INTRODUCTION

Type 2 diabetes is a global public health crisis, which threatens all economies especially those of developing countries (Hu, 2011). The number of people with diabetes is increasing due to rapid ageing, population growth and rapid urbanisation (Wild et al., 2004). As of now type 2 diabetes (T2D) has become one of the most challenging health problems of the 21st century. There have been several previous estimates predicting the epidemic rise in number of people with diabetes worldwide (King et al., 1998; Wild et al., 2004). The recent studies estimated that more than 171 million people are affected with T2D and predicted figures are to touch 366 million by the year 2030 (Wild et al., 2009). Other comprehensive studies also predicted that there would be 439 million diabetic people by the year 2030 (Shaw et al., 2010; International Diabetes Federation (IDF), 2012) and these studies further reveal that between 2010-2030, developing countries will be worst hit where the number of people with T2D will increase by whopping 69% while increase in developed countries will be about 20% (Shaw et al., 2010).

Diabetes mellitus is recognized as a constellation of heterogeneous disorders characterized by chronic hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or no feedback inhibition of hepatic glucose production, which leads to characteristic abnormalities in the metabolism of fuel (carbohydrate, lipid, and protein). On the basis of aetiology and clinical presentation diabetes mellitus is classified into four types (ADA, 2011) Type 1 diabetes (T1D), Type 2 diabetes (T2D), gestational diabetes, and other forms of diabetes (Kuzuya et al., 2002). Traditionally, diabetes mellitus is classified into two major categories namely T1D and T2D. T1D is characterized by destructive lesions of pancreatic β-cells by either an autoimmune mechanism or unknown cause and accounts for about 5–10% of all cases of diabetes (Cooke, 2004). T2D is characterized by combination of decreased insulin secretion and decreased insulin sensitivity (insulin resistance). Among all the forms of diabetes T2D is a major health problem in developed and developing countries, which accounts for approximately 90-95% of
diabetic individuals (de la Sierra et al., 2009). The heterogeneous, multifactorial/polygenic, late onset and progressive nature of T2D has made it more difficult for researchers to identify the root cause of this complex disease. In early stages T2D can be asymptomatic due to mild metabolic abnormality but in later stages it becomes symptomatic because of elevated hyperglycaemia due to abnormal glucose homeostasis (Scheen, 2003). The classical symptoms of T2D include thirst, polydipsia, polyuria and weight loss whereas, in severe cases, ketoacidosis or hyperglycemic–hyperosmolar states occur which lead to disturbances of consciousness, coma and even death unless treated appropriately (WHO, 1999).

As the duration of T2D increases, there is steep rise in its complications, which are broadly classified as, microvascular and macrovascular (Campbell, 2000). Microvascular complications are specific to T2D with the principal sites affected being eyes (retinopathy), kidneys (nephropathy) and the nervous system (neuropathy), leading to clinical consequences like blindness, renal failure and foot problems with risk of amputation. On the other hand, macrovascular complications are nonspecific and are not unique to T2D but occur more commonly in T2D patients, giving rise to heart attack, stroke and gangrene. Both macrovascular and microvascular complications are significant cause of morbidity and mortality among diabetic subjects (Zargar et al., 1999).

Pathomechanism of T2D is very complex and controlled by multiple factors leading to chronic hyperglycemia caused by insulin resistance of peripheral tissues (skeletal muscle, liver, adipose tissue) (Singh, 2011), altered balance of central nervous system pathways controlling food intake and energy expenditure (Porte et al., 2005; Schwartz and Porte, 2005; Seeley and Tschop, 2006) and insufficient compensatory insulin secretion by pancreatic β-cells (Scheen, 2003). Multiple mechanisms have emerged as potential cause of T2D progression with impaired mitochondrial capacity and/or function; altered insulin signaling due to cellular lipid accumulation, proinflammatory signals, and endoplasmic reticulum stress and reduced incretin-dependent and independent β-cell insulin secretion being more common. Thus, these T2D related quantitative traits including insulin secretion, insulin sensitivity and β-cell function
(Rodriguez et al., 2010), mitochondrial dysfunction (Lowell and Shulman, 2005) have been studied in relation to genes involved in various metabolic pathways.

On the other hand, it is also commonly accepted that T2D results from adverse environmental factors of the modern world (i.e., high-caloric diet, physical inactivity and a sedentary lifestyle) which favour the development of obesity. In fact, excess body weight represents a major risk factor for T2D (Hu et al., 2011), however, about 10% of T2D patients display normal weight and many obese subjects never develop T2D, indicating that disease is not exclusively caused by environmental factors. Thus, making it evident that pathophysiology of T2D does not involve environmental factors alone but genetics also play a role in its development, which have been are supported by ample evidences. Firstly, exceptionally high T2D prevalence have been observed in certain ethnic minorities and indigenous groups with low population admixture (Pima Indians, Micronesians and other Pacific Islanders, Australian Aborigines, and Mexican-Americans) (Knowler et al., 1978; Brosseau et al., 1979; Serjeantson et al., 1983; Stern, 1999). Secondly, first-degree relatives have shown up to 3.5 fold higher risk to develop T2D, which indicates its clustering within families (Gloyn and McCarthy, 2001; Lyssenko et al., 2005; Weires et al., 2007). Thirdly, the lifetime risk of developing T2D is about 40%-70% in offspring, if one or both parents are affected (Köbberling and Tillil, 1982; Ridderstråle and Groop, 2009) and greater, if the mother is affected with T2D (Groop et al., 1996). Finally, higher concordance rate of T2D in monozygotic (70%) as compared to dizygotic (10%) twins (Newman et al., 1987; Kaprio et al., 1992) suggested that T2D has a strong genetic component.

T2D being a complex disease does not follow simple Mendelian inheritance, but is considered as a polygenic disease caused by the simultaneous occurrence of common DNA sequence variations in many genes (Collins et al., 1997). Each of these DNA alterations is supposed to exert only moderate effects on the affected gene’s function and/or expression, whereas in additive manner these variations confer an increased susceptibility towards the adverse environmental factors. Exactly, how many genes and what their relative contributions are still remains uncertain (Rich, 1990; Kahn et al., 1996; Doria et al., 2008). The rapid increase in T2D during the past 50 years must be
ascribed to changes in the environment rather than to genes as the genetic background has not changed during this period (Ridderstråle and Groop, 2009), however, the genetic background determines how we respond to the environment (Luan et al., 2001; Memisoglu et al., 2003; Ylönen et al., 2007).

In the last 10 years strenuous efforts with different study designs and strategies have been made to identify various susceptibility loci for T2D (Hirschhorn and Daly, 2005; Bonnefond et al., 2010). Linkage and positional cloning of genes was more labor-intensive approach because of non-Mendelian mode of inheritance of T2D; non-availability of family samples (Teare and Barrett, 2005) and the size of the chromosomal areas linked to T2D often encompassing up to hundreds of genes. No doubt this approach helped in finding few candidate genes like CAPN10, ENPP1 and TCF7L2, which were validated and replicated in several populations and ethnicities. Candidate gene approach was the most preferred approach to find a genetic variant with modest effect (Kwon and Goate, 2000) within a gene providing risk in complex disorders. This approach was most successful in finding, validating and replicating variants in different genes associated with T2D. With the advent in high throughput genotyping technology, the era of genome wide association started which changed the genetic landscape of T2D. The first GWAS in T2D (Sladek et al., 2007) provided important evidences that GWAS would work for complex diseases (Wheeler et al., 2011). The maximum number of such studies have been conducted in European populations (Hayes et al., 2007; Salonen et al., 2007; Scott et al., 2007; Rampersaud et al., 2007), and only a few in Asian populations (Yasuda et al., 2008; Unoki et al., 2008; Tsai et al., 2010; Yamauchi et al., 2010). So far 44 T2D susceptibility loci have been identified by successive rounds of GWAS and GWA meta-analysis (Loos et al., 2008; Zeggini et al., 2008; Willer et al., 2009; Heid et al., 2010; Speliotes et al., 2010; Voight et al., 2010). The recent GWA meta-analysis also identified some additional susceptibility loci for T2D (Strawbridge et al., 2011).

It was known earlier that risk of developing T2D is a combination of genetic risk for β-cell dysfunction superimposed on genetic and environmental factors (e.g. obesity, western diet, sedentary lifestyle) that promote insulin resistance, but recent genetic
discoveries have identified numerous variants that appear to influence insulin secretion rather than insulin resistance (Billings et al., 2010). In addition, the patho-mechanistic investigations found that most of the risk SNPs affect β-cell and favour involvement of β-cell centric view on the genetics of T2D (Staiger et al., 2009). The TCF7L2 and KCNJ11 are two such putative candidate genes which were discovered by linkage and candidate gene studies respectively, play a central role in insulin secretion whose variants have shown strong association with T2D in almost every European (McCarthy and Zeggni, 2009) and in few Asian populations (Chauhan et al., 2010; Gupta et al.; 2010). So, it becomes necessary to explore the risk of T2D provided by variants in these two genes in Indian populations.

**TCF7L2**

The strongest signal of T2D is within a region on chromosome 10q25 located in a 92 kb linkage disequilibrium block in the TCF7L2 gene (Grant et al., 2006). TCF7L2 is also known as TCF-4/ β-catenin interacting protein which is a high-mobility group box-containing transcription factor that is involved in the wnt signaling pathway and also acting as a nuclear receptor for β-catenin (Prunier et al., 2004; Yi et al., 2005). Wnt signaling is critical for cell proliferation and involved in many aspects of embryogenesis, including adipogenesis (Prestwich and MacDougald, 2007), myogenesis (Cossu and Borello, 1999), and pancreatic islet development (McLin et al., 2007). TCF7L2 is believed to develop T2D mainly by affecting three mechanisms, such as, glucose stimulated insulin secretion, incretin stimulated insulin secretion (incretin sensitivity or secretion) and proinsulin to insulin conversion (McCaffery et al., 2011). Activation of TCF7L2 gene induces a variety of genes downstream, including those for intestinal proglucagon and glucagon-like peptides-1 and glucagon-like peptides-2 (Fehmann et al., 1995). Expression studies in β-cells have given conflicting results TCF7L2 with one case showing a blunting of glucose-stimulated insulin secretion (Lyssenko et al., 2007) and another showing a beneficial effect to protect islets from glucose and cytokine-induced apoptosis and impaired function (Shu et al., 2008). Other studies suggest roles for TCF7L2 in T2D via control of the incretin axis, hepatic glucose production and adipocyte function (Lyssenko et al., 2007; Cauchi et al., 2006).
The variants in this gene contribute more powerfully to the risk of developing T2D than any other gene discovered (Zeggini and McCarthy, 2006). One study revealed that islets from carriers of the risk genotypes have increased TCF7L2 mRNA compared to non-carriers (Lyssenko et al., 2007). TCF7L2 could exert its effect in a variety of ways, since it is expressed at high levels in a wide variety of tissues, including the hypothalamus. Clinically, carriers of the high-risk TCF7L2 genotype have reduced insulin secretion (Florez et al., 2006), suggesting a possible role of the gene in the β-cell dysfunction for T2D. The association of the TCF7L2 locus with T2D is by far the strongest and most consistent signal across the GWA studies. Meta-analysis of (WTCCC, Fusion, and DGI) studies have provided 1.37 fold risk to develop T2D with a combined p value of $10^{-48}$ (Zeggini et al., 2007; Saxena et al., 2007; Scott et al., 2007). Variants within TCF7L2 remain the basis for the strongest T2D-susceptibility signal in Europeans (McCarthy and Zeggini, 2009). However, by far, very little information about association of variants in TCF7L2 gene with T2D is available in Indian population and almost none in population of Punjab.

**KCNJ11**

KCNJ11 (Potassium inwardly rectifying channel, subfamily J, member 11 gene) together with ABCC8 gene encodes for an octamer protein that regulates transmembrane potential and glucose-stimulated insulin secretion by pancreatic β-cells (Ridderstråle and Groop, 2009). However, the pancreatic β-cell may not be the only important target organ but it is also expressed in wide variety of tissues and organs like heart, pituitary gland, skeletal muscle and smooth muscle (Staiger et al., 2009). Closure of the potassium channel is a prerequisite for insulin secretion and blood glucose uptake. Severe defects in glucose induced insulin secretion were verified by KCNJ11 knockout mice (Seino et al., 2000), also expression study in transgenic mouse model suggested that defects in KCNJ11 cause loss of glucose sensing in β-cell and POMC neurons, leading to impairment in insulin action and secretion in whole body (Parton et al., 2007). A study on human subjects also revealed that rare loss of functional mutations in KCNJ11 cause decreased function of the KATP channels, leading to persistent hyperinsulinemic and hypoglycemia of infancy (Lin et al., 2008). On the
contrary, activating mutations lead to permanent neonatal diabetes due to over activity of KATP channel (Gloyn et al., 2004).

A common glutamate (E) to lysine (K) change at position 23 (E23K) has been consistently associated with T2D, which leads to modest reductions in ATP sensitivity and insulin secretion (Schwanstecher et al., 2002; Nielsen et al., 2003; Florez et al., 2004; Saxena et al., 2007), impaired insulin sensitivity and process of glucose metabolism disturbance (Wang et al., 2011). E23K (rs5219) polymorphism of KCNJ11 has been highlighted as a candidate gene loci because of its consistent association with T2D in populations of different ethnicities (Schwanstecher et al., 2002; Gloyn et al., 2003; Love-Gregory et al., 2003; Florez et al., 2004) which has also been verified by genome wide association studies (Zeggini et al., 2007; Saxena et al., 2007; Scott et al., 2007). Although many replications have been performed to determine the relationship between the KCNJ11 gene and T2D, there are still few studies that explore the association of the KCNJ11 gene in Indian population (Chauhan et al., 2010; Gupta et al., 2010) making it important to decipher its role in T2D development in population of Punjab.

**MT-ND3**

In addition to nuclear genome, mitochondrial genome serves multiple essential cellular functions apart from energy generation via oxidative phosphorylation (OXPHOS) which support various metabolic reactions of human body. The mitochondrial dysfunction (Lowell and Shulman 2005) due to defects in mitochondrial genome are directly influenced by increase in ROS production or altered ATP/ADP ratio, essential for molecular signaling and release of insulin from pancreatic β-cells respectively. The G10398A (rs2853826) polymorphism in MT-ND3 is believed to increase ROS production in stress condition like hyperglycemic which could be one of the reasons for T2D development (Bhat et al., 2007). Many case-control studies were carried out to genotype this polymorphism in various populations. The A allele of 10398 (rs2853826) polymorphism is supposed to increase ROS production which results in less ATP production, required for first phase of insulin secretion in β-cell, resulting in direct influence of this polymorphism in development of T2D (Bhat et al., 2007), whereas,
conflicting results have been obtained with few, reporting the association of G allele with the disease. The G carriers had 1.26-fold increase in risk of developing metabolic syndrome compared to A allele carriers in a Chinese population (Juo et al., 2010) but another study by Liao et al. (2008) reported that T2D in Chinese Han population. As inadequate information is available for G10398A in relation to T2D, it becomes necessary to find the status of this polymorphism in T2D patients in India.

**Rationale of Study**

India has now become one of the largest populous countries of the world, with the most distinct feature being its varied religious communities, hierarchical castes, sub castes and isolated tribal groups. Many of these groups have strict social rules governing mating patterns demonstrating their extensive genetic diversity (Bamshad et al., 2001; Roychoudhury et al., 2001; Basu et al., 2003; Kivisild et al., 2003; Cordaux et al., 2004; Kashyap et al., 2006; Sahoo et al., 2006; Sengupta et al., 2006; Thanseem et al., 2006), thus, providing a unique resource for dissecting complex disease etiology and pathogenesis. T2D is one of those diseases which has increased at an alarming rate all over the globe (IDF, 2011), with India being the most affected region often known as diabetes capital of the world (Mohan et al., 2007) where prevalence is reaching epidemic proportions (Ramachandran et al., 2001; Bjork et al., 2003; Anjana et al., 2011). The reason of increase in number of diabetic patients in India is due to adaptation of western lifestyle, urbanization, mechanization, poor dietary habits and sedentary lifestyle/ physical inactivity. Researchers have made many strides to observe the link of T2D with behavioural and environmental factors such as BMI, physical inactivity and dietary habits, hereditary or genetics factors, aging, race or ethnicity (Hu et al., 2001; Narayan et al., 2001; Meisinger et al., 2002; Kriska et al., 2003; Hu et al., 2004; Wild et al., 2004; Chaturvedi, 2007; Hussain et al., 2007;). Among these risk factors physical inactivity, diet and obesity are predicted as major contributors to T2D in India too. In addition to the strong environmental component, there is compelling evidence that genetic factors are also involved in the pathogenesis of T2D.
From past 10 years, the advancement in genotyping technology and increasing prevalence of T2D has changed its genetic landscape large extent by simply raising number of susceptible loci for this complex disease. Looking at the overall outcomes of all genetic studies (linkage, candidate gene approach, GWAS and GWAS meta-analysis), most of the genes identified belong to insulin secreting pathway (Schäfer et al., 2011) but only three genes TCF7L2, KCNJ11, PPARG (Visscher et al., 2012) are believed to be strong putative candidate genes for T2D. According to Indian Genome Variation Consortium (2008), the genetic basis of several diseases in Indian populations is different from that of Europeans, which could be due to differences in the risk allele frequency and pattern of linkage disequilibrium. The above contrary observations make it pertinent to study the genetic variability of the implicated genes, involved in insulin secretion pathway, in Indians endogamous groups, as such a data is merely present for Indian populations and if present, it is confined to only few population group of India.