6.0 Summary

Diabetes Mellitus (DM) has been defined as the collection of metabolic disorders which occurs due to defects in insulin secretion, insulin action or both leading to malfunction in carbohydrate, protein and fat metabolism further leading to the state of chronic hyperglycaemia (American Diabetes Association, ADA 2011).

Current estimates have found that 246 million people are affected with DM worldwide. Among these, Indians are believed to have the largest number of cases and highest prevalence of DM (Garduño-Diaz and Khokhar, 2012).

Traditionally DM has been classified into two major types: Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). T1D is an autoimmune form of DM characterised with absolute insulin deficiency due to destruction of pancreatic β-cells. It affects only 5% of the diabetic cases while T2D affects approximately 95% of the cases reported with DM. T2D is a heterogeneous group of disorders marked by prominent symptoms of insulin resistance in peripheral tissues, insulin deficiency and defects in insulin secretion leading to impaired glucose homeostasis and chronic hyperglycaemia.

Chronic hyperglycaemia is the primary cause of long term damage to various important organs of the body like eyes (diabetic retinopathy), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), heart and blood vessels (cardio vascular disorders).

Insulin and glucagon are the two key hormones involved in the regulation of normal glucose homeostasis in the body (Rosen and Spiegelman, 2006). In normal individuals glucose levels is maintained between the narrow ranges of 65-140 mg/dl which is tightly linked with glucose production during fasting state and glucose utilisation during fed state. It involves delicate balance between three pathways i.e. rate of endogenous hepatic glucose production (HGP), proper insulin action for glucose disposal and insulin secretion.
During fasting in normal physiological conditions, under the action of glucagon, liver produces glucose by metabolic process called gluconeogenesis and glycogenolysis. In fed state, the elevated glucose levels are sensed by pancreatic β-cells and the first phase of insulin is secreted. Insulin works antagonistically to glucagon hormone. Release of insulin stops hepatic glucose production from liver and causes disposal of glucose into skeletal muscles and adipose tissue. But in patients with T2D the glucose disposal into skeletal muscles and adipose tissues is impaired and liver continues to produce glucose by breakdown of glycogen reserves leading to fasting hyperglycaemia. This phenomenon is known as insulin resistance.

Insulin resistance is regarded as the first symptom in pathophysiology of T2D. To overcome insulin resistance, pancreas increase in cell mass and secrete more insulin. This mechanism is called compensatory mechanism of pancreas. Thus, the levels of insulin increase in blood leading to hyperinsulinemia and increased insulin secretion, for prolonged period causes pancreatic dysfunction and pancreas no longer can compensate for insulin resistance. Therefore, due to defects in insulin action and insulin secretion overt T2D develops in the individuals (Scheen, 2003).

T2D is a complex disorder which involves complex interplay between environmental risk factors and genetic risk factors. Obesity, hypertension and high cholesterol levels are well known environmental factors associated with T2D (Ahdi et al., 2012). Waist Hip Ratio (WHR) and waist circumference (WC) are commonly used as the cut off based indicators of obesity, which have also been associated as diabetic risk factors in various studies, along with Hypertension for T2D development.

In addition to environmental factors, multiple genes or polymorphisms belonging to different pathways work with an additive effect, each insufficient in themselves to cause T2D. Using approaches like candidate gene approach and linkage analysis number of SNPs in diabetogenic genes has been screened for having contributory roles in various populations.
Impaired insulin secretion and insulin resistance are the two major defective pathways in patients with T2D. PGC-1α, UCP2 and SIRT1 genes play important role in insulin secretion and insulin resistance. Therefore studying polymorphisms in these genes can be informative with regard to T2D.

Association of PGC-1α (Gly482Ser), UCP2 -866 G>A and SIRT1 -1400 T>C polymorphisms has been confirmed in many populations (Sesti et al., 2003, Soyal et al., 2006; Weyrich et al., 2008). The data regarding the association of these polymorphisms in population of Punjab is either scanty or not available. Therefore, the present study, analysed the association of PGC-1α (Gly482Ser), UCP2 -866 G>A and SIRT1 -1400 T>C polymorphisms with T2D in population of Punjab.

The population structure in India is very complex. There are several population groups which have remained mostly endogamous from ages which may have resulted in conservation of genetic pool. Moreover, it has been known that association of SNPs varies among populations and ethnicities. Therefore it becomes pertinent to replicate these markers in various endogamous groups and in pooled population of Punjab.

Therefore, the present study was focused on:

- Analyse the association of WHR, WC and hypertension with the development of T2D.
- Screen and estimate the co-relation of UCP2 (-866G/A), PGC 1α (GLY482SER) and SIRT1 (-1400C/T) polymorphisms in T2D patients and controls.
- Analyse the risk assessment of these polymorphisms in development of T2D in total population of Punjab and in endogamous population groups after thorough statistical analysis.
- Explore the SNP-SNP interactions, if any, to understand the disease biology.
The present case-control study was carried out on 1813 samples (859 diabetic cases and 954 controls) belonging to seven endogamous groups (Banias, BCs, Brahmins, Jat Sikhs, Khtaris, Rajputs and SCs) from Punjab. This sample size was attained by calculating the power (>95%) of the study. For data collection, a detailed questionnaire was prepared having all the desired details relevant for the study.

The criteria for selection of Patients was based on 2007 ADA criteria (FPG ≥126 mg/dl or OGTT ≥200 mg/dl). Participants with very early age of onset (<30 years), history of ketoacidosis, exocrine pancreatic disease or continuously on insulin supplementation were excluded from the study.

For sampling of controls, the individuals with normal blood glucose levels (FPG <99 mg/dl or OGTT <139 mg/dl). Controls were also age, sex and ethnically matched. Individuals with positive family of T2D, very young age were not taken as controls.

After obtaining informed consent, the blood samples were collected randomly from hospitals, clinics and by arranging medical camps in various villages and towns with the help of resident doctors. 3-4 ml of venous blood was collected from the individual and immediately transferred to pre-labelled EDTA (anti-coagulant) vials. The samples were transported from place of collection to the laboratory on ice and stored at -20°C till further processing. The study has been approved by ethical committee of the university. The clinicians did the diagnosis of the diabetes according to the criteria recommended by American Diabetes Association (ADA), 2007.

Anthropometric measurements were taken following standard procedures and protocols (Singh and Bhasin, 1968; Weiner and Louire, 1981). The patients with history of antihypertensive medication or Systolic blood pressure (SBP) of ≥135 mmHg and Diastolic blood pressure (DBP) of ≥85 mmHg were taken to be clinically hypertensive (Asayama et al., 2006). Estimation of the castes and sub-castes was made by asking questions about the surnames/subcastes/gotras of all the subjects. Other parameters which were relevant for the study such as family history of T2D, age of onset, duration of T2D and type of medication was also carefully noted.
DNA was isolated using standard phenol: chloroform method with minor changes according to lab conditions (Kunkel et al., 1977). The isolated genomic DNA was quantified using Nanodrop. Quantified DNA was then diluted to a concentration of 25ng/µl. PGC-1α (Gly482Ser) and UCP2 -866 G>A polymorphisms were genotyped using PCR-RFLP method whereas SIRT1 -1400 T>C polymorphism was typed by direct sequencing method.

The statistical power of the study was calculated by using the software Quanto. Two – tailed students t-test was used to calculate the mean difference between the continuous variables among cases and control group. χ² test was employed to assess the difference in allelic and genotypic frequency distribution. The magnitude of association providing risk or protection was detected by calculating odds ratio (OR) and population attributable risk (PAR). Logistic regression models [dominant (W vs H+M), recessive (W+H vs M) and heterozygous (H vs W+M)] were applied to test the association of genotypes with T2D after adjusting for confounding factors like age, sex and dietary pattern. Statistical significance threshold was adopted to be p<0.05. Interaction analysis was also carried out to see the combined effect of genotypes in development of T2D in pooled population of Punjab.

The analysis was carried out and discussed in four categories: (i) Comparison of clinical parameters among cases and controls, (ii) Comparison of WHR, hypertension and WC in cases and controls (iii) Molecular analysis of PGC-1α (Gly482Ser), UCP2 -866 G>A and SIRT1 T>C polymorphism and (iv) Interactive analysis of the polymorphisms. Analysis was carried out in various endogamous groups (Bania, BCs, Brahmins, Jat Sikhs, Khatris, Rajputs and SCs) and in pooled population of Punjab.

(i) **Comparison of clinical parameters among cases and controls**

Analysis of clinical parameters revealed that there is higher percentage of central obesity in endogamous population groups and in pooled samples. The percentage of central obesity as indicated by WHR and WC was higher among cases than controls in various endogamous groups. The mean values of WHR was statistically different in
Banias (0.96 vs 0.92; p=0.004), Brahmins (0.93 vs 0.88; p<0.001), Jat Sikhs (0.94 vs 0.91; p<0.001), Khatris (0.94 vs 0.90; p=0.02) and SCs (0.94 vs 0.92; p=0.04) and in overall pooled population (0.94 vs 0.92; p<0.001).

The mean values of SBP and DBP were also significantly higher in cases than controls of Banias (135.37 vs 130.04; p=0.01) and Jat Sikhs (88.70 vs 85.20; p<0.001) respectively while in rest of the groups no significant difference in mean values of SBP and DBP was observed. In overall pooled population there was higher values of SBP (129.85 vs 128.31; p<0.001) and DBP (87.15 vs 85.64; p<0.001) among cases than in controls.

The mean values of fasting glucose levels and random glucose levels were significantly (p<0.0001) higher in cases in all the endogamous groups and in pooled population of Punjab.

The epidemic increase in the prevalence of T2D mainly involves interplay of both environmental as well as genetic components.

The changing environmental attributes like urbanisation (Ramachandran et al., 2008) and economic growth in form of green and white revolutions lead to improved socioeconomic status, rural to urban migrations, improved nutrition and sedentary lifestyles leading to obesity and diabetes epidemic in India (Ramachandran et al., 1992; Reddy and Yusuf, 1998; Chan et al., 2009; Matharoo, 2008). Studies for Asian Indian populations have also indicated that central obesity poses greater risk than general obesity toward T2D and associated complications (Daousi et al., 2006; Kumar et al., 2008; Agyemang et al., 2012). WC and WHR are the commonly used as the cut off based indicators to assess central obesity. The strong association of WC and WHR with increased risk for developing CVDs, also indicates that increased WC and WHR may act as a risk factor for developing T2D as well.
Similarly environmental factors such as modernisation, physical inactivity, obesity, stress have lead to Hypertension (Gupta, 2004). Hypertension has also been identified as a major confounding factor which is associated with T2D (Gress et al., 2000).

Hence, the results of various studies across the globe confirm both central obesity and hypertension as robust risk factors for T2D across populations (McKeigue et al., 1991, McKeigue et al., 1992, Ramachandran et al., 1992, Nyamdorj et al., 2008, Mehta et al., 2009).

(ii) Comparison of WHR, WC and Hypertension in cases and controls.

WHR provided significant risk in Brahmins \([p=3.9\times10^{-5}, \text{OR}- 3.5 (1.8-6.3)]\), Khatris \([p=1\times10^{-3}, \text{OR}-4.4 (1.9-8.6)]\) and SCs \([p=2\times10^{-2}, \text{OR}- 2.8 (1.13-4.2)]\). However, no significant association was observed in Banias, BCs, Jat Sikhs and Rajputs. Analysis carried out in pooled population revealed statistically significant association of WHR \([p=1\times10^{-5}, \text{OR}-1.9 (1.4-2.6)]\) as risk factor.

Hypertension did not provide risk in any of the endogamous groups while hypertension was found significantly associated \([p=3\times10^{-3}, \text{OR}- 1.7 (1.2-2.4)]\) as risk factors in pooled population of Punjab.

WC provided risk in BCs \([p=0.012, \text{OR}-2.7 (1.2-6.2)]\), Brahmins \([p=0.016, \text{OR}-2.3 (1.17-4.8)]\), Jat Sikhs \([p= 2.5\times10^{4}, \text{OR}- 2.22 (1.4-3.4)]\), Khatris \([p=0.005, \text{OR}-3.4 (1.47-8.1)]\), SCs \([p=0.015, \text{OR}-2.09 (1.15-3.8)]\) as well as in pooled population \([p=1.6\times10^{-6}, \text{OR}-1.93 (1.50-2.47)]\) of Punjab.

The differential action of various contributing risk factors among the endogamous groups strongly indicate towards the ethnic differences in terms of lifestyle, profession and dietary pattern. All the epidemiological risk factors were not equally associated as risk factors in each group rather different combinations of risk factors predisposed different endogamous groups towards T2D development. Central obesity which is measured by WC and WHR has emerged as the major driving risk factor. Adaptation to sedentary lifestyle and fat rich diet has led to elevated central obesity. Asian Indians are
known to have ethnic susceptibility towards T2D development (Ramachandran et al., 2001). The results of the present study have also confirmed central obesity as one of the major confounding factors predisposing the population of Punjab to an increased susceptibility towards T2D development.

Studies on different populations of the world have well established that WHR and WC are strong predictors of T2D (Hamman, 1992; Chan et al., 1994; Molarius and Seidell, 1998; Field et al., 2001; Grundy, 2004; Steinbrecher et al., 2012). Few studies also investigated the combined association of BMI, WC and WHR and suggested WHR as a better predictor of T2D and cardiovascular disease (CVD) (Folsom et al., 2000; Wang et al., 2005). Kaur et al., in 2008 investigated the association of obesity indices like BMI, WC, WHR and Waist to stature (WSR) with T2D in Industrial population of South India. They found that prevalence of central obesity using WC and WHR was 70%. They also reported WHR as better predictor for T2D among male industrial population from Chennai. A recent study by Kamath et al., in 2011 also observed that larger percentage of diabetics had central obesity as compared to general obesity (68.1% vs 48.9%) as measured by WC. WC is one of the parameters used by International Diabetes Federation (IDF) to define metabolic syndrome (Stevens et al., 2010). Studies have suggested that individuals with elevated WC and WHR have increased risk of development of T2D and CVDs (Pischon et al., 2008). Abdominal obesity reflects increased amount of intra abdominal fat which also includes visceral adipose tissue (VAT). In addition to this, hepatic fat cells have also been identified with respect to increased risk of T2D and other metabolic diseases. Thus, increased values of WC and WHR may reflect increased VAT and hepatic fat leading to increased risk (Lear et al., 2010).

The present study also demonstrated hypertension as a major risk factor for development of T2D in pooled population of Punjab whereas no statistically significant association was observed in any of the endogamous groups. However, the trend was almost similar in all groups, with increased blood pressure values in cases than in controls. Statistically significant association of hypertension could not be observed in
any of the endogamous groups, However hypertension provided an almost two fold increased risk in the pooled population of Punjab.

It has been documented that concomitant presence of hypertension and T2D in patients, increased the chances of morbidity and mortality (Sowers et al., 2001; Chew et al., 2012). The series of pathophysiological changes occurring in the patients with hypertension in T2D are very complex. It involves interplay of genetic predisposition and environmental factors along with some biological factors such as sedentary lifestyle, unhealthy diet leading to sodium retention, abdominal obesity, autonomic derangements, premature arterial stiffening and endothelial dysfunction (Campbell et al., 2011). Not only are the patients with hypertension more likely to develop T2D, but at any given values of BP diabetic patients have two fold increased risk of death due to CVDs (Stamler et al., 1993). So, confirming hypertension as the major risk factor for T2D.

Therefore, it can be observed from the present discussion that WC, WHR and hypertension all are diabetogenic risk factors.

On segregating the samples into males and females revealed that males were at risk due to increased WC \([p=0.005, \text{OR}=1.12 (1.03-1.21)]\) and hypertension \([p=0.004, \text{OR}=1.56 (1.15-2.12)]\), however in females all the three risk factors WHR \([p=0.000001, \text{OR}=3.53 (2.2-5.6)]\), WC \([p=0.00005, \text{OR}=1.16 (1.08-1.22)]\) and hypertension \([p=0.0001, \text{OR}=1.79 (1.32-2.33)]\) were associated.

There have been few studies which have documented that women have stronger risk of developing T2D than in men. This can be due to large differences in the subcutaneous as well as visceral adipose tissue distribution in men and women. Visceral adipose tissue is strongly associated with T2D in men while no such association has been seen for women (Frederick et al., 2011). A study by Lois et al., in 1996 evaluated that women (25%-30%) have more fat deposits than men (18%-25%), even within normal weight range.
Similar findings were also reported in many research publications. The strong correlation between central adiposity and T2D has further pointed out towards the usefulness of these parameters in designing predictive models and preventive programmes for T2D in population of Punjab.

Together with obesity indices, no difference in association of hypertension was observed among males and females. This can be attributed to the association of WC and WHR. Both WC and WHR are risk factors for hypertension as well. Several studies have documented the association of central obesity with hypertension (Pimenta et al., 2008; Aline et al., 2011). Moreover, changing lifestyle, sedentary habits, smoking and increased intake of alcohol act as add on into the increasing incidence of hypertension in India (Gupta, 2004).

Therefore, the gender disparities with respect to central obesity and hypertension indicated towards the need of preventive programmes for averting T2D which should be gender specific.

(iii) Molecular analysis of PGC-1α (Gly482Ser), UCP2 -866 G>A and SIRT1 -1400 T>C polymorphism

The Ser variant of PGC-1α provided significant risk only in Banias [p=3.3×10^{-4}, OR=3.3 (1.7-5.5)], Jat Sikhs [p = 3.0 × 10^{-3}, OR= 1.7 (1.20-2.4)] and in pooled population [p = 1.6 × 10^{-4}, OR= 1.46 (1.20-1.78)] of Punjab. No association of PGC-1α (Gly482Ser) polymorphism was observed in Brahmins, BCs, Khatris, Rajputs and SCs.

Ser482 variant has also been significantly associated with increased risk of T2D among various populations including Danish Caucasians (Ek et al., 2001), Japanese (Hara et al., 2002), Slovene (Kunej et al., 2004), North Indians (Bhat et al., 2007) and Chinese (Zhang et al., 2007).

Positive association of PGC-1α (Gly482Ser) polymorphism among population of Punjab in the present study (North Indians) and lack of association of this
polymorphism among South Indians (Vimaleswaran et al., 2005) may be taken as a reflection of population specific influence.

The frequency distribution of 482Ser variant varies among various global populations. It ranges from being completely absent in Africans from New Guinea and reaches 0.85 in western Samoan and Niue Islanders. However, all throughout Europe, East Asia and America, the allele frequency of Ser variant is either very low or intermediate. Moreover, the highest frequency is observed in Polynesians (Myles et al., 2011). It is also suggested that the large difference in frequency distribution of risk alleles between populations may account for very huge differences in prevalence and susceptibility of a disease in a population (Myles et al., 2008). As evidenced from the present study, in Brahmns, BCs, Rajputs and SCs, the allele or genotype of PGC-1α (Gly482Ser) polymorphism did not show any significant association with T2D. However, in SCs the distribution of Ser482 (AA) genotype showed similar pattern of increased frequency among cases as compared to controls (12.1 vs 9.6). Findings again indicate this polymorphism might have an important role in T2D, although statistically significant association could not be obtained in some groups.

It may also be possible that PGC-1α being a stimulator of mitochondrial biogenesis and respiration can play a role in genetic predisposition of T2D in combination with mitochondria or independently (Bhat et al., 2007). St-Pierre et al., in 2006 has suggested the role of PGC-1α in metabolism and absence of ROS detoxification agents in PGC-1α null cells. Further, ROS is also associated with insulin resistance (Houstis et al., 2006). Another study by Choi et al., (2006) also proposed a functional link between Gly482Ser variants of PGC-1α gene and mitochondrial function by observing the activity of Tfam promoter after transfection of Gly482 and Ser482 plasmid constructs.

Thus, it can be inferred from the present results that PGC-1α (Gly482Ser) variant may help in exploring the presumed link between central obesity, insulin resistance, β-cell dysfunction and development of T2D.
The AA genotype of UCP2 -866 G>A polymorphism provided significant risk in Banias \( [p=4.7\times10^{-6}, \text{OR}=4 \ (2-5.9)] \), BCs \( [p=1.2\times10^{-2}, \text{OR}=2.2 \ (1.1-4.2)] \) and SCs \( [p=1.1\times10^{-2}, \text{OR}=1.9 \ (1.16-3.2)] \) while no significant association was observed in Brahmins, Khatris, Jat Sikhs and Rajputs. AA genotype of UCP2 -866 G>A polymorphism also provided risk in pooled population \( [p=1.6\times10^{-4}, \text{OR}=1.46 \ (1.2-1.78)] \) of Punjab.

-866 G>A promoter polymorphism of UCP2 gene is the most extensively studied polymorphism of this gene. It acts as binding site for insulin promoter factor 1(IPF-1) and pancreatic transcription factors paired box-containing 6 (Esterbauer \textit{et al.}, 2001; Krempler \textit{et al.}, 2002). It is also associated with higher UCP2 mRNA levels; decreased GSIS and T2D risk (Sesti \textit{et al.}, 2003; D’Adamo \textit{et al.}, 2004; Sasahara \textit{et al.}, 2004).

The UCP2 -866 A allele and AA genotype conferred increased risk in Banias, BCs, SCs as well as in pooled population of Punjab. These findings are in concordance with previous studies where A allele of UCP2 -866 G>A polymorphism has been associated with increased risk \( [p=0.0004, \text{OR}=1.71 \ (0.97-3.01)] \) of T2D among Austrians and Italians (Krempler \textit{et al.}, 2002; Sesti \textit{et al.}, 2003). A-allele was also found associated with increased risk of T2D in women \( (p = 0.037 \text{ and OR of } 1.84 \ (D’Adamo \textit{et al.}, 2004; Hsu \textit{et al.}, 2008)). Besides T2D, A-allele of UCP2 -866 G>A polymorphism was found associated with impaired β-cell function, low GSIS and early age of onset of T2D in Japanese with \( p \) value of 0.012 and odds of 2 (Sasahara \textit{et al.}, 2004). Moreover, it was also reported to be associated with diabetic polyneuropathy in Japanese (Yamasaki \textit{et al.}, 2006), obesity (0.001) and hyperinsulinaemia in North Indians (Srivastava \textit{et al.}, 2010).

Various studies have also identified the association of -866 AA genotype with obesity. In addition to obesity, it should also be noticed that frequency distribution of alleles and genotypes among Brahmins and Jat Sikh groups showed higher percentage of A allele and AA genotype among controls as compared to cases, although no statistically significant association can be obtained. The frequency distribution of UCP2 -866 alleles is in concordance with the frequencies reported by Rai \textit{et al.}, (2007) in North Indians. Thus, the difference in results obtained in the present study and the results reported by
(Rai et al., 2007) can be explained in the background of obesity and ethnic diversity prevailing among North Indians.

Various studies have also documented the effects of UCP2 -866 G>A promoter polymorphism on the transcriptional activity of UCP2 gene (Krempler et al., 2002; Daldaard et al., 2003).

The association of minor allele A of UCP2 gene with T2D in various populations can help to establish the crucial link between obesity, β-cell dysfunction and pathogenesis of T2D. Our study also confirmed the association of UCP2 -866 A allele and AA genotype with T2D in population of Punjab. These results are in concordance with many studies which have reported the decreased GSIS during presence of UCP2 -866 A allele.

SIRT1 -1400 T>C polymorphism did not provide risk in any of the endogamous groups except for Khatris \( [p=4.0\times10^{-3}, \text{OR}-2.4 (1.3-4.5)] \). T allele or TT genotype provided risk in Khatris of Punjab. SIRT1 -1400 T>C polymorphism did not provide any risk in pooled population of Punjab.

Although, SIRT1 -1400 T>C polymorphism did not confer risk in population of Punjab but the SIRT1 -1400 TT genotype and T allele conferred risk among Khatris of Punjab. The frequency of TT genotype and T allele was high among cases as compared to controls (77 vs 61). Similar, distribution was also observed among Banias, Brahmins and SCs, although statistically significant associations could not be obtained. This shows that TT genotype and T allele genotype of SIRT1 gene may be involved in pathogenesis of T2D among some endogamous groups of Punjab. The mechanism by which SIRT1 polymorphism confers susceptibility to T2D remains unclear. Since SIRT1 is involved in various metabolic pathways, so the effect of polymorphism can be mediated through obesity, blood pressure and glucose levels.
Furthermore, SIRT1 is also associated with PGC-1α and UCP2 genes. Therefore, SIRT1 can play role in pathophysiology of T2D by modulating UCP2 and PGC-1α genes.

In pancreatic β cells SIRT1 positively regulates GSIS (Moynihan et al., 2005; Bordone et al., 2006). SIRT1 causes enhancement of GSIS by increasing the release of insulin during the first-phase. This is done by repression of UCP2 gene by SIRT1 (Imai and Kiess, 2009). Similarly, SIRT1 modulate PGC-1α in liver, skeletal muscle and adipose tissue and thus, acting as a therapeutic target for T2D. During fasting conditions, SIRT1 deacetylates PGC-1α, in an NAD-dependent manner (Rodgers et al., 2005). SIRT1 leads to both induction of gluconeogenic genes and the repression of glycolytic genes by its effect on PGC-1alpha in vitro and in vivo (Rodgers et al., 2005; Rodgers et al., 2007). Further investigation is needed, but these effects of SIRT1 to improve glucose tolerance and insulin sensitivity in the liver can partly be understood by stimulatory effect of SIRT1 on PGC-1α function.

Therefore, it can be concluded that although no significant association of SIRT1 -1400 T>C polymorphism can be observed in population of Punjab, however, SIRT1 gene might contribute to protect against T2D through its action on UCP2 and PGC-1α genes.

On gender based analysis, it was found that in males PGC-1α (Gly482Ser) polymorphism \([p= 4.4 \times 10^{-5}, \text{OR-} 1.81 (1.36-2.43)]\) and UCP2 -866 G>A polymorphism \([p=1.0 \times 10^{-2}, \text{OR-}1.45 (1.09-1.94)]\) provided significant risk under dominant models. However, no significant association was observed with SIRT1 -1400 T>C polymorphism \((p=0.59)\) in males of population of Punjab. Ser variant of PGC-1α (Gly482Ser) and AA genotype of UCP2 -866 polymorphisms provided almost two fold risk in males of Punjab.

In females also, PGC-1α (Gly482Ser) polymorphism \([p= 1.3 \times 10^{-2}, \text{OR-} 1.36 (1.07-2.43)]\) and UCP2 -866 G>A polymorphism \([p=3 \times 10^{-3}, \text{OR-}1.49 (1.14-1.95)]\) provided risk under dominant models and no association of SIRT1 -1400 T>C polymorphism was also observed in females \((p= 0.71)\) of population of Punjab. In females also, Ser variant
of PGC-1α (Gly482Ser) and AA genotype of UCP2 -866 polymorphisms provided 1.5 fold risk in females of Punjab.

As PGC-1α (Gly482Ser) polymorphism provided risk in both males and females, this can be attributed to positive association of hypertension in both males and females. T2D and hypertension both are involved in metabolic syndrome (Timar et al., 2000). In patients with hypertension both insulin resistance and hyperinsulinemia has been identified, which also contributes to the pathophysiology of T2D (Pollare et al., 1990; Zavaroni et al., 1996).

PGC-1α (Gly482Ser) polymorphism is also associated with T2D and hypertension (Okauchi et al., 2008). The probable link between PGC-1α (Gly482Ser) polymorphism, hypertension and T2D can be explained by hypoadiponectinemia (Ek et al., 2001, Hara et al., 2002; Esterbauer et al., 2002; Cheurfe et al., 2004).

The gender influences in association of PGC-1α (Gly482Ser) polymorphism with T2D has also been documented in few studies. However, none of the studies reported the association in both the genders as in the present study. Esterbauer et al., 2002 studied the sex specific association of PGC-1α (Gly482Ser) polymorphism with obesity indices in Austrian men and women. They found positive association in women while Cheurfa et al., 2004 reported significantly positive association among men [p=0.0064, OR- 2.52 (1.35-5.00)]. Similar finds were also reported by Okauchi et al., 2008, where they also found the significant association of PGC-1α (Gly482Ser) polymorphism with lower plasma adiponectin levels (p=0.006) in men.

Thus, it can be summarised that PGC-1α (Gly482Ser) polymorphism is an important polymorphism, which may act as a link between complex disorders like obesity, hypertension and T2D.

The present study demonstrated the gender differences with respect to association of UCP2 -866 G>A polymorphism also. UCP2 -866 G>A polymorphism was observed to provide risk only in women while no association was observed for men. Similar
findings were also reported by A’Damo et al., 2004. They also found positive association of UCP2 -866 G>A polymorphism with T2D in women from Rome. These results may indicate that UCP2 -866 G>A polymorphism confers increased risk of T2D in sex specific fashion. Another, study by Krempler et al., 2002 also reported the sex specific association of UCP2 -866 G>A polymorphism in obese middle aged subject. The sex specific risk association may also be obesity modulated. UCP2 -866 G>A promoter polymorphism is also associated with obesity. Moreover, UCP2 is considered a gene which plays role in development of obesity and T2D (A’Damo et al., 2004). UCP2 is expressed in tissues related with obesity like adipose tissue as well as in tissues related with T2D like skeletal muscle, liver and pancreatic β-cells (Fleury et al., 1997; Gimeno et al., 1997).

Moreover, women are also known to have more fat deposits even at normal weight conditions (Lois et al., 1996). Therefore, it can be possible that UCP2 -866 G>A polymorphism is conferring risk to females of Punjab by obesity. However, further detailed studies may be required to reach a conclusive association.

Therefore, it is evident from the following discussion that difference in association of PGC-1α (Gly482Ser) polymorphism and UCP2 -866 G>A polymorphism can be observed in males and females. However, no such gender disparities in association of SIRT1 -1400 T>C promoter polymorphism was observed in the present study. SIRT1 -1400 T>C polymorphism did not confer risk in both males and females of Punjab.

(iv) Interactive analysis of PGC-1α (Gly482Ser) polymorphism and UCP2 -866 G>A polymorphism.

Interactive analysis between PGC-1α (Gly482Ser) and UCP2 -866 G>A polymorphisms showed increased risk in patients carrying risk genotypes of both the polymorphisms. The risk genotypes of both polymorphisms, PGC-1α (Gly482Ser) (XA) and UCP2 -866 (G/A) (XA) were compared against all the other genotype combinations. The interactive analysis revealed highly significant association \([p=4.9 \times 10^{-10}, \text{OR}=2.3 (1.70-3.0)]\) and increased risk of T2D in patients with combined PGC-1 α XA and UCP2 -866 XA
72% of the patients had risk combination of PGC-1 α XA and UCP2 -866 XA genotypes as compared to 28% in controls. The results depict that the individuals carrying both risk genotypes of PGC-1α and UCP2 had 2.3 fold increased risk of developing T2D.

Biologically this interaction can be explained by keeping in account the various physiological effects of the studied polymorphisms in insulin secretion pathway. PGC-1α is a transcriptional co-activator with pleiotropic effects on various genes in different organs. In pancreatic β-cells, studies have found that over expression of PGC-1α is correlated with decreased insulin secretion. Similarly, studies have also found that over expression of UCP2 also diminishes release of insulin from β-cells.

So it can be postulated that the group of individuals in the study with UCP2 -866 G>A (XA) genotype secrete less insulin or may be insufficient amount of insulin is produced in individuals with PGC-1α (Gly482Ser) (XA) backgrounds, leading to more severe hyperglycaemic phenotype and finally overt T2D (Rai et al., 2007).

Further studies with more combinations and larger sample size are required to understand the complex aetiology of T2D in a better way and reach conclusive results.

From the above results and discussion, it can be concluded that to understand the complex aetiology of T2D in population of Punjab, both epidemiological risk factors and genetics factors should be taken into consideration. The role of central obesity has emerged as very important risk factors predisposing various endogamous groups and pooled population of Punjab. Along with central obesity, PGC-1α (Gly482Ser) and UCP2 -866 G>A polymorphism were also found highly associated with T2D in population of Punjab. Various endogamous groups were at risk due to combination of different risk factors.

Thus, the present study, in line with the literature, confirms that both environmental factors and genetic factors play important role in pathogenesis of T2D. Understanding
the interplay between these factors is very crucial to better understand the aetiology of T2D.

Therefore, having the background knowledge of ethnicity in association with the risk factors is very important to identify the specific polymorphisms predisposing a particular population towards T2D development and to design better medical interventions.