1.0 Introduction

Complex diseases result from the cumulative and interactive effects of large number of loci. Each locus imparts a modest marginal effect on expression of the phenotype (Marian, 2013). In the last few years, complex diseases especially diabetes mellitus (DM) has attained an epidemic proportion. DM is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, action or both. Chronic hyperglycaemia results in long term damage, dysfunction and failure of different organs especially heart, eyes, kidneys and blood vessels. Broadly there are two major forms of DM; Type 1 diabetes (T1D) and Type 2 diabetes (T2D). T2D accounts for 90-95% of all forms of diabetes and involves insulin resistance and relative insulin deficiency. It results from progressive defect in insulin secretion on the background of insulin resistance (American Diabetes Association, 2012). The worldwide prevalence of diabetes is expected to increase upto 552 million by 2030 and at that time Indians would comprise 20% of the total diabetic population with the highest number of people suffering from diabetes in the world (IDF Diabetes Atlas, 2011, www.idf.org/diabetesatlas).

As a complex disorder, T2D follows a polygenic inheritance as genetic factors as well as non genetic factors plays an important role in its pathogenesis (Kommoju and Reddy, 2011). There are a number of factors like dietary excess, physical inactivity (obesity), genetics and aging (mitochondrial dysfunction) etc. which result in an increase in insulin resistance and decrease in insulin secretion, hence predispose an individual to T2D (Doria et al., 2008). There are over 60 loci identified for the genetic susceptibility to T2D (Hara et al., 2014). The pathophysiology of T2D involves multiple defects in insulin action and secretion. Insulin is the key hormone that regulates blood glucose homeostasis. The important actions of insulin in glucose homeostasis involve inhibition of glucose production from the liver and stimulation of peripheral glucose uptake by the muscles. Reduction in biological action of insulin due to defects in insulin signalling pathway leads to insulin resistance (Spellmann, 2010). The key signalling molecules that effect insulin action include insulin, insulin like growth factors (IGF), insulin receptor substrate (IRS) proteins and various downstream proteins including signalling...
molecules. Insulin resistance and impaired insulin secretion are the major causes of T2D (Stumvoll et al., 2005). Obesity and physical inactivity play a major role in the progression of insulin resistance. Increased prevalence of T2D and obesity parallels each other and ‘diabesity’ is the new term given to this dual epidemic Various circulating hormones, cytokines and non esterified free fatty acids (NEFA), originating in adipocytes are responsible for modulating insulin action (Yaturu et al., 2011). Mitochondria and endoplasmic reticulum have a major role in maintaining cellular homeostasis, thus play an important role in the aetiology of T2D (Rieussset, 2011).

T2D is associated with a number of vascular complications which includes microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (Coronary artery disease, stroke and peripheral vascular disease) complications (Nigro et al., 2006). Of all the complications of T2D patients, cardiovascular diseases (CVD) are the leading cause of morbidity and mortality. Different CVD risk factors like insulin resistance, obesity, central obesity, low high density lipoprotein cholesterol (HDL-C), elevated triglycerides and elevated blood pressure are clustered in prediabetic subjects. CVD and T2D patients have insulin resistance in several tissues, which results in hyperglycaemia and hence predisposes an individual to atherosclerosis. Insulin resistance regulates almost all the mechanisms known to be associated with CVD in T2D individuals (Laakso, 2010).

Coronary artery disease (CAD) is the most common and life threatening cardiovascular complication of T2D subjects. It is characterized by the presence of atherosclerosis in the epicardial coronary arteries; the atherosclerotic plaques progressively narrow the coronary artery lumen and impair blood flow to heart which may lead to myocardial infarction (MI) (Marchetti et al., 2007). Atherosclerosis has been previously considered as a cholesterol storage disease but is now considered as an inflammatory disorder. Various biochemical stimuli trigger the immunological response in the vascular smooth muscle cells and result in CAD (Nigro et al., 2006). Risk for CAD is high among diabetic subjects by the factor of 2 to 4 as compared to non diabetic subjects (Deepa et al., 2002). CAD and diabetes have many risk factors in common like age, hypertension, obesity, dyslipidemia and physical inactivity. Thus, high prevalence of diabetes directly or indirectly increases the risk of CAD (Haffner et al., 1998). In India CAD occurs
prematurely i.e one or two decades earlier than west. There is a high prevalence of CAD and diabetes in affluent migrant Indians as well as those living within the Indian subcontinent. There is a strong association between CAD and diabetes despite wide ethnic and geographic variations in their prevalence. Thus, there is an urgent need for studies on CAD in diabetic and non diabetic subjects from India (Mohan et al., 2010).

Apart from the various environmental risk factors, both CAD and T2D have a strong genetic component. Genetic component in T2D is validated from the increased risk in the first degree relatives of the affected individuals (Pierce et al., 1995), high lifetime risk in the offspring if both the parents are affected (Ridderstrale and Groop, 2009) and high concordance in monozygotic than dizygotic twins (Kaprio et al., 1992). Similarly, there is a high concordance rate of atherosclerosis and MI in monozygotic twins than dizygotic twins (Zdravkovic et al., 2007). The results of adoption studies have further validated the contribution of genetic component in atherosclerosis and thereby in CAD (Peterson et al., 2002).

To identify the various susceptibility loci for T2D and CAD, strenuous efforts with different study designs and strategies have been made in the past few years. As both T2D and CAD are the multifactorial diseases, candidate gene approach has been the most preferred approach over linkage and positional cloning (Kwon and Goate, 2000). In the recent years, the search for the determination of the various genetic variants in the common diseases has changed dramatically by the introduction of high throughput technology of Genome wide association studies (GWAS). The first GWAS for finding the susceptibility loci for T2D was conducted on European populations with the finding of few susceptibility loci but by increasing the sample size and wider coverage of genome, a number of loci have been found (Sousa et al., 2011). While in GWAS for CAD, fewer genetic variants met the threshold of p< 5.10^{-8} as compared to T2D. The most replicated locus ANRIL was found on chromosome 9p2.3 (McPherson et al., 2007; Mohlke et al., 2008). There is as such no comprehensive report of GWAS for patients with both T2D and CAD.

As insulin resistance best links CAD and T2D, genes of insulin signalling pathway are the major candidate genes for both the diseases. The cause of insulin resistance
primarily results from defects in insulin signalling pathway. Binding of insulin or
insulin like growth factors on insulin receptors triggers tyrosine autophosphorylation of
β-subunit of insulin receptor substrates (IRS1 and IRS2). Phosphorylation of insulin
receptors activates downstream signalling cascade for glucose uptake through glucose
transporters (GLUTs) by activating Phosphatidylinositol 3-kinase (PI3-K) which further
phosphorylates and activates Akt. Peroxisome proliferator-activated receptor-γ (PPARγ)
is a transcription factor that promotes insulin stimulated tyrosine phosphorylation of
IRS1, IRS2 and PI3-K activity associated with insulin receptor substrate proteins
(Zhang et al., 1994; Smith et al., 2001; Jiang et al., 2002).

One of the mechanisms that link diabetes with vascular damage is endothelial
dysfunction which is characterized by defects in vascular relaxation in response to
increased blood flow. Reduction in bioavailability of nitric oxide (NO) which is a potent
vasodilator, leads to endothelial dysfunction. NO is produced in the endothelial cells by
the stimulation of nitric oxide synthase (eNOS). Several pathways can result in eNOS
dysfunction, of which insults in PI3-Akt pathway leads to diabetes and impairment of
both endothelial function and NO production. Blockade of Akt is known to reduce
eNOS activity by inhibiting phosphorylation of eNOS serine residues (Kobayashi et al.,
2005).

1.1 Insulin receptor substrate 1 (IRS1)

IRS1 is located on chromosome 2q36 and has an important role in insulin action in
skeletal muscle, adipose tissue and pancreatic β cells (Nandi et al., 2004). Apart from
peripheral insulin sensitivity it has also been found to be associated with regulation of
insulin secretion by pancreatic β cells (Sesti et al., 2001). IRS1 is involved in the insulin
signalling in adipose tissue and skeletal muscle cells. It regulates muscle glucose
transport, brown adipocyte differentiation and insulin induced β cell insulin secretion
(White, 2002). G972R (Glycine to Arginine) is the most common polymorphism of
IRS1. The carriers of this variant have 25% increased risk of developing T2D (Jellema
et al., 2003). It has been found that this variant causes insulin resistance in vasculature
and directly impairs NO formation in the endothelial cells by direct impairment of Akt/
eNos pathway (Federici et al., 2004). Apart from T2D and cardiovascular diseases,
G972R variant is found to be associated with various diseases like colorectal cancer, T1D, non dipper hypertension, gestational diabetes mellitus and polycystic ovary disease (Morrison et al., 2004; Slattery et al., 2004; Pappa et al., 2008; Valdes et al., 2008; Dziwura et al., 2011).

1.2 Insulin receptor substrate II (IRS2)

It is present on chromosome 13q34 and has a very important role in the development and survival of pancreatic β cell, lipolysis of fat, glucose production by liver and pituitary ovarian axis function (Sesti et al., 2001). It also has a potential role in atherosclerosis as IRS2 deficiency in macrophages, leads to insulin resistance. As a result the accumulation of macrophages is enhanced in vascular wall as well as there is increased expression of proinflammatory mediators in macrophages (Mita et al., 2011). Glycine to Asparagine (G1057D) is a common missense variation, which might lead to T2D by interacting with obesity (Mammarela et al., 2000; Stefan et al., 2003; Bodhini et al., 2007). Inspite of its potential role in vasculature, G1057D polymorphism is less extensively studied in the context of CVDs. G1057D polymorphism has been found to be associated with various diseases like colorectal cancer (Slattery et al., 2004), gastric cancer (Zhao et al., 2012) and ovarian cancer (Cayan et al., 2011).

1.3 Peroxisome proliferator activated receptor gamma (PPARγ)

It is a transcription factor and is the most extensively studied Peroxisome proliferator activated receptor (PPAR). It is present on chromosomal location 3p25. It is highly expressed in adipose tissue and macrophages, where it is involved in adipocyte differentiation, triglyceride synthesis, differentiation of adipocytes and glucose homeostasis (Semple et al., 2006). It has various direct and indirect metabolic effects which are antiatherogenic. It regulates the release of various adipokines including tumor necrosis factor alpha (TNFα), angiotensinogen (AGT), interleukin-6 (IL-6) and plasminogen activator inhibitor 1 (PAI-1) (Ahima and Flier, 2000). It is widely expressed in endothelial cells, vascular smooth muscle cells, macrophages and T cells. It has a potential effect on vasculature by decreasing cytokines, vasoconstrictors like endothelin 1 and lox-1, increasing cholesterol efflux and NO release (Plutzky, 2004). In PPARγ, Proline to Alanine (Pro12Ala) is a loss of function variation that has been
found to provide protection for T2D (Gauda et al., 2010) and CAD (Galgani et al., 2010). Another polymorphism C1431T is a silent variation in exon 6 and is also found to be associated with CAD (Liu et al., 2007) and insulin resistance (Moffett et al., 2005). As \( \text{PPAR}_\gamma \) has anti-inflammatory action, thus it may have protective role in various diseases like sepsis, atherosclerosis, cancer and other inflammatory diseases (Schmidt et al., 2010).

1.4 Rationale of the study

India is a vast country with different ethnic groups inhabiting different geographical regions of the country (Thangaraj et al., 2006). Lifestyle diseases like CAD and T2D are influenced by genetic and environmental factors. Ethnic heterogeneity plays an important role in determining the genetic susceptibility to both the diseases. Indians are racially more predisposed to T2D and premature CAD as explained by their Asian Indian phenotype, insulin resistance and greater abdominal obesity (Higher waist circumference despite lower body mass index) (Mohan et al., 2010). The prevalence of T2D and CAD in Punjab is further confounded by higher prevalence of obesity, physical inactivity and consumption of fat rich diet (Kaur et al., 2010; Kaur et al., 2013). All these factors have led to a drastic increase in the prevalence of T2D and its associated complications especially CAD in the region. In population of Punjab, prevalence of CAD has been found out to be 18% among diabetes patients (Bhatti et al., 2007). In the recent years, rural to urban migration has led to the adoption of sedentary lifestyle by the majority of people, hence is responsible for the high prevalence of obesity in the region. The population of Punjab is also known for specific dietary pattern and eating habits (Kaur et al., 2013; Matharoo et al., 2013). A survey conducted by the Government of India (GOI), National Sample Survey Organisation, Ministry of Statistics and Programme Implementation has documented higher average calorie consumption in Punjab on account of the intake of fewer cereals, more fat and sugar. Calorie rich dietary pattern and intake of fat rich diet is responsible for obesity and upsurge of T2D and CAD in the region (GOI National Sample Survey Organisation, 2001)

The majority of genetic studies for the search of genetic variants in T2D and CAD are on the western population or migrant Indian population. Little information is available
for Indian populations, especially North Indian population groups. There is a complete lacuna for the genetic studies in T2D with CAD patients. The prediction of individuals at risk for developing premature atherosclerosis or CAD is important to select patients at high risk for more aggressive intervention strategies at an early stage of T2D. The reason for enhanced susceptibility for CAD among T2D subjects is still not clear. Thus, it becomes imperative to study the role of genetic factors along with obesity and dyslipidemia in predisposition to T2D and CAD as well as their coexistence in the population of Punjab.

For the search of various pathways involved in pathophysiology of CAD and T2D, it was found that the insulin resistance plays an important role in the pathogenesis of these diseases. It is the common mechanism which best links obesity, T2D and CAD. PI3-K/Akt pathway has a potential role in insulin signalling as well as NO formation in endothelium. Thus for the present study genes from this pathway are selected. Various authors have studied the *IRS1* (G972R), *PPARγ* (Pro12Ala, C1431T) and *IRS2* (G1057D) polymorphisms separately in T2D or CAD and there are no comprehensive reports about their relation with T2D and CAD and their relative contribution in predisposition of CAD in T2D Patients. To the best of my knowledge, this is the first study in population of Punjab for the search of the genetic link in CAD and T2D by studying CAD and T2D together.

Thus, the present study was proposed with a **hypothesis** that polymorphisms; Pro12Ala and C1431T in *PPARγ*, G972R in *IRS1* and G1057D in *IRS2* are among the genetic factors predisposing population of Punjab to T2D and CAD.

### 1.5 Objectives

- To find out the association if any, of CAD and T2D with the selected polymorphisms in *PPARγ*, *IRS1* and *IRS2*.
- To analyse the interaction of selected genes for better understanding of their role in the predisposition to CAD and T2D.
- To study the association of obesity as estimated by anthropometric factors with selected polymorphisms in CAD and T2D.