# University of Alberta

Pharmacogenomics of Sulfonylureas and Glinides on ATP-Sensitive Potassium Channel

by

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## Abstract

The common ATP-sensitive ($K_{ATP}$) channel variants E23K and S1369A, found in the *KCNJ11* and *ABCC8* genes respectively, form a haplotype that is associated with an increased risk for type 2 diabetes. Our previous studies showed that $K_{ATP}$ channel inhibition by the A-site sulfonylurea gliclazide was increased in the K23/A1369 haplotype. Therefore, I studied the pharmacogenomics of eight more clinically used sulfonylureas and glinides to determine their structure activity relationships in $K_{ATP}$ channels containing either the E23/S1369 non-risk or K23/A1369 risk haplotypes by utilizing patch-clamp technique. The results demonstrate that the ring-fused pyrrole moiety in several A-site drugs likely underlies the observed inhibitory potency of these drugs on $K_{ATP}$ channels containing the K23/A1369 risk haplotype. It may, therefore, be possible to tailor existing therapies or design novel drugs that display increased efficacy in type 2 diabetes patients homozygous for these common $K_{ATP}$ channel haplotypes.

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## List of Abbreviations

| | |
|---|---|
| ADP | Adenosine Diphosphate |
| AMP | Adenosine Monophosphate |
| ATP | Adenosine Triphosphate |
| $Ca^{2+}$ | Calcium |
| $Ca_V$ | Voltage-gated Calcium Channel |
| cAMP | Cyclic Adenosine Monophosphate |
| cGMP | Cyclic Guanosine Monophosphate |
| CNS | Central Nervous System |
| DEND | Developmental Delay, Epilepsy, and Neonatal Diabetes |
| DMSO | Dimethyl Sulfoxide |
| DPP4 | Dipeptidyl Peptidase-4 |
| EGTA | Ethylene-glycol-tetra-acetic Acid |
| GIP | Glucose-dependent Insulinotropic Polypeptide |
| GLP | Glucagon-like Peptide |
| GLUT | Glucose Transporter |
| $Hb_{A1C}$ | Glycated Hemoglobin |

| | |
|---|---|
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic Acid |
| $IC_{50}$ | Half Maximal Inhibitory Concentration |
| IRS | Insulin Receptor Substrate |
| $K_{ATP}$ | ATP-sensitive Potassium Channel |
| $K_{Ca}$ | Calcium Activated Potassium Channel |
| $K_{ir}$ | Inwardly-rectifying Potassium Channel Family |
| $K_V$ | Voltage-gated Potassium Channel |
| MgADP | Magnesium Adenosine Diphosphate |
| MgATP | Magnesium Adenosine Triphosphate |
| MODY | Maturity Onset Diabetes of the Young |
| NBD | Nucleotide Binding Domain |
| ND | Neonatal Diabetes |
| PGx | Pharmacogenomics |
| PHHI | Persistent Hyperinsulinemic Hypoglycemia of Infancy |
| PNDM | Permanent Neonatal Diabetes Mellitus |
| PPAR | Peroxisome Proliferator Activated Receptor |
| sarc$K_{ATP}$ | Sarcolemma ATP-sensitive Potassium Channel |

| | |
|---|---|
| SNP | Single Nucleotide Polymorphism |
| SU | Sulfonylurea |
| SUR | Sulfonylurea Receptor |
| T1D | Type 1 Diabetes |
| T2D | Type 2 Diabetes |
| TMD | Transmembrane Domain |
| TNDM | Transient Neonatal Diabetes Mellitus |
| TZD | Thiazolidinediones |
| $V_m$ | Membrane Potential |

# Chapter 1

# General Introduction

Currently, type 2 diabetes (T2D), which is one of the most common chronic diseases, represents a major public health issue worldwide, affecting over 200 million individuals by 2010 [1]. The pathophysiology of T2D is not fully understood so far, but is known to be a disease characterized by inadequate insulin secretion from pancreatic β-cells and diminished tissue responsiveness to insulin action, which is also known as insulin resistance. Insulin, a kind of endogenous hormone, lowers the blood glucose levels by promoting plasma glucose uptake and inhibiting hepatic glucose output. Therefore, either insufficient insulin secretion or impaired insulin action will lead to hyperglycemia and diabetes.

It's commonly believed that aging and environmental factors contribute most to T2D pathogenesis [2]. Indeed, obesity, a result from high-caloric diets and a sedentary lifestyle, represents a major risk factor for T2D [3]. Nevertheless, cases of many obese individuals who don't develop T2D and many T2D patients who are with normal weights, suggest that environmental factors are not exclusive risk factors for the development of T2D. An emerging risk factor brought to light is that some variations in individual genetic makeup can predispose carriers to T2D. Since the identification of first T2D risk gene *KCNJ11* in 1998, at least 40 risk genes have been recognized that associate with the development of T2D by genetic research through the last decade [4-6]. Most risk genetic variants are believed to exert only a moderate effect on the following expression or function of the affected gene, but when coupled with aging and environmental factors mentioned above, they will increase T2D risk to genetic variant carriers.

The goal of current management of T2D is to avoid poor glycemic control and maintain $Hb_{A1C}$ level less than 7%. A number of treatments, with different mechanisms of action to lower blood glucose level, have been made available to T2D patients. However, inter-individual variability is always observed in patients treated with pharmacotherapies. The idea of genetic variants carried by patients affecting drug response has emerged in the research field.

The term "pharmacogenomics" refers to the study of relationship between common genetic variants in patients and variability in drug response. Therefore, the objective of this thesis is to discuss sulfonylurea and glinide pharmacogenomics in T2D, specifically focusing on two genetic variants, which are on T2D risk genes also encoding for the drug target of sulfonylureas and glinides. Before the discussion of their relationship with inter-individual variability in sulfonylurea/glinide response, some background knowledge should be prepared. In this chapter, topics on common risk genes in T2D, current pharmacotherapies for T2D and most pharmacogenomic studies for T2D will be reviewed in detail.

## Section I Risk Gene in Type 2 Diabetes

### *Electrophysiology in Pancreatic Islet*

Type 2 diabetes (T2D) is characterized by elevation of the blood glucose concentration, which is caused by both impaired insulin secretion and insulin resistance at target tissues (liver, fat, and muscle). Additionally, excessive glucagon secretion is also observed.

The islets of Langerhans are pivotal in blood glucose homeostasis. Insulin secreting β-cells constitute 60% to 70% of the islet cell population; whereas glucagon secreting α-cells account for 20% to 30% of the islet [7]. Insulin decreases blood glucose levels by uptake glucose into tissues, such as liver, fat and muscle. Glucagon increases blood glucose levels by increasing hepatic glucose output. Therefore, the balance of insulin and glucagon secreted from islets at appropriate rates maintains blood glucose levels within a relatively narrow range. Both β- and α-cells act as sensors of blood glucose levels. In response to glucose challenge, pancreatic β-cells secret insulin in a biphasic profile, consisting of an initial transient phase followed by a sustained phase. The initial transient phase is generated by the triggering signal, whereas the following sustained phase is generated by the amplifying signal. Both triggering and amplifying signals are produced through distinct pathways [8]. The former is well characterized and will be the focus of this thesis.

Like other excitable cells, such as neurons and muscle cells, pancreatic β-cells are electrically excitable, and can generate action potentials. When exposed

to high glucose levels, β-cells increase excitability that stimulates insulin secretion. Besides glucose, β-cell electrical activity can be also regulated by other nutrients (fatty acids) [9], neurotransmitters (acetylcholine and norepinephrine) [10, 11], hormones (GLP-1 and GIP) [12], and pharmacological agents (sulfonylureas and glinides). Glucose modulates β-cell electrical activity via metabolically induced changes in the activity of a number of ion channels responsible for controlling electrical activity in β-cells.

In the β-cell, membrane potential ($V_m$) is determined by several types of ion channels, pumps, and transporters [13, 14]. These include ATP-sensitive potassium ($K_{ATP}$) channels, L-type voltage-gated calcium ($Ca_v$) channels, voltage-gated potassium ($K_v$) channels and calcium activated potassium ($K_{Ca}$) channels.

When the extracellular glucose concentration is less than 5 mM as in the fasting state, the resting $V_m$ of β-cells is around -70 mV, which is determined mainly by small outward potassium conductance through slightly open $K_{ATP}$ channels [15]. At a $V_m$ around -70mV, L-type $Ca_v$ channels remain closed, so there is no influx of calcium to trigger the release of insulin granules.

When the concentration of extracellular glucose increases that occurs after a meal, glucose metabolism increases in β-cells, and generates more ATP through glucose oxidation in the mitochondria. An increase in ATP/ADP ratio inhibits the activation of $K_{ATP}$ channels, leading to a decrease in the efflux of potassium ions through $K_{ATP}$ channels that causes a depolarization of the β-cell $V_m$ from ~ -70mV to ~ -50mV, which reaches the threshold $V_m$ of activation of L-type $Ca_v$ channels

[13] **(Figure 1-1)**. The opening of L-type $Ca_v$ channels further depolarizes the β-cell $V_m$, and increases calcium influx into the β-cells which is sufficient to trigger insulin granule release [16]. Activation of $K_v$ and $K_{Ca}$ channels, along with inactivation of L-type $Ca_v$ channels, repolarizes the β-cell action potential [17] **(Figure 1-1)**. Once repolarized, L-type $Ca_v$ channels are released from inactivation state and ready to open again, leading to the generation of another action potential and secretion of insulin from granules. When maintained in high glucose, the β-cell $V_m$ undergoes a continued firing of action potentials [18]. The generation of β-cell electrical activity through the closure of $K_{ATP}$ channels is termed as the triggering pathway of insulin secretion [8]. However, this triggering signal can only release immediately available insulin granules which have been already primed / docked to the β-cell membrane ("the readily releasable pool"), and produce the initial transient phase of insulin secretion ($1^{st}$ phase). The triggering signal is augmented by an amplifying signal, in which the increased concentration of cytosolic free calcium ($[Ca^{2+}]_i$) acts as a second messenger further increasing the cytosolic calcium mediated granule recruitment ("reserve pool") and exocytosis, producing the sustained phase of insulin secretion ($2^{nd}$ phase) [8].

Under physiological conditions, insulin secretion is determined by both the triggering and amplifying pathways. The initial closure of $K_{ATP}$ channels triggers the $1^{st}$ phase insulin secretion, while the amplifying pathway serves to enhance the initial secretory response induced by the triggering signal, but remains functionally silent if the triggering pathway has not initially depolarized

the β-cell $V_m$. This mechanism helps to ensure that no insulin is secreted inappropriately in the presence of low glucose concentrations [8].

The presence of the $K_{ATP}$ channels is essential in the triggering pathway which determines the firing of β-cells. In order to trigger an action potential, the depolarization of β-cell $V_m$ should above the threshold potential for activation of L-type $Ca_v$ channels. This process is the essence of electrical excitability in β-cells and controlled primarily by the activity of $K_{ATP}$ channels. As discussed above, the activity of $K_{ATP}$ channels sets the β-cell $V_m$ around -70 mV, which is very close to the equilibrium potential of potassium ($E_K$). The threshold $V_m$ of L-type $Ca_v$ channel activation is -50 mV, so a depolarizing voltage of 20 mV generated by the closure of $K_{ATP}$ channel should be able to trigger β-cell firing. The input resistance of the β-cell membrane is very high (~ 1GΩ). According to Ohm's Law (*V=IR*), 20 pA of whole-cell current can change β-cell $V_m$ by 20 mV. In the case of β-cell, this would be a removal of 20 pA of total $K_{ATP}$ current to depolarize the β-cell by 20 mV.

The amplitude of whole-cell current (*I*) is related to unitary current (*i*) by the relationship $I = NPoi$, where *N* denotes the number of channels per cell and *Po* is the average channel open probability (the fraction of time it spends in the open, current conducting state). *Po* can range in value from 0 (closed all the time) to 1 (open all the time). Each β cell contains ~ 1000 $K_{ATP}$ channels, and the size of unitary current flowing through each channel is around 2 pA. Thus *Po* equals 0.01 by substituting numbers (*N*=1000, *i*=2 pA, *I*=20 pA) into the equation *(I = NPoi)*, which indicates that a change of 1% in *Po* of $K_{ATP}$ can change β-cell $V_m$ by 20

mV. Therefore, β-cell $K_{ATP}$ channels only operate in a very limited range of low open probability (*Po*≈0.01) under physiological conditions.

As $K_{ATP}$ channels control the threshold for β-cell electrical activity, small changes in the open probability of $K_{ATP}$ channels can make the difference between firing and non-firing of β-cells, and consequently between insulin secretion and no secretion at all. In the case of T2D, minor increases in $K_{ATP}$ channels activity may impair β-cell electrical activity, and contribute to the defective insulin secretion observed in T2D patients [19].

Islet α-cells, which secrete glucagon, are equipped with similar channels to β-cells, including $K_{ATP}$ channels. In contrast to β-cells, α-cells can produce action potentials at low glucose concentrations. Although α-cell $K_{ATP}$ channels are not 100% blocked under low glucose concentrations, the low $K_{ATP}$ channel activity can already induce α-cell action potential firing, trigger glucagon secretion, and prevent voltage dependent inactivation of sodium and calcium channels [20, 21]. Persistent α-cell membrane depolarization results in voltage dependent inactivation of sodium and calcium channels, and further leads to impaired action potential firing and glucagon secretion [22]. As a result, α-cell $K_{ATP}$ channels are fully blocked under high glucose concentrations, and the sustained α-cell membrane depolarization finally leads to impaired action potential firing and glucagon secretion.

***Risk Genes in Type 2 Diabetes – Affecting Insulin Secretion***

In people without diabetes, after glucose administration, there will be a biphasic release of insulin. First phase, a rapid secretion of insulin from the pancreas occurs within 3–5 minutes and disappears within 10 minutes. Then, the second phase of insulin release occurs slowly, until glucose levels return to normal. A large amount of T2D patients is characterized with impaired insulin secretion in both of its phases, a defective first phase and a reduced second phase. Most T2D risk genes have been found to involve disruption of pancreatic β-cell function, some of which reduce first phase glucose-stimulated insulin secretion, some of which impair second phase incretin-induced insulin secretion, and some of which diminish the conversion of proinsulin to matured insulin.

Glucose is known to induce the first phase insulin secretion from pancreatic β-cells through a series of cellular process. Glucose is transported into β-cells and is metabolized. Increased ATP/ADP ratio leads to membrane depolarization, which triggers the release of pre-stored insulin granules. Thus, genetic variants in genes involved in glucose sensing / metabolism in β-cells will affect glucose-stimulated insulin secretion. Within this cellular process, common risk genetic variants, such as Gly-30Ala in the promoter region of *GCK* gene encoding glucokinase [23], Leu446Pro in *GCKR* gene encoding glucokinase regulatory protein [24], and SNP rs16856187 in *G6PC2* gene encoding glucose-6-phosphatase catalytic subunit 2 [25], were shown to modulate the provision of fasting glucose level and associate with both first and second phase insulin secretion. Two other crucial genes involved in glucose-stimulated insulin

secretion pathway are *KCNJ11* and *ABCC8* genes encoding ATP-sensitive potassium ($K_{ATP}$) channel, which governs the membrane potential of β-cell to trigger calcium dependent insulin granule exocytosis. Common risk genetic variants Lys23 in *KCNJ11* and Ala1369 in *ABCC8* genes were demonstrated to reduce insulin secretion by hyperpolarizing β-cell membrane potential, hence suppressing β-cell membrane excitability and limiting calcium dependent insulin granule exocytosis [26]. Meanwhile, the $K_{ATP}$ channel that *KCNJ11* and *ABCC8* genes encode acts as a target for a class of insulin secretagogue widely used in the treatment of T2D, which will be discussed later. In fact, glucose-stimulated insulin secretion can also be affected by genetic variants in other genes that are not directly involved in this cellular process. Several variants within *MTNR1B* gene, which encodes melatonin receptor 1B, impair insulin secretion through the effect on the inhibition of cAMP dependent signaling pathway and the reduced formation of cGMP in pancreatic β-cells [27]. Normal processing and crystallization of insulin requires zinc ions. Therefore, *SLC30A8* gene encoding zinc transporter protein in pancreatic β-cells also makes itself a risk gene for T2D. Risk genetic variants Arg325 in *SLC30A8* gene, may result in inadequate zinc transfer from cytoplasm to insulin secretory granules, in which insulin is normally stored as a hexamer coupled with two zinc ions at its core before secretion. The inefficient accumulation of zinc leads to defects in insulin crystallization, storage or secretion [28]. *HHEX* gene encodes a homeobox protein, a transcription factor, which is involved in WNT signaling and is required for early development of ventral pancreas and liver. Risk genetic variants in *HHEX* gene were also

indicated in association with T2D by genome-wide association study, however, besides being implicated in β-cell development, the molecule pathway that *HHEX* gene is involved in glucose-stimulated insulin secretion remains elusive [29].

In addition to glucose stimulus, another important physiological stimulus that induces second phase insulin secretion is incretin hormones including glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). When GLP-1 binds to its G protein coupled receptor, adenylate cyclase becomes activated, leading to an increase of intracellular cAMP level. In pancreatic β cell, cAMP activated protein kinase A triggers the closure of $K_{ATP}$ channel, inducing insulin exocytosis [12]. Thus, genetic variants in genes involved in incretin secretion from endocrine cells or incretin signaling in β-cells will affect incretin-induced insulin secretion. A common genetic variant SNP rs10010131 in *WFS1* gene, which encodes wolframin, an endoplasmic reticulum membrane protein with a role in endoplasmic reticulum calcium homeostasis [30], was demonstrated to reduce GLP-1 induced insulin secretion through GLP-1 signaling in β-cells with unaltered plasma incretin level [31]. Another gene, *TCF7L2* gene, which encodes transcription factor 7-like 2, also demonstrated its involvement in incretin expression in endocrine cells and incretin responsiveness in β-cells, as the expression of GLP-1 gene in endocrine cells and GLP-1 / GIP receptor genes in human islets likewise depend on the transcription factor that *TCF7L2* gene encodes [32]. A good example is that SNP rs7903146 in *TCF7L2* gene was found to impair incretin-stimulated insulin secretion [33]. Furthermore, besides the expression of incretin and its receptor genes, the expression of insulin

gene was also found to be strongly correlated with the transcription factor that *TCF7L2* gene encodes. SNP rs7903146 in *TCF7L2* gene was also shown to be associated with impaired conversion of proinsulin to mature insulin, which was supported by a decreased proinsulin to insulin ratio [34-36].

### *Mutations in $K_{ATP}$ Channel – Affecting Insulin Secretion*

The $K_{ATP}$ channel is a hetero-octameric membrane protein complex comprised of four pore-forming inwardly rectifying potassium-channel (Kir6.x) subunits and four regulatory sulfonylurea-receptor (SURx) subunits **(Figure 1-2)** [37, 38]. Two isoforms of the Kir6.x subunit exist, Kir6.1 and Kir6.2, which are encoded by *KCNJ8* and *KCNJ11* genes respectively. Kir6.2 is more widely expressed than Kir6.1 that is predominately expressed in vascular smooth muscle [39]. Similarly, two isoforms of SURx subunits have been identified, SUR1 and SUR2, encoded by the *ABCC8* and *ABCC9* genes respectively. The *ABCC9* gene is alternatively spliced such that two splice variants exist, SUR2A and SUR2B, which differ only in the distal C-terminal of the protein [37]. The subunit composition of $K_{ATP}$ channel differs between tissue types [40]. In pancreatic β-cells and neurons, $K_{ATP}$ channels are assembled from Kir6.2 and SUR1 subunits [41]. In cardiac tissue and skeletal muscle, $K_{ATP}$ channels are comprised of Kir6.2 and the SUR2A splice variant subunits [42]; whereas in smooth muscle, $K_{ATP}$ channels contain Kir6.1/Kir6.2 and SUR2A/SUR2B splice variant subunits [43].

The Kir6.2 subunit encoded by *KCNJ11* gene contains ~390-amino acid, while the SUR1 subunit encoded by *ABCC8* gene contains ~1,580-amino acid.

Both *KCNJ11* and *ABCC8* genes are located at the same chromosomal locus (11p15.1) and are only 4.5 Kb apart. Each Kir6.2 subunit consists of two trans-membrane helices connected by a pore-forming loop that confers potassium selectivity to the channel [44]. In the Kir6.2 subunit, the α-helix linking trans-membrane helix 1 (TM1) and intracellular N-terminus, termed as the "slide helix", plays an important role in channel gating. Extensive interactions are found between the cytosolic N- and C-terminal of adjacent Kir6.2 subunits that contribute to the formation of the binding pocket for the inhibitory ATP molecule. Each SUR1 subunit consists of three trans-membrane domains (TMD) with a total of 17 trans-membrane segments [45]. Each SUR1 subunit contains two nucleotide-binding domains (NBD1 and 2) that dimerize to form catalytic sites for the intrinsic Mg-ATPase activity of the channel complex. Therefore, the two NBDs regulate channel activity through the binding and hydrolysis of Mg-ATP and the formation of stimulatory Mg-ADP [46]. Each NBD contains two highly conserved "Walker A" and "Walker B" motifs involved in nucleotide hydrolysis **(Figure 1-2)** [47]. TMD0 and the cytosolic loop linking TMD0 and TMD1 of the SUR1 subunit are responsible for the interaction between SUR1 and Kir6.2 subunits [48]. The Kir6.2 and SUR1 subunits each possess an endoplasmic reticulum retention motif that requires masking via subunit co-assembly to enable correct trafficking of the assembled hetero-octameric channel complex to the cell membrane [49].

$K_{ATP}$ channels possess an exquisite ability to sense changes in cellular metabolism, primarily via nucleotide regulation. $K_{ATP}$ channel activities are

inhibited by nucleotides such as ATP through the binding at the interface between neighboring Kir6.2 subunits, whereas channel activities are promoted by the binding and hydrolysis of Mg-ATP in the NBD1/NBD2 dimers on SUR1 subunit, and the resulting Mg-ADP generated antagonizes the inhibitory action of ATP on the Kir6.2 subunits. Therefore, the overall activity of the $K_{ATP}$ channel complex, and hence the excitability of pancreatic β-cells, is primarily governed by the ratio of cytosolic ATP/ADP in the close vicinity of the $K_{ATP}$ channel complex [50-53].

In pancreatic β-cells, $K_{ATP}$ channels sense changes in the cytosolic ATP/ADP ratio as a result of cellular metabolism and are a major regulator of the β-cell membrane potential. As glucose-stimulated insulin secretion is primarily controlled by the β-cell membrane potential, $K_{ATP}$ channels serve to couple glucose metabolism to insulin secretion. When plasma glucose levels are low, the cytosolic ATP/ADP ratio is reduced leading to a basal efflux of potassium ions from the cell via $K_{ATP}$ channel activity that maintains the membrane potential of the β-cell at approximately -70 mV. This polarized membrane potential prevents calcium entry through voltage-gated calcium channels. As elevations in cytosolic calcium are the primary trigger for insulin granule exocytosis, insulin secretion is suppressed when plasma glucose levels are low **(Figure 1-3)** [13]. When plasma glucose levels rise, glucose enters the β-cells via the glucose transporter 2 (GLUT2). Subsequent glucose metabolism leads to an increase in cytosolic ATP/ADP ratio, promoting $K_{ATP}$ channel closure. The resultant decrease in potassium ion efflux depolarizes the β-cell membrane potential leading to activation of voltage-gated calcium channels, calcium influx and calcium-

stimulated insulin granule exocytosis **(Figure 1-3)**. Graded increases in plasma glucose and subsequent metabolism lead to proportional decreases in $K_{ATP}$ channel activity resulting in an appropriate insulin secretory response that is tightly coupled to the plasma glucose concentration. As the electrical resistance of β-cell is high, only small changes in $K_{ATP}$ channel activity are required to change β-cell excitability (and hence insulin secretion) via alterations in the β-cell membrane potential [54]. Mutations within the $K_{ATP}$ channel complex, which change their intrinsic activity and/or ability to sense changes in either ATP or ADP, will result in altered $K_{ATP}$ channel activity (and hence insulin secretion) that is correlated to the specific effects of the individual mutation on $K_{ATP}$ channel activity[55, 56].

In theory, any mutation in the *ABCC8* and *KCNJ11* genes that promotes channel inactivity would lead to depolarization of pancreatic β cells and excessive insulin secretion that is poorly coupled to blood glucose levels. On the other hand, mutations that activate channels would promote hyperpolarization of the β cell membrane and inhibit insulin secretion, leading to hyperglycemia. Indeed, genetic mutations in the *KCNJ11* and *ABCC8* genes have been linked to persistent hyperinsulinemic hypoglycemia of infancy (PHHI) and neonatal diabetes (ND) [54].

The rare metabolic disorder, PHHI, is commonly caused by mutations that cause $K_{ATP}$ channel inactivation, resulting in the loss of appropriate channel activity. $K_{ATP}$ channel inactivity resulting from PHHI mutations leads to sustained membrane depolarization and excessive insulin secretion that is not coupled to

fluctuations in blood glucose levels [57]. **Table 1-1** lists the reported inactivation mutations causing PHHI in both *KCNJ11* and *ABCC8* genes, and their corresponding locations on each subunit. The majority of PHHI mutations identified to date have been found in the *ABCC8* gene encoding the SUR1 subunit. Mutations can either impair trafficking of functional $K_{ATP}$ channel to the cell surface (class 1) or result in functional expression of $K_{ATP}$ channels with abnormal MgADP stimulation (class 2). In addition, several class 1 PHHI mutations have been identified in *KCNJ11* (Kir6.2), and similarly they prevent trafficking of the mature $K_{ATP}$ channel complex to the cell surface [58]. In some class 2 mutations, where mutant $K_{ATP}$ channels remain partially functional, pharmacological treatment with the $K_{ATP}$ channel opener diazoxide may increase channel activity sufficiently to effectively reduce insulin secretion [59]. However, in severe class 1 mutations, near-total pancreatectomy is required to reduce insulin secretion and relieve the hypoglycemia.

In contrast to PHHI mutations that result in loss of $K_{ATP}$ channel activity, ND mutations in pancreatic $K_{ATP}$ channel subunits lead to varying increases in $K_{ATP}$ channel activity, resulting in clinical phenotypes ranging from the most severe developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome and permanent neonatal diabetes mellitus (PNDM) to the less severe transient neonatal diabetes mellitus (TNDM) phenotype. ND mutations act by causing persistent $K_{ATP}$ channel over-activity, leading to a hyperpolarized membrane potential and a reduced insulin secretion despite elevated blood glucose levels [60].

To date, >40 activation mutations in Kir6.2 subunit have been reported at 30 distinct residues **(Table 1-2)**. The locations of these mutations are clustered into three common regions in the Kir6.2 subunit. One cluster of mutations lines the putative ATP binding pocket (e.g. R50, R201, and Y330) and reduces ATP inhibition on the channel by decreasing ATP binding affinity [61, 62]. Another cluster of mutations resides in the regions involved in channel gating such as the slide helix (e.g. V59), the cytosolic mouth of the channel (e.g. I296), or gating loops (e.g. C166) between ATP binding site and the slide helix [63-65]. These mutations decrease ATP inhibition by stabilizing the open conformation of the channel in both the absence and presence of ATP leading to increases in channel activity. The third cluster of mutations locates at the interface between subunits such as the interface between adjacent Kir6.2 subunits (e.g. F35 and E322), and the interface between Kir6.2 and SUR1 subunits (e.g. Q52 and G53) [66-68]. These mutations likely alter channel activity by affecting the interactions between adjacent Kir6.2 and SUR1 subunits that are important for correct channel gating. In general, $K_{ATP}$ channel mutations in Kir6.2 subunit that cause the greatest increase in channel activity also precipitate a deleterious extra-pancreatic phenotype resulting the symptoms of DEND syndrome [69]. The developmental delay associated with DEND syndrome is thought to result from persistent activation of $K_{ATP}$ channels in the CNS encoded by the *ABCC8* and *KCNJ11* genes. The reduced motor function is now considered to result primarily from CNS $K_{ATP}$ channel activation rather than a direct effect on skeletal muscle $K_{ATP}$ channels, as a recent elegant transgenic mouse study using CNS- or skeletal

muscle- specific insertion of a DEND *KCNJ11* mutation (e.g. V59M) indicates that the motor impairment associated with DEND syndrome originates in the CNS [70].

Meanwhile, there are >30 individual activation mutations in SUR1 subunit, which have been reported to cause ND **(Table 1-2)** [59]. Many of these mutations are dispersed throughout the SUR1 subunit sequence, although a large number of mutations resides in two specific regions of the SUR1 subunit. One cluster of mutations is concentrated in TMD0 and the cytosolic loop linking TMD0 and TMD1 [71, 72]. As this region is known to interact with adjacent Kir6.2 subunit, mutations in this region are believed to reduce ATP inhibition via the Kir6.2 subunit. The second cluster of mutations resides in the NBD2 of the SUR1 subunit, where stimulatory Mg-ADP binds [73-75]. Therefore, NBD2 mutations are thought either to increase direct Mg-ADP stimulation or to enhance MgATPase activity in NBD2 leading to increased Mg-ADP stimulation (e.g. R1380L/C) [75]. The increased intrinsic MgATPase activity of the $K_{ATP}$ channel complex is thought to cause elevation of the MgADP levels in the localized environment of the ADP-sensing region of NBD2, resulting in excessive activation of the $K_{ATP}$ channel that is poorly coupled to metabolic status of the β cell. It is therefore likely that more ND mutations in *ABCC8* will be discovered in the future.

***Risk Genes in Type 2 Diabetes – Affecting Insulin Sensitivity***

Most T2D patients have increased insulin resistance; hence genetic defects in β-cells function may only become apparent if peripheral insulin-responding tissue is experiencing an increase in insulin resistance, or in other words a decrease in insulin sensitivity [76]. Therefore, insulin resistance is considered as an early step in the development of T2D. Several T2D risk genes have been found involved in insulin signaling to affect insulin sensitivity throughout the body.

Insulin is known to exert its effects by binding to insulin receptors in responding cells, i.e. skeletal muscle and adipose tissue. The intrinsic tyrosine kinase activity of insulin receptor is switched on upon insulin binding. Once activated, the insulin receptor phosphorylates tyrosine residues of a variety of docking proteins including insulin receptor substrate 1 (IRS-1). The phosphorylation of IRS-1 leads to activation of a series of downstream intracellular signaling components, such as phosphatidylinositol 3-kinase (PI3-K), phosphoinositide-dependent kinase (PDK), and protein kinase B and C (AkT/PKC). An important role of activation of AkT/PKC pathway is to regulate gene expression involved in lipid synthesis, protein synthesis, glycogen synthesis, cell growth and differentiation. As IRS-1 is a critical component in insulin-stimulated signal transduction pathway, *IRS1* gene has been studied extensively as a risk gene for T2D with a primary effect on insulin action [77]. Several studies have shown that genetic risk variant Arg972 in *IRS1* gene impairs insulin-stimulated signaling and contributes to insulin resistance in normal and diabetic populations [78].

Undoubtedly, insulin resistance is strongly associated with obesity such that the degree of insulin resistance rises with obesity. As obesity is a major risk factor for insulin resistance, risk genes involved in the development of obesity are expected to likewise predispose carriers to T2D risk.

Peroxisome proliferator-activated receptors (PPAR), which are nuclear hormone receptors, have been in the scope of studies for years, as they play important roles in regulation of glucose metabolism and lipid storage [79]. There are three subtypes of PPAR that have been identified, PPAR α, β/δ and γ , which with retinoid X receptors (RXRs) will form heterodimers. In the absence of ligands, such as fatty acids, the heterodimers will bind to corepressors at the promoter regions of target genes to repress gene transcription [80]. Once PPARs are activated by ligands, the heterodimers will disassociate with corepressors, associate with coactivators, induce gene transcription, and further stimulate protein synthesis involved in a wide variety of processes, such as cellular lipid metabolism, adipocyte differentiation and insulin sensitivity [81]. Functional studies demonstrated that Pro12 in *PPARG* gene encoding PPAR γ2 isoform, exclusively expressed in adipocytes, had a higher ability to transactivate responsive promoters, resulting in an increased expression of genes involved in lipid storage and adipocyte differentiation, which eventually would increase adipose tissue mass and thereby decrease insulin sensitivity [82]. On the other hand, a common genetic variant Ala12 at the same position in *PPARG* gene exhibited reduced ability of transcriptional activation and improved insulin sensitivity, affording protection from carriers developing T2D, although the

detailed molecular mechanism remains to be elucidated [83]. Furthermore, PPAR γ is the specific molecular target for a class of anti-diabetic medication, thiazolidinediones (TZD), which is used as an insulin sensitizer and will be discussed later.

A growing body of evidence suggests that insulin sensitivity in the brain associates with risk of obesity [84, 85] and T2D [86, 87] across several ethnic groups. In humans, insulin stimulates cerebrocortical activity in lean but not obese subjects. Recently, intronic genetic variants SNP rs8050136 in the *FTO* obesity gene (the fat mass and obesity associated gene), which is highly expressed in the hypothalamus, was demonstrated to impair insulin-stimulated cerebrocortical activity and to increase over all fat mass [88, 89]. Studies in mice suggest that *FTO* gene may have a role in nucleic acid demethylation, and the mRNA level of *FTO* gene is regulated by feeding and fasting [90]. Subsequently, it has been shown that *FTO* gene encodes a nuclear Fe(II)- and 2-oxoglutarate-dependent DNA demethylase, and recent studies have focused on the determination of the exact physiological role of *FTO* gene.

## Section II Pharmacotherapy for Type 2 Diabetes

Long-term hyperglycemia will induce devastating complications, such as retinopathy, nephropathy, neuropathy, cardiovascular disease, peripheral artery disease, and stroke. Many studies have shown a reduction in the risk of neuropathy and cardiovascular complications in T2D patients receiving intensive glucose control, who maintain their plasma glucose level < 8.6mmol/L and glycated hemoglobin ($Hb_{A1C}$) level < 7% [91, 92]. In addition, elevated plasma glucose level can cause loss of insulin secretory function of β-cell itself, a condition known as glucotoxicity, which is reversible if the plasma glucose level returns to normal [93]. Therefore, the sooner T2D is diagnosed and controlled, the lower the risk of developing any deleterious complications. Of course, lifestyle modifications through diet and exercise are effective strategies to achieve normoglycemia, but most T2D patients will ultimately need pharmacotherapy to control their disease. In addition, a portion of T2D patients, who are unresponsive to oral pharmacotherapy, will eventually require additional insulin therapy to maintain blood glucose level [94].

As T2D generally results from impaired insulin sensitivity in peripheral tissues and diminished insulin secretion in pancreatic β-cells, T2D pharmacotherapies can achieve and maintain optimal plasma glucose level by either elevating insulin sensitivity or stimulating insulin secretion. Numerous effective oral and injected hypoglycaemic agents are available as T2D pharmacotherapies, such as insulin secretagogues sulfonylureas (SU) and glinides, insulin sensitizers biguanides and thiazolidinediones (TZD), incretin potentiators

incretin mimetics and dipeptidyl peptidase-4 (DPP4) inhibitors, α-glucosidase inhibitors and amylin analogues [95].

### *Insulin Secretagogues: Sulfonylureas and Glinides*

Sulfonylureas (SUs) were serendipitously discovered in the 1950s, as antibiotic sulfonamide drugs were found to stimulate insulin secretion and cause hypoglycemia [96]. Since then, orally administered SUs serving as insulin secretagogues have been used widely to treat T2D. Current commonly used SUs cover first generation SUs, which include chlorpropamide and tolbutamide, and more potent second generation SUs, which include gliclazide, glibenclamide (glyburide), glipizide and glimepiride, and they all share a sulfonylurea moiety in structure **(Table 1-3)**. After oral administration, the absorptions of SUs are rapid and complete, and peak serum SU concentrations are achieved within 3-4 hours [93]. SUs are extensively metabolized by the cytochrome P450 2C9 enzyme (*CYP2C9*) [97]. Most SUs have a relatively long duration of hypoglycemic activity up to 24 hours [93], and finally they are excreted through urine and feces. SUs can promote insulin secretion by blocking $K_{ATP}$ channels in pancreatic β-cells membrane. This blockage of $K_{ATP}$ channel inhibits potassium efflux and depolarizes the membrane potential, leading to an opening of voltage-gated calcium channels. Calcium influx and a corresponding increase in intracellular calcium levels, cause release of insulin from the β-cells to reduce plasma glucose level **(Figure 1-4)**. In terms of clinical response, SUs, on average, lower $Hb_{A1c}$

level by 1-2% [93]. However, SUs can be effective only with patients who have residual insulin secretory function of β-cells. For those patients with partial loss of insulin secretory function of β-cells, the effectiveness of SUs may be impaired. Given their mechanism of action that SUs stimulate insulin secretion and lower plasma glucose level in a glucose-independent manner, a major side effect of SUs is hypoglycemia. Furthermore, the hypoglycemic effect can be potentiated by prolonged exercise, irregular eating habits and excess consumption of alcohol [93]. Weight gain, approximately 1-4kg, is another concern of SUs, especially that many people with T2D are already obese. Adverse cardiovascular effects have also been reported for some SUs, such as deleterious effect on heart ischemic preconditioning, especially that people with T2D are predisposed to developing cardiovascular complications [98, 99].

Glinides which do not have a sulfonylurea moiety in structure, however, act in a similar way as SUs to promote insulin secretion. Commonly used glinides include repaglinide which is a benzoic acid derivative, nateglinide and mitiglinide which are D-phenylalanine derivatives. As compared with SUs, after oral administration, glinides have a more rapid onset of action, with a time to peak serum concentrations of 1-2 hours. Similarly as SUs, glinides undergo extensive hepatic metabolism by *CYP2C8*, *CYP2C9* and *CYP3A4* [100-102]. In addition, these agents have a much shorter duration of hypoglycemic activity only up to 4 hours, and they are excreted via bile and urine **(Table 1-3)**. Glinides are fully hepatically cleared and are much friendlier on elder patients with impaired renal function [101, 103, 104]. On average, glinides lower $Hb_{A1C}$ level by 1-1.5%.

Because of their rapid and short hypoglycemic activity, glinides reduce the risk of developing hypoglycemia, yet they need to be taken multiple times during the day, compared to the daily medication of SU treatments.

***Insulin Sensitizers: Biguanides and Thiazolidinediones***

Biguanides, a class of insulin sensitizers, can lower plasma glucose level by reducing hepatic gluconeogenesis and increasing glucose uptake in skeleton muscle. Metformin, the most commonly used biguanide agent, is recommended as a first-line option for the management of T2D by Canadian Diabetes Association, American Diabetes Association and European Association for the Study of Diabetes. It can be quickly absorbed and rapidly eliminated via urine without undergoing metabolism. Although the mechanism of action of metformin is complex and only partially understood at this point, evidence suggests that metformin activates adenosine monophosphate (AMP)-activated protein kinase [105], leading to suppression of hepatic glucose production and promotion of glycogen synthesis in the liver [106]. Additionally, metformin can enhance insulin sensitivity in peripheral tissues and decrease intestinal glucose absorption [107]. Based on clinical trial experience, therapeutic response to metformin varies considerably from patient to patient, such that metformin reduces $Hb_{A1C}$ level by 0.8-3% [108]. The side effects of metformin include diarrhea, dyspepsia, nausea and rarely lactic acidosis.

Thiazolidinediones (TZD), the other class of insulin sensitizers, can reduce plasma glucose level by improving insulin sensitivity in liver, skeletal muscle, and

adipose tissue via multiple actions on gene regulation [109]. Currently, there are two TZDs available on the market, pioglitazone and rosiglitazone, which share a thiazolidine-2-4-dione structure in common. After oral administration, TZDs undergo extensive hepatic metabolism, primarily by *CYP2C8* [110]. As mentioned previously, TZDs act as agonists of PPAR γ [79]. Although the mechanism of action of TZDs is only partially understood, it's commonly accepted that the activation of PPAR γ by TZDs promotes the expression of genes involved in the differentiation process of adipose tissue into more insulin-sensitive small adipocytes. These genes encode for lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid binding protein, glucokinase and the GLUT4 glucose transporter [79] [109] [111]. Also, there is considerable evidence suggesting that TZDs improve insulin sensitivity by inhibiting the expression and action of adipocyte-derived tumour necrosis factor-α (TNF-α) [109], which is over expressed in obesity. However, it may seem contradictory that Pro12 in PPAR γ2, with increased ability of transcriptional activation, predisposes carriers to T2D due to decreased insulin sensitivity; on the contrary, TZDs promoting transcriptional activation of PPAR γ2 can be beneficial to T2D as TZDs increase insulin sensitivity. Therefore, the precise molecular mechanism of TZDs is still a matter of debate. As TZDs achieve their hypoglycemic effect through controlling gene expression, TZDs begin their therapeutic effects in a period of 4–12 weeks after their initial use. On average, they reduce $Hb_{A1c}$ levels by 0.5-1.4%. In addition, they also lower free fatty acid and triglyceride levels in plasma. However, a recent safety announcement released from FDA claims that use of

Actos (pioglitazone) for more than one year may be associated with an increased risk of bladder cancer [112]. As the suggestion of increased risk of bladder cancer, France has suspended the use of pioglitazone and Germany has recommended not starting pioglitazone in new patients. Similarly, back in 2007, Nissen group reported a safety concern on the use of rosiglitazone manufactured by GlaxoSmithKline. They claimed that rosiglitazone was associated with a significant increase in the risk of myocardial infarction and with an increase in the risk of death from cardiovascular causes [113, 114]. Besides the risks in bladder cancer and myocardial infarction, other major concerns with TZDs are fluid retention, weight gain and some extent of hepatotoxicity. However, it has been suggested that the weight gain caused by TZD treatment is associated with an increase in subcutaneous fat rather than visceral fat, and it is known that decreased visceral fat relative to total body fat favors insulin sensitivity [79, 115].

***Incretin Potentiators: Incretin Mimetics and DPP4 Inhibitors***

As discussed previously, incretins, like GLP-1 and GIP secreted by intestinal L-cells and K-cells during a meal, are a group of gastrointestinal hormones, which promote second phase insulin secretion from pancreatic β-cells, and they also slow the rate of absorption of nutrients by reducing gastric emptying [116]. However, endogenous GLP-1 and GIP are degraded by serine protease dipeptidyl peptidase-4 (DPP4) a few minutes after they are secreted. Incretin potentiators, including incretin mimetics and DPP4 inhibitors, can therefore enhance the function of incretins by either mimicking incretins or inhibiting DPP4

protease. Exenatide, which is a long-lasting GLP-1 analog, has been used in U.S. since 2005. It acts as a full agonist at GLP-1 receptor, and it is resistant to DPP4 protease, having a longer half-life in vivo. Unlike SU treatments, exenatide improves insulin secretion in a glucose dependent manner as endogenous GLP-1, so users are at a lower risk of developing hypoglycemia compared to SU treatments [117]. However, as exenatide is a peptide, it has to be administered by subcutaneous injection, which is considered as its main disadvantage. Sitagliptin, a DPP4 protease inhibitor which can be taken orally as a tablet, has been developed and approved to use in 2006 [118]. Sitagliptin can reversibly inhibit the DPP4 protease and prolong the action of endogenous GLP-1 and GIP, resulting in an increased glucose-dependent insulin release from pancreatic β-cells. On average, sitagliptin reduces $Hb_{A1C}$ level by 0.5-0.8%, and users are at a lower risk of developing hypoglycemia compared to SU treatments. Some side effects of sitagliptin are abdominal pain, nausea, and diarrhea.

***α-glucosidase Inhibitors***

Other oral hypoglycaemic agents currently available are α-glucosidase inhibitors, such as acarbose, miglitol and voglibose, which are carbohydrate modulators. It has been suggested that delaying the absorption of carbohydrate in the intestine will provide an alternative strategy to compensate for the reduced pancreatic β-cell function. The α-glucosidase enzyme, located in the brush border of the intestine, converts complex carbohydrates and disaccharides into glucose and other monosaccharides before they can be transported [119]. Thus, α-

glucosidase inhibitors, competing with natural substrates sucrose and starch, delay the breakdown of complex carbohydrates, and consequently decrease the amount of glucose absorbed to plasma. On average, the α-glucosidase inhibitors reduce $Hb_{A1C}$ level by 0.5-0.8%, and are therefore considered less effective than other anti-diabetic medications. The most common side effect of this class of drugs is abdominal discomfort.

***Amylin Analogs***

Other injected hypoglycaemic agents available on the market are amylin analogs, such as pramlintide, which are also classified as amylin receptor agonists [120]. Amylin, a 37-amino acid peptide hormone, is synthesized primarily by pancreatic β-cells, and secreted into blood circulation along with insulin [121]. In general, amylin can suppress glucagon secretion, slow gastric emptying time, and reduce food intake. However, prolonged exposure to high plasma glucose level leads to increase of insulin and amylin secretion, and amyloid formation could be induced by the accumulation of amylin, which is observed in patients with T2D [121]. Therefore, in the invention of pramlintide, three amino acid residues Ala25, Ser28, and Ser29 on amylin, which are believed to promote amyloid formation, are replaced by three prolines, such that pramlintide maintains native amylin's biological activity but is not aggregating or adhesive [122]. From clinical trials experience, pramlintide reduces $Hb_{A1C}$ level by 0.4-0.7%. The most frequent side effect of pramlintide is nausea.

***Current Management of Type 2 Diabetes: Mono-therapy v.s. Combined-therapy***

Although numerous effective pharmacotherapies are available for T2D patients, their effectiveness and adverse effects are highly variable in a substantial number of T2D patients. This variability is often observed in both mono- and combined- therapy. While most T2D patients respond well initially to mono-therapy by anti-diabetic agents, in some patients, mono-therapy cannot achieve adequate glycemic control at its first initiation. This is quite common in the case of SU treatments. For example, 10-20% of SU-treated patients do not achieve acceptable glycemic control within their first three months of initiation, which is defined as primary sulfonylurea failure [123, 124]. Secondary failure is defined when patients achieve good glycemic control with initial mono-therapy; however, over time they are unable to maintain adequate glycemic control. Secondary failure rates for SU-treated patients are reported as 7% with gliclazide, 18% with glibenclamide and 25% with glipizide [125]. Meanwhile, T2D patients may also fail to respond to insulin sensitizer therapies. In A Diabetes Outcome Progression Trial (ADOPT), a long-term glycemic control study of T2D, a cumulative incidence of mono-therapy failure at 5 years was found to be 15% with rosiglitazone and 21% with metformin [114]. While some patients develop mono-therapy failure, other patients show higher sensitivity to the hypoglycemic effects of these agents. In the United Kingdom Prospective Diabetes Study (UKPDS), 31% of patients experienced mild SU-induced hypoglycemia during the first year of glibenclamide treatment [123]. Over time, a decrease in effectiveness of anti-diabetic treatments can result from a variety of factors including weight gain, high degree of insulin resistance, declining β-cell function, age of onset and genetic

variability. The most speculated reason of failure is progressive loss of insulin secretory capacity, in particular, β-cell exhaustion [126], which is seen with both sulfonylurea and metformin treatments [93]. As a large percentage of patients will not achieve and maintain adequate glycemic control with a single anti-diabetic agent, therefore, either addition of a second anti-diabetic agent or transition to insulin therapy becomes necessary to restore adequate glycemic control (plasma glucose level < 8.6mmol/L; $Hb_{A1C}$ level < 7% for adults in general ADA guidelines) [92, 94, 110].

Currently, in the aspect of clinical use, the concept of management of T2D has highlighted the combined-therapy, with a focus on the proper choice of agent combinations. As the complicated nature of T2D is the impairment in both insulin secretion and insulin sensitivity, SUs are particularly beneficial when combined with agents such as metformin that decreases insulin resistance. Metformin is normally considered as a first line agent in management of T2D, therefore, when patients experience secondary failure with metformin, adding a SU to metformin would be helpful. Right now, glibenclamide and metformin have been combined in one tablet [127, 128]. Furthermore, in patients with secondary SU failure, exogenous insulin plus SU therapy markedly improves glycemic control, which is in consistent with the most speculated reason of secondary SU failure as the progressive loss of insulin secretory capacity [129]. Although combined-therapy can offer an opportunity to increase effectiveness of mono-therapy, future study on contraindications of drug combination should be carried out more extensively.

## Section III Pharmacogenomics in Diabetes

### *Pharmacogenomics in Type 2 Diabetes*

Pharmacogenomics (PGx) is the study of relationships between genetic variants and inter-individual clinical outcomes, which can be used to achieve a better therapeutic outcome such as maximizing drug efficacy and preventing drug adverse effects. Despite a great number of publications on this subject, the practice of PGx has remained limited to only a few clinical fields such as oncology and psychiatry. It's common in clinical practice that considerable variability exists among patients in response to different T2D pharmacotherapies, which makes selecting an appropriate pharmacotherapy for T2D patients complicated because it's often difficult to predict the efficacy and adverse effects of a T2D pharmacotherapy on different patients. Although various clinical factors have been taken into account to make the decision of choosing the appropriate therapy, such as body weight, age, duration of diabetes, and other complications, a substantial number of patients still do not achieve adequate glycemic control, or they develop unpleasant adverse effects.

Genetic variation has been found to be not only a significant contributing factor to T2D pathogenesis, but also a key factor impacting the effectiveness of T2D pharmacotherapies. In recent years, PGx studies have linked the inter-individual variability in response to different T2D pharmacotherapies to genetic polymorphisms in drug transporters, drug targets and drug-metabolizing enzymes [110, 130, 131]. Therefore, the application of PGx to the field of T2D pharmacotherapies may help improve the management of T2D by determining the

extent of each genetic variant underlying individual difference in drug disposition (i.e. pharmacokinetics) and response (i.e. pharmacodynamics) **(Figure 1-5)**. The following paragraphs will explore the possible impact of genetic polymorphisms in drug-metabolizing enzyme, drug targets and drug transporters on the pharmacokinetics and pharmacodynamics of T2D pharmacotherapies.

To date, a great deal of progress has been made in the area of genetic variants in drug-metabolizing enzyme genes, mainly cytochrome P450 gene (*CYP*), affecting pharmacokinetics of T2D pharmacotherapies, especially sulfonylurea therapy. After oral ingestion of sulfonylureas, most are extensively metabolized in the liver, primarily by cytochrome P450 2C9 (*CYP2C9*) and secondarily by cytochrome P450 2C19 (*CYP2C19*) enzymes. Importantly, genetic polymorphisms in *CYP2C9* and *CYP2C19* are associated with significant differences in pharmacokinetics of sulfonylureas [132]. Recent data have shown that *CYP2C9*3* (Ile359Leu) carriers, who are poor metabolizers, are correlated with decreased oral clearance and increased plasma drug exposure of many sulfonylureas [97, 131]. Therefore, *CYP2C9*3* carriers may have higher chances of developing SU-induced hypoglycaemia, and should require significantly lower doses than normal metabolizers [133].

In recent years, the field of PGx studies on T2D pharmacotherapies has expanded beyond just genetic polymorphisms affecting pharmacokinetics. Several current PGx studies have now focused on genetic polymorphisms in drug targets, which affect pharmacodynamics. The therapeutic target of sulfonylureas, $K_{ATP}$ channel, has recently started receiving attention [134]. The impact of variants in

$K_{ATP}$ channel genes on sulfonylurea pharmacodynamics has been mainly investigated in the neonatal diabetic population, as most genetic variants in $K_{ATP}$ channel are predominately found in ND [135]. Although there haven't been so many attempts to investigate the impact of channel genetic variants on sulfonylurea pharmacodynamics in the T2D population, several clinical studies demonstrated that carriers of Ala1369 variant on SUR1 subunit of $K_{ATP}$ channel were associated with improved response to sulfonylurea gliclazide therapy, such as greater reductions in fasting plasma glucose and $Hb_{A1C}$ level [136, 137].

Lastly, available data suggest that drug transporters also play a vital role in the disposition of some T2D pharmacotherapies, particularly the glinides and metformin [110]. Consequently, genetic variants in drug transporters may contribute to inter-individual variability in the pharmacokinetics of these agents. For example, polymorphisms in organic anion transporting polypeptide 1B1 (OATP1B1) gene (*SLCO1B1*) have been shown to have significant impacts on the plasma concentrations of nateglinide and repaglinide [138]. As genetic variants existing in drug transporters, drug targets and drug-metabolizing enzymes, all have the abilities to affect clinical response of a therapy, future studies may need to take a comprehensive approach (i.e. multiple genes) to understand the inter-individual differences observed in clinical practice.

***Pharmacogenomics in Neonatal Diabetes***

As mentioned earlier, activation mutations in $K_{ATP}$ channel genes will lead to neonatal diabetes. However, prior to the discovery of activation mutations in

*KCNJ11* and *ABCC8* genes underlying neonatal diabetes, daily insulin therapy was the only effective treatment for ND patients in clinical practice. Since 2004, many ND patients with either *KCNJ11* or *ABCC8* activation mutations have been successfully treated with oral sulfonylureas, removing the requirement for insulin injections [139]. The SU dosage for ND patients can be quite high (up to 2.5 mg/kg/day of glibenclamide) compared to the dosage for patients with T2D (~0.2 mg/kg/day) [140]. However, the effectiveness of oral sulfonylureas varies from patient to patient depending on the genetic cause of ND [141]. The key issue remains as to whether each activation mutation in the $K_{ATP}$ channel can be inhibited by sulfonylureas at a reasonable concentration. So far, there are not many studies reporting that ND patients with activation mutations in *ABCC8* gene fail to respond to SU therapy. In contrast, several studies suggest that DEND patients with activation mutations in *KCNJ11* gene may be refractory to SU therapy [142], which is likely related to the underlying molecular mechanisms of mutations that alter $K_{ATP}$ channel function. For example, activation mutations on $K_{ATP}$ channel that reduce binding affinity of inhibitory ATP causing TNDM or PNDM show adequate efficacy of SUs [143]. However, DEND patients carrying Kir6.2 mutations that affect channel kinetics resulting in greatly increased intrinsic channel open probability do not respond well to SU therapy [144]. In general, the greater the ability of a mutation to increase the intrinsic channel open probability, the higher the SU dosage required to achieve the same level of channel inhibition seen in with mutations causing less severe ND, such as PNDM and TNDM. As SUs are unable to sufficiently inhibit $K_{ATP}$ channels with

mutations that cause a greatly enhanced intrinsic channel open probability, DEND patients with activation mutations in Kir6.2 subunit often require a combination of SU and insulin therapy, rather than SU therapy alone. Furthermore, the CNS dysfunction associated with DEND syndrome may also be improved with SU therapy [145, 146]. These latest findings further underscore the importance of molecular diagnosis of these genetic diseases in order to optimize treatment.

**Figure 1-1. Action potential generation in pancreatic β-cell.**

(Upper) In response of high glucose levels, β-cells generate action potentials (firing) to stimulate insulin secretion. (Lower) Deactivation of $K_{ATP}$ channels depolarizes membrane potential (Vm) from -70 mV to -50 mV, which reaches the threshold Vm of activation of $Ca_V$ channels. Activation of $Ca_V$ channels underlies the upstroke of action potential, and activation of $K_V$ and $K_{Ca}$ channels underlies the downstroke of action potential.

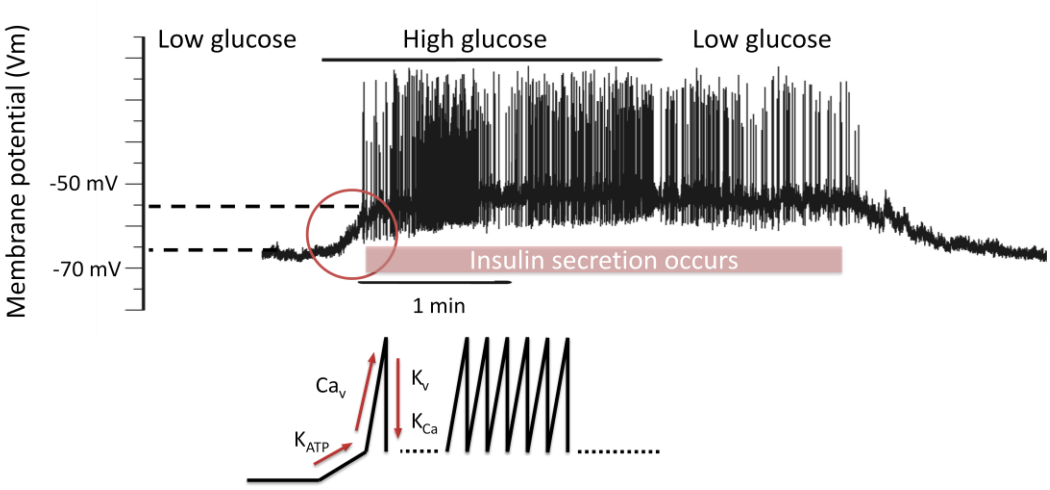

**Figure 1-2. Molecular make-up of the $K_{ATP}$ channel complex.**

(Lower left) $K_{ATP}$ channel is a hetero-octameric complex, comprised of 4 pore Kir6.2 subunits and 4 regulatory SUR1 subunits. (Upper) Membrane topology of SUR1 and Kir6.2 subunits of the $K_{ATP}$ channel. ATP binds to the Kir6.2 subunit, inhibiting $K_{ATP}$ channels. Hydrolysis of MgATP within the SUR1 subunit nucleotide-binding domains (NBDs) leads to generation of stimulatory MgADP. The A and B sites for sulfonylurea drug binding on both subunits are labeled as indicated. [135]

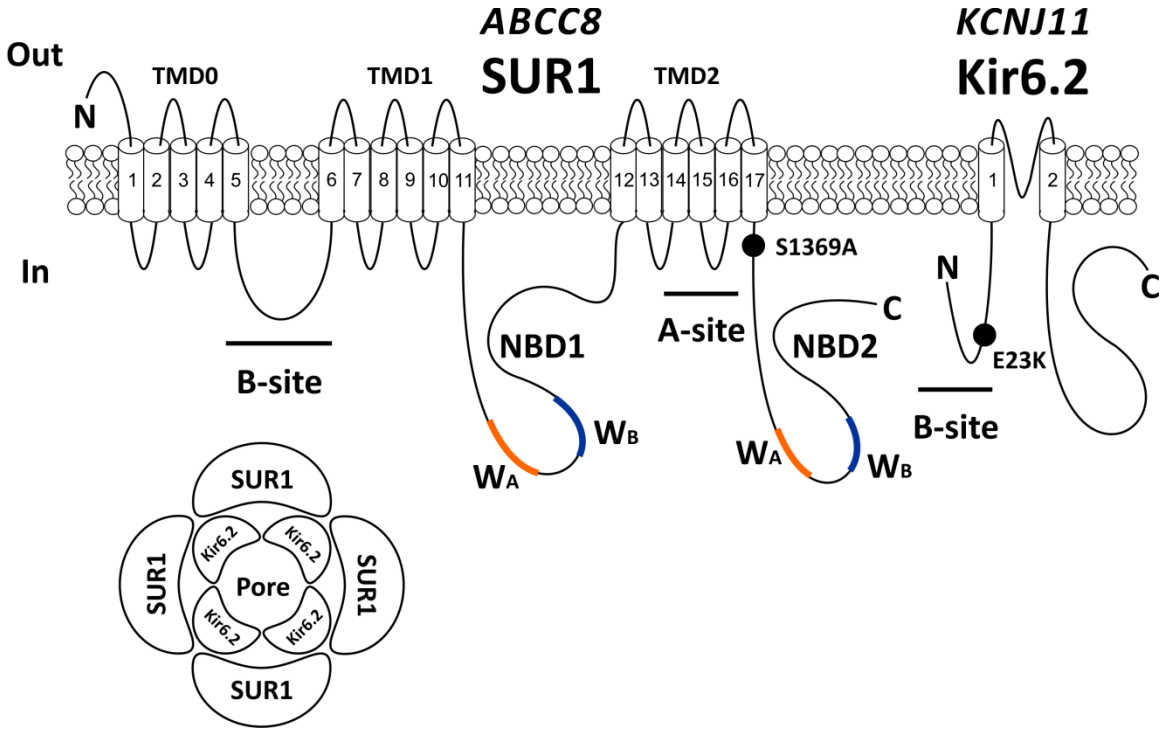

**Figure 1-3. Glucose stimulated insulin secretion in pancreatic β-cells.**

(Left) When plasma glucose is low, the decreased ratio of ATP/Mg-ADP will increase $K_{ATP}$ channel opening. Consequently, the cell membrane is hyperpolarized, preventing voltage-gated calcium channel opening, $Ca^{2+}$ influx and insulin secretion. (Right) When plasma glucose is high, glucose is transported into the cell via GLUT2. Glucose metabolism leads to an increased ratio of ATP/Mg-ADP, resulting in $K_{ATP}$ channel closure, membrane depolarization, opening of voltage-gated calcium channels, $Ca^{2+}$ influx and insulin secretion. [135]

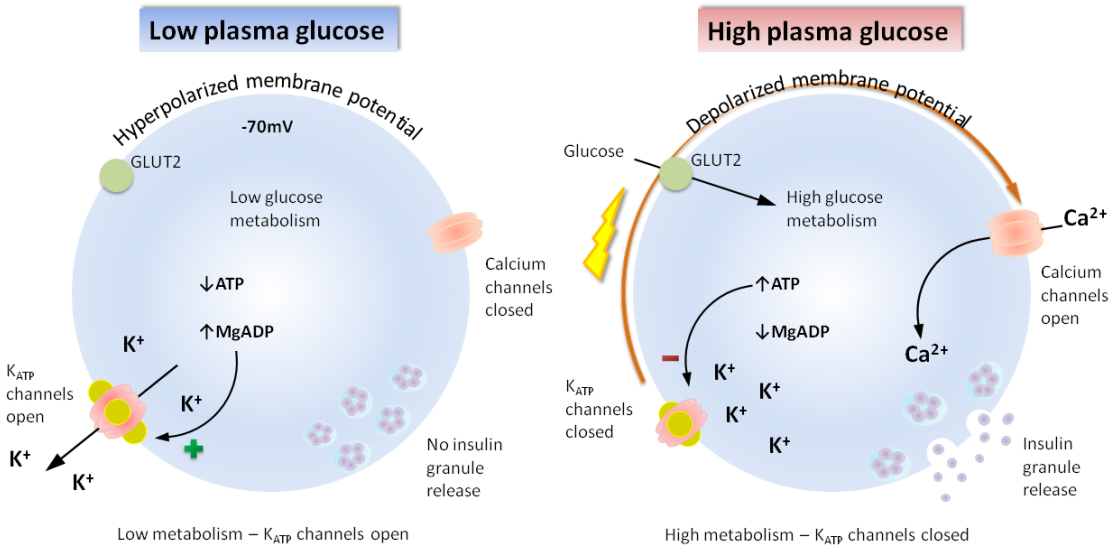

**Figure 1-4. Sulfonylureas stimulate insulin secretion in neonatal diabetes caused by $K_{ATP}$ channel mutations.**

(Left) Activation mutations in the $K_{ATP}$ channel prevent channel closure in response to high plasma glucose. Consequently, the membrane potential remains hyperpolarized even, thereby preventing insulin secretion. (Right) Sulfonylureas bind directly to $K_{ATP}$ channels causing channel inhibition that triggers membrane potential and insulin secretion resulting in a lowering of plasma glucose. [135]

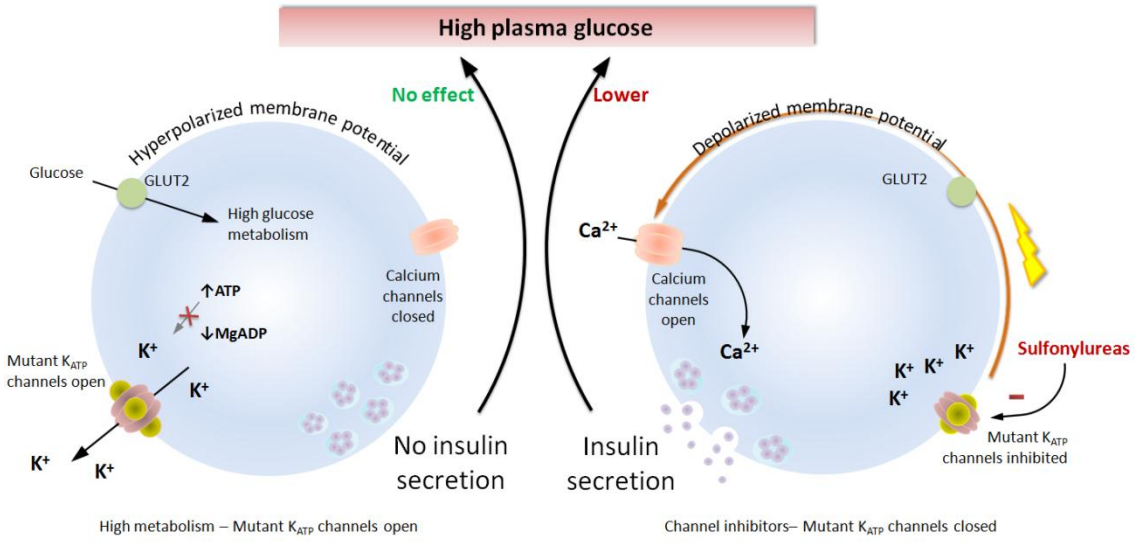

**Figure 1-5. Pharmacogenomics is the study on the relationship between genetic variants in patients and variability in the effects of drugs.**

Genetic variants in drug targets, drug metabolizing enzymes, and drug transporters, each playing a role in the pharmacodynamics / pharmacokinetics, will contribute to the inter-individual differences observed in clinical outcomes.[110]

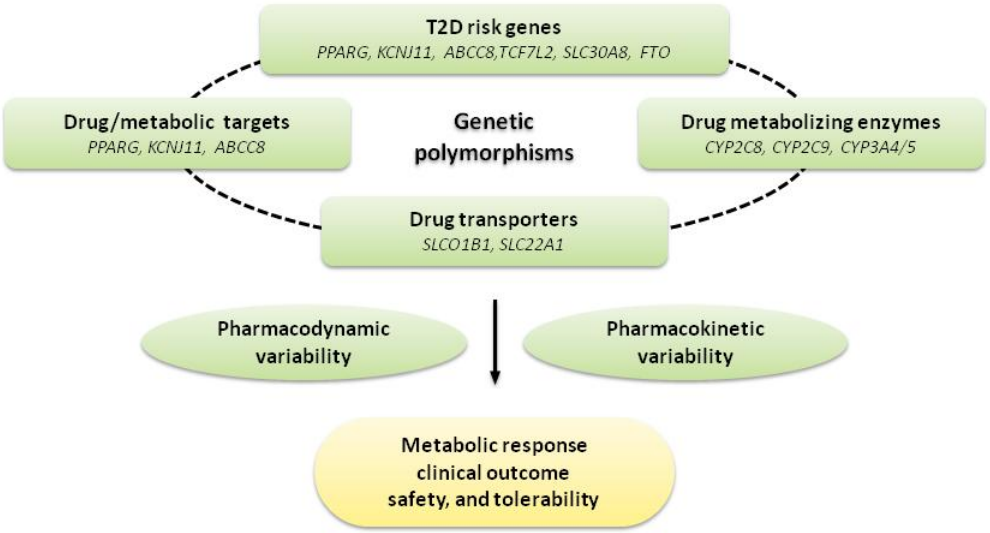

**Table 1-1 Mutations in $K_{ATP}$ channel genes *KCNJ11* and *ABCC8* causing hyperinsulinism of infancy (HI).[135]**

| Genotype | Position in protein | Molecular mechanism | Phenotype |
|---|---|---|---|
| | **Kir6.2 subunit *KCNJ11*** | | |
| **Y12Δ** | N terminus | Immature Kir6.2 subunits | HI |
| **R34H** | Interface between Kir6.2 subunits | | HI |
| **F55L** | Interface with SUR1 subunits | | HI |
| **K67N** | Slide helix | | HI |
| **W91R** | Linker between TM1 and pore region | | HI |
| **A101D** | Linker between TM1 and pore region | | HI |
| **S116P** | Pore region | | HI |
| **G134A** | Linker between pore region and TM2 | | HI |
| **R136L** | Linker between pore region and TM2 | | HI |
| **L147P** | TM2 | | HI |
| **A187V** | ATP binding site | | HI |
| **P254L** | ATP binding site | | HI |
| **H259R** | ATP binding site | Reduced trafficking of the channel | HI |
| **P266L** | C terminus | | HI |
| **E282K** | C terminus | | HI |
| **T294M** | Gating | Reduced channel Po | HI |
| **R301H** | Gating | | HI |
| **C344Δ** | C terminus | Immature Kir6.2 subunits | HI |
| | **SUR1 subunit *ABCC8*** | | |
| **G70E** | Linker between TM1 and TM2 | | HI |
| **R74Q/W** | Linker between TM1 and TM2 | | HI |
| **G111R** | TM3 | | HI |
| **A116P** | TM3 | | HI |
| **H125Q** | Linker between TM3 and TM4 | | HI |
| **V167L** | TM5 | | HI |
| **V187D** | TM5 | | HI |
| **N188S** | TM5 | | HI |
| **Q219Δ** | Linker between TM5 and | Immature SUR1 | HI |

| | | | |
|---|---|---|---|
| | TM6 | subunits | |
| **R248Δ** | Linker between TM5 and TM6 | Immature SUR1 subunits | HI |
| **N406D** | Linker between TM7 and TM8 | | HI |
| **N418R** | Linker between TM7 and TM8 | | HI |
| **L508P** | Linker between TM9 and TM10 | | HI |
| **F591L** | NBD1 | | HI |
| **R598Δ** | NBD1 | Immature SUR1 subunits | HI |
| **R620C** | NBD1 | | HI |
| **G716V** | Walker A in NBD1 | | HI |
| **C717Δ** | Walker A in NBD1 | Immature SUR1 subunits | HI |
| **R837Δ** | NBD1 | Immature SUR1 subunits | HI |
| **R842G** | NBD1 | | HI |
| **K890T** | NBD1 | | HI |
| **Q954Δ** | NBD1 | Immature SUR1 subunits | HI |
| **S957F** | NBD1 | | HI |
| **R999Δ** | NBD1 | Immature SUR1 subunits | HI |
| **T1139M** | Linker between TM13 and TM14 | | HI |
| **R1215Q/W** | Linker between TM15 and TM16 | | HI |
| **K1337N** | NBD2 | | HI |
| **W1339Δ** | NBD2 | Immature SUR1 subunits | HI |
| **G1343E** | NBD2 | | HI |
| **R1353P/H** | NBD2 | | HI |
| **V1361M** | NBD2 | | HI |
| **G1379R** | Walker A in NBD2 | Reduced Mg-nucleotide binding | HI |
| **G1382S** | Walker A in NBD2 | Reduced Mg-nucleotide binding | HI |
| **S1387F** | NBD2 | | HI |
| **F1388Δ** | NBD2 | Immature SUR1 subunits | HI |
| **R1394H** | NBD2 | Impaired trafficking of SUR1 subunits | HI |
| **G1401D** | NBD2 | | HI |
| **R1419H** | NBD2 | | HI |

| | | | |
|---|---|---|---|
| **R1421C** | NBD2 | Reduced Mg-nucleotide binding | HI |
| **R1437Q** | NBD2 | | HI |
| **A1458T** | NBD2 | | HI |
| **G1479R** | NBD2 | Reduced Mg-nucleotide binding | HI |
| **A1493T** | NBD2 | | HI |
| **R1494Q/W** | NBD2 | | HI |
| **E1507K** | Walker B in NBD2 | Reduced Mg-nucleotide binding | HI |
| **L1544P** | NBD2 | Impaired trafficking of SUR1 subunits | HI |
| **V1551D** | NBD2 | Reduced Mg-nucleotide binding | HI |
| **L1552V** | NBD2 | Reduced Mg-nucleotide binding | HI |
| **G1555S** | C terminus | | HI |

**Table 1-2 Mutations in $K_{ATP}$ channel genes *KCNJ11* and *ABCC8* causing diabetes in terms of DEND, PNDM, TNDM, MODY and T2D.[135]**

| Genotype | Position in structure | Molecular mechanism | Phenotype | Sensitivity to SU |
|---|---|---|---|---|
| | | **Kir6.2 subunit *KCNJ11*** | | |
| **E23K** | N terminus | | T2D | Normal sensitivity |
| **R34C** | Interface between Kir6.2 subunits | | TNDM | |
| **F35L/V** | Interface between Kir6.2 subunits | Increased channel Po | PNDM | Normal sensitivity |
| **C42R** | Interface between Kir6.2 subunits | Increased channel Po | PNDM/TNDM /MODY | |
| **H46Y** | Slide helix | Increased channel Po | PNDM | Normal sensitivity |
| **H46L** | Slide helix | Increased channel Po | iDEND | Normal sensitivity |
| **N48D** | ATP binding site | Decreased ATP binding affinity | PNDM | |
| **R50P/Q** | ATP binding site | Decreased ATP binding affinity | PNDM | Normal sensitivity |
| **Q52R** | Interface with SUR1 subunits | Increased channel Po | DEND | Reduced sensitivity |
| **G53R/S** | Interface with SUR1 subunits | Decreased ATP binding affinity | TNDM | Normal sensitivity |
| **G53N/D** | Interface with SUR1 subunits | Decreased ATP binding affinity | PNDM | Normal sensitivity |
| **V59G** | Slide helix | Increased channel Po | DEND | Reduced sensitivity |
| **V59M** | Slide helix | Increased channel Po | iDEND | Normal sensitivity |
| **F60Y** | Slide helix | | DEND | |
| **V64L** | Slide helix | | DEND | |
| **L164P** | Gating | | PNDM | Reduced sensitivity |
| **C166F/Y** | Gating | | DEND | Reduced sensitivity |
| **I167L** | Gating | Increased channel Po | iDEND | Normal sensitivity |
| **K170N/R/T** | ATP binding site | Decreased ATP binding affinity | PNDM | Normal sensitivity |
| **A174G** | ATP binding | | TNDM | |

| | | | | |
|---|---|---|---|---|
| | site | | | |
| **R176C** | ATP binding site | | PNDM | |
| **E179A** | ATP binding site | | TNDM | |
| **I182V** | ATP binding site | Decreased ATP binding affinity | TNDM | |
| **K185E** | ATP binding site | Decreased ATP binding affinity | DEND | |
| **R201C** | ATP binding site | Decreased ATP binding affinity | PNDM/DEND | Normal sensitivity |
| **R201H/L** | ATP binding site | Decreased ATP binding affinity | PNDM | Normal sensitivity |
| **E227K/L** | Gating | Increased channel Po | PNDM | Normal sensitivity |
| **E229K** | Gating | Increased channel Po | TNDM | |
| **V252A** | ATP binding site | Decreased ATP binding affinity | TNDM | |
| **E292G** | Gating | Increased channel Po | PNDM | |
| **T293N** | Gating | Increased channel Po | DEND | Reduced sensitivity |
| **I296L** | Pore | Increased channel Po | DEND | Reduced sensitivity |
| **E322K** | Interface between Kir6.2 subunits | Decreased ATP binding affinity | PNDM | Normal sensitivity |
| **Y330C/S** | ATP binding site | Decreased ATP binding affinity | PNDM/DEND | Normal sensitivity |
| **F333I** | Interface with SUR1 subunits | Increased Mg-ATP hydrolysis by NBD2 in SUR1 subunits | PNDM | Normal sensitivity |
| **G334D** | ATP binding site | Decreased ATP binding affinity | DEND | Reduced sensitivity |
| **I337V** | ATP binding site | | T2D | |
| **R365H** | C terminus | | TNDM | |
| | | **SUR1 subunit *ABCC8*** | | |
| **P45L** | TM1 | | PNDM | Normal sensitivity |
| **N72S** | Linker between TM1 and TM2 | | PNDM | |

| | | | | |
|---|---|---|---|---|
| **V86A/G** | TM2 | | PNDM | Normal sensitivity |
| **A90V** | TM2 | | PNDM | |
| **F132L/V** | Linker between TM3 and TM4 | Reduced ATP inhibitory effect in Kir6.2 subunits | DEND | |
| **L135P** | TM4 | | iDEND | |
| **R176C** | TM5 | | PNDM | |
| **P207S** | Linker between TM5 and TM6 | Reduced ATP inhibitory effect in Kir6.2 subunits | PNDM | |
| **E208K** | Linker between TM5 and TM6 | Reduced ATP inhibitory effect in Kir6.2 subunits | PNDM | Normal sensitivity |
| **D209E** | Linker between TM5 and TM6 | Reduced ATP inhibitory effect in Kir6.2 subunits | PNDM/TNDM | Normal sensitivity |
| **Q211K** | Linker between TM5 and TM6 | Reduced ATP inhibitory effect in Kir6.2 subunits | PNDM | Normal sensitivity |
| **D212I/N** | Linker between TM5 and TM6 | Reduced ATP inhibitory effect in Kir6.2 subunits | TNDM | |
| **L213R** | Linker between TM5 and TM6 | Reduced ATP inhibitory effect in Kir6.2 subunits | DEND | Normal sensitivity |
| **L225P** | Linker between TM5 and TM6 | | PNDM | Normal sensitivity |
| **T229I** | Linker between TM5 and TM6 | | TNDM | Normal sensitivity |
| **Y263D** | Linker between TM5 and TM6 | | PNDM | Normal sensitivity |
| **A269D** | Linker between TM5 and TM6 | | PNDM | |
| **R306H** | TM6 | | TNDM | |

| | | | | |
|---|---|---|---|---|
| **V324M** | TM6 | | TNDM | |
| **Y356C** | TM7 | | T2D | |
| **E382K** | Linker between TM7 and TM8 | | PNDM | |
| **C435R** | TM8 | | TNDM | |
| **L438F** | TM8 | | PNDM | |
| **L451P** | TM9 | | TNDM | |
| **L582V** | TM11 | | TNDM | |
| **R826W** | NBD1 | Increased channel activation by Mg-nucleotide | TNDM | |
| **H1024Y** | TM12 | | TNDM | Normal sensitivity |
| **R1183Q/W** | Linker between TM15 and TM16 | | TNDM | |
| **A1185E** | Linker between TM15 and TM16 | | PNDM | |
| **M1290V** | TM17 | | PNDM | |
| **R1314H** | NBD2 | | TNDM | Normal sensitivity |
| **E1327K** | NBD2 | | PNDM | |
| **S1369A** | NBD2 | | T2D | |
| **R1380C/H/L** | Walker A in NBD2 | Increased ATPase activity in NBD2 | TNDM | Normal sensitivity |
| **G1401R** | NBD2 | | PNDM | Normal sensitivity |
| **I1425V** | NBD2 | Increased channel activation by Mg-nucleotide | PNDM | Normal sensitivity |
| **V1524L/M** | NBD2 | | PNDM | Normal sensitivity |

**Table 1-3. Pharmacokinetics summary of clinically used sulfonylureas and glinides [147]**

| | Plasma half-life (hours) | Mode of metabolism | Rate of renal excretion | Daily dosage |
|---|---|---|---|---|
| First Generation SUs | | | | |
| Chlorpropamide (Diabinese) | 24-48 | Excreted intact by kidney | 80-90% in 4 days | 100-500 mg |
| Tolbutamide (Orinase) | 1-10 | Liver metabolism, Inactive metabolites | 80-90% in 2 days | 1-3 g |
| Second Generation SUs | | | | |
| Gliclazide (Diamicron) | 10-12 | Liver metabolism, Inactive metabolites | 60% in 4 days | 40-320 mg |
| Glibenclamide (Glynase) | 10-16 | Liver metabolism, Active metabolites | 50% in 5 days | 2.5-15 mg |
| Glipizide (Glucotrol) | 3-7 | Liver metabolism, Inactive metabolites | 90% in 3 days | 2.5-30 mg |
| Glimepiride (Amaryl) | 5-8 | Liver metabolism, Inactive metabolites | 60% in 7 days | 1-6 mg |
| Glinides | | | | |
| Repaglinide (Prandin) | 0.6-1.4 | Liver metabolism, Inactive metabolites | <8% by urine | 3-16 mg |
| Nateglinide (Starlix) | 1.5 | Liver metabolism, Inactive metabolites | 75% in 6 hours | 180-720 mg |
| Mitiglinide (Glufast) | 1-1.5 | Liver metabolism, Inactive metabolites | 54-74% in 24 hours | 15-60 mg |

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# Chapter 2

## Pharmacogenomic Analysis of ATP-Sensitive Potassium Channels Co-expressing the Common Type 2 Diabetes Risk Variants E23K and S1369A.

## Introduction

ATP sensitive potassium ($K_{ATP}$) channels were originally identified in cardiomyocytes by Noma in 1983 [1], and the physiological role of pancreatic β-cell $K_{ATP}$ channels was first demonstrated by Ashcroft *et al*. in 1984 [2]. Subsequently, $K_{ATP}$ channels have been found in other excitable cells, such as skeletal muscle, vascular smooth muscle and the central and peripheral nervous system. The molecular identity of the $K_{ATP}$ channel complex remained elusive until 1995 when the β-cell high-affinity sulfonylurea receptor (SUR1) and the inward rectifier subunit (Kir6.2) were cloned by Aguilar-Bryan *et al*. [3] and Inagaki *et al*. [4] respectively and were successfully reconstituted into functional pancreatic $K_{ATP}$ channels [4].

Pancreatic β-cell $K_{ATP}$ channel plays a key role in the control of insulin secretion by acting as the intracellular transducer of glucose metabolism. Elevation in plasma glucose causes an increase in the intracellular ATP/ADP ratio that inhibits $K_{ATP}$ channel activity and depolarizes of the β-cell membrane, triggering the opening of voltage-gated calcium channels. The subsequent increase in intracellular calcium stimulates the exocytosis of insulin-containing secretory granules [2]. It should be noted that this $K_{ATP}$ channel-dependent pathway is thought to be the primary "triggering" pathway for the major glucose-mediated $1^{st}$ phase insulin secretory response [5]. However, separate $K_{ATP}$ channel-independent pathways also exist that contribute to changes in basal insulin secretion [5]. Given the crucial role that $K_{ATP}$ channel plays in regulating insulin secretion, genetic mutations that alter appropriate β-cell $K_{ATP}$ channel

activity can have profound effects on insulin secretion. Furthermore, drugs that either inhibit or activate $K_{ATP}$ channel are used clinically to stimulate or suppress inappropriate insulin secretion respectively.

In 1985, with the advent of the patch-clamp technique, $K_{ATP}$ channel was identified as the molecular target for the action of the sulfonylureas [6, 7], which act as channel inhibitors on $K_{ATP}$ channels. Subsequent research using $I^{125}$-labelled sulfonylureas led to the purification, identification and cloning of the SUR1 subunit as the primary high affinity binding site for these drugs [3, 8]. In the last decade, a new class of insulinotropic drugs – the "glinides", have been developed and these drugs act by also inhibiting the β-cell $K_{ATP}$ channel [9, 10]. In contrast to the sulfonylureas, the glinides are more rapidly and completely metabolized in the liver. They are predominately used to control post-prandial hyperglycemia often observed in older patients and are also more appropriate for patients with impaired renal function [11-13]. The sulfonylurea and glinide drugs can be classified according to their $K_{ATP}$ channel binding sites as A-site, B-site, or AB-site drugs (**Figure 2-1B**) [14, 15, 16]. The A-site is contained within the cytosolic loops linking trans-membrane segments 14-16 of the SUR1 subunit; whereas the B-site, resides in the linker between the trans-membrane segments 5-6 of the SUR1 subunit and the N-terminus of the Kir6.2 subunit (**Figure 2-1A**) [15, 16]. The A-site within SUR1 subunit coordinates with the sulfonylurea moiety of the drug molecules while the B-site coordinates with the non-sulfonylurea carboxamido moiety of the drug molecules.

In direct contrast to rare monogenic mutations that result in overt insulin secretory disorders described in Chapter 1, common genetic variants in the *KCNJ11* and *ABCC8* genes may not manifest a clinical phenotype but are associated with an increased risk for diabetes. A common genetic variant, the K23 single nucleotide variant (E23K, rs5219) within the *KCNJ11* gene (**Figure 2-1A**) is associated with impaired glucose-stimulated insulin secretion in ~20% of the Caucasian T2D population [17, 18]. Moreover, the K23 variant has also been shown to be associated with T2D in almost every ethnic group examined [19-22]. As an estimated 10% of the world's population possesses 2 copies of K23, they may be at increased risk of developing T2D. Despite intense research on the K23 variant, the molecular mechanism(s) that increases T2D susceptibility have not been conclusively established, suggesting perhaps a more complex process underlies K23's association with T2D. It should also be noted that the *ABCC8* and *KCNJ11* genes are located adjacent to each other at the same chromosomal position (11p15.1). In this regard, another common genetic variant, the A1369 variant (S1369A, rs757110) within *ABCC8* gene is tightly inherited with the K23 variant – forming a T2D risk haplotype (K23/A1369), such that >95% of people with 2 copies of K23 also possess 2 copies of A1369 [18, 23-25]. Furthermore, the homozygous K23/A1369 risk haplotype is found in 15~19% in people with T2D, compared to 12~16% in people without diabetes, in nearly every ethnic group tested to date [18, 26-29]. To further highlight the risk of K23/A1369 haplotype, studies have shown that normoglycemic carriers of the K23/A1369 risk

haplotype developed diminished insulin secretory function in response to a glucose challenge [24, 30].

Our laboratory has recently performed mechanistic studies on human $K_{ATP}$ channels containing both E23K and S1369A variants to characterize the contributions of each variant to the intrinsic properties and pharmacology of the $K_{ATP}$ channel complex [31]. We observed a reduced ATP inhibition of channel activity in $K_{ATP}$ channels containing the K23/A1369 risk haplotype compared to the non-risk E23/S1369 haplotype. The precise molecular mechanism for the decreased ATP inhibition observed in $K_{ATP}$ channels expressing the K23/A1369 variant was investigated further. Our results indicate that the stimulatory effects of MgADP are unaltered in the K23/A1369 variant human $K_{ATP}$ channel, suggesting that the molecular mechanism for decreased ATP inhibition does not involve altered MgADP sensitivity. Furthermore, our results showed that the decrease in ATP inhibition in the K23/A1369 variant $K_{ATP}$ channel results from a direct effect of the SUR1 A1369 risk variant [31], perhaps via mild increases in the intrinsic $K_{ATP}$ channel MgATPase activity. Indeed, several rare heterozygous mutations in *ABCC8* that cause overt neonatal diabetes (R1380L/C) act by increasing MgATPase activity [32]. Interestingly, the SUR1 S1369 residue is located in proximity to the MgATPase catalytic site and residue R1380 in the SUR1 NBD2 [33]. Preliminary data from our laboratory has now confirmed that the MgATPase activity of the $K_{ATP}$ channel complex is increased in the presence of the A1369 variant. The substitution of the serine residue (S) with an alanine (A) disrupts a key hydrogen bond with an adjacent glutamine at position 1372 in SUR1, leading

to the observed increase in MgATPase activity. Collectively, these results indicate the importance of the A1369 *ABCC8* variant in the K23/A1369 risk haplotype and provide a rational molecular explanation for the observed increase in $K_{ATP}$ channel activity containing this haplotype and an increased risk for T2D.

As S1369A variant in SUR1 subunit and E23K variant in Kir6.2 subunit are located adjacent to the A-site binding pocket and B-site binding pocket, respectively, it raises a strong possibility that the inhibitory potencies of sulfonylurea and glinide drugs may be altered in $K_{ATP}$ channels containing both variants. Over a decade ago, there was an early report of unaltered sensitivity to tolbutamide (an A-site drug) in patients with the K23 variant [34]. Very recent studies suggest that E23K variant can alter sensitivities to AB-site sulfonylureas glimepiride and glibenclamide. For example, a recent study performed by Sesti and colleagues showed that K23 variant carriers were associated with an increased risk of secondary sulfonylurea failure compared with E23 homozygous carriers in Caucasian patients with T2D who were under AB-site sulfonylurea glibenclamide therapy (15mg per day) [35]. Another study examining the association of E23K with severe sulfonylurea-induced hypoglycemia conducted by Holstein and colleagues demonstrated that homozygous K23 T2D patients were associated with higher $Hb_{A1C}$ levels than T2D patients with E23 variant following AB-site sulfonylurea glimepiride or glibenclamide therapy; therefore, K23 homozygous carriers would have a decreased risk to develop severe sulfonylurea-induced hypoglycemia [36]. A clinical study on repaglinide, a B-site glinide drug, performed by He and colleagues, supported the idea that E23K variant in Kir6.2

subunit was associated with the therapeutic efficacy of repaglinide [37]. They provided the evidence that the decrease in $Hb_{A1C}$ was significantly greater in patients with K23 variant than patients who were E/E homozygous.

Interestingly, several recent clinical studies demonstrated that individuals with A1369 variant in SUR1 subunit displayed a higher therapeutic efficacy to A-site sulfonylurea gliclazide than S1369 carriers. For example, Zhang and colleagues demonstrated that A1369 T2D patients were more sensitive to sulfonylurea gliclazide therapy (40mg twice daily for 8 weeks) such that A1369 carriers had a more efficacious $Hb_{A1C}$ reduction than S1369 homozygous carriers (1.60% ± 1.39 *vs.* 0.76% ± 1.70, respectively; P = 0.044) [38]. A similar study by Feng and colleagues with a larger population cohort was published a year later. They showed that patients with A1369 variant had a significant decrease in fasting plasma glucose ($P < 0.001$) and a 3.5% greater decrease in $Hb_{A1C}$ ($P = 0.06$) than homozygous S1369 carriers after eight weeks of gliclazide treatment (40 mg gliclazide twice daily) [39]. Taken these studies together, it suggests that A1369 in SUR1 subunit is associated with improved therapeutic efficacy of gliclazide.

Collectively, the above mentioned clinical studies all indicate that E23K variant in Kir6.2 subunit and S1369A variant in SUR1 subunit are linked with therapeutic response of sulfonylurea and glinide treatments. Although their results may account for some part of the variability in drug response, there is always a limitation in these clinical studies, which is that they were performed with a primary focus of genetic variants affecting pharmacodynamics, leaving the

genetic variants affecting pharmacokinetics uncontrolled, as discussed in Chapter 1. As such, in order to tease out the pharmacokinetics from the question, it will be determined experimentally in-vitro whether genetic variants would affect sulfonylurea and glinide pharmacodynamics.

With the use of recombinant human $K_{ATP}$ channels, our previous study in 2009 [31] provided direct evidence at the single channel level that $K_{ATP}$ channels containing the K23/A1369 risk haplotype displayed an increased sensitivity to gliclazide that was conferred by the presence of the A1369 variant ($IC_{50}$ for E23/S1369 vs K23/A1369 = 188.7 ±32.6 vs. 52.7 ±11.1 nmol/l). As for this master project, a more thorough pharmacogenomic characterization of the $K_{ATP}$ channel will be established including eight sulfonylurea and glinide drugs used clinically (**Figure 2-1B**), which may provide the molecular basis for the eventual optimization of sulfonylurea/glinide therapy in the ~20% of patients homozygous for the K23/A1369 variants.

## Materials & Methods

### *Molecular Biology*.

The human $K_{ATP}$ channel Kir6.2 and SUR1 subunit clones were kindly provided by Dr. J. Bryan (Pacific Northwest Diabetes Research Institute, Seattle, WA). Clones were sub-cloned into the mammalian expression vector pcDNA3, and site-directed mutagenesis was performed (QuickChange; Strategene) to introduce the E23K variant into Kir6.2 (*KCNJ11*) and the S1369A variant into SUR1 (*ABCC8*). The sequences of generated mutation genes were confirmed through sequence analysis.

### *Cell Culture, Transfection, and Electrophysiology*.

Cultured tsA201 cells (an SV40-transformed version of the HEK293 human embryonic kidney cell line) were transfected, using the calcium phosphate precipitation technique, with human $K_{ATP}$ channel Kir6.2 and SUR1 subunit clones containing either the non-risk E23/S1369 haplotype or the risk K23/A1369 haplotype. Transfected cells were identified by co-expression of a green fluorescent protein plasmid (Life Technologies, Gaithersburg, MD). Macroscopic $K_{ATP}$ channel currents were recorded (pCLAMP 10.2, Axon Instruments) 48-72 hours after transfection, using the excised inside-out patch-clamp technique as described previously [40]. Pipettes were back-filled with solution containing the following components (in mmol/l): 134 KCl, 10 HEPES, 1.4 $MgCl_2$, 1 EGTA, 6

KOH, and 10 glucose. The pH of the pipette solution was adjusted to 7.4 with KOH. Rupture of membrane patches from transfected cells were performed in the bath solution containing (in mmol/l) 140 NaCl, 10 HEPES, 1 $CaCl_2$, 1.4 $MgCl_2$, 5 KCl and 10 glucose. The pH of the bath solution was adjusted to 7.4 with NaOH. Test solutions (same as pipette solution but with pH 7.2 adjusted with KOH) dissolving MgATP and drugs indicated in the context, were directly applied to the cytosolic side of the cell membrane patches using a rapid exchange multi-input perfusion pipette. Time to change solutions was <2s.

***Experimental Compounds.***

MgATP (Sigma, Oakville, ON) was prepared as a 10 mmol/l stock immediately prior to use. Tolbutamide, chlorpropamide, nateglinide, glibenclamide, glimepiride, glipizide, repaglinide (Sigma, Oakville, ON) and mitiglinide (Ryan Scientific Inc, Mount Pleasant, SC) were prepared as 10 mmol/l stocks in DMSO and stored at -20$^{o}$C until use. DMSO concentration did not exceed 0.1% in all experimental solutions. Drug stocks were sonicated prior to use and then diluted to the required concentration indicated in the context.

***Statistical Analysis.***

Recombinant macroscopic $K_{ATP}$ channel currents were normalized to and expressed as an increase in current relative to control current elicited by 5 mmol/l

MgATP. The assessment of drug inhibition was determined by the % of current remaining at steady-state after drug application in the presence of 0.1 mmol/l MgATP (i.e., $I_{drug}/I_{control}$ x 100, where $I_{drug}$ was the current elicited by 0.1 mmol/l MgATP with drug application and $I_{control}$ was the current elicited by 0.1 mmol/l MgATP alone.). Drug concentration–response curves were fitted with the Hill equation. Groups are compared by the unpaired student's t-test to determine statistical significance, with $P< 0.05$ considered statistically significant. Data were graphed as mean ± SEM.

## Results

### *K23/A1369 risk variant $K_{ATP}$ channels showed decreased potencies to A-site SUs tolbutamide and chlorpropamide.*

We have previously shown that the inhibitory potency of the A-site sulfonylurea gliclazide is increased in $K_{ATP}$ channels containing the K23/A1369 risk haplotype ($IC_{50}$ for E23/S1369 vs K23/A1369 = 188.7±32.6 vs. 52.7±11.1 nmol/l) [31]. Therefore, we examined the inhibitory effects of two other A-site sulfonylureas, tolbutamide and chlorpropamide that are in clinical use. Gliclazide, tolbutamide and chlorpropamide share a common sulfonylurea moiety but the latter two possess a distal aliphatic chain rather than a ring-fused pyrrole moiety found in gliclazide (**Figure 2-1B**).

If the previously observed increase in inhibitory potency of gliclazide on $K_{ATP}$ channels containing the K23/A1369 risk haplotype is due to a class effect that is conserved across other A-site drugs, we would expect to see a similar increase in inhibitory potency with tolbutamide and chlorpropamide. However, application of 3 μmol/l tolbutamide inhibited $K_{ATP}$ channels containing the K23/A1369 risk haplotype by only 57.86±2.52%, compared to 68.80±1.61% in $K_{ATP}$ channels containing the E23/S1369 non-risk haplotype (**Figure 2-2A,B**; $p < 0.05$). Similarly, 5 μmol/l chlorpropamide was less effective in inhibiting K23/A1369 $K_{ATP}$ channels compared to E23/S1369 $K_{ATP}$ channels (44.57±2.58 vs 52.95±2.04% inhibition for K23/A1369 vs. E23/S1369, $p < 0.05$, **Figure 2-2D,E**). Tolbutamide and chlorpropamide exhibit a biphasic inhibition with the high

affinity ($IC_{50}$~1 μmol/l) and low affinity ($IC_{50}$>1 mmol/l) binding sites. As only the high affinity inhibition is of therapeutic relevance, we constructed drug concentration-response curves to include the therapeutic relevant range (100 nmol/l–1mmol/l). Our results revealed that the high affinity $IC_{50s}$ for tolbutamide and chlorpropamide were shifted to higher concentrations in $K_{ATP}$ channels containing the K23/A1369 risk haplotype compared to the E23/S1369 non-risk haplotype (tolbutamide $IC_{50}$ for E23/S1369 vs K23/A1369 = 0.71±0.01 vs. 1.15±0.08 μmol/l and chlorpropamide $IC_{50}$ for E23/S1369 vs K23/A1369 = 3.04±0.22 vs. 4.19±0.47 μmol/l, **Figure 2-2C,F**). These results suggest that the non-sulfonylurea region of the A-site drug structure determines the inhibitory potency against $K_{ATP}$ channels containing the non-risk or risk haplotypes.

***K23/A1369 risk variant $K_{ATP}$ channels showed an increased potency to A-site glinide mitiglinide but comparable potency to nateglinide.***

The glinides are another class of A-site drugs in clinical use that are structurally unrelated to the sulfonylureas. For example, mitiglinide and nateglinide lack the sulfonylurea moiety but are able to bind to the A-site binding pocket on SUR1 subunit. Therefore, it is possible to gain further insights into which structural motifs confer the altered inhibitory potency in the $K_{ATP}$ channel non-risk and risk haplotypes. Mitiglinide and nateglinide share the same phenylalanine derivative moiety, although only mitiglinide possesses a ring-fused pyrrole group also found in gliclazide (**Figure 2-1B**).

Macroscopic current recordings of $K_{ATP}$ channels containing the K23/A1369 risk haplotype were inhibited to a greater extent by 10 nmol/l mitiglinide compared to currents recorded from $K_{ATP}$ channels containing the E23/S1369 non-risk haplotype (% inhibition = 41.99±2.82 vs 24.49±2.83 for K23/A1369 vs. E23/S1369 respectively, $p < 0.05$, **Figure 2-3A,B**). Construction of the mitiglinide concentration-response curves confirmed that mitiglinide sensitivity was enhanced in $K_{ATP}$ channels containing the K23/A1369 risk haplotype compared to the E23/S1369 non-risk haplotype ($IC_{50s}$ = 9.73±1.67 vs 28.19±1.04 nmol/l for K23/A1369 vs. E23/S1369 respectively, **Figure 2-3C**). In direct contrast to the observed effects of mitiglinide, macroscopic current recordings from $K_{ATP}$ channel containing either the E23/S1369 non-risk or K23/A1369 risk haplotypes were inhibited to a similar extent by 50 nmol/l nateglinide (% inhibition = 66.55±1.77 vs. 65.55±2.26 for E23/S1369 vs. K23/A1369 respectively, **Figure 2-3D,E**). Construction of nateglinide concentration-response curves (**Figure 2-3F**) also confirmed that there was no significant difference in the effect of nateglinide inhibition on macroscopic currents recorded from $K_{ATP}$ channels containing either haplotype ($IC_{50}$ = 17.12±2.56 vs. 17.18±0.93 nmol/l for E23/S1369 vs. K23/A1369, respectively).

***K23/A1369 risk variant $K_{ATP}$ channels showed a decreased potency to AB-site SUs glimepiride but comparable potency to glibenclamide and glipizide.***

Glibenclamide (glyburide), glipizide and glimepiride are AB-site sulfonylureas currently in clinical use. Glibenclamide, glipizide and glimepiride structures share a sulfonylurea moiety and almost identical A-site binding region but differ significantly in structure in the B-site carboxamido motif (**Figure 2-1B**). Repaglinide, another glinide drug, is the only B-site drug currently in clinical use, whose structure is quite different from other A-site glinide drugs, i.e. mitiglinide, nateglinide. As the N-terminal of the Kir6.2 subunit, where the E23K variant resides, is thought to constitute the B-site binding pocket, we tested the effects of these three AB-site sulfonylureas and one B-site glinide on $K_{ATP}$ channels with non-risk or risk haplotypes.

Macroscopic $K_{ATP}$ currents containing either haplotype were equally inhibited by 3 nmol/l glibenclamide (% inhibition was 58.08±1.51 vs 55.91±2.39 for E23/S1369 and K23/A1369 respectively, **Figure 2-4A,B**); a finding consistent with our previous report. [31] The glibenclamide concentration-response curves (**Figure 2-4C**) also confirmed that there was no significant different inhibition in $K_{ATP}$ channels containing either haplotype ($IC_{50}$ = 1.55±0.26 vs 2.78±0.65 nmol/l for E23/S1369 and K23/A1369 respectively). In contrast to glibenclamide, glimepiride (5 nmol/l) inhibited macroscopic currents from $K_{ATP}$ channels containing the E23/S1369 non-risk haplotype to a greater extent than currents from $K_{ATP}$ channels containing the K23/A1369 risk haplotype (% inhibition = 64.98±0.93 vs 53.70±0.83 for E23/S1369 and K23/A1369 respectively, $p < 0.05$,

**Figure 2-4D,E**). Glimepiride concentration-response curves yielded $IC_{50s}$ of 2.41±0.25 and 4.38±0.44 nmol/l for E23/S1369 and K23/A1369 (**Figure 2-4F**). Finally, glipizide (100nmol/l) inhibited macroscopic $K_{ATP}$ currents equally from either haplotype (% inhibition = 76.21±2.69 vs 70.88±2.56 for E23/S1369 and K23/A1369 respectively, **Figure 2-4G,H**). The glipizide concentration-response curves (**Figure 2-4I**) also verified that there was no significant different inhibition in $K_{ATP}$ channels containing either haplotype ($IC_{50}$ = 22.7±6.5 vs 32.3±10.0 nmol/l for E23/S1369 and K23/A1369 respectively).

***K23/A1369 risk variant $K_{ATP}$ channels showed a decreased potency to B-site glinide repaglinide.***

Interestingly, compared to the currents recorded from $K_{ATP}$ channels containing the K23/A1369 risk haplotype, the macroscopic current recordings from $K_{ATP}$ channels containing the E23/S1369 non-risk haplotype were inhibited to a greater extent by repaglinide only at high concentrations, such as in a concentration greater than 30 nmol/l. (% inhibition = 70.28±1.14 vs 60.17±1.59 for E23/S1369 and K23/A1369 respectively, $p < 0.05$, **Figure 2-5A,B**). Unlike other sulfonylurea and glinide drugs tested, which showed their maximum difference in inhibition effect at the concentration around their $IC_{50}$, repaglinide inhibited $K_{ATP}$ channels containing the E23/S1369 non-risk haplotype to a greater extent than channels containing the K23/A1369 risk haplotype only at higher concentrations. This can be further demonstrated by the construction of

concentration-response curves, which confirmed that repaglinide sensitivity was enhanced in $K_{ATP}$ channels containing the E23/S1369 non-risk haplotype compared to K23/A1369 risk haplotype at high repaglinide concentrations, but this difference was not significant at low repaglinide concentrations ($IC_{50s}$ = 9.32±0.75 vs 10.05±1.59 nmol/l for E23/S1369 vs. K23/A1369 respectively, **Figure 2-5C**).

## Discussion

In our previous study, we established that the A-site sulfonylurea gliclazide displayed an increased inhibitory potency on $K_{ATP}$ channels containing the K23/A1369 risk haplotype [31]. This observation provided the first direct evidence that the K23/A1369 risk haplotype confers altered A-site drug sensitivity to the $K_{ATP}$ channel complex, although whether this is specific to gliclazide alone or common among other A-site drugs was not investigated. In this present study, we performed a comprehensive study on the most commonly prescribed A-/B-site and AB-site drugs with respect to their structure activity relationships and inhibitory potencies on $K_{ATP}$ channels containing either the E23/S1369 non-risk or K23/A1369 risk haplotypes. Our findings provide important information on drug structure that has implications for the clinical management of 15~19% of the population with type 2 diabetes.

Our results on $K_{ATP}$ channels containing either E23/S1369 or K23/A1369 haplotypes confirm that the 2$^{nd}$ generation sulfonylurea gliclazide is a much more potent inhibitor than the 1$^{st}$ generation sulfonylureas tolbutamide and chlorpropamide respectively ($IC_{50s}$ summarized in **Table 2-1**). The structure of gliclazide can be divided into two parts: the sulfonylurea and ring-fused pyrrole moieties. Whereas chlorpropamide and tolbutamide also possess the conserved sulfonylurea moiety, they possess a flexible aliphatic side chain rather than the ring-fused pyrrole group found in gliclazide (**Figure 2-1B**). This structural difference accounts for the increased potency of gliclazide compared to either chlorpropamide or tolbutamide. Furthermore, our observation that tolbutamide is

a less potent inhibitor of K23/A1369 risk haplotype $K_{ATP}$ channels is consistent with previous findings that homomeric K/K variant $K_{ATP}$ channels had a reduced sensitivity to tolbutamide compared to homomeric E/E variant channels [41]. We also observed a similar result with chlorpropamide that is almost structurally identical to tolbutamide.

Taken together, these results strongly infer that it is the individual A-site sulfonylurea structure that determines its inhibitory potency against the $K_{ATP}$ channel non-risk and risk haplotypes rather than a conserved class effect among all A-site sulfonylureas. Specifically, these findings infer that the presence of the ring-fused pyrrole moiety in gliclazide bestows an increased inhibition of $K_{ATP}$ channels containing the K23/A1369 haplotype. To test this notion further, we chose to investigate the inhibitory potencies of two clinically used glinide drugs, mitiglinide and nateglinide. These drugs are $K_{ATP}$ channel inhibitors with $IC_{50s}$ in the nanomolar range (**Table 2-1**) but are d-phenylalanine derivatives rather than sulfonylureas. Mitiglinide, like gliclazide, also possesses a ring-fused pyrrole (**Figure 2-1B**) and displays a significantly increased inhibition of $K_{ATP}$ channels containing the K23/A1369 risk haplotype compared to the E23/S1369 non-risk haplotype (**Table 2-1**). In comparison, nateglinide does not possess the ring-fused pyrrole group and displays no inhibitory preference for either haplotype (**Table 2-1**). These results further support the notion that, in the two structurally distinct drug classes tested, the sulfonylureas and the glinides, it is the presence of a ring-fused pyrrole moiety in both drug classes which underlies the observed increase in inhibitory potency in $K_{ATP}$ channels containing the K23/A1369 risk haplotype.

The clinically used AB-site sulfonylureas glibenclamide (glyburide), glipizide and glimepiride are high affinity $K_{ATP}$ channel inhibitors with $IC_{50s}$ in the nanomolar range. Surprisingly, we observed that glimepiride, but not glibenclamide or glipizide, showed a higher inhibitory potency towards $K_{ATP}$ channel containing the E23/S1369 non-risk haplotype. The major structural differences between these three drugs lie in the non-sulfonylurea carboxamido motif thought to be responsible for binding to the B-site on the $K_{ATP}$ channel complex (**Figure 2-1B**). Furthermore, the B-site glinide repaglinide also displayed a higher inhibitory potency towards $K_{ATP}$ channel containing the E23/S1369 non-risk haplotype at higher repaglinide concentrations. Although in our previous study [31], we found no differences in the inhibitory potency of repaglinide between the E23/S1369 and K23/A1369 haplotypes at 10nmol/l of repaglinide, result from this study showed that the differences in the inhibitory potency of repaglinide wouldn't be significant at low repaglinide concentrations. These results suggest that the K23 variant, located within the putative B-site also contributes to haplotype-specific differences in the inhibitory potency of sulfonylureas and glinides. Interestingly, a recent study demonstrated a reduced inhibition by the A-site sulfonylurea tolbutamide in $K_{ATP}$ channels containing only the K23 variant [41]. Taken together, these results infer that the A- and B-sites must be either physically adjacent or allosterically-linked, and that the close proximity of the A1369 and K23 variants to the A- and B-sites respectively (**Figure 2-1A**) contribute to altered affinities to both A-/B- and AB-site drugs.

The inhibitory potencies of the sulfonylurea and glinide drugs tested in this study have been documented previously using similar techniques [42-45], but have been reported varying $IC_{50}$ values compared to the values obtained in this study. These differences may be explained by species-specific differences between human and rodent $K_{ATP}$ channel clones or by the differences of nucleotide (ATP and ADP) concentration used in the test solutions [46, 47]. In this latter regard, it should be noted that all the drugs tested in this study were applied in the presence of MgATP that represents a more physiological condition. It has previously been shown that $K_{ATP}$ channels comprised of Kir6.2 and SUR1 subunits exhibit enhanced sulfonylurea block in the presence of Mg-nucleotides. Reimann and the colleagues concluded that sulfonylureas inhibited $K_{ATP}$ channel activity by impairment of either MgADP binding or the transduction pathway between the SUR1 NBDs and the Kir6.2 subunits [48]. Therefore, we speculate that the K23 and A1369 variants may contribute to alterations in this transduction pathway and subsequently affect sulfonylurea/glinide inhibition in a drug-structure dependent manner. This concept is further supported by the facts that 1) the A1369 variant is in close proximity to both the A-site and SUR1 NBD2 and 2) the K23 variant is close to both B-site and N-terminal of Kir6.2 thought to directly interact with the SUR1 NBDs [49, 50].

Our findings have potential relevance for the clinical management of diabetes with sulfonylurea and glinide drugs. The concentration range over which we observed significant differences in sulfonylurea/glinide potencies are similar to the plasma concentrations observed in clinical practice for type 2 diabetes [11,

51-55]. It has been suggested that sulfonylureas administered at higher doses are the therapies of choice for patients with neonatal diabetes caused by the most pronounced activating mutations in *KCNJ11* and *ABCC8* genes [56]. Our present study demonstrates that $K_{ATP}$ channels carrying K23/A1369 haplotype are more sensitive to gliclazide and mitiglinide inhibition; whereas channels containing the E23/S1369 haplotype are more sensitive to tolbutamide, chlorpropamide, glimepiride and repaglinide. Although other factors, such as drug metabolizing enzyme, also play important roles in determining the effectiveness of the drugs [57], this doesn't preclude the odds that desired therapeutic efficacy could be achieved simply by adjusting dosage. Thus, a potential clinical application would be that dose escalation could take place for people who are more sensitive to certain drugs to minimize adverse effects and for people who are less sensitive to specific drugs to optimize drug efficacy. From a structural perspective, we have identified the ring-fused pyrrole group as a distinct drug structure motif that directly confers an increased inhibition to $K_{ATP}$ channels containing the K23/A1369 risk haplotype, which supports the finding that patients who are homozygous for this haplotype are more responsive to the A-site sulfonylurea gliclazide [39]. These results suggest that it may be possible to tailor existing therapies according to patient genotype/haplotype to optimize drug effectiveness or to minimize undesired side effects such as hypoglycemia or cardiotoxicity. For example, 1) we observed no differences in glibenclamide's inhibitory potency between the two $K_{ATP}$ channel haplotypes tested, and this AB-site drug also inhibits the cardiac $K_{ATP}$ channel isoform that may contribute to cardiotoxicity

[58]; 2) The A-site sulfonylureas tolbutamide and chlorpropamide are still in clinical use in Europe, and they possess a reduced inhibitory potency against $K_{ATP}$ channels containing the K23/A1369 risk haplotype while exhibiting selectivity towards SUR1 vs SUR2A. As gliclazide displays an increased inhibition of the $K_{ATP}$ channel risk haplotype and is selective for SUR1, then this drug may represent a more effective choice for current sulfonylurea therapy in both $K_{ATP}$ channel haplotypes. Subtle differences in sulfonylurea/glinide efficacies on $K_{ATP}$ channels containing the non-risk or risk haplotypes may in part explain the observed individual variability in sulfonylurea treatment in T2D patients.

The current pharmacogenomic studies of different sulfonylureas in human have been contradictory, which result from the following 3 major limitations existing in conducted studies. First is the relatively small number of patients studied and limited range of ethnicities (i.e. Asians). Second, most studies only examined variants in individual gene of interest, ignoring the contribution of variants from other genes that can also impact clinical outcomes. No human studies accounted for both variants in genes responsible for drug metabolizing enzymes and drug targets comprehensively. Lastly, as this present study demonstrated, sulfonylureas can be classified into detailed class according to their structure, which previous human studies have not accounted for. As we have demonstrated, the efficacies of SUs are highly dependent on their specific structural motifs and certain $K_{ATP}$ channel variants. Compared with the clinical studies mentioned in the introduction section, our findings on glimepiride are partially consistent with the report showing that patients carrying the K23 allele

exhibited a reduced response to sulfonylureas glibenclamide and glimepiride treatments [36]. Our observations that glibenclamide displayed no inhibitory preference between either $K_{ATP}$ channel haplotype instead of what Sesti *et al* claimed in their clinical study [35], and that repaglinide displayed increased sensitivity on $K_{ATP}$ channel with E23/S1369 haplotype rather than on $K_{ATP}$ channel with K23 variant as claimed in the clinical study by He *et al* [37], highlight the fact that many other factors, such as β-cell exhaustion and drug-metabolizing enzyme variants, can also result in variability in the individual sulfonylurea response [57]. However, our findings provide further direct evidence that the $K_{ATP}$ channel haplotype likely contributes to the overall efficacy of the sulfonylurea/glinides which is dependent on the individual drug structure.

In summary, this study represents the first comprehensive pharmacogenomic characterization of the common $K_{ATP}$ channel genetic haplotypes and provides a mechanistic rationale, based on drug structure, for the optimization of drug therapy with respect to the common $K_{ATP}$ channel haplotypes.

**Figure 2-1.** Membrane topology of the $K_{ATP}$ channel SUR1 and Kir6.2 subunits. Nucleotide-binding domains (NBDs) 1 and 2 as well as the A-/ B- binding sites for drugs are labeled as indicated. The common genetic variants E23K and S1369A tested in this study are labeled. The structures and binding-site classification of the sulfonylureas and glinides investigated in this study.

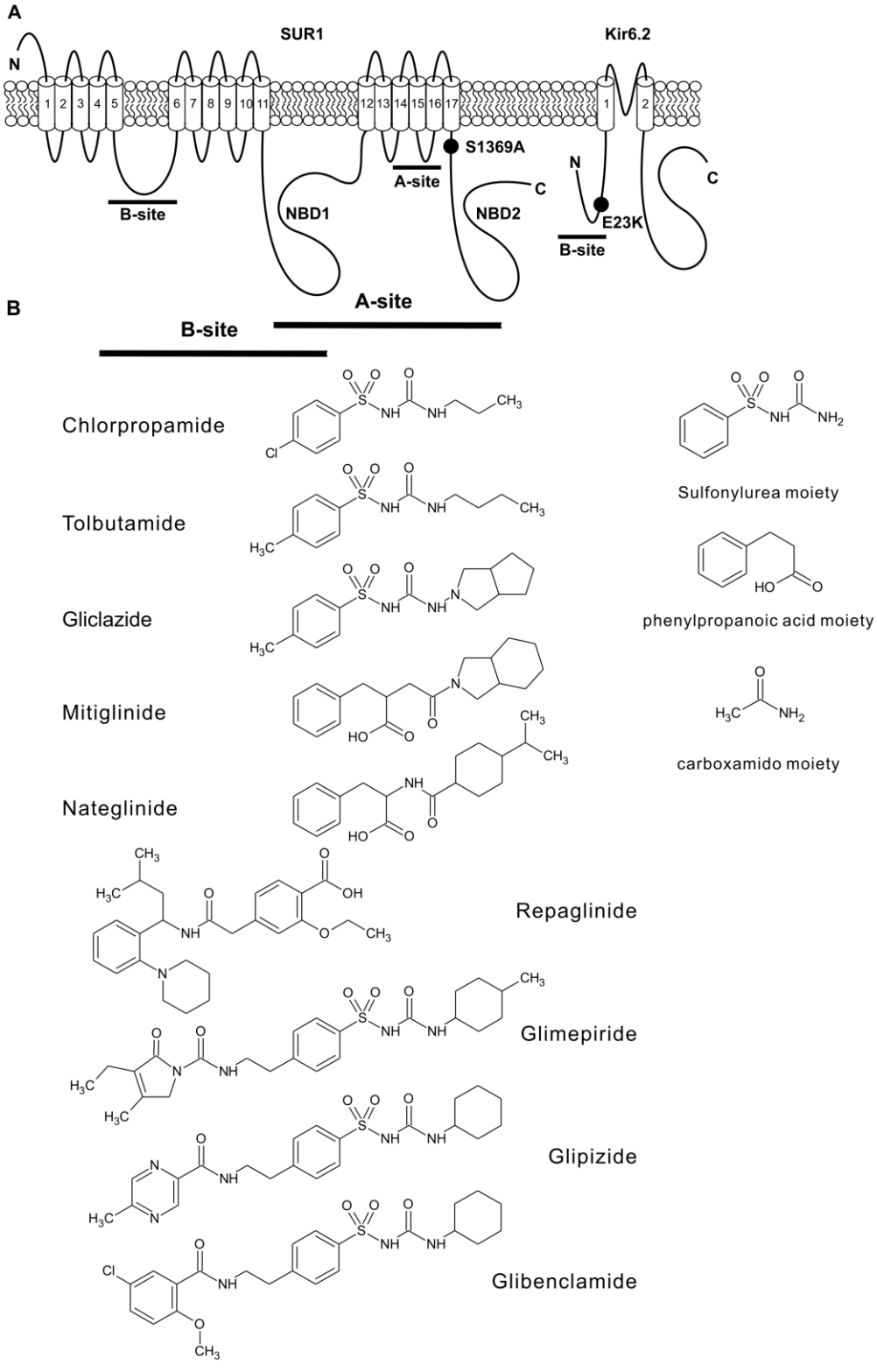
A
SUR1
Kir6.2
N
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 1 2
S1369A
A-site
B-site
NBD1
NBD2
C
N
E23K
B-site
C
B
A-site
B-site
Chlorpropamide
Tolbutamide
Gliclazide
Mitiglinide
Nateglinide
Repaglinide
Glimepiride
Glipizide
Glibenclamide
Sulfonylurea moiety
phenylpropanoic acid moiety
carboxamido moiety

**Figure 2-2.** The A-site sulfonylureas tolbutamide and chlorpropamide are less potent inhibitors of $K_{ATP}$ channels containing the K23/A1369 risk haplotype. **A,B,D,E.** Representative macroscopic current recordings showing tolbutamide (Tolb, 3μmol/l) and chlorpropamide (Chlor, 5μmol/l) inhibition of $K_{ATP}$ channels containing either the E23/S1369 non-risk or K23/A1369 risk haplotype. **C,F** Concentration-response curves illustrating that the K23/A1369 variant channels are less sensitive to inhibition tolbutamide and chlorpropamide (E23/S1369 vs. K23/A1369 $IC_{50s}$ = 0.71±0.01 vs. 1.15±0.08μmol/l (Tolb) and 3.04±0.22 vs. 4.19±0.47μmol/l (Chlor). Solid bar denotes therapeutic concentration range. n=7-11 patches per concentration. * denotes $p<0.05$.)

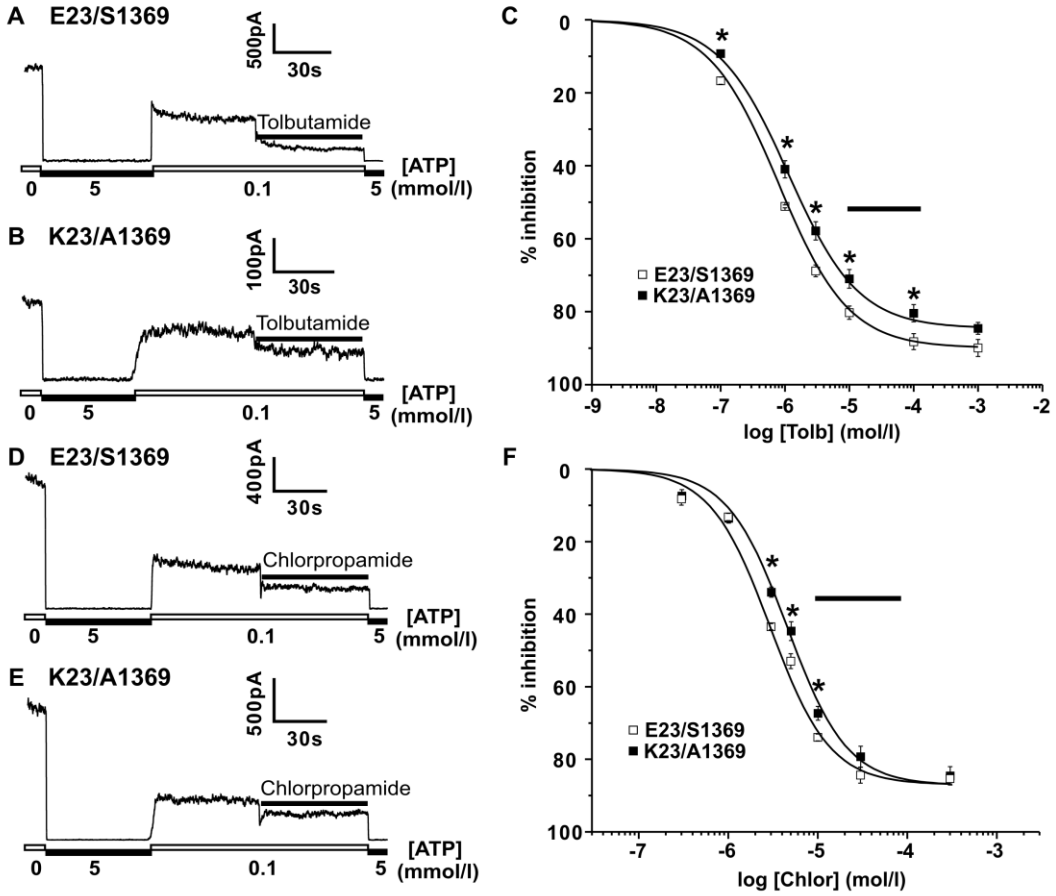

**Figure 2-3.** The A-site drug mitiglinide, but not nateglinide, is a more potent inhibitor of $K_{ATP}$ channels containing the K23/A1369 risk haplotype. **A,B,D,E.** Representative macroscopic current recordings showing mitiglinide (10nmol/l) and nateglinide (50nmol/l) inhibition of $K_{ATP}$ channels containing either the E23/S1369 non-risk or K23/A1369 risk haplotype. **C,F** Concentration-response curves illustrating that the K23/A1369 variant channels are more sensitive to inhibition by mitiglinide but not nateglinide (E23/S1369 vs. K23/A1369 $IC_{50s}$ = 28.19±1.04 vs. 9.73±1.67nmol/l (Miti) and 17.12±2.56 vs. 17.18±0.93nmol/l (Nate). Solid bar denotes therapeutic concentration range. n=6-13 patches per concentration. * denotes $p<0.05$.)

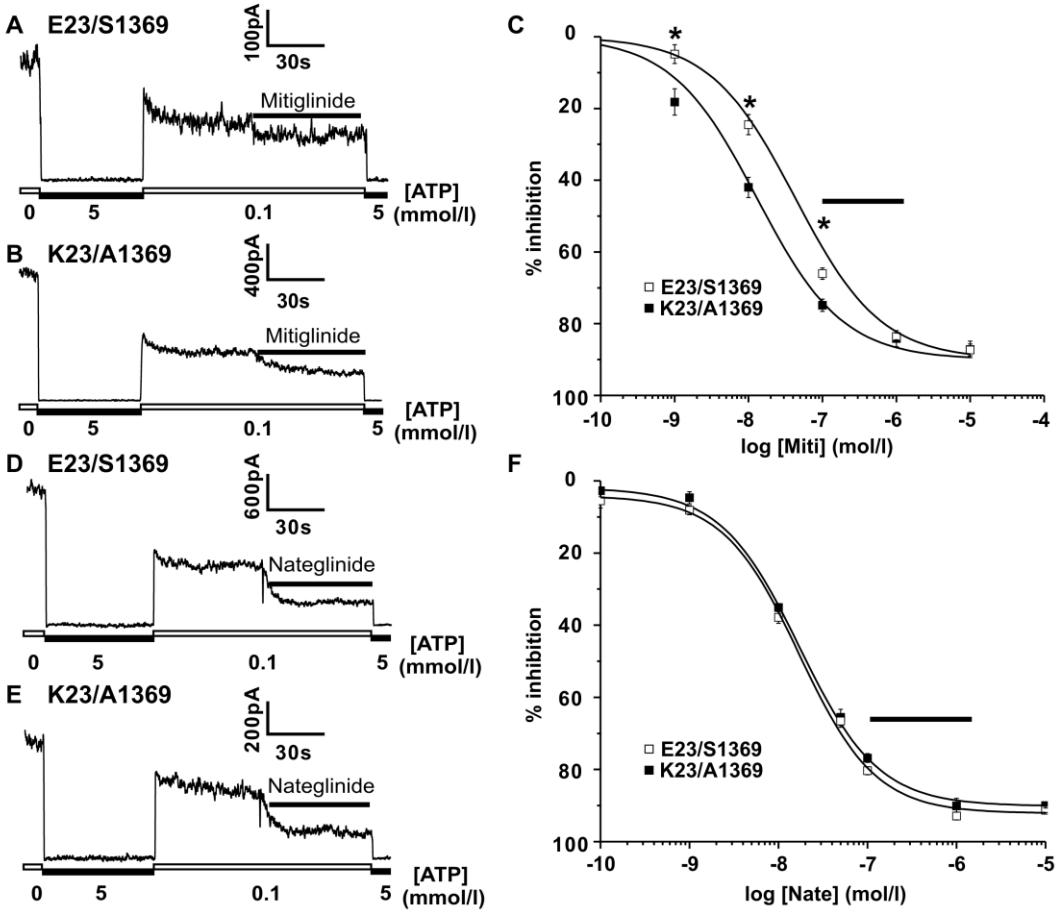

**Figure 2-4.** The AB-site drug glimepiride, but not glibenclamide and glipizide, is a less potent inhibitor of $K_{ATP}$ channels containing the K23/A1369 risk haplotype. **A,B,D,E,G,H.** Representative macroscopic current recordings showing glimepiride (5nmol/l), glibenclamide (3nmol/l), and glipizide (100nmol/l) inhibition of $K_{ATP}$ channels containing either the E23/S1369 non-risk or K23/A1369 risk haplotype. **C,F,I** Concentration-response curves illustrating that the K23/A1369 variant channels are less sensitive to inhibition by glimepiride but not glibenclamide and glipizide (E23/S1369 vs. K23/A1369 $IC_{50s}$ = 2.41±0.25 vs. 4.38±0.44nmol/l (Glim); 1.55±0.26 vs. 2.78±0.65nmol/l (Glib); 22.7±6.5 vs. 32.3±10.0nmol/l (Glip). Solid bar denotes therapeutic concentration range. n=6-13 patches per concentration. * denotes $p<0.05$.)

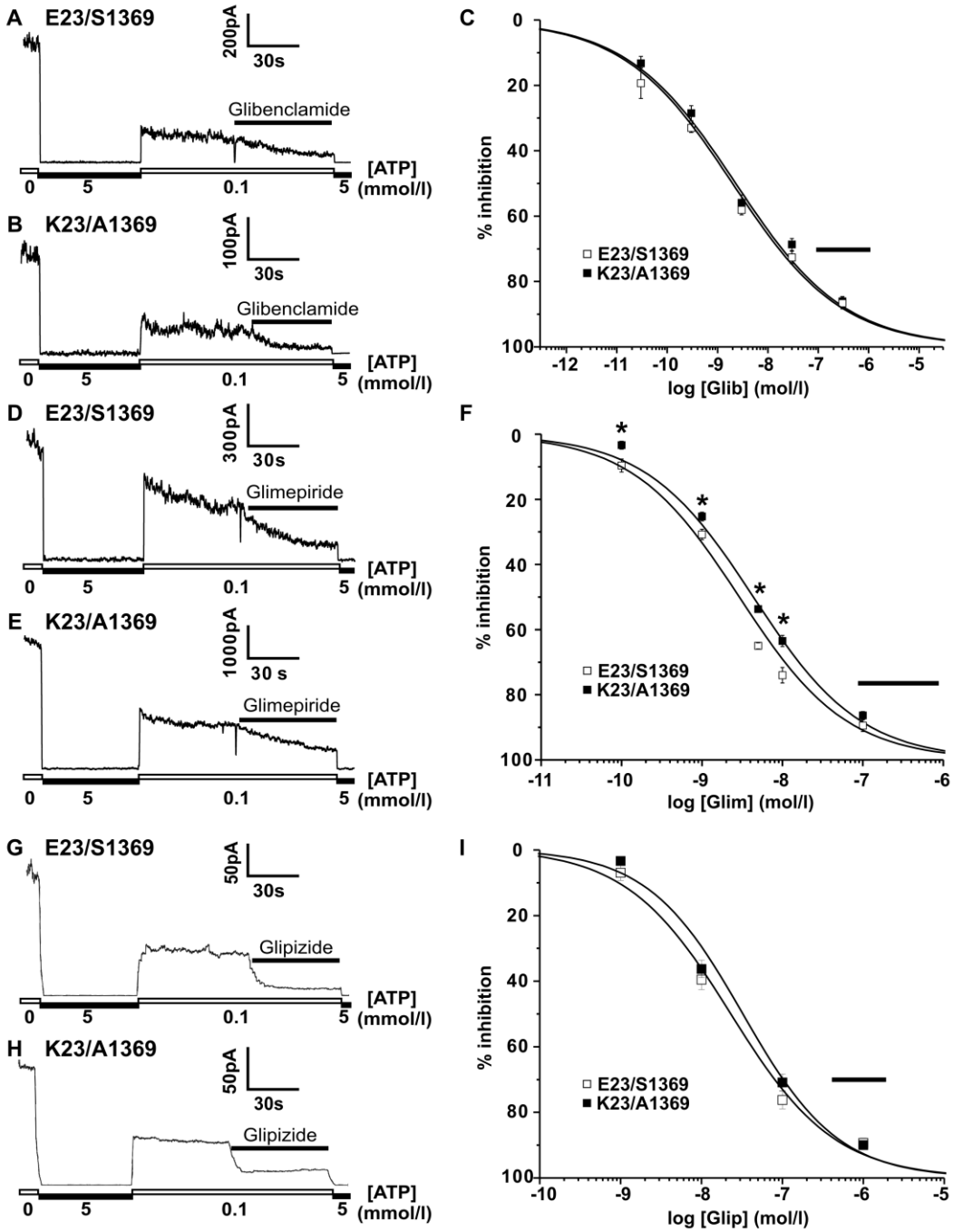
A E23/S1369
200pA
30s
Glibenclamide
[ATP]
0 5 0.1 5 (mmol/l)
B K23/A1369
100pA
30s
Glibenclamide
[ATP]
0 5 0.1 5 (mmol/l)
C
% inhibition
E23/S1369
K23/A1369
log [Glib] (mol/l)
D E23/S1369
300pA
30s
Glimepiride
[ATP]
0 5 0.1 5 (mmol/l)
E K23/A1369
1000pA
30 s
Glimepiride
[ATP]
0 5 0.1 5 (mmol/l)
F
% inhibition
E23/S1369
K23/A1369
log [Glim] (mol/l)
G E23/S1369
50pA
30s
Glipizide
[ATP]
0 5 0.1 5 (mmol/l)
H K23/A1369
50pA
30s
Glipizide
[ATP]
0 5 0.1 5 (mmol/l)
I
% inhibition
E23/S1369
K23/A1369
log [Glip] (mol/l)

**Figure 2-5.** The B-site glinide drug repaglinide is a less potent inhibitor of $K_{ATP}$ channels containing the K23/A1369 risk haplotype. **A,B.** Representative macroscopic current recordings showing repaglinide (30nmol/l) inhibition of $K_{ATP}$ channels containing either the E23/S1369 non-risk or K23/A1369 risk haplotype. **C** Concentration-response curves illustrating that the K23/A1369 variant channels are less sensitive to inhibition by repaglinide (E23/S1369 vs. K23/A1369 $IC_{50s}$ = 9.32±0.75 vs. 10.05±1.59nmol/l (Repa). Solid bar denotes therapeutic concentration range. n=6-17 patches per concentration. * denotes $p<0.05$.)

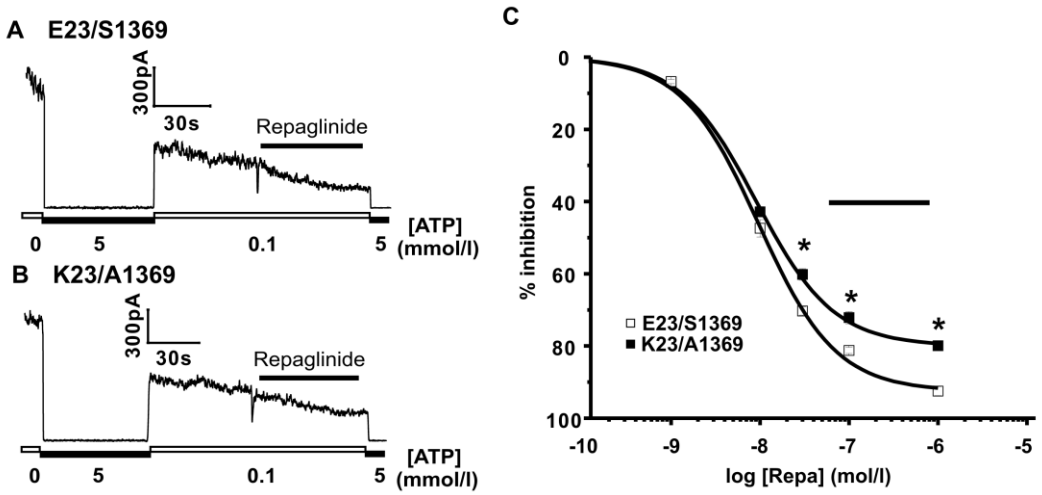

**Table 2-1.** Summary table of drug $IC_{50s}$ (mean ± SEM) determined in this study. * denotes significance difference ($p<0.05$). Gliclazide $IC_{50}$ value obtained from our previous study. [31]

| Drug | Binding site | Haplotype | | | |
|---|---|---|---|---|---|
| | | E23/S1369 | | K23/A1369 | |
| Chlorpropamide | A | 3.04 ± 0.22 | * < | 4.19 ± 0.47 | μmol/l |
| Tolbutamide | A | 0.71 ± 0.01 | * < | 1.15 ± 0.08 | |
| Gliclazide | A | 188.7 ± 32.6 | * > | 52.7 ± 11.1 | **$IC_{50}$** nmol/l |
| Glipizide | AB | 22.7 ± 6.5 | ≈ | 32.3 ± 10.0 | |
| Mitiglinide | A | 28.19 ± 1.04 | * > | 9.73 ± 1.67 | |
| Nateglinide | A | 17.12 ± 2.56 | ≈ | 17.18 ± 0.93 | |
| Repaglinide | B | 9.32 ± 0.75 | * < | 10.05 ± 1.59 | |
| Glimepiride | AB | 2.41 ± 0.25 | * < | 4.38 ± 0.44 | |
| Glibenclamide | AB | 1.55 ± 0.26 | ≈ | 2.78 ± 0.65 | |

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# Chapter 3

# General Discussion

The wide distribution of $K_{ATP}$ channels makes it possible that sulfonylureas and glinides can have any extra-pancreatic effects, as these drugs can also inhibit $K_{ATP}$ channels in other tissues besides their expected target – pancreatic $K_{ATP}$ channels. What even complicates the question is that the molecular composition of $K_{ATP}$ channel is different in different tissues, although it provides a potential to confer any tissue selectivity to sulfonylurea and glinide drugs. Unfortunately, not every sulfonylurea and glinide drug is designed with precise tissue selectivity.

As mentioned in Chapter 1, sulfonylureas may also bring cardiovascular side effects along with their therapeutic hypoglycaemic effect. Furthermore, as the exact role $K_{ATP}$ channels play in the heart is still not clarified, there is debate on whether sulfonylureas have any deleterious or beneficial cardiovascular effects. In this final chapter, the most common extra-pancreatic effects, and whether those effects are genetic depended will be discussed. Moreover, future directions in understanding the unveiled role of $K_{ATP}$ channel playing in regulating metabolic signals, and issues in translating pharmacogenomic studies to clinical practice will also be covered.

## Section I Sulfonylurea and Glinide Tissue Selectivity and Extra-pancreatic Effects

As mentioned earlier, $K_{ATP}$ channels with different combinations of Kir6.x and SURx subunits are also expressed in other tissues besides pancreatic β-cells, such as the sarcolemma of cardiac myocytes, vascular smooth muscle, skeleton muscle, GLP-1 secreting intestinal L-cells and pancreatic α-cells [1]. In different tissues, the sensitivities of sulfonylureas and glinides are determined by the different composition of SUR subunits on $K_{ATP}$ channels. The effect of sulfonylureas on $K_{ATP}$ channels in other tissues besides pancreatic β-cells, which is termed as the extra-pancreatic effect of sulfonylurea therapy, would be either deleterious or beneficial. Patients with T2D are already at high-risk of developing cardiovascular events (mainly myocardial infarction or stroke) [2, 3]. Therefore, understanding the genetic variants of $K_{ATP}$ channel affecting sulfonylurea sensitivities would be helpful in improving pharmacotherapy of treating patients concurrently carrying both conditions.

### *Sulfonylurea and Glinide Tissue Selectivity*

SUs and glinides exhibit differential sensitivities on $K_{ATP}$ channels with different subunit compositions that are often expressed in a variety of tissues [4-6]. The A-site SUs and glinides, such as gliclazide and nateglinide, are highly selective for $K_{ATP}$ channels containing the SUR1 subunit such as found in the pancreas ($IC_{50}$ was 50nmol/L for gliclazide on $K_{ATP}$ channels containing SUR1

subunits) [7]. Thus, $K_{ATP}$ channels containing either the SUR2A or SUR2B subunits (heart/skeletal/smooth muscle) will not be inhibited by these A-site drugs at the same concentration ($IC_{50}$ was 0.8mmol/L for gliclazide on $K_{ATP}$ channels containing SUR2A subunits) [7]. As AB-site SUs and B-site glinides are nonselective, such as glimepiride and repaglinide, they will promiscuously inhibit against all $K_{ATP}$ channels with similar potency ($IC_{50}$s were 3nmol/L, 5.4nmol/L, and 7.3nmol/L for glimepiride on $K_{ATP}$ channels containing SUR1, SUR2A, and SUR2B subunits, respectively) [8].

The observed different tissue sensitivities of SUs and glinides can be explained by the binding site designations of these drugs. The discriminatory tissue-selectivity of SUs and glinides to SURx-containing $K_{ATP}$ channels has been localized to the cytosolic loop linking TM15-16 on SURx subunit, in which Ser at position 1237 (in SUR1) and position 1206 (in SUR2B) are critical for A-site SUs and glinides binding. On the other hand, changing Ser to Tyr at these 2 positions is sufficient to abolish A-site SUs and glinides binding [6, 9-13]. However, B-site glinides are not affected by the alteration in this region [14]. Thus, A-site SUs and glinides are selective for pancreatic SUR1-containing $K_{ATP}$ channel over cardiac SUR2-containing $K_{ATP}$ channel [6, 7, 9, 15-17]; whereas AB-site SUs and B-site glinides inhibit SUR1-/SUR2-containing $K_{ATP}$ channels with similar affinities [5, 8, 14] **(Table 3-1)**.

In clinical practice, the steady-state plasma concentration, which is achieved with the usual therapeutic dose for each drug, is considered as a reference to determine whether the drug will exhibit any extra-pancreatic effect.

For example, the daily gliclazide (A-site) dosage produces a steady-state plasma concentration that falls between the $IC_{50}$ values for pancreatic SUR1-containing $K_{ATP}$ channels and cardiac SUR2A-containing $K_{ATP}$ channels **(Figure 3-1)** [18]. In other words, the steady-state plasma concentration for gliclazide exceeds the level required to inhibit pancreatic SUR1-containing $K_{ATP}$ channels, but doesn't reach the level required to significantly inhibit cardiac SUR2A-containing $K_{ATP}$ channels. Therefore, gliclazide will exhibit inhibitory effects selectively on pancreatic $K_{ATP}$ channels, and have minimal extra-pancreatic effects in cardiac myocytes or smooth muscle. In the case of glibenclamide (AB-site), the steady-state plasma concentration lies above the $IC_{50}$ values for all SURx-containing $K_{ATP}$ channels, suggesting that glibenclamide shows no tissue selectivity when given at the usual therapeutic dosage [18].

### *Sarcolemma $K_{ATP}$ Channel Function*

In cardiomyocytes, sarc$K_{ATP}$ channels containing Kir6.2/SUR2A, instead of contributing to myocardial contractility, serve a cardio-protective role under metabolic stress [19, 20]. Under a normal condition, due to the high concentration of intracellular ATP level, sarc$K_{ATP}$ channels remain closed in cardiomyocytes [21]. Under metabolic stress, such as anoxia, metabolic inhibition, or ischemia, sarc$K_{ATP}$ channels become activated in the adaptive response to the decrease of ATP/ADP ratio, and efflux of potassium leads to membrane repolarization and shortening of the cardiac action potential, which causes the reduction of calcium

influx [22-28]. This protects the cardiomyocytes during metabolic stress, in both ways 1) to prevent calcium overload and excessive contraction, and 2) to help save energy and recovery after metabolic stress [20, 22, 24, 29-31]. Therefore, opening sarc$K_{ATP}$ channels pharmacologically by using either diazoxide or pinacidil has been shown to improve cardiac function after myocardial infarction. Studies of sarc$K_{ATP}$ channel knockout mice have shown that impaired cardiac performance and calcium handling are observed during metabolic stress, and arrhythmias and sudden death are developed in knockout mice [19, 25, 26, 32, 33]. However, activation of sarc$K_{ATP}$ channel under ischemia may also increase the incidence of re-entry ventricular arrhythmia, which is a significant cause of death after myocardial infarction [34]. Besides activation of sarc$K_{ATP}$ channels can protect the heart, some recent studies indicate that transient closing sarc$K_{ATP}$ channels can also have cardiac protection [35]. Before cardiac ischemia-reperfusion, a transient inhibition of sarc$K_{ATP}$ channels leads to a transient moderate increase in cytosolic $Ca^{2+}$, which is known to induce cardiac preconditioning [36]. At the same time, irreversible inhibition of sarc$K_{ATP}$ channels during ischemia results in $Ca^{2+}$ overload and cellular damage.

More recently, studies have linked sarc$K_{ATP}$ channel defects induced by susceptible mutations in *ABCC9* to dilated cardiomyopathy and atrial fibrillation [37, 38]. Inactivation mutations in SUR2A have been demonstrated that mutant $K_{ATP}$ channels can no longer shortening cardiac action potential under metabolic stress, which compromise the ability to protect heart contractility [25]. The identified missense and frameshift mutations were mapped to the region that is

adjacent to NBD2 catalytic ATPase pocket within SUR2A, and they were reported to impair $K_{ATP}$ channel activity by compromising ATP hydrolysis at NBD2 on SUR2A and generating distinct reaction kinetic defects [37, 38]. Meanwhile, mutations in *KCNJ11* have also been linked to sudden cardiac death [39]. As Kir6.2 subunit is also the molecular composition of sarc$K_{ATP}$ channel, common genetic variant E23K in Kir6.2 subunit has been found associated with maladaptive cardiac remodeling and heart failure [40-42]. Greater left ventricular size and impaired heart rate response under exercise stress conditions were detected in hypertensive individuals and heart failure patients carrying of the K/K genotype, respectively [40-42]. Until recently, the sarc$K_{ATP}$ channel was thought to be composed of Kir6.2/SUR2A subunits. However, a recent study has demonstrated that there may be a chamber-specific expression of different $K_{ATP}$ channel isoforms with SUR1 being expressed in the murine atria [43]. Furthermore, the authors also provided evidence that atrial Kir6.2/SUR1 $K_{ATP}$ channels were easier to activate than ventricular Kir6.2/SUR2A $K_{ATP}$ channels, and they further speculated that the activation of atrial SUR1-containing $K_{ATP}$ channels might contribute to atrial fibrillation [43]. Therefore, whether the linked genetic variant S1369A on SUR1 with E23K on Kir6.2 plays a role in the development of atrial fibrillation will be an attractive direction to look into [44]. Moreover, a very recent study suggests that a truncated splice variant of SUR1 (SUR1Δ2) is present in the pancreas and heart which may partially explain why full length SUR1 has not been detected previously in the heart [45]. Future

confirmatory studies in human ventricular and atrial tissues will no doubt provide novel information on the precise role that these channels play in the heart.

***Sulfonylurea Cardiotoxicity v.s. Its Beneficial Effect***

Although SUs and glinides produce the desired hypoglycemic effect on T2D patients, non-selective AB-site SUs may also develop extra-pancreatic side effects in clinical practice as discussed earlier that several AB-site SUs exhibit promiscuous inhibitory abilities against all SURx isoforms in cardiac myocytes and vascular smooth muscle [20]. Interestingly, inhibition of SUR2A-containing $K_{ATP}$ channels in cardiomyocytes by AB-site SUs may have either harmful or protective effects on the heart. Although there has been long-lasting debate on the potential cardiovascular risk of taking AB-site SUs [46], many animal studies have shown that AB-site SUs abolish the protective event of ischemic preconditioning via closure of cardiac $K_{ATP}$ channels in myocardium [47-49]. For example, glibenclamide, an AB-site SU known to have cardiotoxic side effects, irreversibly binds to sarc$K_{ATP}$ channels, and its usage can increase cardiac risk in T2D patients, particularly under myocardial infarction condition [34, 50]. However, gliclazide, an A-site SU, has a high selectivity for pancreatic SUR1-containing $K_{ATP}$ channel over cardiac SUR2-containing $K_{ATP}$ channel, and it has not shown any evidence of increased cardiac risk in large-scale ADVANCE trial [51]. On the other hand, blocking cardiac $K_{ATP}$ channels by AB-site SUs under acute ischemia and reperfusion, which can reduce the incidence of ischemia-

induced re-entry type arrhythmia via lengthening the action potential, has been found possessing antiarrhythmic effect [52-57].

***Other Extra-pancreatic Effects of Sulfonylureas***

In vascular smooth muscle, Kir6.1/SUR2B $K_{ATP}$ channels regulate basal vascular tone [58-60]. In response to certain neurotransmitters and hypoxia, opening of $K_{ATP}$ channels results in membrane hyperpolarization, causing deactivation of voltage gated calcium channel. The reduction of calcium influx leads to muscle relaxation and vasodilatation [22, 60]. Studies of Kir6.1/SUR2B $K_{ATP}$ channel knockout mice have shown that impaired vascular smooth muscle function is developed, such that episodic coronary artery vasospasm and hypertension are promoted [61, 62]. As the ability to inhibit $K_{ATP}$ channels in vascular smooth muscle, AB-site SUs increase vascular resistance and decrease blood flow as vasoconstrictors which would be a potential harmful side effect [59, 63-65]. Conversely, activation of $K_{ATP}$ channels in the arterial wall following an ischemia decreases vascular resistance leading to increased coronary flow [58, 60], therefore, activation of $K_{ATP}$ channels in the condition of systemic hypertension lowers blood pressure [66].

Another beneficial extra-pancreatic effect would be that the closure of SUR2A-containing $K_{ATP}$ channels in skeletal muscle by AB-site SUs can increase peripheral insulin sensitivity [67-69]. Recent studies implicate a role for skeletal muscle (Kir6.2/SUR2A) $K_{ATP}$ channels in peripheral insulin sensitivity [67, 68]. It

has been shown that Kir6.2 activation mutations induced over-active $K_{ATP}$ channels in skeletal muscle may reduce insulin sensitivity in addition to decreasing insulin secretion [70]. Inhibition of skeletal muscle $K_{ATP}$ channels with SUs may increase peripheral insulin sensitivity [69]. This notion is supported by the studies showing that better glycemic control is achieved with AB-site SUs than with A-site SUs in neonatal diabetic patients [71, 72]. This is likely due to the fact that $K_{ATP}$ channels in skeletal muscle and β-cell are inhibited by AB-site SUs; hence, both insulin secretion and insulin sensitivity are achieved.

## Section II Future Directions

### *Future Directions - in-vitro Experiments*

While much is already known about the genetics, physiology and pathophysiology of the $K_{ATP}$ channel complex, our knowledge of this important channel is not complete. In the following, several future areas of research will be discussed, which will likely provide further insights into the $K_{ATP}$ channels in health and disease.

The importance of glucagon in the etiology of T1D and T2D is now appreciated, as inappropriate glucagon secretion is thought to contribute to the hyperglycemia observed in diabetes [73, 74]. It is now known that $K_{ATP}$ channels also play a key role in the control of α-cell excitability and hence glucagon secretion [75, 76]. However, the effects of genetic mutations or common variants in the *ABCC8* and *KCNJ11* genes encoding the $K_{ATP}$ channel on glucagon secretion have not been investigated. Such studies will likely provide additional information on the etiology of rare mutations from the perspective of α-cell function.

The $K_{ATP}$ channel complex, with its regulation by metabolic signals, has largely been considered as a distinct sensor and end effector of altered cellular metabolism. However, evidence over the last decade now suggests that $K_{ATP}$ channels are present with dynamic complexes in the membrane, comprised of kinases and cytoskeletal proteins [24, 31]. Therefore, $K_{ATP}$ channel complex can be viewed as part of a membrane associated "metabolome" consisting of multiple

physically attached proteins. Such proteins include the enzymes adenylate kinase and creatine kinase, both of which catalyze formation or breakdown of high-energy phosphonucleotides that directly alter the ATP/ADP ratio in the vicinity of $K_{ATP}$ channels [77, 78].

Creatine kinase has been shown to physically associate with SUR2A isoform in cardiac muscle [78]. Creatine kinase catalyses the production of ATP from phosphocreatine and ADP, and it maintains a high ATP/ADP ratio in the local environment around the channel, which is sufficient to maintain the channel in a mostly closed state under physiological conditions. Subsequent research has also confirmed a similar interaction between creatine kinase and $K_{ATP}$ channels in pancreatic β cells [79]. Furthermore, Terzic and colleagues have demonstrated the physical association of adenylate kinase, an enzyme which reversibly catalyzes the conversion of ATP and AMP to ADP, with plasma membrane $K_{ATP}$ channels in cardiomyocytes [77]. Therefore, both creatine kinase and adenylate kinase form a phospho-relay system to efficiently transduce metabolic signals to $K_{ATP}$ channel activity via controlling the ATP/ADP ratio in the vicinity of $K_{ATP}$ channel complex, and any disruption of that system may severely impair how $K_{ATP}$ channels respond to metabolic stress [80-82].

Other proteins involved in cellular metabolism may also either form part of the $K_{ATP}$ channel "metabolome" or regulate $K_{ATP}$ channel activity directly. For example, AMP-activated kinase [73] has recently been shown to phosphorylate $K_{ATP}$ channels [83] and also alter trafficking of channels to the plasma membrane [84]. Moreover, mutations and/or variants in the SURx subunit of the $K_{ATP}$

channel may alter the channel's association with key regulatory enzymes/proteins and hence modulate metabolic signaling. For example, Florez *et al.* found that patients carrying two copies of the *KCNJ11* E23K T2D risk variant (K/K) were less protected from developing diabetes by the anti-diabetic drug metformin than patients with E/E homozygotes. Given the fact that metformin is thought to act via activation of AMP-activated kinase, the reduced effectiveness of metformin in K/K homozygotes may reflect altered AMP-activated kinase signaling with the $K_{ATP}$ channel complex that is affected by the E23K variant [85]. Further research is, therefore, required to elucidate other molecules that are associated with the $K_{ATP}$ complex responsible for sensing and perhaps even regulating cellular metabolism.

Interestingly, it is also known that NBDs of SURx subunits of $K_{ATP}$ channels possess intrinsic MgATPase activity [86], raising the intriguing possibility that $K_{ATP}$ channels may also change the localized nucleotide concentration and generate metabolic signals that may alter cellular metabolism. In regard to the first notion that $K_{ATP}$ channels may change the localized nucleotide concentration, the importance of channel intrinsic MgATPase activity has been highlighted since the study conducted by de Wet *et al* in 2007 [87], demonstrating that monogenic R1380L mutation in NBD2 of SUR1 subunit causing neonatal diabetes led to increased MgATPase activity and increased $K_{ATP}$ channel activity. In a very recent study performed by our lab, by utilizing the patch-clamp technique and *in-silico* homology model, it has been reported that T2D-risk A1369 variant in SUR1 subunit of $K_{ATP}$ channel is adjacent to the

catalytic site of MgATPase activity in NBD2, and that the subtle change in the secondary or tertiary structure of the catalytic site increases intrinsic channel MgATPase activity [88]. Therefore, the increased MgATPase activity further elevates local concentration of MgADP leading to increased $K_{ATP}$ channel activity. However, whether A1369 variant increases sensitivities of A-site gliclazide and A-site mitiglinide by direct inhibition of MgATPase activity on $K_{ATP}$ channel in the presence of the drug molecule still requires further experiments to demonstrate.

While little molecular mechanistic data is available to support the second notion that $K_{ATP}$ channels may generate metabolic signals altering cellular metabolism, a recent research on $K_{ATP}$ channel knockout mice suggests that the whole body metabolic phenotype is significantly altered upon genetic ablation of the $K_{ATP}$ channel [89]. Kir6.2 knockout animals exhibited a leaner phenotype than wild type controls, even when fed with a Western high fat diet, and they displayed a marked preference for carbohydrate over fatty acids as a substrate for energy production. Kir6.2 knockout animals also exhibited lower overall voluntary exercise performance capacity and lower physical endurance than their wild-type counterparts upon increasing workload challenge. Despite these interesting findings, the precise molecular mechanisms underlying how $K_{ATP}$ channels regulate cellular metabolism remain to be identified conclusively.

***Future Directions – Implication for Pharmacogenomics***

To date, many genetic variants discovered in drug targets, drug metabolizing enzymes and drug transporters, have been identified their association with the response of sulfonylurea and glinide therapies, although PGx studies on drug targets and drug transporters are relatively fewer than studies on drug metabolizing enzymes. The information gained through PGx studies holds particular promise in improving drug efficacy while minimizing toxicity; however, there are extremely few guidelines linking these results to specific therapeutic recommendations, with one exception that a recent published study developed a series of PGx-based dose recommendations based on systematic reviews [90]. In order to translate these results from studies to clinical practice, such as selecting therapy and correcting dosage, two general limitations have to conquer.

Firstly, most PGx clinical studies were conducted in Asian population. Therefore, the applicability of these findings to patients of other races is unclear. In future, studies involving more ethnic populations should be widely conducted.

Secondly, most current PGx studies focused on the relationship of a single genetic variant and drug response, ignoring the contribution of other genetic variant to drug response. For example, it has been reported that *CYP2C8*3* variant allele is associated with a reduced plasma concentration of repaglinide [91]. Years later, He *et al* showed that T2D patients carrying K23 allele responded better to repaglinide therapy compared with E23/E23 homozygotes [92]; however, in their study, they only examined patients' genotypes on $K_{ATP}$ channel, leaving repaglinide metabolic enzyme *CYP2C8* gene uncontrolled. Interestingly, early in

Chapter 2, I reported that K23 homozygous $K_{ATP}$ channel exhibited a decreased sensitivity to repaglinide inhibition compared with E23 homozygous $K_{ATP}$ channel, which showed a contradictory result to the clinical study conducted by He *et al*. Therefore, it highlights the possibility that *CYP* gene and maybe other genes also play important roles in drug response process. Future studies should consider the contribution of drug target gene, transporter gene and metabolizing enzyme gene comprehensively rather than considering single individual gene.

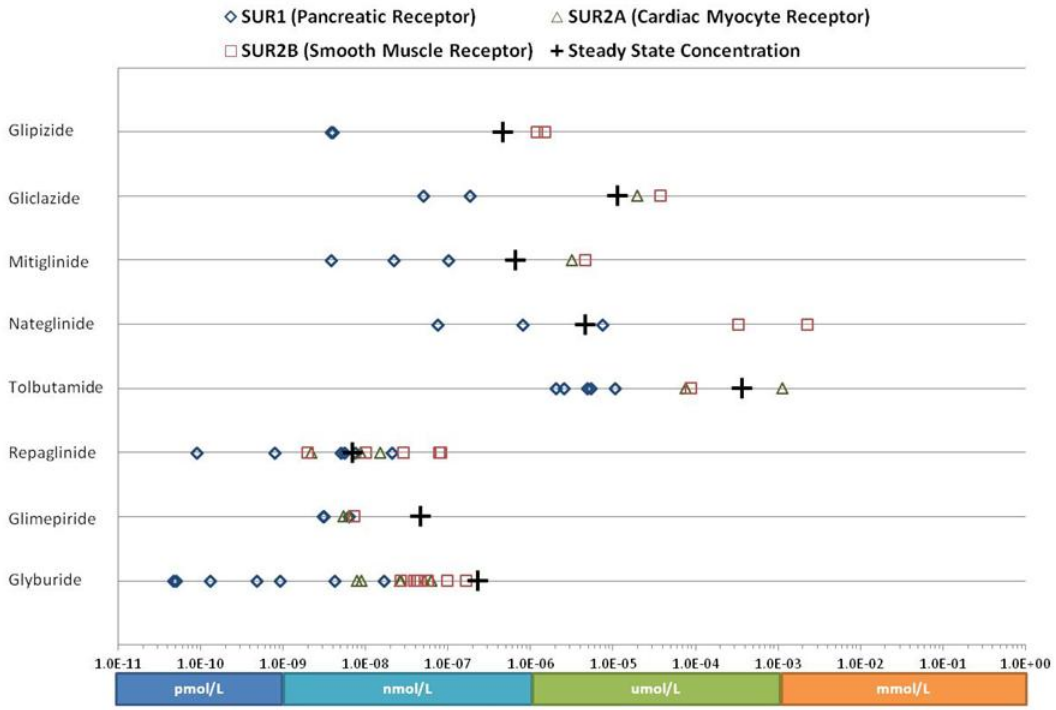
In summary, although there is a huge gap in translation, results from this present study that the contributions of genetic variants on $K_{ATP}$ channel (E23K in *KCNJ11* and S1369A in *ABCC8*) impact on the sensitivities of sulfonylurea and glinide drugs, may still have implications for the development of personalized medicine in the future. Furthermore, future PGx studies, coupled with clinical factors, may help improve the management of type 2 diabetes by aiding in appropriate selection and proper dosing of sulfonylurea and glinide drugs based on the genomic profile of individual diabetic patients.

**Table 3-1 Tissue selectivity of sulfonylureas and glinides based on their binding site designations.**

| SURx-containing $K_{ATP}$ channel targets | Channel blocker |
|---|---|
| Pancreatic Kir6.2 / SUR1 | A-/AB-site SU, A-/B-site Glinide |
| Cardiac Kir6.2 / SUR2A | AB-site SU, B-site Glinide |
| Smooth muscle Kir6.1 / SUR2B | AB-site SU, B-site Glinide |

**Figure 3-1 The selectivity of sulfonylureas and glinides on cardiac and pancreatic $K_{ATP}$ channels.**

$IC_{50}$ values and steady-state plasma concentrations for sulfonylureas and glinide drugs in the pancreas, cardiac myocyte and vascular smooth muscle are plotted. [18]



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