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Pharmacogenomics of cardiovascular complications in diabetes and obesity

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Abstract

Heart disease is major cause of death for population in US and worldwide. The complex interplay of the mechanisms between diabetes, obesity, inflammation causes concerns for therapeutic understanding and developing treatment options for patient. Recent advances utilizing pharmacogenomics has helped researchers to probe in to disease pathophysiology and physicians to detect and, diagnose the disease in patients. The understanding developed in the area primarily addresses the issue focusing on the nature and asks the question ‘Why’ some individuals respond to the standard medication regimen and others do not. The central idea that genomics play a vital part in how the healthcare providers: physician, pharmacist and nurse provide treatment utilizing the best practices available for maximum benefit. Pharmacogenomics is the scientific basis which offers the fundamental understanding for disease based on which therapeutic approaches can be designed and delivered. The discovery that not all humans respond to the drug in the same way is a ‘paradigm shift’ in how current therapies are offered. The area of pharmacogenomics at its core is linked to the genetic basis for the disease and the response to treatment. Given that diabetes and obesity are major metabolic ailments globally wherein patients also often suffer from cardiac disorders, a comprehensive genetic and pharmacogenomic understanding of these conditions enables the development of effective therapeutic strategies. In this review, we discuss various pharmacogenomic approaches with special emphasis on heart disease as it relates to diabetes and obesity. Recent information in regard to relevant patents in this topic are also discussed.

1. Introduction

Diabetes is a complex cardiovascular disease which is diagnosed and detected by increase in blood glucose. Diabetes is among the top 5 pathophysiological conditions with an estimated global prevalence of 6.4% in 2010, and projected to rise to 7.4%, affecting 439 million adults, by 2030 [1]. The high blood glucose level in diabetes is the predominant method for detecting the disease. Even if the blood glucose level is controlled by use of oral anti-

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diabetic agents, there are chronic underlying actions that play a major role in disease development and pathology. The American Heart Association categorizes diabetes as heart disease. Majority of Diabetics (about 95%) are insulin resistant and the rest, ~5% are unable to produce and release insulin from β -cells of the pancreas. Therefore diabetes is predominantly a problem of insulin resistance. However due to the advent of new diagnostic techniques and modern day focus on public health awareness, more people are being detected for diabetic or pre-diabetic stages. Credited to better overall disease management and life style, survival rates in heart disease patients have improved considerably [2]. However, the improved health care also revealed new problems surfacing in terms of disease complications related to age, genetics, environment and co-morbid conditions as diabetes itself. For instance, Type 2 diabetics although are unable to release insulin, the condition eventually manifests as a combination of Type 1 and Type 2 diabetes presenting a much complex situation in terms of treatment strategies that are currently available to the health care providers.

Another major risk factor of diabetes is Obesity, which together with the latter is a significant risk factor of heart diseases. According to Centers for Disease Control (CDC), 35% of the US population is obese, and within the pediatric population obesity is growing at an alarming rate, with children either obese or overweight [3]. Population studies report significant associations between waist length and increased incidences of heart disease and death. The children who are obese and overweight are either pre-diabetic or diabetic. Therefore the disease will eventually predispose into other pathologies and lead to complicated aspects and treatment scenarios. Pharmacogenomics is utilization of genomic information to assess drug efficacy, and allows identification of diseases or risk factors based on genetic markers. The field of pharmacogenomics has provided newer avenues to identify familial or genetic predisposition of diabetics to cardiac diseases.

2. Cardiac complications of diabetes

Heart is a vital organ in the body and performs the basic function of pumping the oxygenated blood to other parts of the body and to itself. The structure and complexity of heart is important to its physiology, which is a synchronized action of the underlying ion channels, muscle contraction, and neurohormonal processes affecting the homeostatic process of heartbeat. However, pathological processes such as ischemic heart disease, congestive heart failure, atherosclerosis, cardiomyopathy and cardiac hypertrophy, leads to deficits in cardiac function. A significant proportion of diabetics suffer from ventricular structural and functional deficiencies, a condition commonly referred to as diabetic cardiomyopathy [4]. The fact that 30% of the US is Diabetic and another significant portion are either pre-diabetic or un-detected calls for immediate attention to garner new understanding and development of tools that are required for better patient treatment and outcomes.

3. Physiological conditions contributing to diabetes

The exact cause or nature of the origin of diabetes remains unknown. However, recent advances in this area of research using animal models, translational approach and clinical

trials have provided useful information for better understanding the pathophysiological basis of diabetes

3.1. Inflammation and atherosclerosis

At the base of the problem is the core issue of the process that ties diabetes and heart disease called as “inflammation.” Diseases such as diabetes and heart attack are often times related to imbalances in the underlying inflammatory processes. The process of atherosclerosis and plaque development and deposition in the coronary artery which leads to ischemic heart disease and heart attack starts at the molecular level with imbalances in inflammatory processes. The events that eventually lead to thrombosis include activation of cytokines (Interleukins, TNF α , etc.) and cell adhesion molecules, increased recruitment of monocytes, conversion of macrophages to foam cells. Ultimately accumulation of cholesterol and oxidative stress processes leads to increased recruitment and rupture of the plaque leading to thrombosis and death [5]. Inflammation is a common link for several diseases such as diabetes, heart disease, infection, ischemic heart disease and other pathophysiological conditions. It was identified several decades ago that inflammation is a plausible link for increased incidence of diabetes. It was observed that higher doses of salicylates were effective in diminishing glycosuria in patients with milder diabetes or type 2 diabetes [6]. The most commonly accepted pathways for linking diabetes and insulin resistance is by I κ B kinase-beta (IKK β)/NF κ B axis [7].

3.2. Endoplasmic reticulum (ER) stress

In recent years another process described as endoplasmic reticulum stress (ER stress) has been linked to diabetes. A review by Hotamisligil [8] illustrates that ER and the associated signaling pathways are an emerging intersection for understanding inflammation and metabolic diseases. The inflammatory basis of diseases is fundamentally linked with protein folding and trafficking and is a central process that links many cellular functions.

Previous studies have been conducted to elucidate the role of ER stress in insulin resistance and obesity. The work published by Ozcan et al [9] probed the idea that obesity causes ER Stress and leads to insulin resistance. The key players in the signal transduction mechanisms are through down regulation of X-box binding protein 1 (XBP-1) which in turn leads to IR (insulin receptor) signaling via downstream signaling disruption of inositol-requiring and ER-to-nucleus signaling protein (IRE-1 α) dependent activation of c-Jun N-terminal kinases (JNK). The study points out at the role of fundamental mechanism for sensing molecular changes in obesity induced metabolic stress. The overall idea was to identify the central molecular pathways in the cluster of diseases called as the metabolic syndrome that encompass high triglycerides, high cholesterol and high glucose in blood, which may be caused by obesity. The models used by the authors are db/db and high fat diet fed mouse models in which the leptin receptor is mutated or mouse is fed with high fat (Western diet), respectively leading to increased food intake and obesity in both cases. Using these and other knockout mouse models the authors specifically show that ER stress is predominantly present in the peripheral regions causing insulin resistance. It is also suggested that pancreatic ER kinase (PERK) mutations cause a rare inherited form of type 1 diabetes in humans. Previous work [10,11] shows that loss of eukaryotic initiation factor 2 α (EIF2 α)

phosphorylation by targeted mutation of S51 to A (alanine) leads to altered pancreatic β -cell functioning resulting in maladaptation impacting liver gluconeogenesis. Based on their findings the authors suggest that chronic ER stress would lead to metabolic changes and insulin resistance and may lead to type 2 diabetes. Therefore novel therapeutics and interventions can be developed based on ER stress response for preventive treatment of type 2 diabetes.

In 2006, Ozcan et al [12] further evaluated the role of chemical chaperons in reducing ER stress and glucose homeostasis, providing mechanistic link between obesity and insulin resistance and opportunities for therapeutic molecules called as chemical chaperons. The use of 4-phenyl butyric acid (PBA), trimethylamine N-oxide dehydrate (TMAO), ursodeoxycholic acid and its taurine conjugated derivative (TUDCA) which improve protein folding and ER function were tested for their effects on ER stress. The report shows that chemical chaperons produce its beneficial effects in models of type 2 diabetes and modulate adaptive phases of the ER stress phenomenon, therefore demonstrating that these molecules are potentially anti diabetic. The study for the first time demonstrates that chemical chaperons alleviate ER stress and restore the normal actions of insulin to establish the normal glucose levels in mouse model of type 2 diabetes and therefore clinical investigations for these compounds may be beneficial in diabetes and obesity.

The role of ER stress in heart is important but unknown and therefore studies were conducted to address this issue and to understand how ER stress may affect the pathology in cardiovascular diseases. The major cause of cardiovascular related deaths is ischemic heart disease. Therefore the study by Azfer et al. [13] investigated the role of ER stress in ischemic heart disease using murine model of cardiac specific overexpression of monocyte chemo attractant protein-1(MCP-1), a major player in ER stress signaling. The authors identified increased expression of genes involved in unfolded protein response (UPR) chaperons, oxidoreductases and protein disulfide isomerases (PDI), the study strongly suggests that activation of ER stress response is involved in development of heart disease due to ischemia.

4. Cardiac ion channels

4.1. Action potential

Ion channels form the basis of electrical conductivity and rhythmic contractility of cardiac tissue. Several currents that these channels conduct have been identified and their functional contribution to action potential (AP) in both the atrial and ventricular myocytes is well studies [14]. As shown in Figure. 1 the AP wave form in cardiomyocytes begins with membrane depolarization due to the influx of extracellular sodium ions (Na^+) into cardiomyocytes through the voltage gated sodium ion channels, $\text{Na}_v1.5$ (I_{Na}). Subsequently, repolarization augments as a transient downward notch of AP due to the efflux of potassium ions (K^+) through activation of voltage gated potassium ion (K^+) channels, $\text{Kv}4.2$ and $\text{Kv}4.3$ ($I_{\text{to,f}}$). Repolarization down stroke progresses further by K^+ ion efflux through delayed rectified voltage gated K_v channels (I_{Kr} and I_{Ks}). Simultaneously, influx of calcium (Ca^{2+}) through high voltage-activated calcium channel, Ca_v ($I_{\text{Ca,L}}$) results in cytoplasmic enrichment of Ca^{2+} and ensues myocyte contraction. Finally, inward rectifier, non-voltage

dependent K^+ channels ($Kir2.x$), is involved in restoring the resting membrane potential. In addition to these, K^+ ions through $Kv1.4$ ($I_{to,s}$) and $Kv1.5$ channels (I_{Kur}) also contributes to repolarization in the cardiomyocytes. Conductance of ions through these channels defines the shape and duration of cardiac AP sequence, and therefore determines the electrical and contractile phenotype of the heart. Abnormalities and changes in the functioning of the ion channel may result in asynchronous beat leading to arrhythmia and death.

4.2. Depolarizing sodium currents (I_{Na})

Action potential starts with the entrance of Na^+ ions into the myocytes (I_{Na}). Underlying molecular determinant of this current is the α -subunit of the voltage gated Na^+ channel ($Na_v1.5$) that *SCN5A* gene encodes, which conducts the sodium ions into the myocyte resulting in membrane depolarization and myocyte excitability [15,16]. Therefore, $Na_v1.5$ driven sodium currents (I_{Na}) determines both the initiation as well as the duration of cardiac AP in myocytes. Several proteins interact, either directly or indirectly, with $Na_v1.5$, and regulate its gating and kinetics. These include the β -subunits ($\beta1-4$, encoded by *SCN1B-4B*) that are expressed in the heart [17,18,19]. Each β -subunit through its extracellular amino-terminus, transmembrane segment and cytoplasmic carboxyl-terminus, regulates $Na_v1.5$ membrane expression and channel gating in addition to forming macromolecular regulatory complexes with other supporting extracellular, membrane, and cytoplasmic proteins [20,21].

Inactivation of $Na_v1.5$ terminates the depolarization upstroke, and subsequent repolarization and restoration of membrane potential is accomplished by at least five voltage gated K^+ channels in human cardiomyocytes. Mutations in the sodium channel genes have been linked to arrhythmias. Brugada syndrome is well documented and mechanisms linking sodium channel mutations are demonstrated by use of animal models as well as human studies have been performed to identify the role of sodium channel mutations in causing arrhythmias [22].

4.3. Transient repolarization currents ($I_{to, fast}$ and $I_{to, slow}$)

The transient repolarization current, $I_{to, fast}$, marks early repolarization downstroke [14]. This current reflects an outward K^+ ion conduction by members of Shal-related subfamily of voltage gated K^+ channels formed by homo-, and/or hetero-, tetramerization of the α -subunits of $K_v4.2$ and $K_v4.3$ in mammals. $K_v4.2$ and $K_v4.3$ are encoded by *KCND2* and *KCND3* genes, respectively, and these channels typically show fast activation and inactivation channel kinetics. In human heart, $K_v4.3$ encodes the major pore forming α -subunit and exists as a multi-protein complex at the plasma membrane. Auxiliary subunit, KCHIP2 that is also expressed in the heart co-assemble with $K_v4.3$ and increases the membrane expression, current amplitude, decelerates channel inactivation, accelerate recovery from inactivation and shifts the inactivation midpoint to more depolarized potentials [23,24]. Another auxiliary protein, dipeptidyl – aminopeptidase-like protein 6 (DPP6) also binds to the voltage sensing domain of $K_v4.3$ and positively regulates the activation and inactivation kinetics of $K_v4.3$ [25]. Additionally, studies conducted in heterologous expression systems such as *Xenopus* oocytes show that Mink-Related peptide 1 (MIRP1), found in native K_v4 channel complexes, slows the activation and inactivation kinetics of $K_v4.2/4.3$ [26].

Another component of transient outward current, referred to as $I_{to, slow}$, represents the K^+ outward current conducted by the voltage gated K^+ channel, $K_v1.4$, in the heart [14]. Encoded by *KCNA4* gene, $K_v1.4$ represents the channel pore forming α -subunit with channel kinetics of fast activation, slower inactivation and slower recovery from inactivation, that are distinct to that of $I_{to, fast}$ currents conducted by the $K_v4.2/4.3$ [27,28]. Previous research utilizing type I and type II models of diabetes and obesity clearly revealed that the action potential is prolonged with depleted repolarization reserve which is primarily due to decreased K_v4 currents [29,30].

4.4. Ultra-rapid delayed rectifier K^+ currents (I_{Kur})

Outward efflux of K^+ ions underlying the ultra-rapid delayed rectifier K^+ currents (I_{Kur}) observed in atrial myocytes is accomplished by $K_v1.5$ channel, which *KCNA5* encodes [31,32]. These fast activating, but slow inactivating, K_v channel is thought to co-exist at the plasma membrane in macromolecular complexes with its auxiliary subunits called the β -subunits. The β -subunits belong to aldo-keto reductase superfamily and bind pyridine nucleotides with high affinity [33,34]. The β -subunits ($\beta1.x$, $\beta2$ and $\beta3$) are expressed in the heart of several species with high abundance. Studies conducted in the heterologous expression systems show that, these auxiliary proteins influence $K_v1.5$ surface expression, channel gating, conduction kinetics and sensitivity to pyridine nucleotide ratios in the cell, coupling the channel conduction to metabolic status of the cell [32,35,36]. Channelopathy in human $K_v1.5$ have been related to atrial fibrillation and therefore there is risk for repolarization deficiency and heart disease. Recent evidence shows that genetic mutations resulting in either loss- or gain, of $K_v1.5$ α -subunit functionality precipitate AF independent of other risk factors. A heterozygous nonsense mutation in exon 4 of *KCNA5* gene was found in atrial fibrillation (AF) patients that encodes a premature stop codon (E375X) resulting in truncated protein and loss of channel function [37]. Similarly, studies also reported several other loss-of-function mutations in *KCNA5* gene; T527M, A576V, E610K, Y155C, D469E, and P488S in AF patients that negatively impacts surface channel expression or current conduction or both [38,39]. Loss of function mutations in *KCNA5* gene, for instance, E375X affects I_{Kur} currents, repolarization and prolongs action potential duration [37]. Also gain of function mutations in *KCNA5* gene were also observed in early-onset lone AF patients. Christophersen et al. (2012) reported three gain of function mutations; E48G, A305T, and D322H in *KCNA5* gene of AF patients [39]. Therefore, genetic variations of structural and functional consequence in $K_v1.5$ (*KCNA5*) gene represent an important etiological factor of AF in human population.

4.5. Delayed rectifier K^+ currents (I_{kr} and I_s)

Delayed rectifier potassium channels encoded by *hERG/KCNH2* gene are responsible for the rapidly activating (I_{kr}) repolarizing currents in ventricular myocytes of mammals including humans. These channels conduct K^+ ions outward, and exhibit rapid activating and fast inactivating channel kinetics [40,41]. A second current in this type is the, I_{Ks} component mediated by heteromeric channel formed by $K_v7.1$ and MINK subunit protein that are encoded by *KCNQ1* and *KCNE1* genes, respectively. Channels that conduct I_{Ks} show slowly activating and inactivating kinetics, and constitute a predominant late repolarization current in the ventricles [42].

4.6. Voltage-gated Calcium Channels (Ca_v)

Influx of calcium (Ca^{2+}) through high voltage-activated calcium channels results in cytoplasmic enrichment of Ca^{2+} and ensues ventricular myocyte contraction. This excitation contraction coupling in ventricular myocytes is accomplished by L-type calcium channels that activates at relatively positive membrane potentials, and inactivate rapidly [43]. While the pore forming α -subunit, Cav1.2 that is encoded by CACNA1C gene is responsible for these calcium currents (I_{CaL}) in ventricular myocytes [14], the cytoplasmic β subunits ($\text{Ca}_v\beta$) regulates the gating and gating and surface expression of the α -subunits [44].

5. Long QT (LQT) syndrome

Long QT syndromes (LQTS) are a complex set of disorders with many causative factors (Figure 2). LQT can be inherited or acquired and lead to massive cardiac abnormalities leading to ion channel abnormalities and arrhythmias and sudden cardiac death. It is ascribed that LQT is a major cause of death for all forms of sudden cardiac death. Research in the area of LQT has provided more evidence for understanding the complex processes, mutations in ion channels, acquired single nucleotide polymorphisms, drug induced abnormalities and activity of ion channels. The recent understanding developed in the area by developing insights with LQT types and diseases has revolutionized the field of cardiac arrhythmias. The basis for LQT is fundamentally hidden in the fact that the heart is architecturally heterogeneous organ. There is a transmural heterogeneous expression of ion channel from the outside of the heart to the inside and different regions of the heart signify specific functions. The atria and the ventricles work in synchrony to pump the blood to the body; however when there is asynchrony or mismatch in the electrical processes, arrhythmogenic situation leads to massive failure and death.

In LQT syndromes, the major problem arises due to repolarization defects. The differential regulation of ion channels such as potassium and delayed rectifier (I_{Ks}) and sodium channels and auxiliary subunits such as potassium channel interacting (KCHIP; isoforms 1, 2 and 3) are electrically asynchronous leading to action potential changes and irregularity.

Action potential in the heart is interplay between the outward and the inward currents. The sodium and the calcium current are inward currents and the potassium is the outward currents that together establish the action potential (AP) the duration of the AP, and its manifestation into the ECG (electrocardiogram) leads to understanding and diagnosis of the diseases for the heart. Potassium channels that are reported to directly contribute to LQT syndrome include KCNE1, KCNE2, KCNQ1 and KCNQ2, KCNJ2. There are also sodium channel increases (SCN5A) and calcium current abnormalities (CACNA1C) that lead to LQT.

The abnormalities can occur at transcriptional, translational and posttranscriptional level, which includes trafficking of proteins to the plasma membrane and surface localization. Based on the problem reported at the respective stages in the Figure 2, the disease is classified as LQT. At molecular level, the ion channels that are responsible for modulation of electrical signaling are many. The factors that change the action potential duration and cause LQT can lead to Torsade de pointes (TdP).

There are four major types of this type of arrhythmia in long QT syndrome (Class 1 – 4). Depending on the level at which the problem occurs, the Class of arrhythmia is categorized. If the problem occurs at the transcriptional or translational level the class is defined as Class 1, the Class 2 is defined as problems or disruptive mechanisms taking place at protein folding, subunit assembly and trafficking and localization of the ion channel and its accessory units. The basis of ER stress is given in the section of this chapter, as it relates to protein folding and stress in diabetes and obesity. Disruption occurring at post-translational level resulting in functional changes is class 3 and disruption occurring at ion channel gating or selectivity or conductance is called as Class 4. For understanding the four classes of LQT in detail, a pictorial depiction of the various steps involved and the associated problems, defining the four classes is given in Figure 2. Ultimately the four classes along with the genetic risk involved are combined with the environmental and epigenetic risk factors which will evolve into a phenotype that may lead to arrhythmias called as LQT syndromes.

6. Diabetes and cardiac repolarization defects

Human and animal studies have shown that cardiac repolarization that is primarily determined by Kv channels slows down markedly in diabetic hearts. Electrocardiographic measurement of cardiac repolarization; QT interval, is frequently prolonged in both type I and type II diabetic patients [45,46]. Animal studies further extended these observations, and showed that diabetes also results in prolonged action potential durations in ventricular [47], and atrio-ventricular nodal myocyte cells [48]. Electrical basis for these changes in both type I and type II models of diabetes and obesity clearly revealed that the action potential prolongation in diabetic hearts involves depleted repolarization reserve which is primarily due to decreased Kv4 expression [49,50] and associated potassium currents [29,30].

7. Diabetes, Obesity and Atrial Fibrillation

Obesity is associated with a 50% increase for the risk of atrial fibrillation (AF)[51]. Furthermore, obesity is associated with an increased prevalence of hypertension, coronary artery disease, diabetes mellitus, obstructive sleep apnea, and congestive heart failure [52,53], most of which are risk factors for thromboembolic stroke in patients with AF.

The propensity of obese people to develop AF may be attributable to left ventricular (LV) diastolic dysfunction and left atrial (LA) dilation [51]. The electrophysiological features that may promote and perpetuate AF in the obese patient have not been studied in detail [54]. A paper published by researchers from the Mayo clinic concluded that increased LA pressure and volume and shortened effective refractory periods in the left atrium are potential factors in facilitating and perpetuating AF [54]. Echocardiographic studies showed that the left atrium of obese patients had higher volumes, increased strain, and lower contractility [54].

8. Pharmacogenomics

The genotype can determine phenotypes and the associated variation. As with any other correlation from transcription to translation related changes, the genotypic determinant may lead to phenotype alteration however the correlation in most cases is poor. Therefore, other determinants are also sought in such cases. Even in families with identical mutations,

variations do occur at phenotype and presentation [55]. It is also well known that in families, some members who carry the mutation do not display any symptom or sign of disease where as other members will have a profound effect of such mutations, raising the importance of asymptomatic management or treatment in such individuals [56]. This variability also raises the question that genotypic changes may be exacerbated by environmental factors that may be influencing the phenotypic changes leading to increased risk and sudden death. It has also been determined that factors such as age, gender, nervous system (sympathetic and parasympathetic tone), drugs, and environment can act as triggers for causing the genotypic identifiers to be manifested as phenotypic alterations. Specifically, differential drug responses correlate to inter-individual variations at one or more genes in the genome. Pharmacogenomics is a scientific discipline that studies these associations at the whole genome level. With the advent of high throughput genome sequencing technology and the availability of draft sequence of human genome, pharmacogenomics offers an unprecedented opportunity towards developing personalized medicine, wherein the inherent genetic uniqueness of each individual can be factored into contemplating effective therapeutic strategies for better outcome [57].

8.1. Pharmacogenomic advances

Use of scientific techniques and state of the art tools to understand the disease have evolved in the last 30 years (Figure 3). Inter-individual variations in the genome appear as single nucleotide polymorphisms (SNPs), indels (insertions/deletions of short stretches of nucleotides) in the coding and/or non-coding regions, which constitute the genetic variance of a population. Understanding and developing the area of pharmacogenomics is based on molecular biology and some fundamental techniques.

The polymerase chain reaction is a groundbreaking technique and an important tool in pharmacogenomics based studies. This technique is based on designing specific primers that can anneal to, and subsequently amplify the, DNA sequence of interest. Several tools that incorporated PCR have been used in pharmacogenomics studies. Real-time PCR based detection of genetic polymorphisms are useful for identification of known SNPs/ polymorphisms in new samples [58]. For instance, TaqMan PCR assays discriminates polymorphic alleles using a pair of PCR primers in addition to two fluorescent DNA oligos (probes) that differ only at the polymorphic base, and this approach has been used in identification of important polymorphisms in drug metabolism related genes [59]. The use of microchip assays has been employed for mRNA and more recently for micro-RNA quantification on a high throughput scale [60]. The speed and rapid nature of these techniques gives researchers the ability to obtain large amounts of information in relatively small time. Further, the fundamental concept of PCR has been used at different levels such as chip assays, deep sequencing, that are used in genome wide association studies (GWAS) [61,62,63]. Additionally, as shown in Figure 3, the use of cloning offers the specific expression of gene of interest in the vector for identification and detection of functional properties. The technique of cloning has helped researchers unravel the properties of ion channels, membrane proteins, kinases, and many more functions in biology. We have utilized some of these techniques in our research for identification of Kv β 1, 2 and 3 subunits roles as enzyme, and as partner for redox modulation of Kv channel [33,36,64].

9. Pharmacogenomics of diabetic cardiomyopathy

Cardiovascular diseases are caused by variety of conditions among which heart failure is one of the serious and most expensive conditions in healthcare due to its high prevalence [65]. In the United States, heart failure accounts for more than 200,000 deaths per year with a mean survival of only 1.7 years (Men) and 3.2 (Women) after diagnosis [66]. Heart failure is leading cause of mortality in people with type 2 diabetes. [67]. The incidences and severity in cardiovascular events and deaths is significantly higher among diabetics compared with non-diabetics [68]. Genetic factors contribute the major portion of primary pathophysiological processes involved in heart failure syndrome. Among these pathophysiological changes, cardiomyopathy or heart muscle disease occupies the large portion, off which most common form is dilated cardiomyopathy (DCM). Recent advancements in cardiovascular genomics and genetic studies identified many genes that can cause DCM (see [69] for details). Personalized medicine of a patient starts with family history which should include at least three generations. Genetic testing of important and recommended genes in DCM is one of the major steps in determining the personalized medicine. As pharmacogenomics is the study of genes that influence the drug responses, individual's genetic profile is therefore essential. To date, there are very few pharmacogenomics studies reported for diabetes induced cardiomyopathy and major part of the research is focused on β -blockers.

Adrenoceptors (AR) are family of G-protein coupled receptors to which stress hormones such as epinephrine and norepinephrine binds. Sympathetic changes inducing responses such as “fight or flight” are consequences of adrenergic receptor activation. This includes increase in heart rate, pupil dilation, vasodilation to muscles and brain, increased myocardial ionotropy and many other effects [70]. The adrenergic receptors are classified into 2 major classes; α and β [71]. Among the β -ARs, β_1 and β_2 are functionally active in human heart [72] and upon activation they induce increased force of contraction (Inotropy), increased heart rate (Chronotropy), and increased rate of relaxation (Lusitropy) [73]. In contrast, β_3 is shown to function opposite to β_1 and 2 ARs by inhibiting inotropy [74,75]. Upon activation of β_1 and β_2 -ARs, activation of intracellular adenylyl cyclase follows the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Then cAMP activates kinases such as protein kinase A and calcium channels that are important for myocardial contraction via phosphorylation [76,77]. Whereas β_3 ARs couple through the inhibitory G protein (G_i) and decreases cAMP production. Also β AR blockers that are currently in use are not only antagonists that block β_1 and 2-ARs but also activate β_3 -ARs simultaneously [78]. As β_3 -ARs have fewer effects on heart biological function [75] [79] [80] and up-regulation of this β_3 -ARs is reported in heart failures in human and also in animal models, this may have interesting implications in cardiovascular disease [81,82].

Over the last decade, many studies have been focusing on single nucleotide polymorphisms (SNPs) on β -ARs and their effect on β -AR blocker therapy in heart failure population. Based on the existing literature it is evident that a genetic variation that is on one hand a risk factor for disease is on the other hand associated with the better response to β AR blockers. Some of these polymorphisms are listed in the Table 1. Recent reports on patients with β_1 AR SNPs especially on Gly389 and Arg389 showed positive effects on pharmacogenomics of

β AR blockers [83,84]. They reported that the patients with Arg389 allele showed enhanced hemodynamic response to β AR antagonist therapy and being homozygous for this genotype was an advantage for patients treating with carvedilol for heart failure. In another study it was shown that the patients with a combination of Arg389 on β 1AR and Gln27 on β 2AR is associated with two-fold increase in mortality when treated with carvedilol when compared to other genotypes [85]. A similar study was conducted on the impact of β AR SNPs on β AR blocker metoprolol treatment after coronary artery bypass grafting in 82 patients [86]. They concluded that the β 1AR polymorphism Arg389Gly is important in predicting the need for and response to β AR blockers as the patients with this genotype needs lower doses and shorter duration of inotropic support after coronary artery bypass grafting.

Lanfear et al. [87] performed one of the largest clinical studies on effect of β AR (1 and 2) SNPs on mortality of patients with acute myocardial infarction and started β -blocker therapy. They tested four common β 1 AR genotypes (Arg389Gly, 1165C/G, Ser49Gly and 145A/G) and found no effect on mortality for either β 1 AR SNPs studied. They also studied four common β 2 AR SNPs (Gly16Arg, 46G/A, Gln27Glu, and 79C/G) and reported that increased mortality with the genotype Gly16Arg and 46A/A. Similarly the 3-year mortality for patients with genotype Gln27Glu and 79C/C is 16% and reduce up to 6% with C > G mutations. Interestingly this effect was observed only in patients taking β -blocker, but not in patients discharged without β -blocker treatment.

In addition to the pharmacogenomics effects of β AR polymorphisms, laboratories have also reported the effect of cytochrome P450 2D6 (CYP2D6) polymorphism on cardiovascular diseases. CYP2D6 metabolizes the β AR antagonist metoprolol in liver and genetic polymorphisms in this gene cause variability in metoprolol pharmacokinetics. The poor metabolism of metoprolol lead to elevated levels of this antagonist in plasma and cause significant reduction of heart rate, diastolic atrial blood pressure, and a higher incidence of bradycardia [88]. In another report, it was suggested that the polymorphisms that can increase CYP2D6 activity cause lower antagonist levels in plasma, poor heart rate control, and increasing risk of arrhythmias after myocardial infarctions [89].

10. Clinical Implications

Diabetes mellitus is a strong predictor of short-term and long-term recurrent ischemic events, including mortality.[90,91]. Diabetic patients without a history of coronary artery disease have the same overall cardiac risk as a non-diabetic patient with a history of a myocardial infarction [92]. They also have a higher risk of cardiovascular complications and recurrent athero-thrombotic events[93].

Platelets of diabetic patients have been proven to be hyperactive with intensified adhesion, activation, and aggregation leading to a higher risk of developing acute coronary syndromes and worse outcomes [94,95]. These patients experience increased platelet turnover, upregulation of P2Y₁₂ signaling, and increased GP expression, which may lead to inadequate responses of such agents as aspirin, thienopyridines, or GPIIb/IIIa inhibitors.

Thienopyridines include such agents as ticlopidine, clopidogrel, and prasugral. Evidence that diabetic patients may not respond to some of these agents as adequately when compared to

non-diabetic patients was demonstrated in the TRITON-TIMI 38 trial in which clopidogrel and prasugrel were compared. Despite compliance, patients with diabetes had a lower rate of ischemic events on prasugrel than clopidogrel [96]. Both drugs are prodrugs that undergo metabolism to their active form. Clopidogrel through the 2C19 enzyme and prasugrel undergoes hydrolysis and then oxidized by 3A4, 2B6, and to lesser extent the 2C19 enzymes. Genetic polymorphisms of the CYP enzymes may affect the drug's conversion to its active form causing variable responses in individuals. Ticagrelor, a newer agent, is a P2Y₁₂ ADP receptor antagonist that binds directly to the P2Y₁₂ receptor and does not require conversion to its active form [97]. Warfarin is a familiar agent and is the oldest agent used in the prevention of thromboembolic stroke in AF patients. Newer agents, such as dabigatran, rivaroxaban, and apixaban have entered the market. Concerns about genetic variants of CYP2C9 and VKORC1 have contributed to the differences in patients' response to various warfarin doses. More information about this translational information are emerging and being utilized in the clinical setting. New evidence is finding that genetic variants could also play a role in newer agents, such as dabigatran [98]. Currently, the data shows that the gene variant seems to influence the bleeding risk, but not its antithrombotic efficacy, but eventually may be utilized clinically to improve outcomes as in warfarin.

10.1. Warfarin therapy in diabetics

A study by Meier et al. comparing the promise of GPI (Glycoprotein IIb/IIIa inhibitors) suggests that the antibody abciximab are not alternatives to the use of heparin in anticoagulant therapy in diabetics suffering from blood clots [99]. But it can be used in addition to the unfractionated heparin (UFH). The basic idea is that diabetics are at higher risk to develop stable or unstable coronary artery disease and need the use of percutaneous coronary intervention (PCI). The study compared the choice between the uses of bivalirudin along with GPI's. The strategy for use is mostly dictated by the patients risk for ischemia and bleeding. The study suggests that bivalirudin is preferred for reasons that it is having a simple protocol and reduces the risk of bleeding. Over all the main conclusion of the study is that for minor bleeding risk use of Bivalirudin is preferred in real-world population while for major risk of bleeding for ischemia it may be comparable between both groups i.e. ischemia and risk of bleeding.

Another study by Schwalm et al. (2010) assessed the feasibility of evaluating the benefit and safety of use of triple therapy (TT-warfarin, ASA, and clopidogrel) versus dual therapy (DT-ASA and clopidogrel) in patients at risk of LVT post aSTEMI (post anterior ST-elevation myocardial infarction (aSTEMI) [100]. The study was designed as open labeled randomized controlled trial with follow-up and discharge and 3 month echocardiographic evaluation. Based on the outcomes the authors conclude that there was no significant difference in the composite endpoint at 3 months between triple therapy vs. the double therapy. STEMI patients have higher incidence of left ventricular thrombus despite routine use of early revascularization and dual antiplatelet therapy. The overall the study concludes that more effective antithrombotic therapies have to be devised and current therapies are inadequate. Therefore use of pharmacogenomics for improving the therapeutic outcomes along with disease detection may be crucial for clinical use.

10.2. Ion Channel blockers: Therapeutic Approaches for diabetes and cardiovascular conditions

Targeting ion channels have emerged as a promising therapeutic approach for the treatment of various diseases including cardiovascular [101] metabolic [102] and neurological disorders [103]. While the molecular basis of ion channel regulation in various diseases is still being elucidated; number of therapies that target ion-channels for prevention and treatment of cardiovascular diseases and diabetes have been proposed. For instance, there is growing evidence that modulation of ion channels such as voltage-gated, potassium (Kv) channels; Kv1.3, Kv1.4, Kv2.1, and calcium channels have strong antidiabetic indications [104].

10.3. Treatment strategy targeting ATP-sensitive potassium channels

The KATP channel comprises of 4 subunits of sulfonylurea receptor (SUR) and 4 subunits of channel protein (Kir6). Many current therapeutic agents for diabetes, such as sulfonylureas, nateglinide and repaglinide promote insulin secretion by binding to the regulatory sulfonylurea receptor subunit (SUR1 or SUR2) and inhibiting KATP channel currents [105]. Certain drugs act as potent stimulators of insulin secretion through direct closure of KATP channels mediated via Kir6.2 subunit [106,107]. Inhibition of insulin secretion via activation of KATP is a more recent approach for treatment of diabetes since reduction of insulin has been shown to increase β cells function. Indeed, KATP channel openers, such as diazoxide and NN414 have been shown to protect β cells and preserve function of islets [108,109,110].

10.4. Kv Channels as potential targets

Pharmacological strategies for targeting KV channels and recent progress in the preclinical and clinical development of drugs targeting KV1.x, KV7.x (KCNQ), KV10.1 (EAG1) and KV11.1 (hERG) channels have been discussed recently [111]. Kv channel is the dominant Kv current of β -cells [112] and its inhibition enhances glucose-induced insulin release by prolonging action potentials. Naturally, Kv channels have emerged as an exciting potential therapeutic target for treatment of type II diabetes [113,114].

10.5. Voltage-gated calcium (Cav) channels

Calcium channels (CaV) channels are ubiquitously expressed in various cell types and composed of at least three subunits; α 1, α 2- δ and β subunits. The α 1-subunit is a pore-forming component and six CaV α 1 subunits (CaV1.2, CaV1.3, CaV2.1, CaV2.2, CaV2.3, and CaV3.1) have been identified in pancreatic β -cells. These pore-forming subunits form complexes with other auxiliary subunits to conduct L-, P/Q-, N-, R-, and T-type CaV currents, respectively. A recent review summarizes current knowledge of CaV channels in β -cell physiology and pathophysiology [115]. A combination therapy using valsartan and a calcium channel blocker (CCB) has been claimed to improve endothelial dysfunction [116], thereby providing benefit in diseases in which normal endothelial function is disrupted such as heart failure, angina pectoris and diabetes, e.g. non-insulin dependent diabetes mellitus (NIDDM). Piperazine substituted compounds have also been used as calcium channel blockers to treat cardiovascular diseases and diabetes [117]. In addition to the above, a

number of formulations targeting sodium channels and beta blockers have also been claimed to be effective for cardiovascular diseases and diabetes (Table 2).

11. Current and Further Developments

In the present review, we describe the importance of pharmacogenomics in heart disease and how diabetes obesity and genomics are interrelated. The recent scientific advances achieved by use of genomic techniques for identification, detection and overall understanding as to *why* individual variations in disease phenotype and responses to drugs is described with examples for diabetes, long QT syndrome and cardiomyopathy. At fundamental level the role of ion channels in regulating the function of heart is described with special emphasis on key ion channels such as potassium (Kv), sodium (Nav) and I_K (HERG). Together, this report offers a review and advances of the topics in the exciting area of pharmacogenomics as it relates to diabetes and heart disease.

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Figure 1. Schematic for human cardiac ventricular action potential

The action potential represents the shape and form depicting the involvement of ion channel in manifesting into a complete waveform.

Figure 2. Phenotype of long QT syndrome

The pathway depicts the steps leading to pharmacogenomic phenotype for long QT syndromes. The flow chart shows the starting point with DNA until the cardiovascular phenotype utilized for identification and detection. Important intermediate points such as the RNA, protein and functional changes are shown as discrete steps. The environmental, drug induced influences, epigenetics and congenital categories influence are shown as pharmacogenomics indicators.

Figure 3. Techniques in pharmacogenomics

Use of different techniques in identification of single nucleotide polymorphisms (SNP) and diseases. The classical use of molecular biology concepts for primer design and use along with cDNA hybridization amplification by polymerase chain reaction (A), development of cloning techniques for identification and specific expression of the gene of interest using vector system is shown in (B), the high throughput technique for automating the PCR technique is shown in (C) with gene chip able to hybridize multiple genes in a short period of time. Panel (D) shows the evolution of current day deep sequencer able to provide whole genome information used for mapping of diseases.

Table 1

Single nucleotide polymorphism in β ARs and their pharmacogenomics effects.

SNP	Receptor	Effect	Reference
Gly389	β 1 AR	Better survival by high-dose β -blocker.	[83]
Arg389/Gln27	β 1/ β 2 AR	Two-fold increase in mortality in heart failure patients treated with carvedilol.	[85]
79 G	β 2 AR	Increase survival rate on β -blocker therapy after acute coronary syndrome.	[87]
46 G	β 2 AR	Increase survival rate on β -blocker therapy after acute coronary syndrome.	[87]
79 GG/46 GG	β 2 AR	Increase survival rate on β -blocker therapy after acute coronary syndrome.	[87]
CYP2D6 mutations	Cytochrome P450 2D6	Low metabolization of metoprolol and higher incidence of bradycardia.	[88]

Note: Three letter nomenclatures refer to amino acid and single letter corresponds to nucleotide.

Table 2

Therapeutic targets and molecular intervention.

	Description	Diseases	Targets	Publication Number
1.	Sodium channel blockers reduce glucagon secretion [118]	Hyperglycemia and related diseases	Sodium Channels	WO2013043925 A1
2.	Fused benzoxazepinones as ion channel modulators [119]	Cardiovascular diseases, diabetes and conditions related to diabetes.	Sodium Channels	WO2013006485 A1
3.	Fused heterocyclic compounds as ion channel modulators [120]	Cardiovascular diseases and diabetes	Sodium Channels	US20130005706 A1
4.	Methylglyoxal-scavenging compounds and their use for the prevention and treatment of pain and/or hyperalgesia [121]	Diabetes and other diseases associated with the formation of methylglyoxal and related reactive carbonyl species (RCS)	Sodium Channels	EP2435465 A1
5.	Human obesity susceptibility gene encoding potassium ion channels and uses thereof [122].	Obesity and associated disorders	Potassium Channels	WO2006092660 A1
6.	Pharmaceutical composition comprising shikonin derivatives from lithospermum erythrorhizon for treating or preventing diabetes mellitus and the use thereof [123].	Diabetes	Potassium Channels	EP2101749 A1
7.	Combination therapies using late sodium ion channel blockers and potassium ion channel blockers [124].	Atrial fibrillation and/or Atrial flutter	Sodium + Potassium Channels (Combination)	WO 2013112932 A1
8.	Potassium ion channel modulators and uses thereof [125].	Cardiovascular and metabolic diseases, including diabetes.	Potassium Channels	EP 2231594 B1
10.	Analog of ShK Toxin and Their Uses in Selective Inhibition of Kv1.3 Potassium Channels [126].	Obesity and Diabetes	Potassium Channels	US 20130274438 A1
11.	Pharmaceutical composition for treatment of diabetic complications [127]	Diabetes	Beta blocker	EP 2444077 A1
12.	Composition including beta-blocker as an active principle for treating or improving obesity, metabolic diseases, and osteopenia [128]	Obesity, metabolic diseases including diabetes mellitus secondarily caused by obesity, and osteopenia/osteoporosis.	Beta Blocker	WO 2013157833 A1
13.	Crystalline forms of sartans like telmisartan with beta blockers [129]	Hypertension, heart failure and myocardial infarction.	Beta Blocker	EP 2649996 A1
14.	Substituted heterocyclic compounds as ion channel modulators [130]	Cardiovascular diseases and diabetes.	Sodium Channels	US 20130102632 A1
15.	Antihypersensitive combination of valsartan and calcium channel blocke [116]	Cardiovascular diseases, diabetes etc.	Calcium Channels	WO2000002543 A2
16.	Piperazine substituted compounds used as calcium channel blockers [117]	Stroke, pain, anxiety disorders, depression, addiction, gastrointestinal disorders, genitourinary disorders, cardiovascular disease, epilepsy, diabetes and cancer	Calcium Channels	WO2005021523 A1