2.0 Review of Literature

Glucose homeostasis in the body is regulated by accurate balance between insulin secretion and insulin action. Defects in insulin secretion, insulin action or both pathways lead to development of chronic hyperglycaemia and finally overt diabetes (Herzberg-Schäfer et al., 2012). Genetic studies provide a means to identify pathways that predispose individuals to development of insulin resistance, hyperglycaemia and associated risk factors. Most of the common human diseases involve complex interactions among multiple genetic variants and environmental risk factors (Yang et al., 2005). Moreover, the modern way of sedentary living and high caloric diet has created new physiological conditions, affecting the level of expression levels of genes involved in metabolic pathways and body weight regulation, resulting in fuel disorders. Therefore, identification of risk loci and putative pathways can help us design better therapeutic interventions (Carlson et al., 2004). Most studies have focused on replicating the associations reported in literature rather than emphasising on the candidate genes interlinked in specific pathways that may have putative role in aetiology of diabetes. Thus, SNP typing in genes involved in pathways like insulin secretion and insulin resistance may help to decipher the molecular genetic basis of these complex traits and finding the potentially susceptible alleles implicated with diabetes and obesity, the two main fuel disorders of the present era.

2.1 Defining diabetes mellitus

World Health Organisation (WHO) and American Diabetes Association (ADA) define diabetes mellitus (DM) as a condition characterised with defect in insulin secretion, insulin action or both leading to malfunction in carbohydrate, protein and fat metabolism resulting in the state of chronic hyperglycaemia (World Health Organisation, 1999; American Diabetes Association, 2011). Several pathogenic processes are known to be involved in the development of DM which include pancreatic beta (β) cell dysfunction, inflammatory cytokine response, mitochondrial dysfunction, increased non-esterified fatty acids (NEFA) and increased adipokines with consequent
insulin deficiency and finally insulin resistance. Diabetic phenotype may result from impaired insulin secretion or insulin resistance which frequently coexists in the same patient making it unclear which is the primary cause of hyperglycaemia (American Diabetes Association, 2011). However, chronic hyperglycaemia remains the primary cause of long term damage to multiple important organs of the body like eyes, kidneys, nerves, heart and blood vessels etc.

2.2 Classification of Diabetes Mellitus (DM)

ADA has recognised two major forms of DM on the basis of fasting hyperglycaemia or elevated plasma glucose levels after oral glucose tolerance test (OGTT), termed as Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D) (American Diabetes Association, 2011).

2.2.1 Type 1 diabetes (T1D):

It is the most common autoimmune disease of childhood mainly characterised by T-cell mediated autoimmune destruction/dysfunction of insulin secreting pancreatic β-cells leading to absolute insulin deficiency (Todd et al., 1987; Morel et al., 1988). It is also known as juvenile onset diabetes because of very early age of onset and affects about 5%-10% of the total reported cases of DM. Components of the inflammatory responses which include CD4+ and CD8+ T-cells, macrophages, dendritic cells, natural killer (NK) cells, cytokines, free oxygen, nitric oxide (NO) radicals, etc., are known to contribute to β-cell destruction in T1D patients (Pietropaolo et al., 2007) who have to be treated by insulin supplementation for survival because of complete insufficiency of pancreas to secrete insulin (Godfrey et al., 2011).

Another subtype of T1D known as latent autoimmune diabetes of the adult (LADA) has also been identified, which occurs in adults and has a late age of onset. Patients with LADA have slow progression of β-cell failure and more preserved β-cell function than T1D patients. Due to production of pancreatic auto-antibodies patients with LADA are
completely on insulin supplementation (Tuomi et al., 1993; Brophy et al., 2008). Patients affected with LADA show symptoms nearly similar to T2D due to intense insulin resistance and late age of onset. It is the presence of β-cell antibodies which differentiates LADA from T2D (Naik et al., 2009). Reinehr et al., (2006) identified another subtype of LADA in youth, termed as latent autoimmune diabetes in youth (LADY).

2.2.2 Type 2 diabetes (T2D)

It is a complex heterogeneous group of disorders mainly characterised by insulin resistance and impaired β-cell function (Wheeler and Barroso, 2011). T2D is a multifactorial disorder which includes both genetic and environmental factors that affect β-cell function and tissue’s insulin sensitivity (Scheen, 2003; Martin-Gronert MS and Ozanne SE, 2012.). T2D is also known as adult onset or maturity onset diabetes. It affects 90% of the total diabetic cases. Major confounding factors which are known to play role in the aetiology of T2D are positive family history, age, obesity, increased abdominal fat, hypertension, lack of physical exercise and ethnic background. Some biological markers such as fasting hyperinsulinaemia, increased fasting proinsulin and decreased HDL cholesterol levels are also important indicators for development of T2D (American Diabetes Association, 2011).

2.2.3 Other forms of diabetes

ADA, (2011) has also recognised several other specific types of diabetes involving genetic defects of β-cell function, genetic defects in insulin action, disease of exocrine pancreas, endocrinopathies, drug or chemical induced diabetes, infections, uncommon form of immune mediated diabetes and some genetic syndromes associated with diabetes. Among these defined categories maturity onset diabetes of young (MODY) and gestational diabetes mellitus (GDM) are the most commonly known types of DM. MODY is a monogenetic form of DM caused due to single gene defect having autosomal
dominant inheritance and accounts for very few cases of DM. It is characterised by hyperglycaemia at young age of <25 years with primary defect of pancreatic β cell dysfunction (Stride and Hattersley, 2002). The pattern of inheritance of MODY is autosomal dominant. There are six types of MODY from MODY1 to MODY6 depending upon the type of genetic defect of pancreatic β-cell function. Abnormalities at 6 loci located on different chromosomes have been identified with MODY. Most common of these, is at chromosome number 12 in hepatocyte transcription factor, also referred as hepatocyte nuclear factor (HNF-1α) known as MODY3. Second, on chromosome 7 in glucokinase gene leading to MODY2. Third, on chromosome 20, HNF-4α known to cause MODY1. Fourth, on chromosome 13, in insulin promoter factor-1 gene (IPF1) leading to MODY4. Fifth, at chromosome 17, HNF-1β causing MODY5 and the sixth and the final one is on chromosome 2, at NeuroD1 gene known as MODY6. Patients with MODY are generally controlled on diet and low dose of oral hypoglycaemic drugs or occasionally insulin supplements (Fajans et al., 2001; Maestro et al., 2007).

GDM is form of hyperglycaemia characterised with impaired glucose tolerance first recognised during pregnancy. The requirement of insulin is high but differs slightly between normal and gestational diabetic women, revealing that reduced insulin response is the key cause of GDM. Moreover, women with GDM have β cell dysfunction occurring in the background of chronic insulin resistance (Buchanan and Xiang, 2005). The condition of hyperglycaemia reverts back to normoglycaemic control after delivery but GDM may progresses to T2D in some cases. Meta-analysis study has revealed that the women with GDM have increased risk of developing T2D later in life as compared to those women who had normoglycaemic pregnancy (Bellamy et al., 2009).
2.3 Epidemiology of diabetes

Changes in human behaviour and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide. Estimates predict that by the year 2030, 366 million people will be suffering from T2D worldwide with 79.4 million Indians with its high prevalence in urban populations (Wild et al., 2004). According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India, which was 35 million in 2003, the world’s largest diabetic population, has reached around 40.9 million in 2006 and is expected to rise to 69.9 million by 2025 (Mohan et al., 2007). Recent estimates have rated India as the top most country with 50.8 million diabetic people in 2010 and will continue to be on the top in 2030 with 87 million diabetic people (Shaw et al., 2010). This would comprise almost 20% of the total diabetic population of the world. Among all South Asians also, Indians have the fastest growing prevalence of T2D and maximum number of reported cases of T2D (Garduño-Diaz and Khokhar, 2012).

The phenomenal rise in prevalence of DM in India can be attributed to the genetic predisposition known as “Asian Indian phenotype” (Joshi, 2003); and green and white revolution which lead to better prosperity, improved socioeconomic status, better economy, improved nutrition and finally rural to urban migrations associated with sedentary lifestyle over a short span of time (Reddy and Yusuf, 1998; Chan et al., 2009)

2.4 Diagnosis of Diabetes

The following tests are used for diagnosis:

1) A fasting plasma glucose (FPG) test measures blood glucose after at least 8 hours without eating. This test is used to detect diabetes or pre-diabetes. The FPG is the preferred test for diagnosing diabetes due to convenience and is most reliable when done in the morning. Classification on the basis of glucose levels is represented in table 2.1. Fasting glucose level of 100 to 125 mg/dl represents a form of pre-diabetes called
impaired fasting glucose (IFG), meaning that the individual is likely to develop T2D. A level of 126 mg/dl or above, confirmed by repeating the test on another day, means that the person is diabetic.

Table 2.1: Fasting Plasma Glucose Test

<table>
<thead>
<tr>
<th>Plasma Glucose Result (mg/dl)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>99 and below</td>
<td>Normal</td>
</tr>
<tr>
<td>100 to 125</td>
<td>Pre-diabetes (impaired fasting glucose)</td>
</tr>
<tr>
<td>126 and above</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>

2) An oral glucose tolerance test (OGTT) measures blood glucose after at least 8 hours without eating and 2 hours after drinking a glucose-containing beverage, 75 grams of glucose dissolved in water. This test can be used to diagnose diabetes or pre-diabetes. Table 2.2 represents the classification based on glucose levels obtained with OGTT. Blood glucose level in the range of 140 - 199 mg/dl, 2 hours after drinking the liquid represents pre-diabetic state called impaired glucose tolerance or IGT. A two hour glucose level of 200 mg/dl or above, confirmed by repeating the test on another day, presents overt diabetes.

Table 2.2: Oral Glucose Tolerance Test

<table>
<thead>
<tr>
<th>2-Hour Plasma Glucose Result (mg/dl)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>139 and below</td>
<td>Normal</td>
</tr>
<tr>
<td>140 to 199</td>
<td>Pre-diabetes [impaired glucose tolerance (IGT)]</td>
</tr>
<tr>
<td>200 and above</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>
3) In random plasma glucose test (RPGT), blood glucose level is checked without reference to the last meal. RPGT along with an assessment of symptoms, is used to diagnose diabetes but not pre-diabetes. A random blood glucose level of 200 mg/dl or more, as well as the presence of the symptoms (increased urination, increased thirst, and unexplained weight loss) indicates that the person is diabetic.

4) Glycated haemoglobin (HbA1c)- ADA in 2010 included HbA1c as a diagnostic criteria diabetes, impaired glucose tolerance and impaired fasting glucose (American Diabetes Association, 2010). The proposed value of 5.7% to 6.4% is considered as increased risk for developing diabetes (American Diabetes Association, 2010; Park et al., 2012).

2.5 Thrifty gene hypothesis

The possible explanation of the interaction between genes and environment can be done on the basis of thrifty gene hypothesis. The growth of human civilisation has created a major change in human lifestyle. Earlier humans used to live a nomadic life, which made their organs and body to work in such a manner so that it could give more energy with limited amount of food but with adoption of agriculture and other technological advancements, there was a transition to more sedentary lifestyle. James Neel in 1962 proposed a thrifty gene hypothesis according to which certain genes in humans have evolved to maximise metabolic efficiency, lipid storage and food searching behaviour, and that in times of abundance these genes predispose their carriers to diseases caused by excess nutritional intake, such as obesity and diabetes. In the past, this genetic predisposition would have been advantageous for puberty or child-bearing women during periods of famine. However, with the advent of high fat, high carbohydrate, and low fibre diets, and relative inactivity, the genotype is no longer advantageous. This has lead to obesity and related health problems. It follows from the theory that populations with a history of food scarcity have undergone a comparatively high evolutionary pressure and hence may harbour more thrifty genes than other populations (Neel, 1962).
Again the procurement of western dietary habits and motorisation made human susceptible to many diseases, obesity and diabetes being the major ones (Takasu et al., 2007). Different genes may act as thrifty genes in different populations. FTO gene is regarded as thrifty gene in Asians and Africans (Prentice, 2009) while Leptin receptor gene (LEPR) has been recently identified as thrifty gene in Pacific islanders (Furusawa et al., 2010). A recent study also identified PGC-1α as a thrifty gene in Tongan population (Myles et al., 2011) while another recent study reported PC-1 Gln 121 gene as thrifty gene in Amerindians (Rey et al., 2012).

2.6 Pathophysiology of T2D

Glycaemia or blood glucose levels are controlled by balanced interplay between insulin action and insulin secretion. In patients with T2D, hyperglycaemia occurs in combination with peripheral insulin resistance and decreased efficiency of pancreatic β-cells to secrete proper amount of insulin needed by the body (Martin-Gronert and Ozanne, 2012). Normal pancreatic β cell adapts to the decreased insulin action by upregulation of insulin secretion and vice versa (Stumvoll et al., 2005). Physiologically there are two key hormones released from pancreas involved in regulation of normal blood glucose in the body which is maintained by coordinated secretion of insulin and glucagon hormones (Rosen and Spiegelman, 2006).

2.6.1 Pancreas

Pancreas is a gland of endodermal origin with both endocrine and exocrine functions and consists of head, neck, body and tail. Exocrine part of pancreas constitutes 80%-90% of pancreatic mass and is composed of acinar cells and ducts. Acinar cells secrete digestive enzymes which are carried by ducts into duodenum. Endocrine part of pancreas constitutes about 2% of the total pancreatic mass and consists of islets of Langerhans. Islets of Langerhans has four majors types of cells namely alpha (α) cells which secrete glucagon hormone, beta (β) cells which secrete insulin hormone, delta (δ)
cells which secrete somatostatin and finally pancreatic polypeptide secreting F cells or PP cells (Thorel et al., 2010).

2.6.2 Insulin

Insulin was first discovered by Fredrick Banting in 1920 for which he was acknowledged with Nobel Prize in 1923. Insulin also known as “hormone of plenty” is an anabolic hormone which stimulates the hepatocytes, adipose tissue and skeletal muscles to take up glucose from blood and store it as glycogen. It is composed of two polypeptide chains, A chain of 21 amino acids and B chain of 30 amino acids linked by two disulphide bonds. Insulin is synthesised in rough endoplasmic reticulum of β cells as preproinsulin and cleaved to proinsulin molecule in golgi bodies and finally stored as secretory granules which are released into circulation by exocytosis (Rorsman and Renstrom, 2003). Half-life of insulin is approximately 5 minutes and it regulates carbohydrate metabolism by reducing the rate of glucose release from liver by inhibiting glycogenolysis and gluconeogenesis by stimulating glycogen synthesis, glucose uptake and glycolysis. Simultaneously insulin has effects on fat and protein metabolism as well (Michael et al., 2000; Guyton and Hall, 2010).

2.6.3 Glucagon

Glucagon mainly works antagonistically to insulin. It stimulates pathways to release glucose from glycogen and fatty acids from stored triglycerides and stimulates gluconeogenesis. Fall of glucose levels below 4 mmol/l decreases insulin secretion and promote glucagon release from pancreatic α cells (Liljenquist et al., 1977; Saltiel and Kahn, 2001; Guyton and Hall, 2010).
2.6.4 Maintenance of normal glucose homeostasis

One of the most important of all the physiological functions is the regulation of energy homeostasis as it determines the ability to perform optimally and face the ever changing and challenging environment. Altered glucose homeostasis leads to development of T2D due to loss of tissues to adapt to changing metabolic demands (Herman and Kahn, 2006). In normal individuals plasma glucose levels are maintained between the narrow ranges of 65-140 mg/dl which involves delicate balance between three pathways i.e. rate of endogenous hepatic glucose production, proper insulin action for glucose disposal and insulin secretion. Type 2 diabetic patients manifest impairments in these three pathways (DeFronzo, 1988; Porte, 1991).

1) Hepatic glucose production (HGP)- Production of glucose from liver arises by break down of glycogen by a process called glycogenolysis and gluconeogenesis. Normal liver glucose production is very crucial as it provides glucose to brain and maintains normal plasma glucose levels during fasting state but these levels are abnormally elevated in T2D (Matsumoto and Accili, 2006). Maintenance of normal plasma glucose levels within the narrow range of 65-140mg/dl is tightly linked with glucose production during fasting state and glucose utilisation during fed state (Bolli and Fanelli, 1999). This is maintained by delicate balance between absorption of glucose from intestine, production of glucose from liver (HGP) and glucose disposal into peripheral tissues (Saltiel and Kahn, 2001).

During over night fasting liver releases glucose into the blood stream to avoid hypoglycaemia by the process of glycogenolysis and gluconeogenesis. Liver and kidneys can release glucose because of presence of glucose-6-phosphatase enzyme. During fed state, insulin plays a crucial role in regulation of HGP by acting directly and indirectly on liver. It directly binds to insulin receptors in liver and activates downstream insulin signalling pathways which suppresses both the processes of glycogenolysis and gluconeogenesis (Rosen and Spiegelman, 2006). Indirect regulation
of HGP by insulin involves coordinated actions on various organs of the body such as decreased production of glucagon in pancreas, reduced lipolysis in adipose tissue and finally decreased protein catabolism in skeletal muscle. Reduced lipolysis and protein catabolism lead to less availability of precursors for both gluconeogenesis and glycogenolysis respectively (Edgerton et al., 2006).

2) Glucose uptake by muscle and adipose tissue- Disposal of glucose load into peripheral tissue like skeletal muscle and adipose tissue has been studied widely and considered as crucial step in regulation of glucose homeostasis. In healthy individuals insulin tightly regulates uptake of glucose in skeletal muscles and adipose tissue by translocation of glucose transporters (GLUTs) from intracellular sites to the cell surfaces (Klip and Paquet, 1990). GLUTs are the sodium (Na) dependent family of integral membrane proteins which facilitates diffusion of glucose (Shepherd and Kahn, 1999). Skeletal muscle comprises 75% of the total insulin stimulated glucose uptake whereas very small quantity of glucose is taken up by adipose tissue (Klip and Paquet, 1990). Knockout mice for insulin dependent glucose transporters in fat showed impaired glucose tolerance, confirming insulin resistance develops in muscle and liver (Abel et al., 2001). Insulin resistance is defined as impaired glucose disposal which is considered as very first step in pathophysiology of T2D. To curve down insulin resistance, pancreas secretes more insulin as compensatory mechanism. Thus, leading to fasting hyperinsulinaemia at an early stage of T2D. Finally development of overt diabetes occurs when pancreas is no longer able to compensate for insulin resistance by producing more and more insulin (Herman and Kahn, 2006; Drews and Dufer, 2012).

3) Glucose stimulated insulin secretion (GSIS)- Insulin secretion is a bi-phasic process. The first phase is marked by rapid increase in insulin secretion occurring immediately after an increase in glucose levels is sensed by pancreatic β-cells. This phase lasts for about 10 minutes. It efficiently switches metabolism from the fasting state to prandial state by suppressing endogenous glucose production and efficient glucose disposal. Subsequently the second phase of insulin secretion begins after 10-20 minutes exposure
of β-cells to glucose. This phase is marked by sustained increase in insulin secretion which may last for several hours (Pratley and Weyer, 2001).

Mechanism of glucose stimulated insulin secretion (GSIS) starts with the ability of β cells to sense elevated glucose levels and adjust secretion of insulin according to the organisms need. Glucose molecules enter into pancreatic β-cell by facultative glucose diffusion by GLUT2 and gets metabolised by glycolysis pathway, where they get phosphorylated by Glucokinase enzyme leading to ATP generation. This step is termed as the rate limiting step of GSIS. For complete oxidation of glucose, glycolysis by product pyruvate undergoes oxidative phosphorylation via Electron Transport Chain (ETC) in mitochondria generating ATPs, termed as oxidative metabolism of glucose (Schuit et al., 1997). This complex reaction begins as electrons within the carbon bonds are transferred to dinucleotide electron carriers, NADH and FADH₂. This in turn donate electrons to mitochondrial ETC, a multiprotein unit grouped into four complexes (I to IV) located within mitochondrial inner membrane. Ultimately, electrons cause reduction of oxygen to water. Complexes I, III and IV are reduction and oxidation driven proton pumps that use energy carried by the electrons to pump protons out of the matrix, creating a proton electrochemical potential gradient across the mitochondrial inner membrane. Energy stored in this gradient is utilised by ATP synthase enzyme to generate ATP from ADP (Lowell and Shulman, 2005). Oxidative metabolism of glucose in pancreatic β-cells leads to increase in ATP/ADP ratio which is a very crucial step as it causes closure of ATP sensitive potassium (K⁺) channel. Closure of K⁺ channel, depolarises the pancreatic β-cell membrane, which opens voltage dependent Ca⁺⁺ channel. Opening of Ca⁺⁺ channel leads to incoming of Ca ions and thus stimulating secretion of stored insulin granules from β-cells via exocytosis (Sesti et al., 2003; Guyton and Hall, 2010).

2.7 Risk factors in T2D

Basically, these risk factors can be divided into three main groups-
2.7.1 Environmental factors

In certain ethnic groups, the environmental and behavioral factors such as a sedentary lifestyle, overly rich nutrition and obesity (Martin-Gronert and Ozanne, 2012) play a major role in the susceptibility to T2D but presence of positive family history seems the most constant factor (Mokdad et al., 2000, Lyssenko et al., 2005). The prevalence of T2D varied from one population to another. This difference in prevalence rate can be attributed to the variable environmental conditions prevailing in different parts of the world (Zimmet et al., 2001). In virtually all populations, higher fat diets, decreased physical activity and sedentary occupational habits accompanied by migration from one environment to other, have resulted in the doubling of the prevalence of T2D. Hence genetic predisposition to T2D may or may not lead to the development of the disease every time (Chan et al., 2009).

2.7.2 Metabolic factors

There are number of metabolic factors, which are responsible for the occurrence of T2D besides environmental factors. The main factors include levels of insulin and glucagon hormone, adipokine’s concentration and cytokine levels in the body. Insulin and glucagon help in maintenance of normal blood glucose levels and any kind of impairing in the secretion of these hormones or the response of the target cells to these hormones lead to the development of T2D.

Another such factor is Adiponectin, an adipocytokine, which accounts for 0.01-0.03% of total plasma proteins. Adiponectin is produced by adipocytes, which affects the
body’s energy balance (Qiao et al., 2012) and the regulation of insulin and glucose in the body. Decreased levels of adiponectin are independently associated with insulin sensitivity and are negatively correlated with plasma glucose and adipose tissue metabolites, fasting plasma insulin (FPI), triglycerides (TGs) and body mass index (BMI) (Sharma et al., 2006).

Adiponectin also have antisuppressive properties because of its ability to induce production of anti-inflammatory cytokines, thus inhibits the production of pro-inflammatory cytokines (Wolf et al., 2004, Wulster-Radcliffe et al., 2004). This long-term low-grade inflammation further plays a key role in insulin resistance, hyperglycemia, oxidative stress and endothelial dysfunction that fin turn leads in renal damage (Navarro-Gonzalez and Mora-Fernandez, 2008). The acute-phase reactants and pro-inflammatory cytokines are positively correlated with measures of insulin resistance and moreover, levels of inflammatory parameters were elevated in diabetic cases as compared to non-diabetic control subjects (Pickup et al., 1998; Rivero et al., 2009). Examples of inflammatory cytokines include MCP-1, TGF-β, TNF-α and interleukins like IL-6, IL-4, IL1.

In addition to adiponectin, Adrenocorticotropic hormone (ACTH) and growth hormone, released from the pituitary that acts to increase blood glucose by inhibiting uptake by extra hepatic tissues. Glucocorticoids, mainly Cortisol, also act in the same manner to ACTH (Hollis and Huber, 2011).

2.7.3 Genetic factors

T2D is an extremely heterogeneous disorder, phenotypically and pathogenetically. Multiple genes or polymorphisms work with an additive effect, each insufficient in themselves in order to cause diabetes. These genes might affect β-cell apoptosis, regeneration, glucose sensing, glucose metabolism, ion channels, energy transduction.
These genes can also cause changes in islet proteins necessary for synthesis, packaging, movement and release of secretory granules.

A number of candidate genes have been screened for association with T2D. The genes involved in susceptibility to T2D are not very well defined (Ridderstrale and Groop, 2009), except in the few populations in whom specific genetic mutations have been identified (Saxena et al., 2012). Genes regulating various metabolic pathways could contribute to pathophysiology of T2D. Table 2.3 shows T2D susceptibility genes investigated recently for association with T2D.

2.8 Estimation of genetic risk

To establish the genetic influence on a disease, familial aggregation studies are performed. Influence of heredity on disease can be assessed by twin studies, sib-pair analysis, parent-offspring studies, concordance studies and migrant studies. Twin studies have been very useful to determine the genetic component of any disease. Identical twins (monozygotic twins-developing from single fertilised egg) share 100 percent genetic content, while fraternal twins (dizygotic twins-developing from two fertilised eggs) share only 50 percent genetic content (Falconer, 1989); but at the same time both monozygotic and dizygotic twins share same intrauterine environmental influences.

Twin studies carried out with respect to T2D reported that in individuals older than 60 years had concordance rates of 35-58% in monozygotic twins and 17-20% in dizygotic twins (Newman et al., 1987; Kaprio et al., 1992). It was also observed that positive family history confers 2.4 fold increased risk for T2D. Moreover, 15-25% of the first degree relatives of the patients develop impaired glucose tolerance or T2D and the life time risk of 38% was calculated when one parent is affected with T2D (Pierce et al., 1995). Furthermore, if both the parents are affected then the prevalence of T2D may rise to 60% in offspring by the age of 60 years (Tattersal and Fajans, 1975).
<table>
<thead>
<tr>
<th>Candidate gene</th>
<th>rsIDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOTCH2</td>
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</tr>
<tr>
<td>NOTCH2</td>
<td>rs2641348</td>
</tr>
<tr>
<td>GCKR</td>
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</tr>
<tr>
<td>THADA</td>
<td>rs7578597</td>
</tr>
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<td>RBMS1</td>
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<td>PPARG</td>
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<td>CDC123/CAMK1D</td>
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</tr>
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<td>DUSP9</td>
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</tr>
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</table>
As T2D is a complex disorder involving combination of genetic and environmental factors which contribute in disease causation, it becomes all the more difficult to elucidate the candidate genes responsible for T2D (Elbein et al., 1994). Various combinations of the techniques such as linkage and association studies are required to dissect the genetic component responsible for T2D (Ghosh and Schork, 1996). CAPN 10 is the only gene that has been identified by linkage studies in T2D whereas association studies have found genes like PPARG and KCNJ11 associated with T2D (Ridderstrale and Groop, 2009). Therefore, based on the type of study design, different approaches can be selected to conduct the study and elucidate the risk genes.

2.9 Approaches for genetic analysis

Complex or multifactorial diseases are determined by number of genetic and environmental factors playing role in disease aetiology. The factors like polygenic inheritance, locus heterogeneity, epistasis, environmental influences, gene-environment interactions, developmental and time dependent expression and characterisation of genes responsible for complex diseases like T2D is very difficult (Schork, 1997).

The putative genes influencing complex disorders can be identified using linkage analysis, candidate gene approach and whole-genome scans.

2.9.1 Linkage analysis:

Linkage analysis is the positional cloning based approach to identify the genes and markers associated with the disease which has been the dominant choice of design for studying inherited disease for past few decades. Linkage analysis is a statistical approach which searches for regions of the genome with higher than expected number of shared alleles among affected individuals within a family, indicating the presence of disease predisposing allele (Carlson et al., 2004). The rationale behind linkage analysis states that the genes which reside in close proximity on a chromosome remain linked
during meiosis due to less chances of cross over and are passed on together from one generation to another (Pulst, 1999). The major prerequisite to carry out successful linkage analysis is to identify regions on the chromosome that are likely to segregation with diseased gene. After identifying such regions, genes located in the nearby regions are looked for in both normal control samples and diseased samples. Uncertainties about the diseased status may produce noise or may give false positives. Therefore, linkage analysis has been a greater success in Mendelian disorders which have clear pattern of inheritance and one to one genotype-phenotype correspondence but for complex disorders linkage analysis has seen limited success. For linkage studies, lesser sample size may also give accurate results if clear diagnosis can be done between the diseased/non diseased and even large sample size may give ambiguous results if the criteria for diagnosis is not confirmed (Botstein and Risch, 2003). A major drawback of linkage analysis is collection of large number of pedigrees having many affected individuals. Segregation of few markers or genes can be studied by linkage; therefore the marker density is kept low in linkage analysis. Multipoint linkage analysis has been suggested for complex disorders. However, multipoint linkage analysis has also seen limited success as compared to candidate gene approach for complex disorders. Most importantly, linkage analysis is not believed to be a very powerful tool to identify common genetic variants that have modest effect on disease (Risch and Merikangas, 1996; Risch, 2000).

2.9.2 Candidate gene approach:

Candidate genes are the genes with known biological function which can be involved directly or indirectly in pathophysiology of a particular disease. The rationale behind candidate gene approach states that a major component of genetic variation of the disease under investigation is caused by a functional mutation in candidate gene (Zhu and Zhao, 2007). Various association studies with candidate genes have been used to investigate the genetic component of complex disease (Tabor et al., 2002). Candidate gene approach is preferred over linkage analysis as it helps in detecting genes underlying common and more complex diseases where the risk associated with any
given candidate gene is relatively small. Technique used in it includes selecting a candidate gene based on the known physiological function and then choosing a DNA polymorphism which can be useful for testing in an association study (Kwon and Goate, 2000). Role of candidate gene can be confirmed by evaluating the effects of gene variants in an association analysis. Association studies (case-control association studies) are used to evaluate association (risk/protection) of a particular genetic variant in a candidate gene (generally suggested by linkage analysis) with the disease. Case control association compares the allele frequency of polymorphic marker/markers like SNPs in unrelated patients (cases) and healthy controls and evaluates if the distribution of allele differs significantly among cases and controls (Carlson et al., 2004). Such studies use linkage disequilibrium (LD) to choose the marker of interest. A useful marker can either be the causal allele or may be highly correlated (in LD) with the causal allele. LD is non-random correlation between the alleles at a pair of SNPs. Association studies are more powerful than linkage analysis for detecting genes with modest effect, as the complex disorders involves multiple genes of relatively small individual effect. Moreover association studies are the cheaper and simpler way to identify the common variants underlying complex traits. (Risch and Merikangas, 1996)

For example in T2D, genes playing role in insulin secretion, insulin action, β-cell development and other related pathways can be considered as candidate genes and polymorphisms in these genes can be screened for association with T2D. In the present study, PGC-1α, UCP2 and SIRT1 genes were analysed as they play major role in insulin secretion.

2.9.3 Genome wide scans

As a result of systematic and well powered genome-wide surveys, there has been a vast advancement in understanding of genetic basis of many multifactorial diseases (McCarthy et al., 2008). Successful completion of human genome sequence (Venter et al., 2001) and international HapMap project has lead to better understanding of the
human genome and identification of millions of SNPs across the whole genome. These advancements have made it easy to carry out whole genome scans for better understanding of the complex diseases. Genome wide scans can be carried out as genome-wide linkage studies and genome-wide association studies. Genome wide linkage analysis is a traditional method in which markers (minisatellite or microsatellite) which are regularly spaced in whole genome are traced in families and siblings for segregation with T2D (Whittemore, 1996). It has been very successful for mapping monogenic Mendelian diseases. It has also been carried out for complex disorders but success has been limited and the genes discovered explain very less heritability. Lack of success can be because of various reasons like low heritability of most complex traits, ambiguity in definition of phenotypes and less power of the studies to identify true linkage (Evans and Cardon, 2004; John et al., 2004). Moreover, linkage studies are insufficiently powered to detect association of genetic variants with modest effects on disease (Tabor et al., 2002). Therefore, strategic complement to genome wide linkage studies is genome wide association studies. Genome wide association scans helps to identify and characterise susceptibility genes for complex traits and diseases. These studies involve SNP genotyping in large sample size of hundreds and thousands of individuals (Chen and Abecasis, 2007). These studies require knowledge of genetic variations in the plethora of genes selected for the study in large sample size. SNP database with more than 9 million SNPs reported is a good source to select the desired SNP for typing. It is a hypothesis free approach. Markers for these studies can be LD-based markers or some missense SNP markers (Hirschhorn and Daly, 2005). Thus genome wide association analysis carried out comprehensively on larger sample size improves the understanding of genetic basis of complex disease.

2.10 Genome wide association studies in T2D

Due to limitations of classical approaches like linkage studies and candidate gene studies, it has been very challenging to identify the genetic variants associated with T2D until the advent of genome wide association studies (GWAS). Completion of Hap Map project, improvement of high throughput technology for SNP typing and decreasing
Genotyping cost led to the advent of GWAS. GWAS uses the phenomenon of linkage disequilibrium (LD). SNPs with strong LD will be co-inherited because of their close proximity on a chromosome such that recombination at meiosis does not occur. Thus analysis can be carried out on comparatively smaller subsets of SNPs or “tag” SNPs (Imamura and Maeda, 2011).

First GWAS for T2D was conducted in 2007 by Sladek et al., on a French cohort comprising 661 cases and 614 controls. A total of 392,935 SNPs were genotyped in the study and novel association signals were observed at SLC30A8, HHEX, LOC387761 and EXT2. Their study also validated the association of TCF7L2. Soon after Steinthorsdottir et al., (2007) also confirmed the association between T2D and SLC30A8, HHEX and CDKAL1. At the same time, three groups, Wellcome Trust Case Control Consortium/United Kingdom Type 2 Diabetes Genetics Consortium (WTCCC/UKT2D), the Finland-United States Investigation of NIDDM (FUSION) and the Diabetes Genetics Initiative (DGI) replicated the association of SCL30A8 and HHEX with T2D and also reported the novel association of CDKAL1, IGF2BP2 and CDKN2A/B (Saxena et al., 2007; Scott et al., 2007; Zeggini et al., 2007). In addition WTCCC/UKT2D also found strong association between FTO and T2D (Frayling et al., 2007).

To reach more conclusive results in association, WTCCC/UKT2D, FUSION and DGI combined their data to form the diabetes genetics replication and meta-analysis (DIAGRAM) consortium. With increased sample size (4,549 cases and 5,579 controls) five additional novel risk loci namely JAZF1, CDC123/CAMKID, TSPAN/LGR5, THADA and ADAMSTS9 were identified (Zeggini et al., 2008). DIAGRAM has now become DIAGRAM+, as most of the T2D genetics cohort combined with them to further increase the sample size to more than 22,000 subjects of European origin. Recently they identified twelve new risk loci associated with increased susceptibility to T2D (Voight et al., 2010). Nine additional risk loci (ADCY5, MADD, CRY2, ADRA2A, FADS1, PROX1, SLC2A2, GLIS3 and C2CD4B have also been identified.
after replication among 76,558 individuals associated with fasting glucose levels (Saxena et al., 2010).

GWAS studies were also carried out among individuals from Asian descent. Two independent groups in Japan identified strong association of KCNQ1 locus with T2D (Unoki et al., 2008; Yasuda et al., 2008). Two additional risk loci (UBE2E2, C2CD4A-C2CD4B) have also been identified in Japanese GWAS study in 2010. Recently, four new loci (PTPRD, SRR, CDC123/CAMK1D and SPRY2) were identified among Han Chinese in Taiwan and Shanghai (Shu et al., 2010; Tsai et al., 2010).

2.11 Genetic predisposition to T2D among Indians

Numerous twin studies, family studies and epidemiological studies have evidenced the important role of genetics in the aetiology of T2D. It has been a well known fact that prevalence of T2D varies among different population groups. This can be attributed to different environmental factors prevailing in different geographical regions. At the same time, the difference in prevalence of T2D among ethnic groups sharing common environment, strongly indicates contribution of genetic factors (Diamond, 2003). Familial aggregation in T2D further strengthens the implication of genetic component in aetiology of T2D. A study by Meigs et al., (2000) reported that odds ratio (OR) for the offspring with single affected parent is 3.5 which increase to 6.1 if both the parents are affected. Twin studied also identified high concordance rate of 80% in monozygotic twins which declines to 50% in dizygotic twins providing evidence for genetic component in causation of T2D (Vaag et al., 1995; Poulsen et al., 1999). Moreover, studies measuring both insulin sensitivity and insulin secretion are also in support of genetic basis for T2D (Gerich, 1998; Elbein et al., 1999). Several Indian studies also support the role of genetic factors in T2D. A study carried out on 135 Asian Indians and 146 Europeans in UK reported that 45% of Asian Indians had a first degree relatives with T2D as compared to 36% of Europeans (Mohan et al., 1986a). Also, 10% of the diabetic Asian Indians had both the parents with T2D, while this percentage was only
1% for the Europeans diabetic cases. Various studies also identified that 55-60% offspring with two T2D parents had diabetes or impaired glucose tolerance (Viswanathan et al., 1985; Mohan et al., 2003). Thus these findings strongly indicate towards the role of genetic component in making Asian Indians more susceptible for T2D.

Mohan et al. in 1986 reported for the first time that the plasma insulin levels are higher in Asian Indians as compared to Europeans. Later on they also found that Asian Indians develop insulin resistant at lower BMI values than their European counterparts (Ramachandran et al., 1997). Furthermore, at any given value of BMI, Indians also present with higher body fat percentage and greater insulin resistance as compared to other ethnic groups (Banerji et al., 1999; Chandalia et al., 1999; Raji et al., 2001). Various epidemiological studies conducted among migrant Indians also showed high prevalence of T2D indicating towards the strong genetic predisposition of Indians (McKeigue et al., 1992; Abate and Chandalia, 2003). Furthermore, it has been documented that moderate obesity in Asian Indians leads to insulin resistance as more fat tends to accumulate in central region among Indians. Individuals with high WHR or high truncal to peripheral skinfold develop more insulin resistance (Abate et al., 1995). Studies are also available indicating Indians are more susceptible in developing truncal obesity and hence more insulin resistance. This is referred to as “Asian Indian Phenotype”. Thus, both involvement of genetic factors and environmental factors are predisposing Indians for T2D causation.

Various GWAS and case control association studies have identified several genetic risk loci in different candidate genes involved in various biological pathways with risk of developing T2D. To confirm the association of genetic risk variants with T2D genotyping and replication in different populations are required. Therefore, in the present study common SNPs in PGC-1α, UCP2 and SIRT1 genes from insulin secretion pathway will be analysed.
2.12 Peroxisome proliferator-activated receptor gamma, co activator 1 alpha: PGC-1α

PGC-1α is a transcriptional co-activator that regulates genes involved in energy homeostasis, mitochondrial biosynthesis, lipid oxidation and T2D (Li et al., 2012). Co-activators are the protein complexes that increase the rate of transcription by interacting with transcription factors and help in chromatin remodelling but do not itself bind to DNA. Most co-activators help in transcription by having specific enzymatic activity required to remodel chromatin. PGC-1α is a class II co-activator which lacks DNA binding domain and histone acetyl transferases (HATs) activity domain required for histone modification. It does not have much amino acid sequence homology with other transcription factors. It associates via acidic N-terminal activation domain with other co-regulators that acetylate chromatin like cAMP response element binding protein (CREB)- binding protein (CRP/p300) and steroid receptor co-activator. Binding of transcription factors like PPARγ and NRF-1(nuclear respiratory factor) to amino acids 200-400 region to PGC-1α induces conformational changes to recruit histone acetyl transferases like CBP/p300 and SRC-1 into the complex at the N-terminal for increased rate of transcription (Puigserver et al., 1999).

PGC-1α is also known as PPARGC1A. It is located on chromosome number 4, at 4p15.1. It spans 67kb in length and it comprises 13 exons and 12 introns. It codes for 91kDa protein, mainly expressed in tissues like heart, skeletal muscle and kidney Figure 2.5.

It was first discovered in 1998 as a co-activator of PPARγ in brown adipose tissue found to play role in adaptive thermogenesis (Puigserver et al., 1998). Later it was also found to increase the transcriptional activity of PPARα, glucocorticoid, liver X, Vitamin D and Thyroid receptor families (Knutti et al., 2000; Vega et al., 2000; Wu et al., 2002; Oberkofler et al., 2003). It can also bind to nuclear receptors like hepatocyte nuclear factor (HNF) 4α (Rhee et al., 2003). Not only nuclear receptors, it is also believed to be
involved in insulin and glucagon signalling pathway including forkhead helix protein family FOXO1 (Puigserver et al., 2003).

2.12.1 PGC-1α in glucose homeostasis and T2D

PGC-1α integrates metabolic pathways which include increased hepatic gluconeogenesis and β-oxidation, mitochondrial biogenesis and function, insulin independent uptake of glucose and metabolism, reduced insulin secretion providing more glucose for brain and kidney during starvation and other environmental adversities (Storey, 2003; Russell, 2012). Expression of PGC-1α has been broadly described in tissues like heart, liver, brain, adipose tissue and skeletal muscle (Handschin and Spiegelman, 2006). In cardiac cells, to overcome the increased energy requirement more uptake and metabolism of glucose is required. Thus, high expression levels of PGC-1α mRNA have been noticed in cardiac cells which have been known to stimulate the genes of fatty acid oxidation. Genes regulated by PGC-1α in cardiac cells include several genes from ETC, mitochondrial biogenesis and fatty acid oxidation leading to induction of increased fatty acid oxidation in heart (Vega et al., 2000). While in liver, the expression of PGC-1α is low under normal conditions but the expression is rapidly induced by fasting on release of glucagon hormone (Lin et al., 2003). Enhanced PGC-1α levels leads to enhanced glucose output by inducing the expression of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PCK) and glucose-6-phosphatase (G6PC) and also enhanced fatty acid oxidation by co-activation of PPARα (Soyal et al., 2006). Gene expression studies using various animal models demonstrated that PGC-1α stimulated 3 folds increase in secretion of glucose from liver when provided with gluconeogenic precursors (Puigserver and Spiegelman, 2003).

PGC-1α is also known to play role in adipocyte cell fate and thermogenesis. There are two types of adipose tissue i.e. white adipose tissue (WAT) which stores energy as triacylglycerols which can be used during fasting and second brown adipose tissue (BAT) which is involved in thermogenesis and energy dissipation as heat. Increased
expression of PGC-1α in adipocytes can lead to conversion of WAT to BAT (Puigserver, 2005). In skeletal muscle PGC-1α plays role in mitochondrial biogenesis, fat metabolism and carbohydrate metabolism. In β-cells, expression of PGC-1α is up regulated as observed in animal models. In rat islets and INS-1 cells, the up regulated expression of PGC-1α resulted in delayed and reduced insulin secretion (Yoon et al., 2003). Similar effects were seen on UCP2 as UCP2 over expression also diminishes insulin secretion (Zhang et al., 2001; Krempler et al., 2002; Krauss et al., 2003). The expression of PGC-1α diminishes in patients with T2D (Russell; 2012), which makes it a important candidate gene in aetiology of T2D.

2.12.2 Association studies in PGC-1α

The locus where PGC-1α gene is located (4p15.1-2) has been previously associated with insulin insensitivity in Pima Indians (Pratley et al., 1998), with abdominal subcutaneous fat in Quebec family studies (Perusse et al., 2001), systolic blood pressure and dyslipidemia in families form Netherlands (Allayee et al., 2001) and with obesity in Mexican Americans (Arya et al., 2004). Hence, minor imbalance in this co-activator may influence the activity of several genes contributing to pathogenesis of many complex disorders like T2D and hypertension.

A total of seven variants (Ser74Leu, IVS2 + 52C>A, Thr394Thr, Asp475Asp, Gly482ser, Thr528Thr and Thr612 Met) in PGC-1α has been studied in association with T2D (Ek et al., 2001), and Gly482Ser has been studied most extensively in past decade. PGC-1α has been found to have two distinct haplotypes conferring risk to T2D, haplotype I consists of SNPs from promoter region while Gly482Ser belongs to second haplotype. This is the most associated region with insulin secretory pathway and T2D phenotypes. Gly482Ser variant of PGC-1α gene is located at exon 2, two amino acids downstream of the DEAD box motif suggestive of its putative functional role in protein binding (Soyal et al., 2006).
Association of PGC-1α (Gly482Ser) polymorphism with T2D and other related traits has been studied in various populations. Ek et al., (2001) found that individuals with Ser482 allele had 1.34 fold increased risk of developing T2D with population attributable risk (PAR) of 18% in Danish Caucasians. Hara et al., (2002) observed significant association of Thr394Thr and Gly482Ser polymorphisms with T2D in Japanese population while another study by Lacquement et al., in same year (2002) reported no association of Gly482Ser polymorphism with T2D in French Caucasians. Andruilionyte et al., (2004) found 1.6 fold higher risk of conversion from IGT to T2D in carriers of Ser variant when compared with subjects having Gly482 variant in study to prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) trail. In same year, Kunej et al., (2004) also found significant association of Ser482 variant as risk for T2D development in Slovene population (Caucasians). In twins, Ser482 variant was associated with more age-dependent reduction in muscle PGC-1α expression which may suggest the reason for late age of onset of T2D (Ling et al., 2004). However, several studies have yielded conflicting results for association of Gly482Ser polymorphism with T2D. No association of the variant with T2D was reported in Austrian, Pima Indian populations and Asian Indians (Muller et al., 2003; Oberkofler et al., 2004; Vimalaswaran et al., 2005). Furthermore, no association was observed with T2D related traits in non-diabetic German and Dutch populations (Stumvoll et al., 2004). In 2006 Barroso et al., conducted a meta-analysis to confirm the association of Gly482Ser polymorphism with T2D and found modest association with T2D risk. Later on in 2007 a study conducted on Chinese population again confirmed its association with T2D (Zhang et al., 2007). They also reported the higher levels of cholesterol and LDL in patients with GLY482Ser variant. Positive association of Gly482Ser variant with T2D was also reported in 822 subjects by Bhat et al., (2007) among two North Indian populations belonging to Punjab and Jammu and Kashmir regions. Nelson et al., (2007) observed no association with T2D or BMI among Hispanic and non Hispanic whites from Colarado. In Japanese population, association was also reported with lower plasma adiponectin levels in T2D men while no such association was seen in diabetic women (Okauchi et al., 2008). Ruchat et al., (2009) reported significant independent association of Gly482Ser variant with insulin and glucose homeostasis in overweight
subjects belonging to Quebec region of Canada. While Zambrano et al., (2009) found no association of Gly482Ser polymorphism with insulin resistance and T2D in individuals from Maracaibo, Venezuela. In 2010, Gayathri et al., found significant association between Gly482Ser polymorphism with diabetic nephropathy in Asian Indians. Recently significant association of Gly482Ser variant with increased BMI was reported in Tongans (Myles et al., 2011).

The studies around the globe thus present contradictory views on association of PGC1-α (GLY482Ser) variant in different populations of world may be due to their ethnicity. So, further studies in specific endogamous groups are needed to elucidate the role of this polymorphism in T2D.

2.13 Uncoupling Protein 2: UCP2

Uncoupling Proteins (UCPs) are the mitochondrial inner membrane anion-carrier proteins which regulate ATP production by uncoupling oxidative phosphorylation (Lim et al., 2012). Oxidative phosphorylation is a process which takes place in mitochondria that links the oxidation of fuel substrates to the generation of ATPs which are used by cells to carry out various pathways. In bioenergetics the term ‘Uncoupling’ refers to the process of energy release from combustion of substrate in mitochondria. UCP2 is a mitochondrial integral membrane protein that mediates proton leak across mitochondrial membrane causing uncoupling effect. UCP2 gene is located on long arm of human chromosome number 11 (11q13.4). It contains 8 exons and 7 introns. Transcription of this gene produces 12 different mRNAs out of which 10 are spliced and 2 unspliced forms. 5 spliced and one unspliced mRNAs encode proteins, and the remaining 6 mRNA (5 spliced, 1 unspliced) do not encode proteins (Figure 2.6).
2.13.1 The UCPs

Uncoupling proteins are metabolite transporters belonging to the subfamily of the mitochondrial anion carrier family. The genes encoding members of this family have been found across various animal and plant species. Mammals express five UCP homologues, UCP1-UCP5 (Lim et al., 2012). UCP1 was first discovered in BAT tissue as adaptive thermogenesis mediator in mammals (Nicholls and Locke 1984). Two other paralogues of UCP1 (UCP2 and UCP3) were discovered in 1997 (Millet et al., 1997). UCP2 and UCP3 show about 59% and 57% sequence identity with UCP1 and about 73% identity with each other (Echtay 2007). While two other UCPs (UCP4 and UCP5) have much less sequence homology with UCP1. Based on sequence homology and tissue specificity UCPs performs specific functions. UCP1 is mainly involved in adaptive thermogenesis and decrease in ROS production. UCP2 helps in regulation of free fatty acid (FFA) metabolism, protection against ROS mediated β-cell dysfunction and inhibition of insulin secretion. Finally, UCP3 is believed to be involved in FFA metabolism and transport. Out of these three, UCP1 is expressed in BAT only while UCP2 and UCP3 are expressed in variety of tissues. UCP3 is mainly expressed in skeletal muscle and heart tissue as well as in BAT while UCP2 is expressed in varying amounts in several tissues like kidney, pancreas, spleen, immune cells and central nervous system and cell types including β-cells (Rousset et al., 2004).

2.13.2 Oxidative phosphorylation and uncoupling

Oxidation of nutritional substrates leads to generation of ATP in mitochondria, suggesting that respiration and mitochondrial ATP synthesis are coupled. Peter Mitchell’s chemiosmotic theory in 1961, postulated a mechanism by which oxidation of nutritional substrates is coupled to ATP synthesis in mitochondria. During this process, electrons move through the respiratory chain complexes simultaneously ejecting protons from mitochondrial matrix to intermembrane space. This leads to generation of proton electrochemical gradient across mitochondrial inner membrane. Dissipation of
this electrochemical gradient through $F_0/F_1$ ATP synthase, catalyses the generation of ATP from ADP and $P_i$ (Hirono-Hara et al. 2001).

ETC comprises of five complexes. Complexes I, III and IV pump protons outside the inner membrane during reoxidation of coenzymes, generating proton gradient which is consumed by complex V. ATP synthase enzyme catalyses synthesis of ATP from ADP and $P_i$ utilising proton gradient. Mitchell’s theory predicted that any proton leak across the membrane disturbs the gradient and provoke uncoupling of respiration and thermogenesis. Thus, failure of ATP production due to proton leak mediated by UCPs is termed as uncoupling. Uncoupling allows reoxidation of coenzymes, prevents uncontrolled increase in ATP production along with adaptive thermogenesis (Rousset et al. 2004).

UCP2 is expressed in kidneys, pancreas, spleen, immune cells and the central nervous system (Pecqueur et al., 2001). As UCP2 is expressed widely in variety of tissues so it is being implicated in variety of processes like ROS production, insulin secretion, immunity and ailments like T2D, cancer and neuronal injury (Pecqueur et al., 2001; Brand and Esteves, 2005; Affourtit et al., 2007; Derdak et al., 2008). However, the precise function of UCP2 is unclear. Few studies indicate that UCP2 may function as the facilitator of proton leak when activated by ROS and some studies suggest that UCP2 instead is a metabolic switch favouring fatty acid metabolism over glucose metabolism or UCP2 may be involved in calcium import along with UCP3 (Trenker et al., 2007). But UCP2 mediated decrease in ROS production is supposed to occur via dissipation of mitochondrial protonmotive force (Azzu and Brand, 2009). This again suggests the role of UCP2 as an uncoupler.

Insulin is secreted by the pancreatic β-cells into the blood stream after carbohydrate rich meal. Insulin then acts on various tissues to normalise the levels of glucose in circulation. β-cells sense the increased amount of glucose by increased ATP/ADP ratio after glucose metabolism, which closes the ATP-sensitive $K_{ATP}$ channel causing
membrane depolarisation and influx of Ca\(^{2+}\) ions in the cytosol, which triggers insulin secretion. UCP2 mediates proton leak activity, thus decreasing the generation of ATP from glucose. This decrease eventually affects the ATP/ADP ratio and consequently impairs glucose stimulated insulin secretion (GSIS). Thus UCP2 negatively controls secretion of insulin from β-cell. Various studies on mouse models have demonstrated the negative regulation of insulin secretion by UCP2. β-cells from UCP2 deficient mice were observed to have increased insulin secretion as compared to normal wild type β-cells (Zhang et al., 2001). Mice heterozygous for UCP2 genes expressed intermediate amount of serum-insulin and blood glucose levels. Therefore, emphasising that even small amount of change in UCP2 expression effects insulin secretion.

T2D involves interplay between two major pathways- insulin resistance in skeletal muscles, adipocytes, liver and defective insulin secretion from β-cells. UCP2 being negative regulator of insulin secretion, has been evidenced to play role in β-cell dysfunction and eventually T2D. Increased levels of UCP2 m-RNA has been investigated in animal models designed for T2D (Kassis et al., 2000; Zhang et al., 2001; Joseph et al., 2002; Laybutt et al., 2002). Studies have also demonstrated that increased UCP2 levels in humans also down regulate insulin secretion from β-cells (Robson-Doucette et al., 2011). So, UCP2 is regarded as a link between β-cell dysfunction and T2D. A study by Produit-Zengaffinen et al. (2007) proposed a hypothesis to explain the protective as well as detrimental role of UCP2. Experiments conducted on transgenic mice and β-cell line showed that UCP2 proteins play protective role against ROS production and over expression of UCP2 induces negative effects on insulin secretion by decreasing GSIS. Hypothesis states that increase in levels of UCP2 may be due to increased FFA associated with obesity and insulin resistance. This increased UCP2 levels decreases mitochondrial ATP synthesis with oxygen consumption, which in turn may decrease the production of ROS and impair insulin secretion simultaneously (Chan et al., 2001; Brand et al., 2010; Pi and Collins, 2010).
Along with protection against ROS production, UCP2 is also thought to have more protective role in several ways, viz it protects against oxidative stress in liver (Lee et al., 1999) and endothelium (Mattiasson and Sullivan, 2006). It promotes the survival of both β-cells (Emre et al., 2007), pancreatic α-cells (Diao et al., 2008) and neurons (Andrews et al., 2008). UCP2 is also believed to have protective role against induction of cell death by decreasing membrane potential and Ca influx into mitochondria (Mehta and Li, 2009).

2.13.4 Susceptibility studies of UCP2 -866 G>A polymorphism (rs659366)

UCP2 maps to the region 11q13.4 which has been associated with obesity and T2D (Harper and Himms-Hagen, 2001). A common UCP2 promoter polymorphism at -866 position has been the most replicated and associated polymorphism with T2D across populations. Esterbauer et al., (2001) for the first time reported the association of -866G>A polymorphism with obesity in Bavarians and Austro-German descendents. Same group later investigated the association of this polymorphism with T2D and found that UCP2 -866GG genotype conferred enhanced risk for development of obesity and reduced risk for T2D in middle aged obese Caucasian Europeans (Krempler et al., 2002). Sesti et al., (2003) reported impairment of pancreatic β-cell function in carriers of UCP2 -866AA genotype. Another study carried out on Japanese population documented that subjects carrying A allele of -866 G>A polymorphism have greater UCP2 expression and lower GSIS than subjects with G Allele. Their study also reported that patients with A allele had early age of onset of T2D and required insulin supplementation earlier than patients carrying G allele. It was also noticed that frequency of A allele was higher in Japanese population (47.1%) than that reported in Caucasian population (41.3%) and thus it was suggested that this might be the putative reason behind lower insulin secretion in Japanese population (Sasahara et al., 2004). Likewise Reis et al., (2004) reported the association of -866 UCP2 A allele with high lipid levels in T2D patients and D’Adamo et al., in 2004 documented an association of A allele with increased risk of developing T2D in women diabetic subjects. However, discordant association of this polymorphism was also observed among different
populations. A study in Pima Indians failed to report the association of this polymorphism with risk of T2D and obesity (Kovacs et al., 2005). While, another study on the Italian Caucasia observed the association of -866 UCP2 AA genotype with reduced risk of developing T2D rather than the G allele (Bulotta et al., 2005). Similar findings were also reported by Rudofsky et al., (2006) where A allele was found to be associated with reduced prevalence of diabetic neuropathy in type 1 diabetic subjects. While Shen et al., (2006) reported the significant association of UCP2 -866 A allele with central obesity and metabolic syndrome in Asians and with peripheral nerve function in Japanese T2D patients (Yamasaki et al., 2006). Another study by Gable et al., in 2006 reported the association of A allele with decreased age of onset of T2D. They also noticed that under the background of Obesity (BMI>30 kg/m²), the effect was exacerbated. Rai et al., in 2007 studied the interactive effect of UCP2 -866 polymorphism with other diabetogenic SNPs and reported GG genotype as risk in North Indian population while Ochoa et al., (2007) found no independent association of this polymorphism with obesity and insulin resistance in Spanish children and adolescents. In 2008, Hsu et al., again reported UCP2 -866 A allele as risk for developing T2D in postmenopausal women belonging to diverse ethnic backgrounds (Caucasians, Hispanics, African and Asian Americans). UCP2 -866 GA/AA genotypes have also been associated with poor survival of the diabetic patients (Palmer et al., 2009). No association of this polymorphism was also found in Tehran population (Heidari et al., 2010) while Srivastava et al., (2010) reported significant association of A allele of UCP2 gene with obesity and hyperinsulinaemia in obese subjects from North India. Recently, a case control study conducted on population from Chennai also found no association of this polymorphism with T2D in Chennai Urban Rural Epidemiology Study (CURES) (Vimaleswaran et al., 2011). In the same year, Xu et al., (2011) conducted a meta-analysis study and found no association of this polymorphism in individuals from Asian and European decent. Conclusively, the association of UCP2 -866 G>A polymorphism has been inconsistent among populations but as per its functional importance it becomes pertinent to replicate it in our population.
2.14 SIRTuin1: SIRT1

SIRTuis or Sir2 (silent information regulator 2)-related enzymes have originally been defined as a family of nicotinamide adenine dinucleotide-dependent enzymes that deacetylate lysine residue on various proteins including NAD\(^+\)-dependent histone deacetylase (HDAC) that regulates chromatin silencing (Imai \textit{et al.} 2000; Landry \textit{et al.} 2000; Smith \textit{et al.} 2000; Gasser and Cockell 2001; Denu, 2003; He \textit{et al.}, 2012). SIRT1 gene is located on long arm of human chromosome number 10 (1q21.3) and comprises of 9 exons and 8 introns. Transcription of SIRT1 gene produces 6 transcripts. Alternate splicing generates 2 more transcripts from this gene. SIRT1 encodes for 81681 DA protein comprising 747 amino acid residues (Figure 2.9).

2.14.1 The Sirtuins

The SIRTuis have been conserved throughout evolution from archaeabacteria to eukaryotes. Sir2 was first identified in yeast for its role in chromatin remodelling associated with gene silencing and prolonged life span. SIRT1, the mammalian ortholog of yeast Sir2, is one of seven mammalian SIRTuis (SIRT1–7). The mammalian SIRTuis SIRT1–SIRT7 are implicated in a variety of cellular functions ranging from gene silencing, control of the cell cycle and apoptosis, to energy homeostasis. On a whole body level, the wide range of cellular functions performed by SIRTuis suggests that they could constitute therapeutic targets for metabolic, neurodegenerative, and proliferative diseases (Yamamoto \textit{et al.}, 2007).

\textit{Sir2} controls the longevity in lower eukaryotes like \textit{S.cerevisiae} and \textit{C.elegans} (Kauberlein \textit{et al.}, 1999; Tissenbaum and Guarente, 2001). In \textit{S.cerevisiae}, Sir2 extends the lifespan through suppression of formation of extra chromosomal ribosomal DNA circles in the nucleoli (Kauberlein \textit{et al.}, 1999). The Sir2 protein also plays a critical role in heterochromatic gene silencing through regulation of histone modifications at telomeres, ribosomal DNA clusters, and mating-type loci (Lustig, 1998). The NAD\(^+\)
dependency of Sir2 activity suggests that the control of lifespan is highly associated with metabolic state. Calorie restriction (CR) not only affects the metabolic processes but also extends the lifespan in a wide range of organisms from yeast to mammals (Heilbronn and Ravussin, 2003; Hursting et al., 2003). The insulin/IGF-I signalling pathway, a mediator of aging effects by calorie restriction (Barbieri et al., 2003), has been linked to the expression of a mammalian Sir2 homologue (Cohen et al., 2004).

An increasing number of studies from *S. cerevisiae*, *C. elegans*, *Drosophila melanogaster*, and mouse models have linked CR and metabolism with longevity (Bordone and Guarente, 2005). CR could affect metabolism and longevity in humans as well mediated through SIRT1 (Martin-Montalvo and de Cabo, 2012).

*SIRT1*, the mammalian Sir2 homologue is the best characterised SIRTuin family member. Human SIRTuins (*SIRT1-SIRT7*) share catalytic domains with Sir2 (North and Verdin, 2004; Shan et al., 2012). *SIRT1*, has highest sequence similarity to Sir2 (Frye, 2000). *SIRT1* can modulate cellular stress response and survival through regulation of p53 (Luo et al., 2001; Vaziri et al., 2001; Langley et al., 2002) NF-κB signalling (Yeung et al., 2004), and FOXO transcription factors (Brunet, 2004; Motta et al., 2004). *SIRT1* has also been reported to play role in mammalian development and differentiation (Fulco et al., 2003; Takata and Ishikawa, 2003).

*SIRT2* is a cytoplasmic protein that deacetylates α-tubulin (North et al., 2003) while *SIRT3* is localised to mitochondria and becomes activated by the proteolytic processing at N-terminus (Onyango et al., 2002; Schwer et al., 2002). *SIRT3* expression is induced in mice in both WAT and BAT by CR and upon cold exposure. *SIRT3* furthermore activates known mitochondrial genes such as PGC-1α and UCP-1 suggesting an important role of *SIRT3* in thermogenesis (Shi et al., 2005). *SIRT4* is also a mitochondrial protein and lacks in vitro deacetylase activity (Haigis et al., 2006). However, *SIRT4* ribosylates the proteins in the presence of ADP and inhibits the mitochondrial glutamate dehydrogenase (GDH). GDH controls amino acid-stimulated insulin secretion by regulating glutamine and glutamate oxidative metabolism.
Inhibition of GDH activity by SIRT4 decreases insulin secretion in mouse pancreatic β-cells in response to amino acids (Haigis et al., 2006). SIRT1 and SIRT4 seem to work in opposite directions to control insulin secretion. Furthermore, SIRT4 expression is downregulated in response to CR in β-cells, which is opposite to the regulation of SIRT1 during CR. These findings indicate towards the possible role of SIRT4 and SIRT1 in the pathophysiology of T1D and T2D. SIRT5 is also found to be localized in mitochondria (Onyango et al., 2002; Michishita et al., 2005). Human SIRT5 has been shown to have weak deacetylase activity in vitro (North et al., 2003). On a co-expression of SIRT3 and SIRT5, it has been observed that the expression of SIRT3 changes from mitochondria to nucleus (Nakamura et al., 2008). SIRT6 is suggested to control genomic DNA stability and DNA repair. Although SIRT6 was originally described as an exclusive ADP-ribosyltransferase (Lisz et al., 2005), it was recently demonstrated that SIRT6 deacetylates histones and the DNA repair enzyme (Mostoslavsky et al., 2006). SIRT7 is the only SIRTuin to be localized in the nucleolus and is a component of the RNA polymerase I (Pol I) transcriptional machinery. SIRT7 interacts with RNA Pol I and histones, and positively regulates the transcription of r-DNA during transcriptional elongation, which accounts for about 60% of total transcription in metabolically active cells in mammals (Grummt and Pikaard, 2003). Important information about human SIRTuin homologues has been compiled in the table 2.4 below.

SIRT1 is the best characterized human SIRTuin whose substrates include proteins primarily but not exclusively involved in transcriptional regulation, thus influencing diverse aspects of human physiology such as differentiation, cell survival, and metabolism. This is a protein deacetylase, located in cell nucleus. It requires oxidised NAD$^+$ as a cofactor and is negatively regulated by either NADH or the deacetylation product nicotinamide. A decrease in NAD/NADH ratio inhibits SIRT1 activity (Leibiger and Berggren, 2005, Jang et al., 2012).
Table 2.4: Compiled information about Human Sirtuins (SIRT1-SIRT7). Source: (Dali-Youcef et al., 2007)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzymatic activity</th>
<th>Subcellular localisation</th>
<th>Function</th>
<th>Putative target genes</th>
<th>Link with diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIRT1</td>
<td>Deacetylase</td>
<td>Nuclear, cytoplasmic</td>
<td>Glucose production (liver), Fatty-acid oxidation (liver, skeletal muscle) Cholesterol regulation (liver), Fatty-acid mobilisation (WAT), Adipokine regulation (WAT), insulin secretion (β-cells), neuroprotection (brain), regulation of cellular differentiation, stress resistance and apoptosis</td>
<td>p53, Ku70, NFκB, PGC-1α, MEF2D, MyoD, PPARγ, FOXO, p300, AceCSI.</td>
<td>Ageing, obesity, Insulin resistance, inflammation, diabetes, heart failure, axonal degeneration, AIDS</td>
</tr>
<tr>
<td>SIRT2</td>
<td>Deacetylase</td>
<td>Nuclear, cytoplasmic</td>
<td>Tubulin deacetylation, cell cycle control</td>
<td>A-tubulin</td>
<td>Down regulated in human gliomas</td>
</tr>
<tr>
<td>SIRT3</td>
<td>Deacetylase</td>
<td>Mitochondria</td>
<td>Protein deacetylation, acetate metabolism regulation, ATP production, regulation of mitochondrial Fatty-acid oxidation</td>
<td>PGC-1α, AceCSI</td>
<td>Adaptive thermogenesis, over expressed in node-positive breast cancer</td>
</tr>
<tr>
<td>SIRT4</td>
<td>ADP-ribosyltransferase</td>
<td>Mitochondria</td>
<td>Amino-acid stimulated insulin secretion</td>
<td>Glutamate dehydrogenase</td>
<td>Inhibits amino acid stimulated insulin secretion</td>
</tr>
<tr>
<td>SIRT5</td>
<td>Deacetylase</td>
<td>Mitochondria</td>
<td>Urea cycle regulation</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>SIRT6</td>
<td>ADP-ribosyltransferase, Deacetylase</td>
<td>Nuclear</td>
<td>Base excision repair, Telomeric chromatin structure, NF-βB regulation</td>
<td>DNA polβ</td>
<td>Age-related diseases</td>
</tr>
<tr>
<td>SIRT7</td>
<td>Deacetylase</td>
<td>Nuclear</td>
<td>Pol I transcription</td>
<td>RNA polymerase I</td>
<td>Highly expressed in thyroid cancer, and node-positive breast cancer</td>
</tr>
</tbody>
</table>
2.14.2 SIRT1 and Cancer

Cancer is widely related with genetic and epigenetic changes. A relationship between ageing and cancer is also well defined (DePinho, 2000). It has been implicated through many evidences that SIRT1 plays a role in epigenetic regulation of gene expression in cancer cells. SIRT1 deacetylates histone H1-K26 and promotes heterochromatin formation through spreading of hypomethylated histone H3-K79 (Vaquero et al., 2004; Kuzmichev et al., 2005). Histone H1 is a linker histone that primarily has a structural role in maintaining chromatin structure and through this regulates genomic stability and ageing (Harvey and Downs, 2004).

Besides histone modifications, SIRT1 deacetylates several transcription factors involved in the regulation of cell cycle progression and apoptosis consistent with a role in the fundamental processes underlying cancer. SIRT1 deacetylates the tumour suppressor p53 to inhibit its transcriptional activity, resulting in reduced apoptosis in response to various genotoxic stimuli (Luo et al., 2001). MEFs (mouse embryonic fibroblasts) lacking SIRT1 have an increased resistance to senescence induced by chronic oxidative stress, a phenomenon associated with decreased levels of the tumour suppressor p19ARF and thus p53 levels (Cheng et al., 2003; Mostoslavsky et al., 2006).

SIRT1 associates with the tumour suppressor HIC1 (Chen et al., 2005). SIRT1 has been shown to regulate the transcription factor NF-κB as well as several forkhead family transcription factors, including FOXO1, FOXO3a, and FOXO4 (Brunet et al., 2004; Daitoku et al., 2004; Motta et al., 2004; van der Horst et al., 2004; Yeung et al., 2004; Yang et al., 2005) SIRT1 deacetylates and inactivates NF-κB, leading to enhanced cell death in response to the inflammatory cytokine TNF-α (Yeung et al., 2004). The effects of SIRT1 on FOXO target gene transcription are heterogeneous, including both activation and repression through an NH2 terminal domain (Ramaswamy et al., 2002; Giannakou and Partridge, 2004; Yang et al., 2005).
2.14.3 Neuroprotective and Cardioprotective Roles of SIRT1

Experimental evidence suggests that SIRT1 has a protective role against neuronal and cardiac damage. In the Wallerian degeneration slow (wld<sup>s</sup>) mice, increased nuclear NAD<sup>+</sup> underlies the protection exhibited in the neurons of these mice against neurodegenerative agents (Araki <i>et al.</i>, 2004). Importantly, the neuroprotective effects of NAD<sup>+</sup> require SIRT1. Moreover, SIRT1 inhibition by either nicotinamide or SIRTinol induced cell death in a p53-dependent manner (Araki <i>et al.</i>, 2004). SIRT1 over expression also caused an increase in cardiomyocyte size and protected cells from apoptosis following serum starvation (Alcendor <i>et al.</i>, 2004). Importantly, SIRT1 levels were dramatically increased in a dog model of heart failure, possibly a result of a failed attempt to prevent cell death (Crow, 2004). A causative role for the devastating outcomes of ischemic conditions is also connected to how promptly they are treated so as to minimise tissue damage. This, in combination with lifestyle factors such as diet, which is proposed to affect SIRTuin function, renders SIRTuins an important potential target for preventive treatments in the context of these diseases.

2.14.4 SIRT1 and Muscle Mass Maintenance

SIRT1 deacetylates and thus negatively modulates the transcription factor MyoD, which is one of the key executors of the muscle differentiation programme (Fulco <i>et al.</i>, 2003). SIRT1 activity in turn is dictated by the progressively decreasing levels of NAD<sup>+</sup> during muscle differentiation, thus alleviating SIRT1-mediated MyoD suppression and allowing differentiation (Fulco <i>et al.</i>, 2003). SIRT1 was also found acting in conjunction with HDAC4 (histone deacetylase 4) to regulate the activity of another transcription factor myocyte enhancing factor 2 (MEF2) with roles in muscle differentiation (Zhao <i>et al.</i>, 2005). Since SIRT1 was found to modulate both NF-κB and FOXO transcription factors in heterologous systems, in conjunction with its role in regulation of MyoD and
MEF2, a broader role of SIRT1 in the control of muscle mass maintenance during injury and ageing is conceivable.

### 2.14.5 SIRT1 and Reproduction

*SIRT1* is highly expressed in the developing spermatocytes, and deletion of the SIRT1 gene leads to severe sperm abnormalities and sterility (McBurney *et al.*, 2003). In this context, SIRT1 would appear to be important to the reproductive capacity of the animal, thus defying its role in longevity based on the above.

### 2.14.6 SIRT1 in Metabolism and T2D

Energy homeostasis is achieved by the coordinate action of central and peripheral signals that control appetite and dictate efficient nutrient distribution among tissues to sustain body functions (Flier, 2004).

Ageing is associated with several pathological conditions resulting in aberrant metabolic functions (Luchsinger, 2006). T2D has been the most prevalent among them which shows almost an exponential increase in rate of incidence after the age of 20–30 years (Moller *et al.*, 2003, Gardu’no-Diaz and Khokhar, 2012). Pathogenesis of T2D involves compromised balance between insulin sensitivity and secretion which results in insulin resistance and eventually β-cell dysfunction. SIRT1 contributes to both the aspects of pathogenesis of T2D in liver, skeletal muscle, adipose tissue and pancreatic β-cells as shown in the figure 2.10 below (Imai and Guarente, 2010).

### 2.14.7 SIRT1 in liver

SIRT1 regulates three main processes of (i) glucose production, (ii) oxidation of fatty acids and (iii) cholesterol flux in liver. SIRT1 acts as a molecular switch which
enhances gluconeogenesis and represses glycolysis. In response to fasting SIRT1 interacts and deacetylate PGC-1α, FOXO1, CRTC2 and STAT3 (Erion et al., 2009; Liu et al., 2008; Nie et al., 2009; Rodgers et al., 2005; Rodgers and Puigserver, 2007). SIRT1 also promotes fatty acid oxidation through PGC-1α and PPARα (Purushotham et al., 2009). Moreover, to regulate homeostasis of cholesterol SIRT1 deacetylates and activates LXRα, which is a critical nuclear receptor (Li et al., 2007). Thus, SIRT1 acts as a negative regulator of insulin secretion.

2.14.8 SIRT1 in skeletal muscle

SIRT1 is found to improve insulin sensitivity in skeletal muscle by deacetylation of PGC-1α (Gerhart-Hines et al., 2007), inducing fatty acid oxidation in mitochondria in response to fasting and by transcriptional repression of protein tyrosine phosphatase 1B (PTP1B) gene in skeletal myotube cells (Sun et al., 2007).

2.14.9 SIRT1 and white adipose tissue (WAT)

During fasting conditions, SIRT1 represses PPARγ, triggers lipolysis and promotes free fatty acid mobilisation (Picard et al. 2004). SIRT1 also regulates adiponectin through regulation of FOXO1 and PPARγ, in turn regulating the secretion of insulin (Hammes et al., 2012).

2.14.10 SIRT1 in pancreatic β-cells

SIRT1 positively regulates secretion of insulin by repressing the expression of UCP2 gene (Bordone et al., 2006). SIRT1 also plays protective role against metabolic stress and cytokine induced β-cell death by deacetylating FOXO1 (Kitamura et al., 2005) and NF-κB (Lee et al., 2009) respectively.
Thus, recent literature and evidences suggest that SIRT1 has protective role against T2D in humans.

2.14.11 Susceptibility studies of SIRT1 -1400 T>C polymorphism (rs12778366)

SIRT1 is found to play a major role in energy homeostasis and glucose metabolism but its relation with T2D is not clear and needs further studies. SIRT1 is expressed ubiquitously in many tissues including insulin sensitive organs like adipose tissue, pancreatic β-cells, liver, skeletal muscle. Keeping all this in mind, it becomes pertinent to ask if human SIRT1 genetic variants may also play role in predisposing individuals for T2D. Studying SIRT1 promoter variations may lead to an early diagnosis of the disease at genetic level and thus help reduce the impact of the disease by taking precautionary measures as promoter functionally controls regulation of gene expression. Any alterations in the promoter region might cause change in gene expression.

SIRT1 -1400 T>C polymorphism is a promoter SNP, causes no amino acid change. This polymorphism is one of the very first SNPs of the 11 tag SNPs identified in the promoter region of SIRT1 gene. 11 Tag SNPs searched by HapMap database in SIRT1 gene are rs12778366, rs3740051, rs2236318, rs2236319, rs10823108, rs10997868, rs2273773, rs3818292, rs3818291, rs4746720, rs10823116. Out of these we genotyped rs 12778366 for association with T2D in Punjabi population.

Lagouge et al., in 2006 for the first time assessed the association of genetics variants in SIRT1 gene with basal energy expenditure in Finnish type 2 diabetic patients. They found the significant association of three SNPs i.e rs3740051, rs2236319 and rs2273773 of SIRT1 with energy expenditure in humans. Another study by Weyrich et al., in 2008 studied the association of SIRT1 promoter polymorphisms in T2D Caucasians with lifestyle interventions. They found that subjects with minor allele of rs12413112 (G/A) had low basal energy expenditure and increased respiratory quotient. Their study concluded that SIRT1 plays role for the subjects with T2D with lifestyle interventions.
and therefore may act as a good pharmaceutical target for individuals undergoing calorie restriction and increased physical activity as lifestyle intervention. However, no association of rs12778366 with T2D was observed in their population. Zillikens et al., in 2009 investigated the associated of three SIRT1 variants (rs7895833, rs1467568 and rs497849 with obesity. They found significant association of rs7895833 and rs1467568 with lower BMI among Dutch population. They did not investigate the association of SIRT1 -1400 T>C promoter SNP with obesity in their study. Similarly, a recent study by Maeda et al., in 2011 also typed 11 SNPs of promoter region of SIRT1 gene in Japanese subjects with diabetic nephropathy. No significant association of individual SNP was observed with diabetic nephropathy but haplotypes analysis revealed strong association of haplotypes consisting of 11 SNPs suggesting the putative role of SIRT1 variants in diabetic nephropathy in Japanese population. Worldwide there are very limited studies documenting the association of SIRT1 promoter variants with T2D or some T2D complication.

Few studies have investigated the association of SIRT1 variants with other disorders like bipolar disorder, Alzheimer’s disease, ageing and other neurodegenerative disorders in various populations (Helisalmi et al., 2008; Kishi et al., 2010; Zhang et al., 2010). However, no significant association has been reported. A recent study conducted on Japanese population reported no association of SIRT1 variants with psychosis (Kishi et al., 2011). There is negligible data documenting the association of SIRT1 promoter variants with T2D and related complications. Therefore, to fill the existing lacuna SIRT1 promoter polymorphism -1400T>C was analysed for association with T2D in the present study.