum control of glycemic status results in micro and macro vascular changes and end organ damage. Hence choosing the right drug in the right dose at the earliest is an essential requirement in the treatment of diabetes mellitus. In spite of several developments in the treatment of diabetes mellitus, the response to treatment varies significantly between individuals which can be attributed to modifiable and non-modifiable risk factors. Several investigators have addressed the modifiable factors such as change in life style, diet, physical activity, reduction of body weight.\textsuperscript{5–8} Among non-modifiable factors, genetics play a crucial role. Genetic markers with a predictive potential can play a crucial role in adopting appropriate alternative
Pharmacogenetics of sulphonylurea drugs

Sulphonylurea drugs such as glibenclamide, gliclazide, glipizide, glimepiride produce their action by blocking sulphonylurea receptors (SUR1) in the β cells of pancreas. These receptors are one of the units of inward rectifier potassium channels. Blockade of these potassium channels results in depolarisation of the cell and secretion of insulin. A genetic polymorphism resulting in altered protein structure of SUR1 or the potassium channel can theoretically affect sulphonylurea mediated insulin release and variability in response.

Sulphonylurea receptor SUR1 is coded by the gene ABCC8 (ATP binding cassette subfamily C member 8) and the inwardly rectifying potassium channel is coded by the gene KCNJ11 (Potassium inwardly rectifying channel subfamily J member 11). Further, sulphonylurea drugs are largely metabolized by CYP2C9 enzyme which is coded by the gene CYP2C9. Studies on genetic polymorphisms in all these genes have been done to explain the variability in drug response and hypoglycaemic adverse effects.

CYP2C9

The major two variant alleles of CYP2C9 include CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu), the latter resulting in a more pronounced decrease in enzyme activity than the former. Studies done with tolbutamide and glimepiride among the genotypes of CYP2C9 had shown decreased oral clearance by 6.5 times with tolbutamide and 50% with glimepiride, among patients with CYP2C9*3 variant alleles compared to patients with normal allele. A study on 7983 elderly diabetic patients had demonstrated a lower dose requirement of tolbutamide among carriers of CYP2C9*3 allele with no significant effect caused by other alleles. In another population based study, it was found that, in patients with genotypes of variant alleles (CYP2C9 *2/*2, *2/*3 and *3/*3) the reduction of Hba1c was 0.5% higher than patients with normal genotype and this difference was statistically significant. Further, these patients had a higher chance (3.4 times) of reaching the target HbA1c value of less than 7% compared to patients with normal genotype (p = 0.0009). It was also demonstrated that patients receiving sulphonylurea drugs and possessing at least one variant allele (*2 or *3) were less likely to have treatment failure (Hazard ratio per allele 0.79; 95% CI 0.63-0.99, p = 0.04). On the other end of clinical spectrum, patients with variant genotypes CYP2C9 *2/*3 and *3/*3 were more likely to encounter hypoglycemic adverse effects compared to patients with normal genotype of CYP2C9 [OR 5.2; 95% CI 1.01-27].

ABCC8

In a study evaluating the effects of ABCC8 SNPs, Thr759Thr (exon 6) and C>T at position -3 of intron/exon 16 splice acceptor, on insulin response and serum C peptide levels following tolbutamide administration, insulin secretion dropped by 40% and serum C peptide levels were lesser by 50% in the presence of the genetic variants. However, there were no differences in the same parameters following glucose administration. The influence of these SNPs was due to its effect on ligand-receptor interaction rather than on endogenous response. However the study had limitations of small sample size, and lack of measurement of pre-prandial and post-prandial values of blood glucose and insulin. The study results were based on fasting values of blood glucose and insulin. This study was one of the earliest to explore the role of ABCC8 genetic polymorphism on response to sulphonylurea drugs.

Zhang et al have described increased insulin secretion in the presence of ABCC8 Ser1369Ala genetic polymorphism in response to gliclazide 40 mg BD. Gliclazide caused a greater lowering of Hba1c (-1.6%) among 1369Ala variant allele carriers as compared to those who were with Ser/Ser genotype (-0.76%). Feng et al studied the influence of 25 SNPs in 11 candidate genes on response to gliclazide. Among them heterozygous and homozygous variant genotype patients
of Ser1369Ala genetic polymorphism demonstrated a greater decrease in fasting plasma glucose values compared to normal genotype individuals. Similar effect was also observed in values of 2 hour post prandial plasma glucose measurement. A better response rate was seen in patients with Ala/Ala genotypes (OR – 2.2; 95% CI 1.4 – 3.6) and Ser/Ala (OR – 1.4; 95% CI 1.0 – 2.1) compared to patients with Ser/Ser genotype. However, there were no differences in insulin levels between the genotype groups.19

**KCNJ11**

*KCNJ11* gene codes for the inward rectifier potassium channel in the β cells of pancreas.20 Polymorphisms in this gene may result in altered protein structure of the channel and affect the functioning of this channel. The most studied genetic polymorphism, E23K (Glu23lys), has also been shown to be in linkage disequilibrium with Ser1369Ala genetic polymorphism of *ABCC8* gene.21–23 These two genetic polymorphisms have also been associated with risk of developing diabetes mellitus and response to therapy with sulphonylurea drugs. An early study done by UKPDS had shown lack of association between E23K genetic polymorphism and response to sulphonylurea drugs.24 However, another study by Sesti *et al* had explored the role of E23K genetic polymorphism on secondary sulphonylurea failure among 525 Caucasian diabetic patients. It was seen that 23K variant allele was associated with secondary sulphonylurea failure compared to patients with homozygous Gly972 variant allele as compared to those with homozygous Gly972 variant allele (OR 2.16; 95% CI 1.0 – 4.2). Ex-vivo, islet cells of pancreas with 23K after exposure to 24 hour high glucose concentration, produced significantly less insulin in response to glibenclamide compared to islet cells homozygous with E23 allele.25 In an in-vitro study, islet cells with 23K of *KCNJ11* and 1369Ala of *ABCC8* showed an increased inhibition of K_{STR} channels compared to E23 and Ser1369 of *ABCC8* in response to gliclazide. However this effect was not seen with glibenclamide.26 A case control study evaluating the association of E23K genetic polymorphism with risk of hypoglycaemia, it was seen that 23K allele was associated with decreased risk of severe hypoglycaemic adverse effects when treated with sulphonylurea drugs compared to E23 allele. However, after adjustment of covariates, there were no significant influence on the same. It was also observed that patients with 23K allele had significantly higher HbA1c levels compared to E23.27

Although *ABCC8* 1369Ala and *KCNJ11* 23K variants are in linkage disequilibrium, their effect on sulphonylurea response are contrasting to each other. While *ABCC8* 1369Ala variant is associated with increased response, *KCNJ11* 23K variant allele is associated with secondary sulphonylurea failure. The net effect of these two polymorphisms is based on the effect of *ABCC8* polymorphism. In a study where effect of gliclazide was studied on recombinant *ABCC8* 1369Ala and *KCNJ11* 23K, there was increased sensitivity to gliclazide in the presence of these variant alleles.28 Prospective studies are needed to validate the findings of earlier studies to understand the net effect of *ABCC8* 1369Ala and *KCNJ11* 23K variants.

**TCF7L2**

The gene TCF7L2 codes for a transcription factor in WNT signalling pathway in the pancreas. This factor is associated with normal functioning of the pancreatic β cells and insulin secretion.29 Two intronic SNPs namely, rs7903146 and rs12255372, were associated with 5 fold increase in levels of TCF7L2 expression and decreased insulin secretion. Further, in a meta-analysis, it was shown that presence of homozygous variant alleles of the two SNPs is associated with a two fold increase in risk of developing diabetes mellitus than normal genotypes.29–32 In a study by Pearson *et al* these two SNPs were associated with failure to respond to sulfonylurea in the early phase of therapy. Individuals with the homozygous variant allele genotype (TT) of rs12255372 had a two-fold increased risk of failure to early therapy with sulfonylureas compared to individuals with GG genotype (OR 2.16; 95% CI 1.21 – 3.86). Similarly, individuals with homozygous variant (TT) genotype of rs7903146 have an increased risk of failure with sulfonylures (OR 1.90; 95% CI, 1.09 – 3.33) compared to normal genotypes of each SNP.33

**IRS1**

Insulin receptor substrate 1 (IRS1) is a signal transduction molecule involved in the functioning of insulin receptor. The SNP Gly972arg results in altered binding of p85 subunit of phosphatidylinositol 3-kinase (PI3K) in beta cells of pancreas. This may result in altered secretion of insulin and predispose to risk of type 2 DM.34 Sesti *et al* studied IRS1 Gly972arg polymorphism among 477 diabetic patients and described a twofold increased risk of secondary sulfonylurea failure among carriers of 972arg variant allele as compared to those with homozygous Gly972
allele. Gene – gene interactions also play a major role in the aetiology and pathogenesis of a polygenetic disorder such as DM. Vergotine et al have demonstrated in mixed ancestry South African population, gene interaction between PPARG Pro12Ala and IRS1 Gly972arg. Individually, both SNPs were not associated with type 2 DM. However, in regression models, in the presence of Gly972arg, PPARG 12Pro was found to confer a 64% higher risk of the disease. They have also shown lack of association between IRS1 Gly972arg polymorphism and DM in the absence of gene interaction in the same population. In a study done in 2,148 South Indian subjects, there was no significant association between Gly972arg polymorphism and type 2 DM. However, in a study done in a different South Indian population among 1379 individuals, Gly972arg was found to be associated with type 2 DM, even after adjustment of co-variates. Association of this variation with gestational DM has also been described by many authors. A systematic review has demonstrated an association between IRS1 Gly972arg and gestational DM (OR: 1.39; 95% CI 1.04–1.85, p = 0.027).

**NOS1AP**

NOS1AP codes for nitric oxide synthase 1 adapter protein (NOS1AP). This protein is involved in the functioning of neuronal nitric oxide synthase (nNOS) which in turn regulates the voltage gated calcium channel in the β cells of pancreas. It is located at chromosome 1q23.3. Early studies have generated evidence for association of chromosome region 1q21-q25 and type 2 DM. Becker et al studied rs10494366 T>G SNP in NOS1AP and their influence on efficacy of sulfonylurea therapy and mortality risk. A difference in dose requirement of glibenclamide was identified between the genotype groups. In patients on therapy with glibenclamide, carriers of G allele had an increased mortality risk compared to carriers of T allele (HR 2.8, 95% CI, 1.09 – 7.22). However, with tolbutamide and glimepiride there was a decreased risk of mortality with hazard ratios 0.30 (95% CI: 0.14-0.63) and 0.18 (95% CI: 0.04-0.74), respectively. The reason for the variation in hazard ratio between drugs of the same group is not well understood. In a later study, Becker et al described an association between type 2 DM and rs10494366 SNP in NOS1AP among incident type 2 DM patients who are on therapy with calcium channel blockers. Chu et al replicated the study demonstrating the influence of rs10494366 T>G SNP on type 2 DM among calcium channel blocker users. A lower risk of the disease was observed in the presence of G allele of rs10494366 (HR 0.57, 95% CI 0.35-0.92, p = 0.016). However, the level of association was reduced after adjustment of co-variates (HR 0.63, 95% CI 0.38-1.04, p = 0.052). Further, this association was not present among patients who are not on treatment with calcium channel blockers and in African Americans. A summary of pharmacogenetic factors of sulphonylurea drugs is given in Table 1.

**Table 1 : Pharmacogenetic factors of sulphonylurea drugs**

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Polymorphism</th>
<th>Effect/Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>Arg144Cys (*2) Ile359Leu (*3)</td>
<td>Decrease in enzyme activity and drug clearance. Increased drug sensitivity and chance of hypoglycemia</td>
</tr>
<tr>
<td>ABCC8</td>
<td>Ser1369Ala</td>
<td>1369Ala allele associated with increased insulin secretion in response to sulphonylurea drugs</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>E23K (Gly23Lys)</td>
<td>23K allele associated with secondary sulphonylurea failure</td>
</tr>
<tr>
<td>ABCC8 &amp; KCNJ11</td>
<td>1369Ala with 23K haplotype</td>
<td>Increased sensitivity to sulphonylurea drugs</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs7903146 rs12255372</td>
<td>Decrease in insulin secretion and failure to sulphonylurea therapy</td>
</tr>
<tr>
<td>IRS1</td>
<td>Gly972Arg</td>
<td>972Arg associated with secondary sulphonylurea failure</td>
</tr>
<tr>
<td>NOS1AP</td>
<td>rs10494366</td>
<td>Glibenclamide dose variation between genotypes Increased mortality risk with glibenclamide Decreased mortality risk with tolbutamide and glimepiride</td>
</tr>
</tbody>
</table>
Pharmacogenetics of Biguanides

Metformin and phenformin were the two biguanides that were introduced in the market. Phenformin was widely used initially but was soon withdrawn from market in late 1970s due to the association of phenformin with lactic acidosis. Metformin was found to be unique in that, its anti-diabetic properties were dissociated from the toxicity profiles, making it as the first drug of choice for patients with type 2 diabetes mellitus. UKPDS 34 study has concluded that metformin has the potential to reduce the cardiovascular mortality in high risk diabetic patients. There exists a wide variability in the bioavailability of metformin which ranges from 20 to 70%. This broad range of variability is mostly attributed to the differences in absorption rather than in the first-pass metabolism. Studies have shown that almost more than 20% of patients may fail to achieve glycemic control with metformin monotherapy and the contribution of factors such as age, gender, body weight or BMI is minimal. This highlights the importance of studying the genetic variations influencing the pharmacokinetics or pharmacodynamics of metformin.

Pharmacogenetics of metformin

Chemically, metformin is a hydrophilic base which exists predominantly as cation (>99.9% of the drug) in the physiological pH. Therefore the passive diffusion of the drug across the biological membranes is limited. The organic cation transporters belonging to the solute carrier (SLC) family plays an important role in distribution of metformin across the biological membranes. These transporters mediate the oral absorption of the drug in the gut, transport across hepatocytes, renal tubular cells and biliary epithelium. The unique feature about the pharmacokinetics of metformin is that, it is not metabolized in the body and is excreted unchanged in the urine by tubular secretion. This implies that there is no role for drug metabolizing enzymes in the pharmacokinetics of metformin. However there are certain pharmacokinetic and pharmacodynamic targets, the polymorphisms of which were found to significantly influence the dosage and toxicity profile of the drug. (Table 2)

Role of polymorphisms in transporters on metformin response

Organic Cation Transporter (OCT) polymorphisms:

Metformin is an organic cation molecule that is transported into the liver, intestinal and renal cells by organic cation transporters (OCT1, 2, 3). These transporters are encoded by SLC22A1, SLC22A2, SLC22A3 genes and the polymorphisms of these genes are known to significantly alter the efficacy of the drug. The SLC22A1 gene is located in the chromosome 6q25.3 and consists of 11 exons spanning 37kb. The encoded transporter [OCT1] contains 554 amino acids with 12 transmembrane domains. In a systematic mutation screening of OCT1 gene in 57 Caucasians by Kerb et al, 25 SNPs of SLC22A1 including 8 non-synonymous ones were identified. Among them, 3 non-synonymous SNPs (G401S, C88R, and R61C) showed reduced transport activities. In a study done by Shu et al 15 protein-altering variants of SLC22A1 from diverse ethnic backgrounds were described. Among them, 5 SNPs (R61C, G220V, P341L, G401S, and G465R) showed reduced transport activities and one SNP (S14F) showed increased activity. The variant polymorphisms found in highest frequencies among Asian Americans were 1022C>T (Pro341Leu) and 1222A>G (M408V). Two non-synonymous SNPs (P283L and P341L) of SLC22A1 found in Korean and Japanese populations showed reduced transport activity. Itoda et al in a search for novel SNP’s in OCT1 identified 20 genetic variations including 7 novel ones. Many of the polymorphisms differed among Caucasians and Japanese populations in frequencies. However, the SNPs in Asian Americans were comparable (P341L, P160L, M408V) with the Japanese. Shikata et al performed the genotype-phenotype correlational analysis of OCT1 and OCT2 variants in 33 type 2 diabetes mellitus patients (24 responders and 9 non-responders, with respect to the decrease in HbA1c levels from baseline) and concluded that the polymorphisms in OCT1 and OCT2 have little contribution to the clinical response of metformin.

The highly polymorphic nature of OCT1 transporter and its role in metformin response was comprehensively analyzed by Shu et al. They performed an In-Vitro assay to analyze the effect of oct1 polymorphisms in mouse hepatocytes on metformin uptake into the cells which was then extended to In-vivo studies done on oct1+/+ and oct1-/- mice and subsequently made a genotype-phenotype correlation in healthy volunteers. Reduced transport function of OCT1 has been observed in the presence of 7 out of 12 SNPs evaluated by cellular studies. A higher frequency distribution has been observed for 2 SNPs (420del and R61C with frequencies of 19% and 7.2%, respectively) among the seven. Thus genetic variations in SLC22A1 gene can have a significant role in modulating the response to metformin.
The functional significance of genetic variation in \textit{OCT2} gene in Chinese population was later investigated by Wang \textit{et al}, who identified 14 genetic variants of which 13 had a frequency of \textgreater1\%. Among these, 808 G$\rightarrow$T polymorphism in \textit{OCT2} gene was found to be associated with decrease in metformin clearance.\textsuperscript{62} One other group led by Chen \textit{et al} studied the efficacy and disposition of metformin in Chinese and Japanese population and identified 6 non-synonymous variants of which 3 [Q97K, P117L, R206C] were functionally characterized and were found to influence metformin pharmacokinetics.\textsuperscript{79}

**MATE1, MATE2 and PMAT polymorphisms**

Metformin is excreted out of the liver and renal cells through specific transporters called MATE 1 (liver and renal cells) and MATE 2 (renal cells). These transporters are encoded by \textit{SLC47A1} and \textit{SLC47A2} gene, the polymorphisms of which can increase the blood levels of metformin and contribute to increased risk of toxicity. Jablonski \textit{et al} assessed the common variants in 40 genes for association with incidence of diabetes, metformin response and life-style intervention. A total of 1590 SNPs were analyzed of which \textit{SLC47A1} gene polymorphisms were found to be associated with metformin response.\textsuperscript{80}

In the intestine PMAT (encoded by \textit{SLC29A4}) is another transporter expressed on the luminal surface, which primarily takes metformin into the intestinal cells, the polymorphism of which also contributes to the efficacy of metformin. Christensen \textit{et al} evaluated the influence of genetic variants in \textit{OCT1}, \textit{OCT2}, MATE1, MATE2 and PMAT on plasma levels of metformin and HbA1c. An 80 fold variability in plasma levels of metformin was attributed to \textit{OCT1} variations. Loss of function mutation in \textit{OCT1} gene is found to be associated with diminished pharmacodynamic (HbA1c) activity of the drug after 6 to 24 months.\textsuperscript{81}

**Polymorphisms related to pharmacodynamics of metformin**

The mechanism of action of metformin is not completely understood. It probably acts by inhibiting complex 1 of respiratory chain in the mitochondria of liver cells that creates an oxidative stress leading to activation of adenosine monophosphate activated protein kinase (AMPK). Interestingly polymorphisms in \textit{ATM} gene (ataxia telangiectasia mutated gene) is encoded by \textit{SLC47A2} and \textit{PMAT} (encoded by \textit{SLC47A1}) gene polymorphisms are encoded by \textit{SLC47A1} and \textit{PMAT} (encoded by \textit{SLC47A1}). These transporters are encoded by \textit{SLC47A1} and \textit{SLC47A2} gene, the polymorphisms of which can increase the blood levels of metformin and contribute to increased risk of toxicity. Jablonski \textit{et al} assessed the common variants in 40 genes for association with incidence of diabetes, metformin response and life-style intervention. A total of 1590 SNPs were analyzed of which \textit{SLC47A1} gene polymorphisms were found to be associated with metformin response.\textsuperscript{80}

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The pharmacological mechanisms of metformin includes increased clearance of blood glucose which is not mediated by insulin, reduction of glucose production from the liver, an increase in peripheral utilization of glucose which is mediated through insulin and decrease in gut absorption of insulin.\textsuperscript{73-75} Loss of function variants of \textit{SLC22A1} results in increased plasma levels of metformin\textsuperscript{46} and result in increased activity of metformin.

The Rotterdam study by Becker \textit{et al} a population based cohort study done in 7983 subjects of Caucasian origin.\textsuperscript{76} The correlation between genotypes of \textit{SLC22A1} and response to metformin was evaluated among 152 incident metformin users with DM. Among 12 SNPs studied, one SNP (rs622342) was associated with HbA1c reduction of 0.28% for each variant C allele. The same group also studied the effect of 12 SNPs in \textit{MATE1} transporter gene and found that the SNP rs2289669 was significantly associated with greater response to metformin with greater reductions in HbA1c of 0.30% for each minor allele.\textsuperscript{77} They also confirmed the interaction between the rs2289669 (\textit{MATE1}) and rs622342 (\textit{SLC22A1}), with respect to HbA1c reduction.\textsuperscript{89}

Tzvetkovel \textit{et al} studied the effect of \textit{OCT1}, \textit{OCT2}, \textit{OCT3}, \textit{OCTN1} and \textit{MATE1} polymorphisms on renal clearance of metformin in 103 healthy male Caucasians and showed that the renal clearance of metformin had substantial inter-individual variability which was reflected in the bioavailability of the drug. The genetic variation in \textit{OCT1} gene affected the renal clearance of metformin but the common variants in \textit{OCT2} and \textit{OCT3} did not have any influence.\textsuperscript{61} But a study done by Song \textit{et al} showed that non-synonymous variants in \textit{OCT2} gene significantly decreased the transport activity of metformin which contributes to the inter-individual variation.\textsuperscript{78}
ATM gene belonging phosphatidylinositol 3 kinase related kinase (PIKK) family, encodes serine-threonine kinases that are involved in cell-cycle regulation. As a part of Wellcome Trust Case Control Consortium 2 study (WTCCC2), the GoDARTS [Genetics of Diabetes Audit and Research Tayside] and UKPDS [United Kingdom Prospective Diabetes Study] Diabetes Pharmacogenetics Study Group had performed the first genome-wide association study (GWAS) on patients with type 2 DM to gain insight into the mechanism of action of metformin and to identify the potential genetic factors that can predict the efficacy and safety of metformin.

This study included a discovery cohort of 1024 Scottish patients and 2 replication cohorts of 1783 and 1113 Scottish patients from the UKPDS Prospective Diabetes Study. Around 705,125 SNPs were analyzed in 1024 patients included in the discovery cohort, of which 14 SNPs were identified with p value of <1x10^-6. Among them, the most strongly associated SNP rs11212617 was located in the loci around ATM gene. Carriers of the minor C allele of rs11212617 (with frequency of 44%) had a higher rate of achieving HbA1c value below 7% (OR: 1.64; 95% CI: 1.37-1.99, P=1.9x10^-7). A similar effect was observed in the two replication cohorts, with ORs: 1.21 (95% CI: 1.05-1.38) and 1.37 (95% CI: 1.1-1.72). ATM gene with the SNP rs11212617, was found to be a possible candidate gene, among six other genes (CUL5, ACAT1, NPAT, C11ORF65, KDELC2, EXPH5) which were in linkage disequilibrium. The investigators performed gene analysis in a cohort of non-diabetic individuals and found lack of influence of rs11212617 with glycemic parameters. So metformin was the link between the observed effect (lowering of HbA1c) and the studied polymorphism (rs11212617). To confirm the causal link between ATM gene and AMPK activation, KU-55933, ATM inhibitor was used on rat hepatoma cell lines and it was shown that in the presence of this compound the metformin mediated AMPK activation was decreased. This was similar to other studies that have demonstrated altered AMPK activation with activation or inhibition of ATM. The authors have concluded that rs11212617 is the most robustly replicated variant that is strongly associated with metformin response. However, this explains only 2.5% variation in response to metformin therapy.

Subsequent paper contradicted this hypothesis by claiming that the compound KU-55933 used as ATM inhibitor in the GWAS study can also directly inhibit OCT1 transporters in the hepatocytes. Hence metformin uptake will be reduced and consequently the response will be decreased. HEK-293 cells stably expressing OCT1 transporters were used in the In-vitro analysis to prove the suggested hypothesis. Metformin mediated decrease in AMPK activation was confirmed in the H4IIE rat hepatoma cells. Other pharmacological inhibitors of OCT1 transporter, like cimetidine, imatinib, verapamil were also tested and the results were replicated. The authors also confirmed that OCT1 mediated decrease in metformin uptake is independent of ATM and this gene does not have any detectable effect on OCT1 activity.

Florez et al genotyped the SNP rs11212617 in Diabetes Prevention Program (DPP) including 2,994 overweight or obese, pre-diabetic people belonging to 5 U.S ethnic groups including Whites, African-Americans, Hispanic, Asian and American-Indians. In contrast to the findings of GWAS, this study concluded that the C allele in ATM gene conferred no advantage on diabetes prevention among metformin users. In this study metformin response was considered in terms of incidence of diabetes rather than reduction of HbA1c values. Further, metformin may be more effective in individuals with higher HbA1c at baseline, and hence genotype association can be easily assessed in disease setting rather than in a pre-diabetic cohort. Moreover, the GWAS study was done based on retrospective evaluation of clinical records, while the control of confounders would have been difficult.

Van Leeuwen et al replicated the association established in the GWAS study in 3 different cohorts predominantly of Caucasian origin, including metformin users from Diabetes Care System (DCS) (n = 929), Rotterdam study, Netherlands (n = 192) and from CARDS trial, United Kingdom (n = 254). The association of the genotype with metformin response was based on HbA1c reduction as end-point. In the DCS cohort, the association with treatment success was replicated, while in both the Rotterdam study and the CARDS study, the association was not significant. A meta-analysis was performed by the authors including the three cohorts combined with the previously described GoDARTS and UKPDS stage 2 replication cohorts. The meta-analysis confirmed that the carriers of minor C allele of rs11212617 had higher therapeutic response to metformin in the Caucasian population. A study by Tkac evaluated this research and has concluded that this SNP might belong in the future to the panel of SNPs evaluated for metformin treatment. However, the SNP rs11212617 was studied in Iranian type 2 diabetic patients and found not to be associated with metformin response.
Table 2: Pharmacogenetic factors of metformin

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<tr>
<td>SLC22A2</td>
<td>808 G&gt;T</td>
<td>Associated with decreased metformin clearance</td>
</tr>
<tr>
<td></td>
<td>Q97K, P117L, R206C</td>
<td>Influence metformin pharmacokinetics</td>
</tr>
<tr>
<td>SLC22A3, OCTN</td>
<td></td>
<td>Influence the renal clearance of metformin</td>
</tr>
<tr>
<td>SLC47A1</td>
<td>rs2289669</td>
<td>Associated with increased HbA1c reductions and increased metformin response</td>
</tr>
<tr>
<td>ATM</td>
<td>rs11212617</td>
<td>Attenuate the activation and phosphorylation of AMPK and hence decrease the hypoglycemic effect of metformin</td>
</tr>
</tbody>
</table>

Pharmacogenetics of thiazolidinediones

Thiazolidinediones are insulin sensitizers that activate peroxisome proliferator activated receptor (PPARγ). It regulates gene transcriptions involved in glucose and lipid metabolism. The unique property about this class of drugs is their influence on their cardiovascular outcome which is the major cause of mortality among the patients with diabetes.94 Pioglitazone and rosiglitazone are the two drugs with contrasting features with respect to cardiovascular outcome. Pioglitazone is known to exert a number of pleiotropic effects including favorable redistribution of body fat, blood pressure reduction, improvement in endothelial function, reduction in the circulating levels of inflammatory cytokines and prothrombotic factors.95,96 Though the therapeutic use of the drug is limited due to increased risk of edema and bladder cancer, a recent risk-benefit critique has concluded that this drug is the most suitable one for those diabetes patients in higher insulin resistant states.97 On the other hand, rosiglitazone has been shown to have an adverse effect on the cardiovascular system, and is withdrawn from the European markets and is put under restriction in the United States.98,99

CYP2C8

Pioglitazone and rosiglitazone are mostly metabolized by CYP2C8 enzyme coded by CYP2C8 gene. In addition, minor pathways of metabolism include CYP3A4 and CYP1A1 for pioglitazone and CYP2C9 for rosiglitazone.100,101 CYP2C8 gene polymorphisms were shown to influence the plasma drug concentrations which in turn can influence the risk of type A (augmented) adverse effects such as weight gain and edema. Considering the seriousness of these adverse effects in patients with diabetes and cardiac disease, the genetic variations affecting these drugs require considerable attention.

Kirrcheiner et al extensively studied rosiglitazone in relation to CYP2C8 gene polymorphisms in a group of healthy volunteers of German origin.12 Homozygous carriers of CYP2C8*3 had a 35% lower area under curve (AUC) of rosiglitazone. Further, their drug clearance was 39% higher compared to those with normal genotype. Multivariate regression analysis revealed that around 48% of variability in AUC of rosiglitazone were due to CYP2C8*3 genotype and body weight. Though no influence of this genetic variation was found on the glucose lowering effect of the drug, this study was the...
first to find an association of CYP2C8 genetic variation with the pharmacokinetics of rosiglitazone. Aquilante et al conducted a similar study to analyze the effect of CY2C8 and SLC01B1 polymorphisms on rosiglitazone pharmacokinetics in healthy volunteers of Caucasian origin and concluded that CYP2C8*3 polymorphism influences the AUC of the drug; body weight and the genetic variation predicts 42% of the variability in AUC of rosiglitazone. Stage et al studied the influence of CYP2C8 polymorphisms on steady-state plasma concentration of rosiglitazone and on its glucose lowering effect assessed by change in HbA1c levels. The study has concluded that CYP2C8*3 polymorphism was found to be associated with lower plasma levels of the drug and hence with the reduced therapeutic response.103

Yeo et al performed the sequencing of CYP2C8 genomic DNA in Korean population and identified 17 variants of which CYP2C8*11 variant, which was found only in Asians, had a lower activity level in metabolizing rosiglitazone.104 Torno et al analyzed CYP2C8*3 allele and its effect on the pharmacokinetics of pioglitazone among healthy subjects and were able to elucidate a significant influence of the genetic variation on the AUC of the drug.105 On the other hand, a study done by Pedersen et al to evaluate the effect of CYP2C8 genotype on rosiglitazone pharmacokinetics has found that there was no significant difference in the parameters among the different genotypes and hence the role of CYP2C8*3 polymorphisms could not be confirmed.106 Similar results were depicted in the study done by Hruska et al, when they tried to examine the effect of trimethoprim on CYP2C8 medicated rosiglitazone metabolism.107 Smaller sample size and insufficient power were the two major factors found in common in the two studies that has reported the negative correlation between rosiglitazone pharmacokinetics and CYP2C8*3 polymorphism. However, the inconsistency of the results obtained needs to be addressed with a larger study group sufficiently powered to clarify the association the genotype with the pharmacokinetic and pharmacodynamics parameters of rosiglitazone.

PPAR-γ

Peroxisome proliferator activated receptors have been implicated in conditions such as diabetes mellitus, obesity, and insulin resistance. Among the 3 receptor sub types, PPAR α, PPAR β/δ, PPARγ, thiazolidinediones act as synthetic ligands of PPARγ receptors that are highly expressed in the fat tissues. The receptor has a hydrophobic ligand binding pocket that binds with the drug and activates a number of genes to bring the downstream effects such as increase in glucose and lipid uptake, increased glucose oxidation and decrease in insulin resistance.108

The pharmacogenetic research on thiazolidinediones gained its momentum with the initial research related to the gene variants coding for this receptor. P12A polymorphism (rs1801282) was the variant which was extensively studied in relation to incidence of type 2 diabetes mellitus. Yen et al performed the screening for mutations of the entire PPARγ gene with the DNA of 26 diabetic Caucasians with or without obesity and reported the diversity of P12A genetic variant.109 The initial studies were mainly focused on the association of this variant with incidence of type 2 diabetes mellitus as discussed in a meta-analysis done by Gouda et al in 2010.110 Later on studies linking the polymorphism with the glucose lowering response of thiazolidinediones came up. Two of these studies by Hsieh et al and Kang et al analyzed the effect of Pro12Ala polymorphism on the pioglitazone and rosiglitazone response and concluded that the gene variant influences the therapeutic response of both the drugs.111,112 A similar kind of study by Namvaran et al in Iranian population also reasoned that the carriers of this variants have a significantly reduced risk of diabetes. In diabetes patients with this variants the therapeutic response to pioglitazone was better.113 However, Bluher et al, inferred that PPARγ Pro12Ala variant does not influence the therapeutic efficiency of the drug pioglitazone.114

Adiponectin gene

Adiponectin (ACDC) is a novel polypeptide that has a role in regulating glucose homeostasis and fatty acid oxidation. Genetic variations in adiponectin gene and decreased levels of this protein is associated with insulin resistance, diabetes, atherosclerosis and cardiovascular events.115-117 Studies have shown that rosiglitazone treatment can increase the levels of adiponectin in the body which in turn can help overcoming the insulin resistance in patients with PCOD.118 Kang et al studied common adiponectin gene polymorphisms and the effect of rosiglitazone on plasma levels of adiponectin and glucose. SNP 45 and SNP 276 in ACDC gene affected the plasma levels of adiponectin and blood glucose in patients with type 2 diabetes in response to rosiglitazone.119 Among Chinese type 2 DM patients, adiponectin SNPs 45T/G and -11377C/G, significantly influenced the efficacy of rosiglitazone.120 Subsequently studies
analyzed the effect of pioglitazone on adiponectin gene polymorphisms.\textsuperscript{121} Li \textit{et al} correlated the association of -11377 C>G polymorphism in ACDC gene to the response of pioglitazone in patients with diabetes.\textsuperscript{122} Namvaran \textit{et al} studied the effect of 45T/G SNP of adiponectin gene in the Iranian patients. However, no significant association could be identified with response to pioglitazone.\textsuperscript{123} Studies correlating the association of leptin gene polymorphism (G-2548A), resistin gene polymorphism (rs18625130) and TNF \textgreek{a} gene polymorphism (G-308A) with the therapeutic efficacy of rosiglitazone are being carried out.\textsuperscript{124}

**Pharmacogenetics of GLP-1 analogues**

Glucagon like peptide – 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) mediate insulin secretion following the ingestion of food. The process is termed as incretin effect and the hormones are called the incretins. These incretin hormones are rapidly inactivated in the body by an enzyme called Dipeptidyl peptidase 4 (DPP 4) and hence DPP-4 inhibitors, that can prolong the action of incretin hormones and hence can increase insulin secretion in the body were developed. Other than this GLP 1 receptor agonists or GLP 1 analogues like, exenatide, liraglutide, albiglutide and dulaglutide are in the market. These drugs were generally considered more potent than the DPP 4 inhibitors in the maintaining the glucose homeostasis.

GLP-1 analogues binds to the GLP-1 receptors expressed on the surface of pancreatic \textbeta;-cells and stimulates the downstream adenyl cyclase pathway to increase the synthesis and secretion of insulin. Polymorphism in GLP-1 receptor, by the substitution of methionine residue in place of threonine 149, has been reported in patients with diabetes. This was later replicated in an In-Vitro study done by Beinborn \textit{et al}, who functionally assessed the variant by reproducing the expression of the receptor in COS-7 and HEK 293 cells. The variant had markedly decreased potency in stimulating the downstream cAMP pathway.\textsuperscript{125}

The molecular mechanism of exendin-4, a GLP-1 receptor agonist, involves Wnt signaling in pancreatic \textbeta;-cells that in turn regulates the TCF7L2 transcription factor to increase the proliferation of these cells.\textsuperscript{126} Dominant negative TCF7L2 decreases both basal and exendin-4 induced \textbeta;-cell proliferation, which confirms the association of the Wnt signaling in the process. A study by Florez \textit{et al} has shown that common variants in TCF7L2 were shown to be associated with increased risk of diabetes. Further the risk-conferring genotypes were associated with impaired \textbeta;-cell function but not with insulin resistance.\textsuperscript{127} Further well-regulated studies are needed to confirm this association in a pharmacogenetic perspective. With the increasing availability and use of newer GLP-1 analogues, many such studies are keenly awaited.

**Conclusion and future perspective**

Currently more studies are being done on the genetic aspects of diabetes mellitus in disease causation as well as on pharmacogenetics. Till date, the clinical role of pharmacogenetics of diabetes mellitus is still at its infancy and the utility of genetic information in the treatment of diabetes is primitive. Diabetes mellitus being a polygenic disease involves multiple genetic factors in its causation as well as in the prognosis of treatment. It points towards the fact that the potential and immediate use of pharmacogenetic knowledge would be at the level of generation of novel hypothesis and development of new strategies for drug design targeting diabetes mellitus related gene products. However, the scope of clinical utility of genetic testing for a polygenetic disorder such as diabetes mellitus is still questionable considering the amount of genetic variations that needs to be studied. Greater the number of genetic factors, greater will be the complexity of framing treatment policies. The bigger hurdle would be educating the health care professionals in the aspects of interpretation of pharmacogenetic tests and prudent use of such information.

**CONFLICTS OF INTEREST**

None.

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India bans commercial use of stem cells for therapy

*Announces revised national guidelines for stem cell research*

"No stem cell administration to humans is permissible outside the purview of clinical trials," according to the revised National Guidelines for Stem Cell Research, jointly prepared by the Department of Biotechnology (DBT) and the Indian Council of Medical Research (ICMR) and announced on 11 October 2017.

"We are committed towards stem cell treatments that are safe and have proven efficacy," the government’s guideline says.

But any stem cell use in patients, other than that for treating approved blood (hematopoietic) disorders is "investigational at present" and can be conducted only in the form of a clinical trial after obtaining regulatory approvals from Central Drugs Standard Control Organization (CDSCO). A list of approved indications has been provided in the guidelines.

Genome modification – including gene editing of stem cells, germ-line stem cells or gamete and human embryos – is restricted only to in vitro studies. Only spare embryos or gametes can be used and genome modified human embryos "should not be cultured beyond 14 days of fertilization".

The guidelines prohibit research related to human germ line gene therapy, reproductive cloning, and clinical trials involving "xenogeneic" cells – those derived from different species. "Breeding of animals in which any type of human stem cells have been introduced is prohibited."

According to the guidelines, only umbilical cord blood banking is permitted for the present, but not banking of all other biological materials "until further notification".

All international collaborations in stem cell research and import of any type of stem cells require approvals from the respective funding agencies and the CDSCO.

Clinical trials sponsored by multinationals, employing cell products developed outside India, additionally require Indian health ministry’s screening committee’s permission.

*Reference: National Guidelines for Stem Cell Research, 2017*