Chapter – 5

Discussion
DISCUSSION

T2D has long been known to be a multifactorial disease with a complex aetiology, including both genetic and non-genetic factors. Studies in India focused only on either the genetic contribution or environmental factors when estimating the risk of T2D. In the present study, we investigated genetic, biochemical and environmental risk factors involved in the pathogenesis of T2D and glucose metabolism. The overall aim was to establish a research design to investigate genotype-phenotype relations between common genetic variants of T2D and related traits.

The early life conditions may likely contribute to the gender differences in T2D prevalence. In the first half of the 20th century, a pronounced female excess in T2D was evident in USA and Europe. Presently, T2D is equally prevalent in men and women in most Western populations, with some evidence of male preponderance in early middle age (Gale & Gillespie, 2001). The opposite pattern prevails in developing countries: the prevalence of obesity and T2D are higher in women compared to men (Jafar, Chaturvedi, & Pappas, 2006; Luo et al., 2007; Reddy, 2004; Roglic, 2009). Such gender differences can be at least partly explained by the secular trend in socioeconomic conditions in developed countries. Thus, it is well established that the adverse socioeconomic conditions in early life can profoundly affect individual development and produce lasting increase in the risk of metabolic and cardiovascular disease later in life (Kuh & Ben-Shlomo, 1997). Our study is in agreement with earlier studies, where the prevalence of T2D is more in males than females (54.2% vs. 45.6%).

This investigation showed that Mysore district population is at considerable risk of T2D and have one of the highest prevalence rates of T2D. Results from this investigation also confirm that among this group, T2D occurs at a much younger age and at lower body mass index than in other ethnic groups, including Whites. Population surveys in India have also concluded the highest prevalence of T2D in the 40 to 60 years age group with a steep increase in prevalence prior to age 50 (Mohan,
Sandeep, Deepa, Shah, & Varghese, 2007). King, Aubert, and Herman (1998) reported that in developing countries, the middle age group (45-64 years) has the largest number of T2D in contrast to the 65plus age group in the developed countries. The differences observed in the incidences T2D among different populations can possibly be attributed to ethnicity, population dispersion, physical characteristics and the multiple definitions and surveillance procedures adopted for T2D in the previous studies.

In this study, one measure of mixed food habit diet consumption was assessed but its association with T2D or ethnicity was inconclusive even though the prevalence was higher. South Asian's food habit consisting of higher and faulty fat intake, higher carbohydrates and lower proteins-coupled with reduced physical activity levels is considered a major factor in their increased susceptibility to T2D, cardiovascular disease and other associated diseases (Landman & Cruickshank, 2001; Raheja, Bhoraskar, & Narang, 1996). Maternal and paternal diabetes conferred equal risk of overt T2D among offsprings (Papazafiropoulou et al., 2009). Family histories reflect both inherited genetic susceptibilities and shared environments, which include cultural factors such as preferences, values and perceptions and behavioral factors such as diet and physical activity (Baptiste-Roberts et al., 2007). The results of the present study showed that a family history of diabetes is more pronounced in patients with T2D compared to non diabetic controls. Non diabetic controls had a less incidence of personal history and family history of T2D. Further, the present study showed that family history of diabetes and sedentary lifestyle are more pronounced in patients with T2D compared to non diabetic controls. These two factors may be the driving forces in leading type 2 diabetic patients to become diabetic.

T2D showed a positive and independent association with body mass index (BMI), waist to hip ratio (WHR), family history and sedentary physical activity (Jali, Kambar, Jali, & Gowda, 2009). BMI is a screening tool and not a diagnostic tool (Flegal, Tabak, & Ogden, 2006). The World Health Organization (WHO) has suggested that BMI, the ratio of body weight in kilograms to height in meters squared, is a simple anthropometric index of overweight and obesity and is suitable for use in population surveys. Although it is acknowledged that more precise methods to
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quantify body fat exist. BMI is easy to measure and is widely used for categorizing individuals as overweight \((25 \text{kg/m}^2 \leq \text{BMI} < 30 \text{kg/m}^2)\) or obese \((\text{BMI} \geq 30 \text{kg/m}^2)\) (Razak et al., 2005). While WHO puts the ‘normal range’ for the general population at 18.5 to 24.99, the WHO Western Pacific Region Office (WPRO) considers a BMI of 23.0 as overweight in people of Asian origin and 25.0 or above as obese. The risk of diabetes increases progressively upwards of a BMI of 20.0 (Bhattarai & Singh, 2007).

Obesity and diabetes are increasing globally in epidemic proportions across all age groups (Metzger, 2006). Abdominal obesity is associated with both type 2 diabetes and metabolic complications, including elevations in several circulation biomarkers of cardiovascular risk (Haffner, 2007). Insulin resistance occurs in a wide variety of pathological states and is commonly associated with obesity, T2D, accelerated atherosclerosis and hypertension (Iwai et al., 2006). Treatments that not only reduce HbA1c, but also improve other associated changes such as abdominal obesity are urgently needed (Yeh et al., 2009).

BMI and WHR are two important anthropometric measures proposed to define obesity (Nyamdorj et al., 2008). BMI was found to be lower in non diabetics compared to type 2 diabetic patients. In the present study, BMI and WHR were measured to understand the influence of these parameters directly on obesity and indirectly on diabetes. The mean BMI of type 2 diabetic patients were found to be high compared to non diabetics. Abdominal obesity as per WHO criterion in terms of waist to hip ratio was found to be >0.90cm for males and >0.85cm for females (Sachdev et al., 2005). In the present study, the increased waist to hip ratio and thereby increase in abdominal obesity was seen in a greater percentage in type 2 diabetic patients compared to normal subjects, this may be due to the greater percentage of type 2 diabetic patients adopting the sedentary lifestyle.

Obesity is a complex disorder, where genetic predisposition interacts with environmental exposures to produce a heterogeneous phenotype (Meigs et al., 2006). Studies suggest that the presence of maternal diabetes during pregnancy substantially increase the risk of T2D and obesity in childhood and early adult life (Lindsay et al., 2010). Increasing evidence suggests that prenatal exposure to a hyperglycemic
environment can alter growth trajectories and homeostatic regulatory mechanisms, thus causing lifelong changes that result in an increased risk of overweight and obesity (Pirkola et al., 2010). The results of the present study showed that the obesity parameters namely, BMI and waist-to-hip ratio was found to be increased in type 2 diabetic patients, which confirmed the fact that obesity is a risk factor for T2D. Obesity observed in T2D in the present study might be due to interaction of the environmental exposures with genetic predisposition and or due to prenatal exposure to a hyperglycemic environment.

Results from this investigation confirm that with higher BMI, the prevalence of T2D among cases was higher than controls. This has also been shown to be true in a recent Canadian study showing that this group was also more likely to develop diabetes at a lower BMI and body weight than the Whites and Aboriginals (Anand et al., 2000). Consequently, revisions to BMI cut-offs for South Asians have been proposed to increase the sensitivity of this measure by reclassifying overweight to BMI of 23 or higher (instead of 25) and obesity to BMI of 25 or higher (instead of 30) (Lovegrove, 2007).

Patients with hypertension are at 2-3 times higher risk of developing diabetes than patients with normal blood pressure (Fisman & Tenenbaum, 2008). Age-related arterial stiffness is more pronounced in diabetics and with increasing age, there is a shift from diastolic to systolic blood pressure and pulse pressure as predictors of cardiovascular disease (Os, Gudmundsdottir, Kjeldsen, & Oparil, 2006). Baseline blood pressure data from several recent trials indicate that, in diabetic subjects, there is nearly a fourfold excess in systolic pressure (the difference between baseline pressure and target pressure) over diastolic pressure with respect to the recommended systolic/diastolic target pressure of 130/80mmHg. A recent cross sectional case-control study showed that type 1 diabetic subjects had a higher systolic blood pressure in all age groups. Compared to non diabetic persons, whereas diastolic blood pressure was higher in individuals over 40 years of age as reported by Osher and Stern (2008). There was no difference in blood pressure levels between type 1.5 diabetes and type 1 diabetes or T2D though diastolic pressure seemed to be lower in type 1.5 diabetics than in type 1 diabetics as reported by Biesenbach et al. (2005). High blood pressure
in patients with type 2 diabetes was found in 30.5% of men and 24.5% of women in a study conducted by Shi et al. (2006). Excess body weight increases the risk of hypertension (Slynkova, Mannino, Martin, Morehead, & Doherty, 2006). The present study also confirmed this fact. In the present study, both systolic and diastolic pressures were found to be higher in type 2 diabetic patients compared to the non diabetic control group.

India, in recent times there has been a significant rise in urbanization and lifestyle pattern (sedentary, exercise, diet, smoking, alcoholism). Our study found that a majority of non diabetic subjects did not have an alcohol addiction, smoking and tobacco chewing whereas T2D subjects had mentioned addiction. Non diabetics who are alcoholics had a still lower WHR than non diabetic non alcoholics. This may be due to their lower age or poor nutritional intake associated with alcoholism. These findings are in consonance with that of various previous investigators who found that anthropometric measurements are often high in diabetics or persons with impaired glucose tolerance (Carlsson, Midthjell, Grill, & Nord-Trondelag, 2004; Willi, Bodenmann, Ghali, Faris, & Cornuz, 2007).

Glucose monitoring is a key component in diabetes (Zhou et al., 2009). Human C-peptide provides an accurate assessment of residual β-cell function and thus it has been widely used as a marker of insulin secretion in patients with diabetes (Wiedmeyer et al., 2007). Assay of HbA1c serves as a reliable measure of chronic glycemia and correlates well with the risk of long term diabetes related complications (International Expert, 2009). HbA1c assay is the test of choice for the chronic management of diabetes and is now being recommended for its diagnosis. The Diabetes Complications and Control Trial (DCCT) identified a linear correlation between HbA1c and average blood-glucose concentrations. Linear regression analysis carried out by the A1c-Derived Average Glucose (ADAG) study also revealed a good correlation between HbA1c and average blood glucose (Sacks, 2007).

Our study in consonance with the mentioned studies, where a significant decrease was observed in fasting and postprandial blood glucose levels among non diabetic controls compared to the levels of diabetic subjects with a considerable
difference between mean HbA1c scores of diabetic subjects. Insulin reduces hepatic glucose output and increases peripheral glucose utilization (Rahman, Ismail, & Rahman, 2009). The decrease in both fasting blood glucose and postprandial blood glucose level in the present study might be due to the hypoglycemic effects of insulin which in turn would have been mediated by decreased hepatic glucose output and increased peripheral glucose utilization.

The increase in fasting C-peptide levels were associated with increased plasma glucose due to insulin resistance in type 2 diabetics (Abdullah et al., 2010). C-peptide is secreted from islet cells into the circulation in equimolar concentrations with insulin and is not extracted by the liver. Hence, C-peptide levels are used as a biomarker of \( \beta \)-cell function (Ko et al., 2009). A moderate correlation was observed by Abdullah et al., (2010) between body mass index (BMI), HbA1c and fasting C-peptide levels. According to Leslie, Williams, and Pozzilli (2006), patients with type 1.5 diabetes had reduced fasting C-peptide level at diagnosis, although the levels of C-peptide were higher than those found in type 1 diabetes. Also, according to Kirk and Namak (2009), C-peptide may be low or absent in type 1 diabetes and it may be normal or elevated in type 2 diabetes. The present study confirms this fact, C-peptide levels of type 2 diabetic patients is higher than the non diabetic subjects suggesting that insulin resistance and not destruction of \( \beta \)-cells is the mechanism of occurrence of T2D. The results of the present study suggest that fasting blood glucose, C-peptide and HbA1c status of diabetics might serve as suitable biomarkers in the diagnosis of T2D.

Diabetes alters various metabolic and enzymatic functions of liver. It may also be concluded that the diabetic complication in the liver may be attributed to alterations in the liver enzyme activities (Zafar et al., 2009).

Dyslipidemia is prevalent in diabetic patients (Shamir, Kassis, Kaplan, Naveh, & Shehadeh, 2008). Cardiovascular disease occurs earlier and with greater frequency in people with diabetes. These observations are especially true for young adults with autoimmune diabetes, in whom coronary artery disease is increased tenfold or greater. Much of the risk for coronary artery disease in autoimmune diabetes lies in the presence and severity of atherosclerosis and its risk factors namely dyslipidemia/hyperlipidemia (Prince et al., 2010).
Insulin inhibits cholesterol synthesis and decreases oxidative stress (Dandona, Aljada, Chaudhuri, Mohanty, & Garg, 2005). Glucose is known to increase cholesterol synthesis and oxidative stress (Hayek et al., 2005). Cholesterol may directly impair β-cell function, and glucose stimulates insulin secretion (Hao, Head, Gunawardana, Hasty, & Piston, 2007). Recent evidence suggests that lipid and lipoprotein concentrations are related to nephropathy, retinopathy and neuropathy in diabetes (Petitti et al., 2007). Aggressive therapy of diabetic dyslipidemia will reduce the risk of cardiovascular disease in patients with diabetes. The initial therapy is to improve glycemic control and lifestyle intervention (American Diabetes, 2007). The increase in the total cholesterol levels observed in the present study might be related to cholesterol synthesis by an increase in glucose level and oxidative stress.

According to Suryawanshi, Bhutey, Nagdeote, Jadhav, and Manoorkar (2006), increased levels of lipid and lipoprotein in diabetics may be due to abnormal lipid metabolism. High levels of cholesterol, triglyceride, LDL-cholesterol and low HDL-cholesterol may also be due to obesity, increase calorie intake and lack of muscular exercise in the diabetic patients. Triglyceride levels were found to be higher in diabetic patients with vascular complications as reported by Mohammadi et al. (2009). The reason for the elevated triglycerides in diabetes is complex and stems from a disturbance in fatty acid metabolism, a derangement that is so profound that it has been suggested that diabetes should not be called mellitus but rather lipidus as reported by Tomkin (2008). Hyper triglyceridermia is also a risk factor for cardiovascular disease and triglyceride level is highly influenced by lifestyle factors such as diet and physical activity (Ginsberg, Zhang, & Hernandez-Ono, 2005). An increasing triglyceride level accompanied by low high density lipoprotein (HDL) is a surrogate marker of insulin resistance (McLaughlin et al., 2005).

High-density lipoprotein (HDL) cholesterol levels are a strong, independent inverse predictor of cardiovascular disease. An analysis of data from four large studies conducted by Barter et al. (2007) concluded that each increase of 1mg per deciliter in HDL cholesterol is associated with a decrease of 2 to 3% in the risk of future coronary heart disease. In the present study, HDL cholesterol levels were within the normal range indicating that the patients were not at risk for developing coronary heart disease.
The cardiovascular role of HDL particles is mainly due to reverse cholesterol transport and potentially to the antioxidative, anti-inflammatory, anti-thrombotic and endothelial-dependent vasorelaxant effects (Link, Rohatgi, & de Lemos, 2007). In the present study, the abnormalities of lipid metabolism observed in T2D are one of the major factors contributing to vascular risk. The decrease in HDL cholesterol noted in patients with type 2 diabetes is due to increased catabolism of HDL particles. Reduced HDL cholesterol has shown to be correlated with both hypertriglyceridaemia and obesity (Verges, 2009).

Elevated plasma low-density lipoprotein (LDL) cholesterol is associated with increased risk of atherosclerosis. LDL particles are modified in the presence of diabetes to become more atherogenic. These modifications include glycation in response to high plasma glucose levels, oxidative reactions mediated by increased oxidative stress, transfer of cholesterol ester, which makes the particles smaller and denser. Oxidatively and non-oxidatively modified LDL is involved in plaque formation and may thus contribute to the accelerated atherosclerosis (Scheffer, Teerlink, & Heine, 2005). Atherosclerosis observed in the patients might be due to atheroma related with glycation, oxidative stress and transfer of cholesterol ester.

VLDL carries the highest amount of triglycerides, and this may be the reason for very high level of VLDL in diabetic patients (Ghosh et al., 2006).

The American Diabetes Association (ADA) recommended goal of therapy aiming LDL cholesterol <100mg/dl, as a treatment option in patients with type 2 diabetes and overt cardiovascular disease (Shepherd et al., 2006). An increase in the LDL cholesterol levels observed in type 2 patients might be a risk factor for hypertension. The abnormalities in LDL cholesterol might have interacted with other lipid abnormalities thereby increasing the risk of cardiovascular disease. Mohammadi et al. (2009) reported a positive correlation between VLDL cholesterol and total cholesterol, LDL cholesterol and triglycerides in diabetic patients. The increase in total cholesterol, LDL cholesterol and triglycerides with a decrease in HDL cholesterol in the present study proved the above correlation. Incidence of heart disease among the diabetics in the present study was found to be associated with each of the lipid parameters namely total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol.
CRP may directly promote atherosclerosis and endothelial inflammation by attenuating the release of nitric oxide, a key molecule in the endothelium that plays a pivotal role in the maintenance of vascular tone. CRP levels might be independently related to the degree of insulin resistance, hypertension and dyslipidemia. Multifactorial drug treatment in patients with type 2 diabetes reduces the risk of cardiovascular events, suggesting that these factors are interrelated, numerous and complex (Nystrom, 2007). The present study is in agreement with this fact.

Elevation of the plasma urea and creatinine which are the significant renal function markers, may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, increased triacylglycerol and cholesterol levels (Chandramohan et al., 2009). Due to continuous catabolism of amino acids, high urea is likely to be formed from urea cycle (Lal et al., 2009). After many years of diabetes, the delicate filtering system in the kidney becomes destroyed. Increment of blood urea level with the increment of blood glucose level clearly indicates that the increased blood glucose level causes damage to the kidney (Shrestha et al., 2008). Hypoproteinemia, an abnormally low level of protein in blood may occur because of long standing diabetes and can be a medical sign of nephrotic syndrome (Lal et al., 2009). In the present study, urea creatinine and total protein were in above normal levels among type 2 diabetic patients indicating risk of renal function in near future.

Diabetes disturbs the liver function, due to which the activities of SGOT and SGPT were increased in the blood as observed by Ahmad et al. (2008). In diabetic patients, elevated enzymatic activity of SGOT with only moderately increase in SGPT activity suggested cardiac damage while elevated activity of SGPT with only moderate increase in SGOT suggested liver damage as reported by Sundaram, Reddy, and Singh (2009). SGOT and SGPT enzymes are responsible for production of ketone bodies from aminoacids. The higher activities of SGOT and SGPT were suggested to be the cause for a high concentration of glucose. The gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as aminoacids (Asaduzzaman et al., 2010). In the present study, both SGOT and
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SGPT activities were found to be within normal values indicating that there was no liver damage (Nannipieri et al., 2005). GGT is independently and dose-responsively associated with cardiovascular mortality (Ruttmann et al., 2005).

The endocrine and locally acting (paracrine and autocrine) adipokines can modify insulin sensitivity, and many will contribute to the development and maintenance of insulin resistance in overweight patients with insulin resistance (Rabe, Lehrke, Parhofer, & Broedl, 2008).

The pro-inflammatory cytokines TNFα and IL6 are produced in increasing amounts by expanding adipose depots. Although these cytokines are produced by adipocytes, within adipose tissue, there are large numbers of macrophages, which may also be a source of these adipokines. Animal studies have shown that insulin sensitivity improves after TNF 'knockout' whereas raising TNF levels accentuates insulin resistance. TNF impedes insulin receptor phosphorylation and also impedes some post-receptor insulin signaling reactions. Both TNFα and IL6 reduce the activity of LPL and increase intracellular lipolysis, raising circulating fatty acid concentrations (Antuna-Puente, Feve, Fellahi, & Bastard, 2008). In the present study, there was a decrease in the total level of TNFα and IL6 indicating circulating concentrations of these pro-inflammatory cytokines are relatively low among diabetic subjects. It is possible that their main effects on insulin resistance and lipid metabolism are mediated locally in a paracrine manner.

Resistin, also a known pro-inflammatory cytokine is produced by adipocytes and macrophages. Resistin can increase the production of TNFα and IL6, and concentrations of resistin are raised in some genetic and diet-induced animal models of obesity and diabetes. Also, administration of antibodies to resistin, or resistin gene knockout, has been shown to improve insulin sensitivity and reduce hepatic glucose production. However, in the present study, the resistin level between diabetic subjects is statistically equal with non diabetic subjects. The result is in agreement with the fact that, the expression levels of resistin in human adipocytes are very low and there is no apparent increase in expression of resistin in human obese insulin resistant states leaving the role of resistin uncertain (Sul, 2004).
Adiponectin is produced only by adipocytes. The receptors for this adipokine are expressed in muscle, liver and other tissues, including islet β-cells and endothelium where they bring about tyrosine phosphorylation and signalling of the insulin receptor resulting in improved insulin sensitivity. Activation of adiponectin receptors stimulates AMPK, which increases fatty acid oxidation and glucose uptake in muscle. Adiponectin also increases PPARα activity and reduces inflammation. Additionally, adiponectin exerts anti-atherogenic actions, including a reduction in the expression of several adhesion molecules, increased endothelial nitric oxide production, which improves vasodilation, and reduced vascular smooth muscle proliferation (Kadowaki & Yamauchi, 2005). In the current study, the adiponectin concentration of the diabetic subjects is decreased, which is in agreement, where adiponectin with its reduced production and secretion as the adipose mass expands such that concentrations are reduced in obese diabetic states. Thus, a reduction of adiponectin appears to contribute to the development of insulin resistance in obesity and T2D.

The adipocyte hormone leptin acts mainly via central mechanisms to reduce food intake and increase metabolic energy expenditure. Although leptin concentrations may increase in obesity, this tends to be associated with the development of leptin resistance, which appears to reduce its effectiveness. The study is in agreement with the fact that leptin levels in diabetic subjects are significantly increased. This may be due to, leptin act on skeletal muscle to improve insulin action possibly via activation of AMPK and increased fatty acid oxidation suggesting that it probably does not play a significant role in insulin resistance (Antuna-Puente et al., 2008).

In accordance with previous studies, the absolute HOMA-IR in individuals with T2D was also significantly greater than in individuals without T2D. We have found that insulin resistance measured using the HOMA model is greater and has more intrinsic variability in individuals with T2D compared with those without, suggesting a direct relation between insulin resistance and the degree of variability present in both health and disease (Bonora et al., 2000; Stoney et al., 2001).
In this study, using a combination of genetic and biochemical data we provide further genetic support for the primary contribution of 21 SNPs variations that may affect the pathogenesis of T2D or related parameters.

In recent years, identifying the genetic causes of T2D got extensive support from the advances in molecular biology, which facilitated researchers to identify various loci that are associated with the disease (Kann, 2010). The advent of genome-wide association (GWA) analysis has transformed the potential for researchers to uncover variants influencing complex, common phenotypes, including T2D, and resulted in the identification of a growing number of susceptibility loci (Salonen et al., 2007; Wu et al., 2008; Zeggini et al., 2007).

In the past 10-15 years, huge resources have been devoted to finding T2D genes. These efforts have included many candidate-gene studies and extensive efforts to fine-map linkage signals. Linkage analysis and subsequent positional fine-mapping of candidates have been mostly inconclusive, despite the detection of multiple genomic regions putatively linked to diabetes (Lillioja & Wilton, 2009). The South Indians are genetically distinct population with very less genetic similarity with the rest of the world population and even with a North Indian population (Reich, Thangaraj, Patterson, Price, & Singh, 2009). Some studies showed that the SNPs which showed significant association with diseases in the European populations may not be showing similar effects in Indian population (Chandak et al., 2004). Common polymorphisms in TCF7L2 are strongly associated with T2D in all major world populations (Cauchi et al., 2008; Tong et al., 2009). SNPs in this gene is reported to be associated with BMI in the European population (Cauchi & Froguel, 2008) where as in Indian populations the significance was marginal (Chandak et al., 2007). Hence, in the present, 21 SNPs has been selected from the different genes (MTNR1B, G6PC2, GCKR PPARG, IGF2BP2, CDKAL1, GCK, SLC30A8, CDKN2A/B, TCF7L2, HHEX, CDC123-CAMK1D) which are already found to be associated with T2D in different populations and the same to know the association in South Indian population.

Glucokinase (GCK; EC 2.7.1.1) is a structurally and functionally unique member of this family. Glucokinase is expressed only in mammalian liver and pancreatic islet β-cells. The phosphorylation of glucose at the sixth carbon position is the first step in glycolysis. The reaction is catalyzed by a family of enzymes called
hexokinases, types I through IV (glucokinase). Because of its unique functional characteristics, the enzyme plays an important regulatory role in glucose metabolism. The rate of glucose metabolism in liver and pancreas is a function of the activity of the enzyme (Matsutani, Janssen, Donis-Keller, & Permutt, 1992). The inhibitory effect of GCKR depends on the presence of fructose-6-phosphate (F6P) and is antagonized by fructose-1-phosphate (F1P). Mutations in GCKR might be diabetogenic if they resulted in the synthesis of proteins with increased inhibitory activity, perhaps reflecting increased sensitivity to fructose-6-phosphate or reduced susceptibility to antagonism by fructose-1-phosphate (Warner, Leek, Intody, Markham, & Bonthron, 1995). Warner et al. (1995) determined the complete sequence of human GCKR cDNA. Given the role of glucokinase in the causation of maturity-onset diabetes of the young (MODY) type II, GCKR had been considered a candidate gene for a form of MODY.

CDKAL1 gene product is similar to CDK5 regulatory subunit-associated protein 1 (CDK5RAP1) gene product. CDK5RAP1 is expressed in neuronal tissues and inhibits CDK5 activity by binding to the CDK5 regulatory subunit p35. In pancreatic β-cells, CDK5 shows to act in the loss of β-cell function under glucotoxic conditions (Wei et al., 2005). Inhibition of the CDK5/p35 complex prevents a decrease of insulin gene expression and glucotoxicity (Ubeda, Rukstalis, & Habener, 2006). It is proposed that CDKAL1 may act in the inhibition of the CDK5/p35 complex in β-cells similar to CDK5RAP1 in neuronal tissue. Reduced CDKAL1 expression or inhibitory function could lead to an impaired response to glucotoxicity.

Peroxisome proliferator-activated receptor gamma (PPARG), a member of the nuclear receptor super family, PPARG act by controlling networks of target genes. PPARG can be activated by both dietary fatty acids and their metabolic derivatives in the body, and thus serve as lipid sensors that, when activated, can markedly redirect metabolism. PPARG is involved in adipocyte differentiation and are predominantly expressed in liver and adipose tissue respectively. Consistent with their expression profiles, the PPARG has a unique function in the regulation of energy metabolism. It binds to chemicals that induce proliferation of peroxisomes, organelles that contribute to the oxidation of fatty acids (Evans, Barish, & Wang, 2004). The C-to-G transversion in the PPARG2 gene, resulting in a pro12-to-ala (P12A) substitution.
Thus the product of the PPARG gene is a nuclear receptor that regulates adipocyte differentiation and possibly lipid metabolism and insulin sensitivity, all of which are relevant to the development of T2D (Yen et al., 1997). Functional studies showed that the ala12 isoform of PPARG2 were less effective in activating transcription, which may lead to lower levels of adipose tissue mass accumulation (Deeb et al., 1998). Altshuler et al. (2000) suggested that the risk allele (pro12) occur at such high frequency that the modest effect may translate into a large population-attributable risk which may influence as much as 25% of type 2 diabetes in the general population. The over expression of PPARG in a mouse insulinoma cell line inhibited glucose-stimulated proinsulin biosynthesis and insulin release (Nakamichi et al., 2003). Further, the CDK5-mediated phosphorylation of PPARG may be involved in the pathogenesis of insulin resistance and presented an opportunity for development of an improved generation of antidiabetic drugs through PPARG (Choi et al., 2010).

SLC30A8 gene encodes ion channel zinc transporter protein member 8 (ZnT-8), which is thought to be the β-cell zinc concentration regulator. ZnT-8 is a critical molecule during the insulin maturation and release process that carries zinc from the cytoplasm into insulin secretory vesicles (Chimienti, Devergnas, Favier, & Seve, 2004). Therefore, its polymorphisms may affect its activity, which in turn correlates with T2D susceptibility and therapeutic efficacy. Fu et al. found that reduced ZnT-8 expression in cultured pancreatic β-cells gives rise to the reduced insulin response to hyperglycemia and that SLC30A8 polymorphism could affect insulin secretion and glycemic response (Fu et al., 2009). Another two studies indicated that patients with the rs13266634 C allele showed decreased first-phase insulin release following an intravenously administered glucose load (Boesgaard et al., 2008; Wu et al., 2008). Furthermore, it has been found that the C alleles of rs13266634 at SLC30A8 were associated with increased FPG and decreased insulin during the OGTT. An investigation also showed SNP rs13266634 increased the risk for T2D by 1.24-fold in Chinese Han population (Wu et al., 2008).

The SNP rs563694 in G6PC2 gene is found to be associated with the present study. The association of this SNP remained same upon logistic regression in a dominant model even after adjusting for age, sex and BMI. Our result is in support with the previous studies (Chen et al., 2008). Two genome wide association scans in a
total of 5,088 nondiabetic individuals from Finland and Sardinia and found a
significant association between the SNP rs563694 and fasting glucose concentrations,
which was confirmed in additional 18,436 nondiabetic individuals of mixed European
descent from 7 different studies; combining results from all the studies yielded an
overall p-value of 6.3 x 10^-33. Across these studies, fasting glucose concentrations
increased 0.01 to 0.16mM with each copy of the major allele, accounting for
approximately 1% of the total variation in fasting glucose (Chen et al., 2008).

The G6PC2 gene encodes the enzyme islet-specific glucose-6-phosphatase
catalytic subunit-related protein (IGRP) (Ebert et al., 1999; Hutton & O'Brien, 2009).
IGRP probably acts as a counter-player to glucokinase by dephosphorylating glucose-
6-phosphate. This substrate cycle modulates glucose-stimulated insulin secretion
(Iizuka et al., 2000; Newgard et al., 2002; Petrolonis et al., 2004). Alterations in the
substrate cycle due to variation in the G6PC2 gene may explain the association with
insulin secretion. Interestingly, dephosphorylation of glucose-6-phosphate towards
glucose is enhanced in the islets of animal models for T2D (Khan et al., 1990;
Ostenson et al., 1993) and is accompanied by reduced glucose induced insulin
secretion (Ostenson et al., 1993). The situation in IFG/IGT subjects may be
comparable. They might have altered substrate cycling and accordingly reduced
insulin secretion per se, independent of genetically determined IGRP content or
activity. This would explain how increased blood glucose levels blunt the effect of
G6PC2 genotype on decreased insulin secretion.

In contrast, G6PC2, the β cell-specific isoform of glucose-6-phosphatase is a
highly relevant candidate gene for glucoregulation. The mouse homolog G6pc2 has
been previously implicated as an autoantigen in the NOD mouse model of type 1
diabetes (Mukherjee, Wagar, Stephens, Lee-Chan, & Singh, 2005). Y. Wang et al.
(2007) recently generated G6pc2-null mice and noted that at 16 weeks of age, fasting
glucose concentrations had decreased approximately 13% in both male and female
G6pc2-null mice when compared with wild-type mice. This modest decrease in
glucose concentration was observed despite the absence of any differences in body
weight, fasting insulin, or fasting glucagon concentrations. The characteristics of
these G6pc2-null mice closely paralleled our observations that rs560887 and rs563694
were associated with modest changes in fasting glucose but not in BMI or fasting
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insulin, which are consistent with the hypothesis that the presence of a C allele results in lower G6PC2 expression and therefore, lower glucose concentrations. Interestingly, G6pc2 mRNA levels appear to increase with increasing glucose concentration in isolated mouse islets (Petrolonis et al., 2004). Molecular cloning of G6pc2 identified 2 splice forms that differ by the presence or absence of exon 4 in BALB/C and ob/ob mice and in insulinoma tissue (Arden et al., 1999). The longer cDNA, including exon 4 has approximately 50% homology with glucose-6-phosphatase catalytic subunit (G6pc) across a variety of species, including humans and is a membrane bound in the endoplasmic reticulum (Shieh, Pan, Mansfield, & Chou, 2004). The corresponding G6PC2 splice forms have been observed in the human pancreas (Dogra et al., 2006).

Taken together, our data confirmed previously recognized associations of genetic variation in SNP rs563694 in G6PC2 locus with T2D. Based on our findings, we postulate that these effects are independent.

There was a significant association of rs4402960 of IGF2BP2 in our South Indian population, the association of this SNP showed an association in logistic regression in all three models, even after adjusted for age, sex and BMI. In agreement with our finding, several large-scale population studies reported a lack of association in many populations (Le, Sorrell, & Siddle, 2012; Scott et al., 2007; Sladek et al., 2007; Takeuchi et al., 2009). The majority of subsequent association studies of IGF2BP2 with T2D were focused on intron 2 SNPs; rs4402960 and rs1470579 (Cauchi et al., 2008; Jia, Yu, Jiang, & Ji, 2011; Nemr et al., 2012; Takeuchi et al., 2009).

The IGF2BP2 protein is encoded by 16 exons. The IGF2BP2 gene, located at chromosome 3q27.2, has a very long 125kb intron, which is by far the largest intron among mammalian species. In genome-wide association studies (GWAS), several single nucleotide polymorphisms (SNPs) situated in this intron showed significant association with T2D (T2D) (Diabetes Genetics Initiative of Broad Institute of et al., 2007; Scott et al., 2007). The IGF2BP2 association has been widely replicated in various Caucasian (Grarup et al., 2007; Hertel et al., 2008; Lyssenko et al., 2008) and Asian (Chauhan et al., 2010; Horikawa et al., 2008; Omori et al., 2008) populations. Among IGF2BP2 intron 2 SNPs, rs4402960 G>T showed the most significant association with the disease. According to the results of two recent meta-analyses, the
rs4402960 polymorphism of the IGF2BP2 gene was related to an increased risk of T2D for T vs. G allele (Jia et al., 2011; Zhao et al., 2012). In the analysis of different ethnicities, significantly increased risks were found in East Asian, Caucasian, and Indian populations.

The diabetes-associated allele G of rs4402960 has been found to be linked to decreased early-phase insulin release, reduced homeostatic model assessment of β-cell function (HOMA-β), lowered fasting insulin, and other indices of impaired pancreatic β-cell function (Groenewoud et al., 2008; Rodriguez et al., 2010; Stancakova et al., 2009). Also, rs4402960 of IGF2BP2, showed associations with abdominal total fat and visceral total fat in Canadian Caucasians (Ruchat et al., 2009) and Mexican Americans (Li et al., 2009), suggesting a possible role of IGF2BP2 in insulin resistance (IR). In agreement with earlier studies, the genotype-phenotype analysis in our study also revealed the association of SNPs rs4402960 with fasting glucose, postprandial glucose, HomaIR and triglycerides even after adjusting for sex, age, BMI, smoking and alcohol. Conclusive evidence that polymorphisms within the IGF2BP2 gene affect diabetes susceptibility through changes in the activity of the IGF2BP2 protein per se is lacking, but it seems plausible that IGF2BP2 might influence the development and/or function of the pancreas or adipose tissue through effects on the expression of IGF2 and/or other proteins (Christiansen, Kolte, Hansen, & Nielsen, 2009).

It has been reported that SNP rs4402960 is in high linkage disequilibrium (LD) with rs11705701 G>A, a polymorphic marker located in the promoter region, ~1.48kb upstream from exon 1 of IGF2BP2 (Li et al., 2009). Similar to the present study in Diabetes Genetics Initiative of Broad Institute of et al. (2007), detected altered expressions of IGF2BP2 in adipocytes of T2D subjects compared with healthy people. In two other studies, augmented expression of IGF2BP2 has been shown in the pancreas of T2D patients (Cotsapas et al., 2010; Marselli et al., 2010). Jin et al. (2011) reported unregulated expression of IGF2BP2 in peripheral monocytes of monkeys fed with a high-fat diet.

IGF2 plays a pivotal role in regulating fetal growth and organogenesis, including adipogenesis (Louveau & Gondret, 2004) and pancreatic development (Miralles & Portha, 2001). In Goto-Kakizaki rats, a spontaneous model for T2D,
suppression of IGF2 production in embryonic pancreas results in lowered $\beta$-cell mass that precedes the onset of hyperglycemia (Calderari et al., 2007). Since IGF2BP3s repress translation at late developmental stages (Nielsen et al., 1999), fetal overproduction of IGF2BP2 in pancreatic and adipose tissues could downregulate the translation of IGF2 mRNA at earlier prenatal steps, and thus lead to abnormalities in the development of the pancreas and adipocytes. Generally, IGF2 shortage may contribute to reduced birth weight that is considered an independent risk factor for T2D (Freathy et al., 2009).

van Hoek, Langendonk, de Rooij, Sijbrands, and Roseboom (2009) detected a nominally significant role of rs4402960 of IGF2BP2 and its interaction with intrauterine malnutrition to early induction of glucose. Impaired islet $\beta$-cell development leads to impairment in insulin secretion, thereby promoting the manifestation of hyperglycemia and IR (Meier, 2009). Association of rs4402960 with T2D may be realized not only through impaired $\beta$-cell development and function, but also through alterations in adipose tissue (Li et al., 2009). In murine models, studies suggest that the rs10811661 polymorphism located upstream of the CDKN2B and CDKN2A genes may confer increased risk for T2D by affecting $\beta$-cell function (Moritani et al., 2005; Rane et al., 1999; Tsutsui et al., 1999).

In the present study, we validate the association of the SNP rs10811661 variant in CDKN2A/B gene with T2D. Furthermore, our subjects show that variations in CDKN2A/B loci confer an impairment of glucose-induced insulin release pointing to pancreatic $\beta$-cell dysfunctions.

The rs10811661 variant is located 125kb upstream of the CDKN2A/B genes. In the present study, we find a substantial impact of this variant on T2D risk with an OR of 0.45 per risk allele, and given a risk-allele frequency >40%, this variation contributes considerably to the population-attributable risk. In two population based samples, Grarup et al. (2007) found an impaired glucose and tolbutamide induced insulin release in risk-allele carriers; both studies pointing to a recessive mode of inheritance. Yet, the study of the association with T2D is also consistent with an additive genetic model. The CDKN2A/B genes are expressed in adipocytes and pancreatic islets (Zeggini et al 2007). CDKN2A encodes p16INK4a-a tumor
suppressor influencing pancreatic β-cell proliferation (Krishnamurthy et al., 2006; Rane et al., 1999) thus making it likely that a causal variant is situated in CDKN2A, possibly increasing the susceptibility of T2D through a decreased β-cell mass and subsequent decreased insulin release in conditions with a high insulin demand.

Recent GWAS and meta-analysis provide convincing evidence for the CDKN2A/2B gene region to be involved in T2D (Bao, Xie, & Yang, 2012; Diabetes Genetics Initiative of Broad Institute of et al., 2007; Scott et al., 2007; Shea et al., 2011; Sladek et al., 2007; Zeggini et al., 2007). Meta-analysis of genotype data from GWAS in northern Europeans have confirmed that SNPs rs10811661 and rs564398 in the CDKN2A/2B region are T2D susceptibility variants, although the combined evidence for rs10811661 is far stronger than that for rs564398 (Diabetes Genetics Initiative of Broad Institute of et al., 2007; Scott et al., 2007; Zeggini et al., 2007).

GWAS in French-Canadian obtained nominal association signals for proxies of rs10811661 (Sladek et al., 2007). A strong association between the major allele of rs10811661 and T2D was reported in French Europids (Duesing et al., 2008), Chinese Hans population (Wu et al., 2008), and Korean population (Lee et al., 2008). In a Danish population, variants of CDKN2A/2B were found to be highly associated with T2D and the SNPs within CDKN2A/2B loci impaired glucose induced insulin release in healthy Danes (Grarup et al., 2007). An association of variant of CDKN2A/2B was modestly replicated in Asians but not replicated in African Americans and Pima Indians (Rong et al., 2009). Recent GWAS in Diabetes Prevention Program (DPP) has shown CDKN2A/2B (rs10811661) as a potential intervention-interaction site showing response to treatment with troglitazone by improving insulin sensitivity (Moore et al., 2008).

A strong association between CDKN2A/2B (rs10811661) and T2D has been reported in Chinese population (Hu et al., 2009). A similar result has been reported for CDKN2A/2B (rs10811661) by Chauhan et al. (2010) in the populations of a region of North-Western India. Singh et al 2012 reported a high association for the same SNP rs10811661 of CDKN2A/2B in the Eastern region of India. India represents one of the largest human diversity, consisting of 4635 culturally and anthropologically well defined populations with little or no gene flow between them (Indian Genome Variation, 2008). The deviation in our results from those of Chauhan
et al. (2010) may probably be due to this genetic heterogeneity. Bao et al. (2012) have also reported OR 1.28 for CDKN2A/2B rs10811661 in a recent meta-analysis. The association of common variants of CDKN2A/2B rs10811661 (C/T) is well established with T2D in the population of South India. Interestingly, our data for of the SNP rs10811661 show larger effect size than those reported in European populations.

The SNP rs7903146 in TCF7L2 gene is found to be associated with the present study. The association of this SNP remained same upon logistic regression in the dominant model even after adjusting for age, sex and BMI. Our findings are in agreement with earlier research among different populations (Chauhan et al., 2010; Dupuis et al., 2010; Helgason et al., 2007; Saxena et al., 2012).

Among all the loci, TCF7L2 so far has shown the strongest association with the largest effect size for T2D in Europeans (Grant et al., 2006; Scott et al., 2007; Sladek et al., 2007; Zeggini et al., 2007), Amish (Damcott et al., 2006), and Indians (Bodhini, Radha, Dhar, Narayani, & Mohan, 2007; Chandak et al., 2007; Sanghera et al., 2008), but not in Chinese (Chang et al., 2007) and Japanese (Horikoshi et al., 2007) subjects. The present study confirms the association of TCF7L2 with T2D with the largest effect size. The TCF7L2 gene product has been implicated in blood glucose homeostasis (Grant et al., 2006; Yi, Brubaker, & Jin, 2005), and the variant rs7903146 is reported to be associated with measures of glucose metabolism (Damcott et al., 2006). Consistent with these observations, we also found a strong association of TCF7L2 with HOMA-IR and a nominal association with FPG, 2-h PPG and HbA1c confirming the physiological role of TCF7L2 in glucose homeostasis.

Most recently published studies, it has been suggested that TCF7L2 is associated with impaired insulin secretion, but not with increased insulin resistance (Florez et al., 2006; Loos et al., 2007; Lyssenko et al., 2007) (Florez et al., 2006, Loos et al., 2007, Lyssenko et al., 2007). In addition, a multiplicative interaction between this variant and obesity or high BMI was inferred from two previous studies (Yan et al., 2009). Both imply that the risk of T2D is greater in lean individuals carrying this polymorphism, whereas no significant association was noted in those obese or overweight. Therefore, it seems that the SNP rs7903146 is a much more influential risk factor for lean individuals than for obese (Yan et al., 2009). Helgason et al. (2007) reported that the rs7903146 T allele, probably an ancestral and not causative variant, tags an unidentified functional variant lying outside the screened locus.
We have identified and replicated an SNP rs2166706 near MTNR1B that associated with increased risk of T2D among south Indian populations. Our findings extend the results of recent studies in European Caucasian populations that identify genetic variation near MTNR1B as an important determinant of glucose levels (Bouatia-Naji et al., 2009; Prokopenko et al., 2009). MTNR1B encodes a high-affinity receptor for melatonin, a circulating hormone released by the pineal gland (Brzezinski et al., 2005). Secretion of melatonin is tightly regulated by the hypothalamic suprachiasmatic nucleus, the anatomic center of the mammalian circadian clock (Reppert & Weaver, 2002; Saper, Scammell, & Lu, 2005). Melatonin release is one of the key mechanisms by which the circadian clock maintains synchronization and regulates the biologic activities of peripheral tissues (Brzezinski, 1997). Melatonin receptors (including MTNR1B) are highly expressed in the hypothalamus, where they contribute to melatonin-induced negative feedback and phase timing of circadian activity (Liu et al., 1997). MTNR1B is also expressed in human pancreatic islet cells and may mediate the effects of melatonin on basal and glucose-induced insulin release (Ramracheya et al., 2008).

Our findings of an association between SNP rs2166706 and rs3847554 near MTNR1B and raised glucose, reduced pancreatic β-cell function, and increased risk of T2D is consistent with accumulating evidence that circadian clocks play a key role in the regulation of carbohydrate and energy metabolism. Clock/Clock null mice develop hyperglycemia, and in human sleep loss and depression are associated with circadian desynchronization and increased risk of T2D (Golden et al., 2008; Knutson & Van Cauter, 2008; Turek et al., 2005). Although further functional studies will be required to clarify the contribution of peripheral and central melatonin signaling pathways to glucose metabolism and T2D, our observations suggest that MTNR1B signaling may be a therapeutic target for the development of agents to improve glucose regulation and to prevent or treat T2D.

The SNP rs757210 in TCF2 gene is found to be associated with the present study. The association of this SNP remained same upon logistic regression in the dominant model even after adjusting for age, sex and BMI. These results are confirmed with previous findings (Gudmundsson et al., 2007; Winckler et al., 2007; Zhang et al., 2012).
Human transcription factor 2 gene (TCF2) consists nine exons and encodes the 557-amino hepatocyte nuclear factor 1β (HNF1β). HNF1β is a POU (Pit-1Oct-1/2-UNC-86) domain transcription factor closely related to HNF1α. Both are expressed in the pancreas, liver, and kidney (Maestro et al., 2007; Murphy, Ellard, & Hattersley, 2008). Mutations in TCF2 were initially described in a monogenic form of diabetes, namely maturity onset diabetes of the young type 5 (MODY5) (Horikawa et al., 1997). In humans, TCF2 variants are also associated with several diseases such as, defective kidney development (Gresh et al., 2004), disturbed liver function (Lokmane et al., 2008), pancreatic atrophy (Haumaitre et al., 2005), defective insulin secretion (L. Wang et al., 2004), malformations of the genital tract (Edghill et al., 2008; Fischer & Pontoglio, 2008) and diverse cancers (Elliott et al., 2010). The broad spectrum of HNF1β affected phenotypes is possibly also linked to its ability to interact with many other regulatory molecules (Dudziak et al., 2008).

In recent years, there are several studies explored the relationship of TCF2 polymorphisms and T2D. Some studies have indicated that SNPs on TCF2 were associated with the risk of T2D in Swedish, Finnish and Canadian populations (Bonnycastle et al., 2006; Winckler et al., 2007). The association signals were located in intron 2 (rs757210), intron 6 (rs1008284) and intron 8 (rs3110641). Moreover, a genome-wide association study identified that the G allele of rs7501939 (in intron 1) and A allele of rs4430796 (in intron 2) were associated with reduced risk of T2D in Caucasians and Africans (Gudmundsson et al., 2007). Similarly, a Chinese study using a Southern population observed TCF2 was significantly associated with T2D (C. Wang et al., 2009). However, another Caucasian analysis using the subjects from Sweden and Finland could not replicate the association of TCF2 loci with future risk of T2D in two prospective studies (Holmkevist et al., 2008). The major reasons for this discrepancy may be related to ethnicity and/or environmental factors. Population substructure was also a potentially important cause of different results in case-control genetic studies (Pritchard & Donnelly, 2001). To improve our understanding of the role of TCF2 in T2D predisposition, it is extremely important to understand the consequences of inheriting the variants in other ethnic populations. Therefore, in the present study, we conducted a case-control study to investigate the role of TCF2 SNPs on T2D risk in a South Indian Population.
CDC123 gene encodes a protein involved in cell cycle regulation and nutritional control of gene transcription (Bieganowski, Shilinski, Tsichlis, & Brenner, 2004; Zeggini et al., 2008). CAMK1D regulates granulocyte function (Verploegen et al., 2005), it is also possible that a causative variant in this region is related to CAMK1D and affects pancreatic β-cell function through increased apoptosis.

The SNP rs12779790 is found associated with T2D in several studies. G risk allele of rs12779790 was associated with a lower insulinogenic index, corrected insulin response, and area under the insulin/glucose curve during OGTTs and a lower DI in carriers of the G allele (Grarup et al., 2008). In Asian Indian descent, subjects also found the β-cell defect (Sanghera et al., 2009) (Sanghera et al., 2009). Trend towards lower β-cell function could be observed in Caucasian's population (Lyssenko et al., 2008; Staiger et al., 2008; Stancakova et al., 2009). SNP rs12779790 variation carriers showed a lower insulin response to glucose stimulation and noted a trend towards a reduced insulin response after arginine stimulation. Arginine stimulation during hyperglycemia is a measure of (near) maximal insulin secretion and has been suggested as a proxy for β-cell mass. This gene variant affected β-cell function by causing reduced β-cell mass due to enhanced apoptosis (Verploegen et al., 2005). In contrast to earlier studies, the genotype-phenotype analysis in our study revealed the association of rs12779790 with cholesterol and triglycerides. It is possible that a causative variant in this region is related to CAMK1D and affects through lipid homeostasis.

HHEX encodes the transcription factor hematopoietically expressed homeobox protein, which is expressed in the embryonic ventral-lateral foregut that causes the ventral pancreas and the liver (Bort, Martinez-Barbera, Beddington, & Zaret, 2004). Knockout of HHEX gene showed to impair proliferation of endodermal epithelial cells, positioning of ventral foregut endoderm cells relative to the mesoderm, and budding and morphogenesis of the ventral pancreas (Bort et al., 2004). This genetic manipulation finally provoked lethality during midge station (Bort et al., 2004).

HHEX was a common T2D-susceptibility gene across different ethnic groups. The significant association of HHEX with T2D has been confirmed in the Japanese population (Horikoshi et al., 2007). HHEX variant was associated with impaired proinsulin conversion (Stancakova et al., 2009). SNP rs1111875 and rs7923837 were associated with T2D independent of body fat (Schulze et al., 2007). The SNP
rs1111875 showed a significant association with an increase in total cholesterol levels HDL-C and in LDL-C levels, suggesting that carriers of this variant may be prone to develop lipid metabolism alterations. This is contrast with recent results for the Mexican population (Cruz et al., 2010). The HHEX gene regulates pancreatic development by controlling endodermic cell proliferation and the positioning of the cells that will later turn into pancreatic tissue. This probably occurs through the effects of HHEX on the transcription and translation of the cell proliferator. HHEX expression remains activated in the adult pancreas, which suggests that HHEX may have additional functions in differentiated pancreatic cells. Because the HHEX gene plays a role in pancreas development as described above, it may be involved in glucose metabolism (Podlowski et al., 2000). Some HHEX gene variants have been strongly associated with T2D and insulin-stimulated glucose release (Staiger et al., 2008). In agreement with earlier studies, the genotype-phenotype analysis in our study revealed the association of rs1111875 with HDL and LDL and cholesterol.

Thus, a total of 21 SNPs are genotyped by using taqman technology in the present study. 9 SNPs are found to be associated with T2D. Out of which, two SNPs (rs563694 and rs10811661) are present in the 3’UTR of G6PC2 and CDKN2A/B gene respectively. Two SNPs (rs4402960 and rs757210) are present in the Intron 2-3 of IGF2BP2 and TCF2 genes respectively. An SNP (rs7903146) is present in the Intron 4-5 of TCF7L2 and at the last one, SNP (rs2166706) is present in the 5’UTR of MTNR1b gene.

Further, the genotype-phenotype analysis revealed the association of SNPs rs3847554 (5’UTR of MTNR1b gene), rs4402960, rs7903146 with fasting and postprandial glucose. The SNP rs7903146 association with HbA1c and rs4402960 association with HomalIR and triglycerides. The SNP (rs1111875) is located at HHEX gene is consistently associated with cholesterol, HDL and LDL, and the SNP (rs12779790) is located at CDC123-CAMK1D gene on chromosome 10 associated with cholesterol and triglycerides.

The SNPs (rs560887, rs780094, rs1402837, rs1260326, rs1801282, rs10946398, rs1799884, rs730497, rs13266634, rs10830962, rs10830963 and rs1387153) are not associated with T2D in the present study population. However, above-mentioned SNPs are present in the G6PC2, GCKR, PPARG, CDKAL1, GCK, SLC30A8, HHEX, CDC123-CAMK1D, TCF2, MTNR1b genes, whereas G6PC2 and MTNR1b are found to be associated with T2D from other SNPs as mentioned earlier.
Hence, our data confirm the nine associations of this widely replicated variant with T2D risk in Indian population, in contrast with previous reports in different populations. The remaining 12 SNPs not found any association with T2D, this discrepancy is may be differences in ethnic background or the effect of environmental factors, such as the lifestyle.