Review of Literature
2.0 REVIEW OF LITERATURE

2.1 DIABETES MELLITUS – AN OVERVIEW

Diabetes mellitus is a metabolic disorder defined by a disturbance in glucose metabolism leading to chronic hyperglycaemia. It is diagnosed by increased glucose levels in the fasting state or by a reduced glucose clearance after an oral glucose tolerance test (Alberti and Zimmet, 1998). Diabetes mellitus represents a spectrum of disorders with heterogeneous aetiology but similar clinical signs. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. Based on plasma glucose measurements, two conditions have been identified with increased risk of the disease (Zimmet et al., 2003): a) impaired glucose tolerance (IGT) is defined as hyperglycemia intermediate between normal and diabetic levels following a glucose load; b) impaired fasting glucose (IFG), like IGT, is associated with increased cardiovascular disease (CVD) and future diabetes. Because complications of diabetes may develop years before overt disease, many consider the disease part of a cluster of CVD risk factors that include hypertension, hyperinsulinemia, dyslipidemia, visceral obesity, hypercoagulability, and microalbuminuria. This collection of risk factors is also known as the metabolic syndrome (Meigs, 2003a, b; Misra and Vikram, 2004). While insulin therapy can reverse many of the metabolic disturbances, and numerous improvements in management have been introduced, the disease has reached epidemic proportions. The prevalence of diabetes is increasing worldwide and is a serious public health problem in many countries (Zimmet,
Type 2 diabetes is likely to be one of the most substantial threats to human health in the 21st century (Engelgau et al., 2003; Ramachandran, 2005).

2.2 CLASSIFICATION OF DIABETES MELLITUS

The diagnosis and classification of diabetes have been revised by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA, 2004). The new classification system emphasizes etiology and pathogenesis rather than modalities of treatment. Diabetes is divided into four major categories depending on etiology:

1. Type 1 diabetes mellitus
2. Type 2 diabetes mellitus
3. Gestational diabetes mellitus
4. Other specific types of diabetes

2.2.1 Type 1 Diabetes Mellitus (T1DM): This form of diabetes, which accounts for only 5-10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes (IDDM), type I diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas (Bach, 1994). As a result, the pancreas produces little or no insulin (Figure 2.1), which leaves the patient dependent on insulin injections for survival (Porte et al., 2003). It is one of the most serious chronic diseases, affecting young children and adolescents. We do not know exactly what causes the body’s immune system to attack the beta cells and destroy them. It is believed to be caused by a combination of genetic factors and environmental stressors. T1DM usually results in a drastic reduction in the quality of life and shortens the average life span by 15 years.

2.2.2 Type 2 Diabetes Mellitus (T2DM): Type 2 diabetes mellitus (T2DM), previously referred to as non-insulin-dependent diabetes (NIDDM), type II diabetes, or adult-onset diabetes, is the most common form of diabetes, which accounts for more than 90% of diabetic population. It is a multifactorial disease in which environmental factors interact with genetic variants in the predisposition to the disease (Kahn et al., 1996; Zimmet et al., 2001).
NORMAL CELL
This is a normal cell. Insulin is present and is taken into the cell to facilitate proper glucose uptake and metabolism.

TYPE 1 DIABETES CELL
In Type I diabetes, insulin is not produced. So, there is nothing to signal the cells to take in glucose and metabolize it.

TYPE 2 DIABETES CELL
In Type 2 diabetes, insulin is present but the signal for proper glucose uptake and metabolism is lost.

Figure 2.1: Cell biology of insulin response.
T2DM is characterized by a dual defect of insulin resistance and β-cell dysfunction (DeFronzo et al., 1992). There is impaired insulin secretion and insulin action in target tissues such as muscle and liver (Bonadonna, 2004). Symptoms of type 2 diabetes may include frequent urination, blurred vision, increased thirst, slow healing of cuts or sores, frequent infections, increased hunger, tiredness, dry and itchy skin etc.

Many risk factors have been identified which influence the prevalence of type 2 diabetes (Gerich, 1998; Barroso, 2005). Factors of particular importance are a family history of diabetes mellitus, age, overweight, increased abdominal fat, hypertension, ethnic background and lack of physical exercise. Several biochemical markers have also been identified as risk factors, including fasting hyperinsulinemia, increased fasting proinsulin, and decreased HDL-Cholesterol, increased LDL-Cholesterol (DeFronzo, 1997b). Type 2 diabetes exhibits familial predisposition, indicating strong genetic components associated with the susceptibility to the disease. Several numbers of susceptibility genes are involved in pathogenesis of type 2 diabetes. Also, the interaction between genetic and environmental factors contributes to the pathogenesis of type 2 diabetes (Rich, 1990). Type 2 diabetes is now widely considered to be one component in a group of disorders called the metabolic syndrome, which includes insulin resistance, dyslipidemia, obesity and hypertension. Life expectancy is reduced by about 5 to 10 years among middle-aged adults with type 2 diabetes.

2.2.3 Gestational Diabetes Mellitus (GDM): Gestational diabetes is another form of diabetes, defined as a state of glucose intolerance during pregnancy that usually subsides after delivery but has major implications for subsequent risk of T2DM, as pregnancy serves as an "environmental" stressor that reveals a genetic predisposition (Metzger et al., 1998).

2.2.4 Other Specific Types of Diabetes: There are many other types of diabetes which includes genetic defects of the β-cell, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies and drug or chemical-induced diabetes.
2.3 DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS

Diabetes mellitus is diagnosed based on American Diabetes Association, 2004 criteria incorporating both fasting and 2-h after glucose load (75 g) into a practicable diagnostic classification.

Following categories of Fasting Plasma Glucose (FPG) values are used for the diagnosis of diabetes:

- FPG levels <100 mg/dl or 5.6 mmol/l) = Normal Fasting Glucose (NFG).
- FPG levels 100-126 mg/dl or 5.6-7.0 mmol/l) = Impaired Fasting Glucose (IFG).
- FPG levels ≥126 mg/dl or 7.0 mmol/l) = Provisional diagnosis of diabetes.

Following are the corresponding categories when the Oral Glucose Tolerance Test (OGTT) is used:

- 2-h post-glucose load <140 mg/dl (or 7.8 mmol/l) = Normal Glucose Tolerance (NGT).
- 2-h post-glucose load 140-180 mg/dl (or 7.8-11.1 mmol/l = Impaired Glucose Tolerance (IGT).
- 2-h post-glucose load ≥180 mg/dl (or 11.1 mmol/l) = Provisional diagnosis of diabetes.

2.4 GLOBAL PREVALENCE OF TYPE 2 DIABETES

Diabetes mellitus has become a major public health problem, associated with enormous personal, social, and economic burden in both developed and developing countries. The worldwide prevalence of diabetes for all age-groups was estimated to be 2.8% in 2000 and is predicted to be 4.4% in 2030 by the WHO (Wild et al., 2004). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. WHO has predicted that India would experience the largest increase (48% increase in total population and 168% increase in population with >65 years of age) in type 2 diabetes and would have the greatest number of diabetic individuals in the world by the year 2030 (31.7 million in 2000 to 79.4 million in 2030). By 2030, India, China and
United States will have the largest number of diabetic people (King et al., 1998; Wild et al., 2004) (Figure 2.2).

Figure 2.2: Countries with the highest numbers of estimated cases of diabetic patients (in millions) for year 2030 (Wild et al., 2004).

2.5 EPIDEMIOLOGY OF TYPE 2 DIABETES – AN INDIAN SCENARIO

India is a vast and heterogeneous country, not only in its geographical spread, but as importantly as the second most populous country in the world. It is home to significantly diverse groups of people as far as ethnicity, caste and religion, habitat, socioeconomic status, education levels, lifestyles and food habits are concerned. Rapid globalization and industrialization occurring in developing countries have produced many advancements in the social and economic front. Although it has resulted in economic prosperity and better living standards to many, it has also resulted in considerable increase in lifestyle related diseases such as type 2 diabetes and cardiovascular disease. South East Asian countries have the highest burden of diabetes and impaired glucose tolerance (IGT) (World Diabetes Foundation, 2003; Mohan, 2004). India comprises 85% of the adult population of South East Asia and therefore, the major contribution to diabetic population in South East Asia is from India (Ramachandran, 2005) (Table. 2.1).
Diabetes in India is reaching to an epidemic scale. Data on the prevalence of T2DM in subcontinental Indians is limited considering the socio-economic and rural-urban disparity and the great cultural, geographical and racial diversity of our country. There has been no systematic, scientific and truly nationwide survey on the prevalence of diabetes in India, which takes into account this diversity. In the most recently reported
studies, the megacity bias was again inherent as it was mostly confined to the four large metropolis and two other cities (Ramachandran et al., 2001). Various studies in urban populations reported rising trend in incidence and prevalence of type 2 diabetes in India (Table. 2.2).

Table 2.2: Studies showing a rising trend in the prevalence of type 2 diabetes in India (Ramachandran et al., 2003)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Author (year)</th>
<th>Place (region)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>Cuttack (Central)</td>
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</tr>
<tr>
<td>2</td>
<td>Ahuja et al., 1972</td>
<td>New Delhi (North)</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>Gupta et al., 1979</td>
<td>Multicentre</td>
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</tr>
<tr>
<td>4</td>
<td>Murthy et al., 1984</td>
<td>Tenali (South)</td>
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</tr>
<tr>
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<td>Patel, 1986</td>
<td>Bhadran (West)</td>
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<td>Ramachandran et al., 1988</td>
<td>Kudremukh (South)</td>
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</tr>
<tr>
<td>7</td>
<td>Kodali et al., 1989</td>
<td>Gangavathi (South)</td>
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<tr>
<td>8</td>
<td>Rao et al., 1989</td>
<td>Eluru (South)</td>
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<tr>
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<td>Ahuja et al., 1991</td>
<td>New Delhi (North)</td>
<td>6.7</td>
</tr>
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<td>10</td>
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<td>13</td>
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<td>Mishra et al., 2001</td>
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<tr>
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<td>Kerala (South)</td>
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<td>Gupta et al., 2003</td>
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<td>National</td>
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</tr>
<tr>
<td>19</td>
<td>Sadikot et al., 2004</td>
<td>National</td>
<td>5.9</td>
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The WHO 2030 predictions on prevalence of type 2 diabetes in India were extrapolated from the National Urban Diabetes Survey (NUDS) published by Diabetes Epidemiology Study Group in India (Ramachandran et al., 2001). This Study was conducted in six metropolitan cities of India (Chennai, Bangalore, Hyderabad, Mumbai, Calcutta and New Delhi). The prevalence of diabetes and IGT stratified by age were found to be 12.1% and 14.0 % respectively in NUDS study with no gender difference. Subjects under 40 years of age had a higher prevalence of IGT than diabetes (12.8% vs. 4.6%, p<0.0001). Studies conducted in India in the last decade have highlighted that not only is the prevalence of
type 2 diabetes high, but also that it is increasing rapidly in the urban population (Ramankutty et al., 2000; Misra et al., 2001; Mohan et al., 2001; Menon et al., 2006).

The diabetic surveys conducted in Chennai (Ramachandran et al., 1992; 1997; 2001) were compared for the age-standardized prevalence, anthropometric, demographic and lifestyle characteristics of the glucose intolerant groups. A rising trend was reported for diabetes and IGT (Fig 2.4). A younger age at onset of diabetes has been noted in Asian Indians (Ramachandran et al., 2001). The onset of diabetes occurred before the age of 50 years in 54.1% of cases, implying that these subjects developed diabetes in the most productive years of their life and had a greater chance of developing the chronic complications of diabetes (Ramaiya et al., 1990; Ramachandran et al., 2001). Type 2 diabetes showed positive and independent association with BMI, waist to hip ratio, family history of T2DM, sedentary life style etc. The DECODE -DECODA study group on behalf of the European Diabetes Epidemiology Group and the International Diabetes Epidemiology Group found that prevalence of diabetes in Indians starts increasing at a BMI of 15-20 kg/m2 compared with greater than 25 kg/m2 in Chinese, Japanese and
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Europeans (DECODE-DECODA study group, 2003). Lack of proper representation from different socioeconomic and geographic regions is probably a major factor for over or under estimation of prevalence of diabetes in India by different published epidemiological studies. Recently a random multistage cross-sectional population survey was undertaken to determine the prevalence of T2DM in India by the Diabetes India group, Mumbai called Prevalence of Diabetes in India study (PODIS) (Sadikot et al., 2004). This study was carried out in 77 centers (42 urban and 35 rural) to reflect the size and heterogeneity of the Indian population. This study observed a significant difference in T2DM prevalence between rural and urban population. This study also observed significant difference in the prevalence of T2DM between different geographical regions of India. West (4.02% and 4.34%) and South (3.83% and 4.42%) showed significantly higher prevalence of diabetes and IFG compared to North (3.12 % and 3.67 %), East (2.79 % and 2.76 %) and Central (2.58% and 2.39%) region.

Figure 2.5: Prevalence of diabetes in different habitats (Ramachandran et al., 1999).

This difference in the prevalence is more striking in urban regions as compared to rural regions. There was no significant difference in IFG prevalence in rural regions of different geographic zones. The prevalence of diabetes in urbanizing rural population was found to be midway between the rural and urban populations (Ramachandran et al., 1999) (Figure 2.5).
An urban-rural difference in the prevalence of diabetes indicated that environmental factors related to urbanization had a significant role in increasing the prevalence of diabetes in India (Ramachandran et al., 1992). Although the prevalence of type 2 diabetes is 4-6 times higher in urban population of India than in the rural areas, the number of people with IGT is high (7-8%) even in the rural population which may indicate the presence of a genetic basis for type 2 diabetes in the ethnic group (Ramachandran et al., 1992). Various studies on the prevalence and risk factors associated with type 2 diabetes have been carried out in India in the past (Table 2.2) but most of them have been confined to south Indian population. A few authenticated reports have been published on the population residing in New Delhi (Ahuja et al., 1979; 1996; Ramachandran et al., 2001; Mishra et al., 2001) and Kashmir (Zargar et al., 2000). However, a little information is available on the prevalence and risk factors associated with T2DM in North Indian Punjabi population.

2.6 ECONOMIC IMPACT OF DIABETES IN INDIA

Despite such an alarming prediction that the prevalence of diabetes in India is expected to increase by 195% by 2025, there have been few studies of the status and economic burden of diabetes in India (Shobhana et al., 2000). Diabetics use medical resources at a higher rate than average nationwide. Health resources in India and other developing countries are very limited with only 5% of Gross Domestic Product (GDP) being spent on healthcare. The majority of healthcare expenditure was private (4% of GDP) with only 0.9% of GDP spent on public health care. Therefore, careful planning based on health economics assessments is necessary in order to maximize the use of funds for the treatment and prevention of diabetes. As India has no subsidized, coordinated diabetes care programme, reducing treatment costs through raising public awareness, regular monitoring and earlier diagnosis should be a key objective. Of the estimated 25 million persons with diabetes in India in 1999, only 3.6 million received pharmacological treatment (Kapur et al., 1997). The Bangalore Urban Diabetes Study (BUDS) estimated that the annual direct cost for diabetes care in 1998 was about US$ 191, and the mean direct cost per hospitalization for a diabetes-related event was about US$ 208 (Rayappa et al., 1999). A recent study reported that mean estimated direct costs of diabetes in India were Rs.4,724 per individual per annum including drug treatment, monitoring and check-
ups. Mean estimated indirect costs were Rs.12,756 per individual per annum including measures of productivity and income loss through illness in earning and non-earning family members. Estimated hospitalization costs were Rs. 2434 per individual per annum. Mean estimated total costs of diabetes in India were Rs.19,914 per individual per annum (Bjork et al., 2003; Bjork, 2005).

2.7 DIABETES RELATED COMPLICATIONS

The complications of diabetes are strongly related to high blood sugar levels and are mostly correlated with the duration of diabetes (Bowden, 2002). There are two categories of diabetic complications; macrovascular and microvascular. The long-term complications of diabetes affect many parts of the body, decrease quality of life and increase the use of health services. Individuals with diabetes may face shortened life expectancy due to complications from the disease. Life expectancy is influenced by the age at the onset of diabetes.

2.7.1 Macrovascular Complications: Most diabetic complications arise from damage to blood vessels. Those arising from accelerated atherosclerosis particularly affect the coronary, carotid and femoral arteries. Type 2 diabetes is a major risk factor for the development of cardiovascular disease (Meigs, 2003b). The risk of myocardial infarction is 50% greater in diabetic men and 150% greater in diabetic women. The risk of cardiovascular disease is 2-4 times higher in patients with or without diabetes (ADA, 1993). The high level of HbA1c (>6%) has been associated with the long-term macrovascular complications in diabetic subjects (Plante and Nadler, 2003). Diabetes also carries an increased risk for heart attack, stroke and complications related to poor circulation (Meigs, 2003b).

2.7.2 Microvascular Complications

a) Diabetic Neuropathy: Diabetic neuropathy is of two main types: Autonomic and Peripheral neuropathy. Autonomic neuropathy is the most ominous form of diabetic neuropathy. It involves various organs in the body and may result in silent cardiac infarction or sudden death. Autonomic neuropathy may be associated with other forms of neuropathy and be asymptomatic or give rise to most disagreeable symptoms including sweating after eating (gustatory sweating), uncontrolled urination, sexual dysfunctions,
postural hypotension, diarrhea, vomiting from delayed stomach emptying (termed gastroparesis), and impotence.

Peripheral neuropathy affects the extremities, especially the lower legs and feet (Greene et al., 1993), and results in loss of distal sensation that can lead to musculoskeletal injury or to infection. It includes numbness in feet or fingers, tingling, burning or prickling in toes or fingers. Extreme sensitivity of touch and sharp pains or cramps. Several studies have shown that careful glycemic control may reduce the prevalence and severity of diabetic complications. The peripheral nervous system is commonly affected in diabetic patients and the sequelae of nerve dysfunction often influence the final outcome of the disease. Enhanced polyol pathway activity (Greene et al., 1993; Cameron et al., 1997; Carrington and Litchfield, 1999) and increased non-enzymatic glycation (Brownlee et al., 1988; Yagihashi, 1995) have been implicated in the aetiology of diabetic neuropathy. However, the precise mechanisms that link enhanced polyol pathway activity and non-enzymatic glycation to peripheral nerve damage are still unknown.

b) Diabetic Nephropathy: Diabetic nephropathy (DN) is one of the leading causes of chronic renal failure in India. Diabetic nephropathy occurs in approximately one third of individuals with type 2 diabetes (O'Bryan and Hostetter, 1997). DN is a clinical syndrome characterized by persistent albuminuria, a relentless decline in glomerular filtration rate, raised arterial blood pressure and increased relative mortality for cardiovascular diseases. This follows with a more rapid progression of other secondary complications, (retinopathy, neuropathy, diabetic foot and blood pressure (Lovell, 2000). The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels (>30 mg/day) of albumin in the urine, referred to as microalbuminuria, and patients with microalbuminuria are referred to as having incipient nephropathy. DN is a leading cause of end stage renal failure. The pathogenesis of diabetic nephropathy is multifactorial with contribution from metabolic abnormalities, homodynamic alteration, and various growth factors and genetic factors. Epidemiologic and family studies have demonstrated that family clustering and ethnicity plays an important role in the risk of developing this kidney disease (Parving et al., 1996). It is estimated that up to 50% patients with diabetes mellitus will develop renal failure (Harkonen et al., 1977). It is now firmly established that diabetic nephropathy is associated with high morbidity and
mortality (Krolewski et al., 1988). There is marked heterogeneity in the clinical picture seen in long termed diabetes as some diabetic patients even with poor metabolic control may not develop clinical diabetic nephropathy (Shafi, 1992). T2DM and its long-term complications, such as nephropathy have a strong genetic predisposition. Insulin resistance is thought to be a pathogenic factor, predisposing genetically prone individuals to develop the microvascular complication of diabetes (Vijay et al., 1997). Familial aggregation of DN has been reported in European (Johnsen et al., 1992) and American Whites (Froguel et al., 1992) with type 2 diabetes patients.

c) Diabetic Retinopathy: Diabetic retinopathy (DR) is of two types; Proliferative Diabetic Retinopathy (PDR) and Non-Proliferative Diabetic Retinopathy (NPDR). DR is increasingly becoming a major cause of blindness throughout the world in the age group of 20-60 years (Thylefors et al., 1995). Loss of productivity and quality of life for the patient with diabetic retinopathy lead to additional socio-economic burdens on the community. DR is the cause of blindness in approximately 2.5 million of the estimated 50 million blind people in the world. However, DR, as a cause of blindness, is less common in India (Dandona et al., 1999; 2001).

d) Diabetic Foot: Foot ulceration, which depends on the degree of foot insensitivity (Reiber et al., 1995), and amputation are important and costly sequelae of diabetic neuropathy (Young et al., 1994). Long duration of type 2 diabetes causes nerve and vascular damage that can result in loss of protective sensation in the feet, poor circulation, and poor healing of foot ulcers. All of these conditions contribute to the high amputation rate in people with diabetes. Diabetic foot ulcers are common and estimated to affect 15% of all diabetic individuals during their lifetime (Jeffcoate and Harding, 2003). In India, the prevalence of diabetic foot was 3%, which was much lower than the western world. Diabetic foot infection is a common cause for the hospital admission of diabetic patients in India (Vijay et al., 1997). This could be attributed to several sociocultural practices, such as bare foot walking, inadequate facilities for diabetes care, low education and poor socio-economic conditions. Amputation rate in diabetics increase with advancing age and are generally higher in males than females. Higher rates of amputation were reported in Pima Indians and Caucasians but low in Asians (Nelson, 1988; Gujral et al., 1993).
2.8 GENETIC BASIS OF T2DM: CLASSICAL EVIDENCES

Multiple lines of evidence provide support for the role of genetic variation in the pathogenesis of T2DM (McCarthy and Hitman, 1993).

2.8.1 Prevalence of T2DM in Different Ethnic Groups: The prevalence of T2DM varies widely among populations, from 1% in Chile Mapuche Indian, 2% among Caucasians in Europe to as high as 41% in the Nauru Pacific Island and 50% among Pima Indians in Arizona (Diamond, 2003). Part of this observed ethnic variability can be attributed to non-genetic environmental and cultural factors; however, the observation that the disease prevalence varies substantially among ethnic groups that share a similar environment supports the idea that genetic factors contribute to disease predisposition.

2.8.2 Familial Aggregation: Other than genes, families share environments, culture and habits, yet familial aggregation of the disease is another source of evidence for a genetic contribution to the disease. Evidence for a genetic role includes the nearly 4-fold increased risk for T2DM in siblings of a diabetic proband compared with the general population, the odds ratio (OR) of 3.4-3.5 with only a single affected parent, and the increase in the OR to 6.1 if both parents are affected (Meigs et al., 2000a).

2.8.3 Twin Studies: Multiple studies of twin concordance rates have been undertaken in T2DM. Estimates for concordance rates have ranged from 0.29 to 1.00 in monozygotic (MZ) twins, while in dizygotic (DZ) twins the range was 0.10-0.43 (Barnett et al., 1981; Newman et al., 1987; Poulsen et al., 1999; Medici et al., 1999). Concordance among both MZ and DZ twins increases with the duration of follow up period. In spite of several caveats in twin studies, the high concordance in MZ twins and the 50% fall in DZ twins provides compelling evidence for a genetic component of T2DM.

2.8.4 Heritability of Intermediate Phenotypes: Insulin sensitivity and insulin secretion deteriorate in heritability of diabetes parallel in most human T2DM. Both defects predicted subsequent T2DM in several studies and both defects are shown to be present in nondiabetic but genetically identical co-twins of a diabetic proband (Vaag et al., 1995). Data from many laboratories support a genetic basis for measures of both insulin sensitivity and insulin secretion (Gerich, 1998; Elbein et al., 1999). Quantitative phenotypes related to glucose homeostasis are also known to be heritable (Poulsen et al.,
In families with an increased genetic susceptibility to T2DM, heritability estimates for \( \beta \)-cell function and features of the insulin resistance syndrome of 72% and 78% respectively, were calculated (Mills et al., 2004). The heritability of other features of the insulin resistance syndrome, including BMI, blood pressure, and serum lipid and insulin sensitivity levels, was also estimated to be high. Evidence for heritability of these metabolic phenotypes was reported in studies of Pima Indians (Hanson et al., 2001) and nondiabetic Japanese Americans (Austin et al., 2004); in the Insulin Resistance Atherosclerosis Study (IRAS) among family members of African American and Hispanic heritage (Henkin et al., 2002; Mitchell et al., 2004) and in a study of the familial aggregation of the amount and distribution of subcutaneous fat and responses to exercise training in the HERITAGE Family Study (Perusse et al., 2000). These studies strongly support the role of both genetic and environmental factors in the etiology of diabetes and the insulin resistance syndrome.

2.9 ENVIRONMENTAL RISK FACTORS ASSOCIATED WITH T2DM

Type 2 diabetes is a very complex disease, which is caused by the interaction of both environmental and genetic factors (Hamman, 1993; Kahn et al., 1996; Zimmet et al., 2001). However, the exact cause of T2DM is unknown. Although insulin resistance and progressive pancreatic \( \beta \)-cell dysfunction have been established as the two fundamental features in the pathogenesis of type 2 diabetes (Kahn, 1994), the specific molecular defects affecting insulin sensitivity and/or \( \beta \)-cell function remain largely undefined. Various risk factors have been identified in the pathogenesis of T2DM. Environmental factors such as age, obesity, waist-hip circumferences, race and ethnicity, physical inactivity, dietary habits, family history of diabetes, migration and stress, socio-economic factors, insulin resistance, hypertension and dyslipidemia are found to be associated with T2DM (Ramachandran et al., 1992; 1999; Zimmet et al., 2001).

2.9.1 Age at Onset of T2DM: Studies in India and abroad have shown that Indians develop diabetes at a very young age; at least 10–15 years earlier than the white population (DECODE-DECODA study group, 2003). A study carried out by the National Diabetes Urban Survey in India showed that more than 50% of diabetic cases developed the disorder before the age of 50 years (Ramachandran et al., 2001). The prevalence of
maturity onset diabetes in young (MODY) and type 2 diabetes in children is increasing. Indians show a significantly higher age-related prevalence of diabetes when compared with the white population in USA (Ramachandran et al., 2002). A recent analysis by the International Diabetes Epidemiology Group comparing the profile of type 2 diabetes in the European and Asian populations showed that the Indians had the strongest age associated risk for diabetes among all the groups (DECODE-DECODA study group, 2003). Indians had several fold higher prevalence at all age groups in comparison with the Europeans. Indians had a very high prevalence of diabetes adjusted for age and the risk started to increase at very low levels of BMI. It was also interesting to note that this change was evident despite having a significantly low age-related increase in the BMI in the Indians. Therefore, Asian Indians have a low age threshold for the risk of diabetes.

2.9.2 Obesity and Abdominal Adiposity: Obesity is the most powerful environmental risk factor for T2DM (Kissebah et al., 1989). It is defined by an increase in body weight due to accumulation of excess body fat. Quantitative measures of obesity include the BMI, percent body fat and the waist-to-hip ratio (WHR). BMI is a standard predictor of diabetic status in populations at risk for T2DM. The prevalence of diabetes is 2.9 times higher in overweight (BMI > 27.8 kg/m² in men and > 27.3 kg/m² in women) than in normal weight subjects 20 to 75 years of age.

Abdominal adiposity, measured by an elevated WHR, has been shown to be a strong risk factor for T2DM (Lahti-Koski et al., 2000). Abdominal obesity is defined by accumulation of fat in the abdominal region (WHR above 0.85 for women and 0.9 for men). Abdominal obesity is characterized by fat deposition in the viscera, and is strongly associated with insulin resistance (Karter et al., 1996). Prospective studies also support the association of various anthropometric indices of abdominal adiposity and the future development of diabetes (Ohlson et al., 1985; Karter et al., 1996). It has been suggested that abdominal adiposity is an independent predictor of alterations in the plasma lipid, lipoprotein and plasma glucose concentrations (Ohlson et al., 1985; Obisesan et al., 1997; Brochou et al., 2000; DeNino et al., 2001). There appears to be a biologically plausible metabolic basis for the detrimental influence of abdominal adiposity. Central adiposity indicates deposition of large quantities of abdominal fat, which consists of visceral fat and subcutaneous fat. High proportion of upper-body fat or abdominal fat, independent of
overall obesity, is recognized as an important component in the insulin resistance linked to obesity and T2DM (Karter et al., 1996; Abate et al., 1995; Goodpaster et al., 2000). These elevated levels of free fatty acids may induce insulin resistance in the peripheral tissues and liver. Insulin resistance eventually produces sufficient glucose intolerance which results type 2 diabetes (Kannel et al., 1996). The association of fat distribution with insulin resistance and the resultant metabolic de-control may however, differ with ethnicity. A few studies have suggested less influence of fat distribution on the carbohydrate and lipid metabolism for African-Americans compared to non-Hispanic white individuals, while other studies indicate a difference in the association (Karter et al., 1996). Asian Indians, living in the United States, are more susceptible to developing abdominal adiposity and insulin resistance, which might account for the excessive morbidity and mortality from diabetes in this population (Chandalia et al., 1999). Most studies have shown that the prevalence of obesity is 2-15% in urban, (Zargar et al., 2000; Shukla et al., 2002) and upto 6% in rural populations in India (Gupta et al., 1997; Venkatramana et al., 2002) but a higher prevalence of obesity has been seen in migrant Asian Indians (McKeigue et al., 1991). An increasing trend of obesity also has been seen in Asian Indian children, adolescents, (Kapil et al., 2002; Ramachandran et al., 2002; Guleria et al., 2003) and women. (Zargar et al., 2000; Lean et al., 2001; Misra et al., 2001; 2002). Asian Indians have a higher percentage of body fat in relation to BMI, and there is stepwise increase of percentage of body fat in Asian Indians from rural to urban and migrant populations (Banerji et al., 1999; Dudeja et al., 2001; Lubree et al., 2002; Misra et al., 2002; 2003). As compared with whites and other ethnic groups, Asian Indians have a greater amount of intra-abdominal fat (Banerji et al., 1999; Raji et al., 2001) and thicker truncal skinfolds. (Banerji et al., 1999; Chandalia et al., 1999; Kalhan et al., 2001). Excess body fat, abdominal adiposity and body fat patterning appear to be important determinants of insulin resistance and dyslipidemia.

2.9.3 Dietary Habits: Diet plays a very important role in the pathogenesis of type 2 diabetes in Indians. Asian Indians in India consumed relatively more carbohydrates (Shobana et al., 1998; Misra et al., 2001) as compared with the migrant Asian Indians in the United Kingdom (Sevak et al., 1994) and the United States (Yagalla et al., 1996). High carbohydrate intake has been reported to induce hypertriglyceridemia and post
glucose load hyperinsulinemia in Asian Indians (Yagalla et al., 1996). The dietary fat intake in migrant south Asians was higher than in urban Asian Indians in India. The increase in the consumption of fat in the semi-urban and urban areas of India has been an important component of "dietary westernization" and nutrition transition (Gill, 2001; Popkin et al., 2001; Shetty, 2002).

Although the impact of vegetarian diets on insulin sensitivity in Asian Indians remains to be systematically investigated, significantly more post glucose load hyperinsulinemia has been reported in Asian Indians as compared with white vegetarians. (Scholfield et al., 1987). Finally, hyperhomocysteinemia may be more prevalent among the vegetarian Asian Indians (Cappuccio et al., 2002; Misra et al., 2004). Due to acculturation, the dietary habits of migrant Asian Indians are rapidly changing. These dietary changes may contribute to the higher prevalence of obesity in those migrant Asian Indians settled in the United States (Lauderdale et al., 2000). Similar to the "westernization" of dietary habits of urban Asian Indians, intra-country migrants have acquired adverse dietary habits (Misra et al., 2001; Khanna et al., 2002).

2.9.4 Physical Inactivity: Sedentary lifestyle could be an important risk factor for insulin resistance in Indians. Physical activity has an impact on many of the components of the metabolic syndrome. It is one of the principal therapies to acutely lower blood glucose in type 2 diabetes due to its synergistic action with insulin in insulin-sensitive tissues. For decades, exercise has been considered a cornerstone of diabetes management, along with diet and medication. Abnormal insulin secretion and peripheral insulin resistance are primary factors that influence the acute effects of physical activity on metabolic responses in those with type 2 diabetes. Consistently, South Asians and Asian Indians have been shown to be less physically active when compared with other ethnic groups (Hughes et al., 1990; Gopinath et al., 1994; Williams et al., 1994; Lip et al., 1996; Kamath et al., 1999; Dhanjal et al., 2001). South Asian women are particularly sedentary (Misra et al., 2001). Sedentary Asian Indians had higher average values of BMI, serum triglycerides and blood pressure (Dhawan et al., 1997) and higher future risk for development of hyperglycemia.
2.9.5 Migration and Stress: Epidemiological studies carried out in different parts of the world brought out an interesting finding that Indian migrants who were settled abroad have higher prevalence of diabetes (Zimmet, 1999). In a similar way in India, villagers when settle in cities also run the risk of developing obesity, diabetes and other lifestyle diseases. Stress has a major role to play in the causation and progress of diabetes, particularly in developing countries like India. Psychosocial stress may increase the activity of the stress-activated sympathetic nervous system, which affects glucose uptake in skeletal muscle, lipolysis and the development of hypertension (Reaven et al., 1996).

2.9.6 Smoking: Cigarette smoking is the most important cause of preventable morbidity and mortality around the world. In the United States, smoking is responsible for one in five deaths. The prevalence of smoking in diabetic patients is similar to that in the non-diabetic population, but the health repercussions are more severe in diabetic patients (Haire-Joshu et al., 1999). Cigarette smoking has been shown to increase the risk of cardiovascular disease more in diabetic patients than in those without diabetes, and CVD is responsible for 65% of deaths in patients with diabetes (Stamler et al., 1993; ADA, 2006). In addition to increasing the risk of macrovascular complications in diabetic patients, smoking cigarettes also increases the risk of microvascular complications, contributing to nephropathy, retinopathy, and neuropathy (Haire-Joshu et al., 1999).

2.9.7 Intrauterine Growth Retardation ('Thrifty phenotype' hypothesis): Reduced fetal growth and a low birth weight have been associated with an increased risk of type 2 diabetes, cardiovascular disease and features of the metabolic syndrome in later life (Hales et al., 1991; Barker et al., 1993; Valdez et al., 1994). It has been suggested that intrauterine undernutrition may result in developmental adaptations in certain tissues e.g. pancreas, adipose tissue, muscle fibre and predispose individuals to cardiovascular and metabolic disturbances in adult life (Isomaa, 2003), a theory that has been termed the ‘thrifty phenotype’ theory (Hales et al., 1991). Early-life adverse events, mostly malnutrition, and their relations to adult-onset metabolic syndrome and atherosclerosis have been shown in various populations including Asian Indians (Hales et al., 1991; Barker et al., 1993; Stein et al., 1996; Fall et al., 1998; Yajnik et al., 2000). This issue is particularly relevant to India, where approximately 23% of children are born with a low birth weight and 60% of children younger than 3 years have stunted growth. Asian Indian
babies born in India had lower triceps skinfold thickness than did white babies; however, their higher subscapular skinfold thickness was accompanied by more severe hyperinsulinemia (Yajnik et al., 2002). Further, similar to studies in other ethnic groups, (Wilkin et al., 2002), Asian Indian children born with a low birth weight showed insulin resistance and dyslipidemia when they gained weight in early childhood (Wilkin et al., 2002).

2.9.8 Genetic Susceptibility ('Thrifty genes' hypothesis): There is considerable evidence relating insulin resistance with genetic factors. About 45% of first-degree relatives of patients with type 2 diabetes are insulin resistant, compared to 20% of individuals with no family history of diabetes (Groop et al., 1996). Neel (1962) suggested the 'thrifty gene' theory. The author hypothesizes that our ancestors living in earlier environments with uncertain food supplies would have increased their chances of survival if they stored surplus food energy efficiently. In such an environment, genetic selection would have favoured energy-storing genes. Potential problems arise when people bearing 'thrifty genes' are transported into a society where food is always plentiful and exercise is not; the result is often rapid weight gain, obesity and diabetes. Therefore, presence of 'thrifty genes' would predispose an individual to developing the metabolic syndrome.

2.9.9 Dyslipidemia: Dyslipidemia is defined by alterations in blood lipid levels. Lipids are transported in the blood in lipoprotein. Lipids are a group of heterogeneous, metabolically active substances constantly moving in the circulation and existing in a state of dynamic equilibrium between peripheral tissues, gastrointestinal tract and liver. Triglycerides, cholesterol (both free and esterified), free fatty acids (FFA) and phospholipids (PL) constitute the plasma lipids. All such lipids circulate in blood by being incorporated into a very complex combination where non-polar lipids like triglycerides and cholesterol esters form the core while phospholipids and free cholesterol along with certain specific proteins, called apoproteins (Apo) constitute the surface layer of these molecules called lipoproteins.

In patients with T2DM, there is dysfunction of lipoprotein metabolism. The typical dyslipidemia associated with insulin resistance and diabetes mellitus is one that is
characterized by increased concentrations of triglyceride rich lipoproteins (especially VLDL) and reduced concentrations of HDL-cholesterol compared to subjects without diabetes mellitus/insulin resistance. Elevation in LDL-C and VLDL-C also contribute to the increased risk of T2DM and CHD.

In addition to the lipid abnormalities (McKeigue et al., 1992; Misra et al., 2001; 2002), Asian Indians have high levels of small-dense low-density lipoprotein (Kooner et al., 1998; Kulkarni et al., 1999). Interethnic comparison showed higher levels of serum triacylglycerols in adult Asian Indians (Das, 2004; Misra et al., 2004), which manifests at a young age (Kalhan et al., 2001; Misra et al., 2004).

Hypertriglyceridemia in Asian Indians was observed predominantly in people belonging to high socioeconomic strata (Reddy et al., 1999; Lubree et al., 2002) and in migrant South Asians (McKeigue et al., 1991; Hodge et al., 1996) as compared with the low socioeconomic strata rural populations (Reddy et al., 1994). Low HDL-C levels are also characteristically seen in Asian Indians of both sexes (Misra et al., 1998; Kulkarni et al., 1999; Tai, et al., 1999; Misra et al., 2004). The average level of HDL-C in rural populations in India was higher than that in urban Asian Indian and migrant Asian Indians. However, rural Asian Indians had a lower average HDL-C level than did whites (Misra et al., 2004).

2.9.10 Hypertension: Hypertension was defined as a self-reported history of physician diagnosis or subjects who were receiving drug treatment for hypertension or a systolic blood pressure (SBP) of ≥140 mm Hg and/or diastolic blood pressure (DBP) of ≥90 mm Hg. It is extremely common in patients with diabetes. It is 1.5 to 2.0 times more common in diabetics than non-diabetic individuals (Fuller, 1985; UK Prospective Diabetes Study Group-III, 1985). Hypertension is an under-diagnosed condition because it causes damage to the body with no symptoms or only mild symptoms. Coexistence of hypertension and T2DM significantly increases the risk of cardiovascular complications (Epstein and Sowers, 1992). Pharmacological treatment to reduce the blood pressure from elevated to normal level has clearly demonstrated a reduction in cardiovascular morbidity and mortality (Psaty et al., 1997). Hypertension affects up to 70% of patients with type 2 diabetes and is twice as prevalent in diabetics as in non-diabetics.
2.10 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

The natural history of Type 2 diabetes has four stages (Figure 2.6). The first stage begins at birth, when glucose homeostasis is normal but individuals are at risk for Type 2 diabetes because of genetic polymorphisms. During stage 2, decrease in insulin sensitivity emerges probably as a result of a genetic predisposition and lifestyle (environmental), which are initially compensated for by an increase in β-cell function so that glucose tolerance remains normal, but later both the β-cell function and insulin sensitivity deteriorate (stage of IGT). At this point, β-cell function is clearly abnormal, but sufficient to maintain normal fasting plasma glucose concentrations. In stage 3, as a result of further deterioration in β-cell function and increased insulin resistance, fasting plasma glucose can increase due to an increase in basal endogenous glucose production but the patient is still asymptomatic. Finally in stage 4, as a result of further deterioration in β-cell function, both fasting and postprandial blood glucose levels reach clearly diabetic levels and the patient becomes symptomatic.

Figure 2.6: Model of the progressive pathogenesis of type 2 diabetes (Hansen, 2002).

The ability of β-cell to adapt to insulin resistance depends on various genetic factors that determine the total β-cell mass, rates of replication and apoptosis of the cells and the activity of key biochemical components of cells. Environmental factors can probably aggravate the genetic predisposition leading to β-cell failure. Current evidence supports the view that the liver and β-cells are sensitive to insulin and that increased hepatic
glucogenesis and \( \beta \)-cell failure are different facets of the same metabolic phenotype. The exact mechanism of \( \beta \)-cell failure however remains controversial and is probably regulated at the gene level.

2.11 CELLULAR MECHANISMS OF INSULIN RESISTANCE IN T2DM

T2DM is a clinical disorder of glucose and fat metabolism caused by an inability of insulin to promote sufficient glucose uptake into adipose tissue and striated muscle and to prevent glucose output from the liver. Insulin is the most potent anabolic hormone and promotes the synthesis and storage of carbohydrates, lipids and proteins, while inhibiting their degradation and release into the circulation. Insulin stimulates the uptake of glucose, amino acids and fatty acids into cells and increases the expression or activity of enzymes that catalyse glycogen, lipid and protein synthesis, while inhibiting the activity or expression of those that catalyse degradation.

As blood sugar concentrations rise, insulin is secreted into the blood stream by the pancreatic \( \beta \)-cells of the endocrine pancreas. Insulin stimulates glucose uptake into fat and muscle to promote the storage of sugar as intracellular triglycerides and glycogen in fat and muscle. In addition, insulin inhibits the production and release of glucose from the liver (gluconeogenesis and glycogenolysis). In a healthy person, this prevents the rise of blood sugar concentrations that would occur after meal ingestion. However, in the early stages of T2DM, blood glucose concentrations remain increased, despite the presence of normal to high insulin concentrations in the bloodstream. The combined inability of muscle and adipose tissue to facilitate glucose uptake and of the liver to suppress glucose output in response to increasing amounts of insulin are referred to as insulin resistance. It is regarded as the hallmark characteristic of T2DM. The later stages of T2DM are characterized by low insulin concentrations and the need for exogenous insulin due to the eventual exhaustion of insulin secretory ability by the pancreatic beta cells. Up to 75\% of insulin-dependent glucose disposal occurs in skeletal muscle, whereas adipose tissue accounts for only a small fraction (Klip and Paquet, 1990).

Insulin resistance means an impaired biological response to insulin by one or more of its target tissues, the consequence is reduced glucose disposal in response to insulin (Kahn, 1995). It is a major contributor to the pathogenesis of type 2 diabetes and plays a key role
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in associated metabolic abnormalities, such as dyslipidemia and hypertension (Goldstein, 2002). Insulin resistance has been demonstrated to be a characteristic feature of Asian Indians (Ramachandran et al., 1997). Comparison of Asian Indians, Europeans and other ethnic groups has shown that the former have higher insulin response than others, at fasting and in response to glucose (Mohan et al., 1986; McKeigue et al., 1991). A study
in the normoglycaemic urban population showed that they had hyperinsulinaemic responses, when compared with the reported values in Europids (Ramachandran et al., 1997). It has also been noted that a clustering of the risk factors for cardio-vascular diseases (CVD) occurs in urban Indian population (Ramachandran et al., 1998; Mohan et al., 2001). In all the studies in Indian population, BMI has been strongly associated with glucose intolerance although the mean BMI is much below the obesity level. Even small increments in the BMI adversely affect insulin resistance (Ramachandran et al., 1998; Mohan et al., 2001).

Although the primary factors associated with the pathophysiology of type 2 diabetes are unknown, it is clear that insulin resistance plays a major role in the pathogenesis of type 2 diabetes and other lifestyle related diseases (DeFronzo, 1988; DeFronzo and Ferrannini, 1991). Except in a few rare cases involving antibodies to the insulin receptor or mutations in the insulin receptor gene, the insulin resistance of type 2 diabetes results from impairments in cellular events distal to the interaction between insulin and its surface receptor (Wallace and Matthews, 2002). Significant progress has been made in recent years toward an understanding of the intracellular signaling events that mediate insulin action (Shepherd and Kahn, 1999; Khan and Pessin, 2002; Reusch, 2002).

Insulin exerts its biological effect via binding to its receptor, which resides on the cell surface of target tissues (Reaven, 1998). The insulin receptor consists of two α chains and two β chains, which are connected by disulphide bridges. The binding of insulin to one of the extracellular α chains leads to the autophosphorylation of multiple tyrosine molecules in the intracellular domain of the β chain. The phosphorylated receptor then transfers the message inside the cell by phosphorylating tyrosine residues on insulin receptor substrate-1 (IRS-1). This intracellular protein is considered to play a central role in the intracellular signal cascade that is involved in glucose uptake and glycogen synthesis.
The IRS-1 also transfers the growth-promoting and mitogenic signals of insulin to the nucleus, thereby stimulating protein synthesis. Defects in the insulin-signalling pathway, owing to mutations in the insulin receptor gene or the presence of antibodies to the insulin receptor or insulin itself, are rare causes of insulin resistance (Shepherd and Kahn, 1999). Polymorphisms in the IRS-1 gene have been associated, although not strongly, with insulin resistance in various populations (Sasaoka et al., 1994; Shulman, 1999). Further support comes from studies of knockout animal models (Sasaoka et al., 1996; Jiang et al., 1999). Familial studies in Pima Indians and other populations show the clustering of risk factors related to insulin resistance (Blankard et al., 1997; Cusi et al., 2000), thus suggesting that genetic factors may contribute to the development of insulin resistance. Other factors such as obesity, postmenopausal state, exercise, drug use (glucocorticoids, β-blockers, and adrenergic agents), and infections may also influence the development of insulin resistance (Zecchin et al., 2003; Reaven, 2004). Most studies show that defective glucose oxidation and glycogen synthesis in muscle are the major causes of insulin resistance in type 2 diabetes (Law et al., 1996; Reaven, 2004). These observations may be in part explained by fuel competition—namely, an increased level of free fatty acids competing with glucose as energy substrates. Increased levels of free fatty acids can also induce insulin resistance by reducing the hepatic clearance of insulin and enhancing gluconeogenesis through the Randle cycle (Montagnani et al., 2002). More recent evidence suggests that long-chain fatty acids may modulate gene transcription or directly affect the activity of glycogen synthase (Wang et al., 2003). Furthermore, adipose tissue produces a pro-inflammatory cytokine, tumour necrosis factor, which can inhibit insulin signalling by inhibiting the phosphorylation of the insulin receptor and IRS-1 (Scherrer et al., 1994). Other cytokines such as interleukin-6 may also induce endothelial dysfunction, which in turn can contribute to the clustering of cardiovascular risk factors, as encountered in the metabolic syndrome (Steinberg et al., 1994).

The insulin resistance of T2DM is characterized by defects at many levels, with decreases in receptor concentration and kinase activity, the concentration and phosphorylation of IRS-1 and -2, PI(3)K activity, glucose transporter translocation and the activity of intracellular enzymes (Pessin and Saltiel, 2000). Type 2 diabetes is polygenic and may
involve polymorphisms in multiple genes encoding the proteins involved in insulin signalling, insulin secretion and intermediary metabolism (Stern et al., 2000).

2.11.1 Insulin Signalling: There are two major insulin-signaling pathways activated by insulin binding to its receptor (Figure 2.7). The pathways have been termed the phosphatidylinositol-3'-kinase (PI3K) pathway and the mitogen-activated protein (MAP) kinase, pathway (Reusch, 2002).

Figure 2.7: Simplified schematic of two major insulin-signaling mechanisms (Sivitz, 2004).

The activation of these pathways has different consequences. Although the PI3K pathway is important in mediating the metabolic effects of insulin, activation of MAP kinase is associated with cell growth and proliferation. Any molecular genetic defect in the insulin-signaling cascade could potentially cause the insulin resistance seen in metabolic syndrome and type 2 diabetes.
The pathophysiology of insulin resistance involves a complex network of signalling pathways, activated by the insulin receptor, which regulates intermediary metabolism and its organization in cells. Insulin increases glucose uptake in muscle and fat and inhibits hepatic glucose production, thus serving as the primary regulator of blood glucose concentration. Insulin also stimulates cell growth and differentiation and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis and inhibiting lipolysis, glycogenolysis and protein breakdown (Figure 2.8). Insulin resistance or deficiency results in profound dysregulation of these processes and produces elevations in fasting and postprandial glucose and lipid levels.

Despite this, mice with a knockout of the insulin receptor in muscle have normal glucose tolerance (Bruning et al., 1998), whereas those with a knockout of the insulin-sensitive glucose transporter in fat have impaired glucose tolerance, apparently owing to insulin resistance being induced in muscle and liver (Abel et al., 2001). Both obesity and dyslipidemia also cause insulin resistance and predisposition to type 2 diabetes, demonstrating that adipose tissue is crucial in regulating metabolism beyond its ability to take up glucose. Although insulin does not stimulate glucose uptake in liver, it blocks glycogenolysis and gluconeogenesis, and stimulates glycogen synthesis, thus regulating fasting glucose levels. Insulin action in tissues not normally considered insulin sensitive, including brain and pancreatic β-cell, may also be important in glucose homeostasis (Bruning et al., 1998; Kulkarni et al., 1999; Gavrilova et al., 2000; Abel et al., 2001).

2.11.2 Insulin-Stimulated Phosphorylation Cascades: The integration of multiple transmembrane signals is especially important during development and maintenance of the nervous system, communication between cells of the immune system, evolution of transformed cells, and metabolic control (Hunter, 2000). Tyrosine phosphorylation plays a key role in many of these processes by directly controlling the activity of receptors or enzymes at early steps in signalling cascades, or by coordinating the assembly of multicomponent signalling complexes around activated receptors or docking proteins (Pawson, 1995). The insulin receptor consists of extracellular ligand binding and intracellular tyrosine kinase domains. Binding of insulin to the extracellular portion of the receptor activates its kinase activity resulting in autophosphorylation of specific
intracellular tyrosine residues. This autophosphorylation step enables a variety of scaffolding proteins including insulin receptor substrate (IRS) proteins, Cbl (casitas B-lineage lymphoma) or Cbl associated protein (CAP) to bind to intracellular receptor sites and to become phosphorylated (Pawson et al., 1999; Chiang et al., 2001).

Figure 2.8: Signal transduction in insulin action (Chakraborty, 2006).

These pathways act in a concerted fashion to coordinate the regulation of vesicle trafficking, protein synthesis, enzyme activation and inactivation and gene expression, which results in the regulation of glucose, lipid and protein metabolism (Figure 2.8).
2.12 REGULATION OF GLUCOSE METABOLISM

Glycogenolysis and gluconeogenesis are the two known sources of hepatic glucose production (HGP). HGP is controlled by the integrated metabolite fluxes to and from the liver, hormonal cues and neurotransmitter release. Fasting promotes glucose production through cAMP-dependent mechanisms, whereas feeding inhibits glucose production through insulin.

2.12.1 Regulation of Glycogen Synthesis: Insulin stimulates glycogen accumulation through a coordinated increase in glucose transport and glycogen synthesis. The hormone activates glycogen synthase by promoting its dephosphorylation, through the inhibition of kinases such as PKA or GSK-3 (Cross et al., 1995) and activation of protein phosphatase-1 (PP1) (Brady et al., 1997). Upon its activation downstream of PI(3)K, Akt phosphorylates and inactivates GSK-3, decreasing the rate of phosphorylation of glycogen synthase, thus increasing its activity state (Cross et al., 1995). Insulin does not activate PP1 globally, but rather specifically targets discrete pools of the phosphatase primarily increasing PP1 activity localized at the glycogen particle. The compartmentalized activation of PP1 by insulin is due to glycogen-targeting subunits, which serve as 'molecular scaffolds', bringing together the enzyme directly with its substrates glycogen synthase and phosphorylase in a macromolecular complex and in the process exerting profound effects on PP1 substrate-specific activity (Newgard et al., 2000). Four different proteins have been reported to target PP1 to the glycogen particle. Despite a proposed common function, no two targeting subunits share more than 50% sequence homology, and this is largely confined to the PP1 and glycogen-binding regions. Overexpression of these scaffolding proteins in cells or in vivo results in a marked increase in cellular glycogen levels (Newgard et al., 2000). Although the mechanism by which insulin activates glycogen-associated PP1 remains unknown, inhibitors of PI(3)K block this effect, suggesting that PtdIns(3,4,5)P3-dependent protein kinases are involved. These scaffolding proteins have a critical permissive role in the hormonal activation of the enzyme, perhaps interacting with additional proteins that regulate the interaction of PP1 with glycogen synthase and phosphorylase.
2.12.2 Regulation of Gluconeogenesis: Insulin inhibits the production and release of glucose by the liver by blocking gluconeogenesis and glycogenolysis (Figure 2.9). This occurs through a direct effect of insulin on the liver (Michael et al., 2000) as well as by indirect effects of insulin on substrate availability (Bergman and Ader, 2000). Insulin can also influence glucose metabolism indirectly by changes in free fatty acids generated from visceral fat, the so called 'single gateway' hypothesis (Bergman, 1997). Because visceral fat is less sensitive to insulin than subcutaneous fat, even after a meal there is little suppression of lipolysis by the hormone in this fat depot.

Figure 2.9: The regulation of glucose metabolism in the liver (Saltiel and Kahn, 2001).

The resulting direct flux of fatty acids derived from these fat cells through the portal vein to the liver can stimulate glucose production, thus providing a signal for both insulin action and insulin resistance in the liver. In the hepatocyte, insulin stimulates the utilization and storage of glucose as lipid and glycogen, while repressing glucose synthesis and release. This is accomplished through a coordinated regulation of enzyme synthesis and activity. Insulin stimulates the expression of genes encoding glycolytic and fatty-acid synthetic enzymes, while inhibiting the expression of those encoding gluconeogenic enzymes. These effects are mediated by a series of transcription factors.
and co-factors, including sterol regulatory element-binding protein (SREBP)-1, hepatic nuclear factor (HNF)-4, the forkhead protein family (Fox) and PPAR co-activator 1 (PGC1). The hormone also regulates the activities of some enzymes, such as glycogen synthase and citrate lyase, through changes in phosphorylation state. GK, glucokinase; Glucose-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; F-1,6-Pase, fructose-1,6-bisphosphatase; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PK, pyruvate kinase; ACC, acetyl-CoA carboxylase; FAS, fatty-acid synthase. Insulin directly controls the activities of a set of metabolic enzymes by phosphorylation or dephosphorylation and also regulates the expression of genes encoding hepatic enzymes of gluconeogenesis and glycolysis (Pilkis and Granner, 1992). It inhibits the transcription of the gene encoding phosphoenolpyruvate carboxylase, the rate-limiting step in gluconeogenesis (Sutherland et al., 1996). The hormone also decreases transcription of the genes encoding fructose-1, 6-bisphosphatase and glucose-6-phosphatase, and increases transcription of glycolytic enzymes such as glucokinase and pyruvate kinase, and lipogenic enzymes such as fatty acid synthase and acetyl-CoA carboxylase. Although the transcription factors that control the expression of these genes have remained elusive, new data suggest a potential role for the forkhead family of transcription factors through phosphorylation by Akt-related protein kinases (Nakae et al., 1999) and the PPAR-γ co-activator-1 (Yoon et al., 2001).

2.13 REGULATION OF LIPID METABOLISM

As is the case with carbohydrate metabolism, insulin also promotes the synthesis of lipids, and inhibits their degradation. Recent studies suggest that many of these changes require an increase in the transcription factor steroid regulatory element-binding protein (SREBP)-1c (Shimomura et al., 1999). Dominant negative forms of SREBP-1 can block expression of these gluconeogenic and lipogenic genes (Foretz et al., 1999), whereas overexpression can increase their transcription (Shimomura et al., 1999). Hepatic SREBP levels are increased in some rodent models of lipodystrophy, and this is coordinated with increases in fatty acid synthesis and gluconeogenesis, the exact phenotype observed in genetic models of obesity-induced diabetes (Shimomura et al., 2000). Thus, increased expression of SREBP-1c might contribute to the insulin resistance observed in liver of diabetic rodents, with increased rates of both gluconeogenesis and lipogenesis. The
pathways that account for the changes in SREBP-1c expression in response to insulin or other metabolic changes are not known, but probably lie downstream of the IRS/Pi(3)K pathway. In adipocytes, glucose is stored primarily as lipid, owing to increased uptake of glucose and activation of lipid synthetic enzymes, including pyruvate dehydrogenase, fatty acid synthase and acetyl-CoA carboxylase. Insulin also profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone-sensitive lipase (Anthonsen et al., 1998). This enzyme is acutely regulated by control of its phosphorylation state, which is activated by PKA-dependent phosphorylation, and inhibited as a result of a combination of kinase inhibition and phosphatase activation. Insulin inhibits the activity of the lipase primarily through reductions in cAMP levels, owing to the activation of a cAMP-specific phosphodiesterase in fat cells (Kitamura et al., 1999).

2.13.1 Free Fatty Acids: A major contributor to the development of insulin resistance is an overabundance of circulating free fatty acids (FFAs) derived from adipocytes by inhibiting glucose uptake, glycogen synthesis and glucose oxidation, and by increasing hepatic glucose output (Bergman and Ader, 2000). It is now apparent that elevation of plasma free fatty acids plays a pivotal role in the development of type 2 diabetes by causing insulin resistance. Adipocytes take up and store FFAs. Elevated FFAs might promote accumulation of fat depots in muscle, liver and/or β-cells, and the accumulated triglycerides might provide an environment that could interfere with metabolic signalling and thus action in these different tissues. A link between insulin resistance and triglycerides content in muscle biopsies has been established (Pan, 1997). Moreover, it was shown that elevations in plasma free fatty acid concentrations can lead to an attenuated effect of insulin to stimulate IRS-1 associated PI-3 kinase activity in muscle (Dresner et al., 1999). The reduced PI-3 kinase activity may be due to a direct effect of intracellular free fatty acids or some fatty acid metabolite, or it may be secondary to alterations in upstream signalling events. Recent data have suggested that fatty acid metabolites activate a kinase that phosphorylates serine/threonine sites on IRSs, which in turn may reduce the ability of the IRSs to activate PI-3 kinase and glucose transport (Griffin, 1999). It is well known that FFAs are important substrates for skeletal muscle energy production. In the fasting state skeletal muscle has a high fractional extraction of...
plasma FFAs, and lipid oxidation accounts for the majority of energy production. The capacity of skeletal muscle to utilize lipid or carbohydrate fuels, as well as the potential for substrate competition between fatty acids and glucose, is of interest in insulin resistance. A potential implication of the glucose-fatty acid cycle, originally postulated by Randle and colleagues (Randle, 1963), is that increased lipid availability could interfere with muscle glucose metabolism and contributes to insulin resistance for example in obesity and type 2 diabetes. Several studies support the concept that elevated free fatty acids produce an impairment of insulin-stimulated glucose metabolism (Shulman, 2000). Another concept is that of metabolic inflexibility in insulin resistance. In the fasting condition, skeletal muscle predominantly utilizes lipid oxidation for energy production. Upon insulin stimulation in the fed condition, healthy skeletal muscle rapidly switches to increased uptake, oxidation and storage of glucose and, moreover, lipid oxidation is suppressed (Saltiel and Kahn, 2001). Obese individuals and those with type 2 diabetes manifest higher lipid oxidation during insulin-stimulated conditions as compared to control subjects, despite lower rates of lipid oxidation during fasting conditions. This suggests that a key feature in insulin resistance of skeletal muscle is an impaired ability to switch between fuels.

The mechanisms by which elevated FFA levels result in insulin resistance have been determined in skeletal muscle, where most insulin-stimulated glucose uptake occurs. It is now thought that FFAs induce insulin resistance in human muscle at the level of insulin-stimulated glucose transport or phosphorylation by impairing the insulin-signaling pathway (Boden and Chen 1995; Dresner et al., 1999). Another mechanism by which FFAs can cause insulin resistance is by increasing oxidative stress (Ceriello, 2000). Reactive oxygen species can activate PKC and the NF-κB pathway and thereby contribute to insulin resistance (Itani et al., 2002; Griffin et al., 1999). FFAs also affect the functioning of insulin in the liver and thus contribute to hepatic overproduction of glucose and to elevated circulating blood glucose levels (Ferrannini et al., 1983). The main role of insulin in the liver is control of glucose production. The mechanism by which insulin acutely suppresses hepatic glucose production is by inhibiting glycogenolysis (Gastaldelli et al., 2001). FFAs produce insulin resistance in the liver by inhibiting the acute insulin suppression of glycogenolysis (Boden, 2003). Insulin also
promotes hepatic uptake of FFAs and production of intracellular triglycerides. Thus, insulin resistance in the liver may contribute to elevated plasma FFA levels. An increase in visceral fat could also cause insulin resistance by mechanisms that do not directly involve FFAs.

2.14 STRATEGIES OF THE SEARCH FOR T2DM SUSCEPTIBILITY GENES

The human genome sequence provides us with a tool, which is already transforming biological research. The detailed catalogue of all the genes in the genome and their expression patterns along with comparative genomics using the published rodent genomes will provide researchers with an invaluable resource.

Type 2 diabetes is a very complex metabolic disease where environmental and genetic factors play an important role in favoring or delaying the development of the disease (Kahn et al., 1996; Almind et al., 2001). The susceptibility genes involved in the development of the disease exert only a partial effect; thereby the sole effect of a single gene is not enough to cause diabetes (Hansen and Pedersen, 2005). Only the additive effect of these genes, in certain combinations, confers genetic susceptibility. The disease will then develop only when certain risk factors favoring its expression are present, such as obesity, sedentary lifestyle, or high-fat diets.

In our search for a better understanding of the pathogenesis of T2DM, a genetic approach will help focus on the underlying causes of the disease, and may provide new information for diagnostic treatment and prevention. This genetic information may also form the basis for new drug therapies, such as individually specific or targeted pharmacotherapy (pharmacogenetics). Decades of careful research have failed to clarify a unified model of the pathogenesis of T2DM. Hence, using pathophysiology to predict susceptibility loci is difficult. The pre-sequencing phase of the Human Genome Project provided genetic and physical maps, allowing whole-genome scans to identify chromosomal regions that are linked with T2DM in families. Identification of the genetic components of type 2 diabetes is one of the most important areas of diabetes research because elucidation of the diabetes genes will influence all efforts toward a mechanistic understanding of the disease, its complications, and its treatment, cure, and prevention. Two broad approaches have been used to define the genetic predisposition of T2DM:
(1) Genome-wide scan approach, and
(2) Candidate gene approach

2.14.1 Genome-wide Scan Approach: The genome-wide scans approach is used to identify new susceptibility genes involved in the pathogenesis of T2DM, using polymorphic markers. However, this classical approach of gene localization by linkage analysis in multi-generational families is not the most suitable strategy for T2DM. Because of genetic heterogeneity, non-Mendelian inheritance and hard to obtain families with enough T2DM patients. A commonly used genetic mapping approach is the affected sib pair (ASP) approach using randomly spaced polymorphic markers (usually every 10 cM). Using ASPs in genome-wide scans generally requires large numbers of ASPs to obtain sufficient power for detecting linkage (Risch 1990; Risch and Merikangas, 1996). An efficient study design is an important aspect of any genome-wide scan. Different types of cohorts, consisting of nuclear families, multi-generational families or affected sib pairs, can be used. To date, various research groups have completed or nearly completed genome scans for T2DM using ASPs (Hanis et al., 1996; Zouali et al., 1997; Ji et al., 1997; Ghosh et al., 1999; Hegele et al., 1999; Ehm et al., 2000; Ghosh et al., 2000; Watanabe et al., 2000) or occasionally, multi-generational families (Mahtani et al., 1996; Ji et al., 1997; Hanson et al., 1998; Duggirala et al., 1999; Elbein et al., 1999).

Both types of genome scans (using ASPs or multi-generational families) yield varying levels of evidence. In 1996, a genome-wide significance was found on chromosome 2q37 in a combined data set of 330 Mexican-American ASPs from Starr County, Texas, USA. This locus was designated NIDDM1 (Hanis et al., 1996). In a sample from Botnia, Western Finland, a small number of selected pedigrees with the lowest quartile for mean 30-min insulin levels after oral glucose tolerance tests showed significant evidence for linkage to T2DM on chromosome 12q, and this locus was designated NIDDM2 (Mahtani et al., 1996). More recently, several studies have shown significant evidence for linkage to chromosome 20 (Ji et al., 1997; Zouali et al., 1997; Ghosh et al., 1999; Ghosh et al., 2000) and a recent genome scan in Pima Indians revealed strong evidence that chromosome 11q contains a susceptibility locus influencing both T2DM and obesity (Hanson et al., 1998). Chromosomes 1q and 7q showed some evidence of additional diabetes mellitus susceptibility loci (Hanson et al., 1998).
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families with Northern European ancestry from Utah, significant linkage was found under a model of recessive inheritance on chromosome 1q21-23 (Elbein et al., 1999) and in 49 ASPs of Canadian Oji-Cree Indian origin, both suggestive linkage and suggestive association was found with chromosomes 6, 8, 16, and 22 (Hegele et al., 1999). In Mexican Americans from the San Antonio Family Diabetes Mellitus Study, significant evidence was found that a susceptibility locus on chromosome 10q influences age at onset of diabetes mellitus and this locus also seems to be linked to T2DM itself (Duggirala et al., 1999).

Over 30 genome wide linkage scans have been published in multiple ethnic groups and show more than eight susceptibility regions which may harbor one or more susceptibility genes. Recent genome scan studies have been carried out in Mexican-Americans (Hanis et al., 1996; Duggirala et al., 1999; Hunt et al., 2005), West Africans (Rotimi et al, 2006) US Caucasians (Elbein et al., 1999; Ehm et al., 2000), Pima Indians (Hanson et al., 1998), UK Caucasians (Wiltshire et al., 2001), Finnish Caucasians (Ghosh et al., 1999; 2000; Silander et al., 2004), French Caucasians (Vionnet et al., 2000), Australians (Bushfield et al., 2002), Dutch Caucasians (Van-Tilburg et al., 2003), Chinese (Luo et al., 2001; Xiang et al., 2004; Zhao et al., 2005), Japanese (Mori et al., 2002; Iwasaki et al., 2003; Nawata et al., 2004), Indo-Mauritian (Francke et al., 2001) and African Americans populations (Sale et al., 2004).

2.14.2 Candidate Gene Approach (Association Studies): Conceptually, the simplest strategy for gene discovery in multifactorial traits is the ‘candidate gene study’ (Das and Maji, 1999; Altshuler et al., 2000; Cardon and Bell, 2001). Defects in genes encoding proteins that play a role in pathways involved in insulin control and glucose homeostasis are excellent candidates for T2DM. A powerful approach to finding such defects is the identification of a significant association between diabetes mellitus and a functional polymorphism in a candidate gene. Generally, this is achieved by comparing a random sample of unrelated T2DM patients with a matched control group. This approach may reveal a polymorphic allele that is increased in frequency in the patient group and such a significant association might point towards a disease-susceptibility locus. The usual procedure is to select a gene, usually on the basis of its known or presumed biological function, and the hypothesized relevance of that function to the disease of interest, and
then to look for association between one or more variants in that gene and the disease phenotype. Association studies offer a potentially powerful approach to identifying genetic variants that influence susceptibility to disease but are often plagued by the impression that they are not consistently reproducible. In principle, the inconsistencies may be due to false positive studies, false negative studies or heterogeneity between studied populations. Despite all these problems, a surprisingly large number of candidate genes studies have been replicated and considered positive in meta-analyses (Lohmueller et al., 2003). To date, over 250 candidate genes have been studied for their role in T2DM (DeFronzo, 1997a; 2004).

Figure 2.10: A complex interaction between the genetic and environmental factors (Hansen, 2002).
Many studies have been carried out to study the role for some of the gene products involved in insulin secretion or insulin action, such as IRS-1 (Almind et al., 1993; 1996; Porzio et al., 1999), the glucagon receptor (Hansen et al., 1996), the sulfonylurea receptor (SUR) (Hart et al., 1999), the peroxisome proliferator-activated receptor-γ (PPARγ) (Altshuler et al., 2000; Hegele et al., 2000).

2.14.2.1 Calpain-10 (CAPN10) Gene: A genome-wide linkage mapping study of T2DM in Mexican Americans from Starr County, Texas, USA identified a region with significant evidence for linkage on chromosome 2 (Hanis et al., 1996). The calpain-10 gene (CAPN10) is regarded to be one of the candidate genes for type 2 diabetes. The discovery of calpain-10 by a genetic approach has identified it as a molecule of importance to insulin signaling and secretion that may have relevance to the future development of novel therapeutic targets for the treatment of T2DM. Both genetic and functional data indicates that calpain-10 has an important role in insulin resistance and intermediate phenotypes, including those associated with the adipocyte (Turner et al., 2005). CAPN10 is a ubiquitously expressed member of the calpain-like cysteine protease family that catalyzes the endoproteolytic cleavage of specific substrates and thereby regulates pathways that affect intracellular signaling, proliferation, and differentiation (Sorimachi et al., 1997; Carafoli and Molinari, 1998; Horikawa et al., 2000; Sreenan et al., 2001). This is the first successful positional cloning of a gene for a polygenic disease like type 2 diabetes. CAPN10 gene consists of 15 exons and spans 31 kb in a 66 kb region in chromosome band 2q37.3 within the NIDDM1 region (Hanis et al., 1996). The specific functions of calpain-10 remains to be determined but it is expressed in many tissues including those involved with the pathogenesis of T2DM i.e. pancreatic islets, muscle and fat.

Haplotype combination 112/121 defined by three SNPs (UCSNP-43, -19 and -63) of CAPN10 conferred the highest risk for T2DM in Mexican-American population (Horikawa et al., 2000). Subsequent association and linkage studies of these three SNPs in other populations have produced conflicting results, with association being observed in some populations like South Indian (Cassell et al., 2002), African American (Garant et al., 2002), Polish (Malecki et al., 2002), Finnish/Botnia (Orho-Melander et al., 2002), Pima Indian (Baier et al., 2000) but not in others i.e. Korean population (Xu et al., 2006).
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West African (Chen et al., 2005), Japanese (Daimon et al., 2002; Horikawa et al., 2003; Shima et al., 2003; Iwasaki et al., 2005), Caucasians (Elbein et al., 2002), Finnish (Fingerlin et al., 2002), Danish and Swedish (Rasmussen et al., 2002), British (Evans et al., 2001), Oji-Cree Indians (Hegele et al., 2001), Samoan (Tsai et al., 2001), Chinese (Xiang et al., 2001; Sun et al., 2002). A recent meta-analysis study suggests a role of genetic variation in CAPN10 with the risk of T2DM in European populations (Tsuehiya et al., 2006). Lynn et al., (2002) reported that genetic variation in the CAPN10 gene influences blood glucose levels in nondiabetic British subjects. A novel 111/121 haplotype combination of SNP-43, -19, and -63 of the CAPN10 gene was associated with T2DM and metabolic syndrome in patients with type 2 diabetes in the Korean population (Kang et al., 2006).

SNP-43 may contribute to the risk of diabetes by regulating abdominal obesity in subjects with high risk of type 2 diabetes (Pihlajamaki et al., 2006). SNP-43 is also associated with reduced level of CAPN10 mRNA expression in skeletal muscle (Carlsson et al., 2005). SNP-19 may be associated with elevated BMI and hemoglobin A1c levels in Japanese (Shima et al., 2003), β1-adrenoceptor function in human fat cells and with glucose metabolism in human fat cells (Hoffstedt et al., 2002). Variations in the CAPN10 are associated with elevated triglyceride levels and reduced adipose tissue mRNA expression in obese Swedish subjects (Carlsson et al., 2004). Taking these data together, it appears that variation at the CAPN10 gene does influence susceptibility to T2DM across populations of different racial backgrounds. However, the strength of this influence and the nature of the specific variants do seem to differ between populations.

Several studies examined phenotypes less directly related to type 2 diabetes. The high-risk haplotype combination at CAPN10 are associated with polycystic ovary syndrome and quantitative measures related to type 2 diabetes (Gonzalez et al., 2000; Ehrmann et al., 2002) and microvascular function (Shore et al., 2000).

2.14.2.2 Peroxisome Proliferator-Activated Receptor γ (PPARγ) Gene: PPARs are members of the nuclear hormone receptor superfamily which regulate the number of cellular functions related to lipid metabolism, glucose homeostasis, and adipocyte differentiation (Tontonoz et al., 1994; Spiegelman and Flier, 1996; Beamer et al., 43
1997). Three subtypes have been described: PPAR-\(\alpha\), PPAR-\(\beta\) or \(\delta\) and PPAR-\(\gamma\) encoded by different genes and sharing 60–80% homology in their ligand and DNA binding domains. They are ligand-activated transcription factors which heterodimerize with the retinoid X receptor (RXR) to induce transcription of a number of target genes in adipose tissue after binding to PPAR response elements (PPREs) (Auwerx, 1999). PPAR-\(\alpha\) and PPAR-\(\delta\) regulate genes involved in lipid and lipoprotein metabolism. The human PPAR-\(\gamma\) gene maps to chromosome 3p24, a region implicated in several genome wide scans for type 2 diabetes (Fajas et al., 1997). There are four isoforms of PPAR-\(\gamma\): PPAR-\(\gamma_1\), PPAR-\(\gamma_3\), and PPAR-\(\gamma_4\) that encode the same protein product, and the PPAR-\(\gamma_2\) protein containing an additional N-terminal 28 amino acid exon. PPAR-\(\gamma_2\) and PPAR-\(\gamma_3\) are predominantly expressed in adipose tissue whereas PPAR-\(\gamma_1\) expression is ubiquitous. PPAR-\(\gamma\) regulates the expression of many adipose-specific genes via the binding of the heterodimer PPAR-\(\gamma\)/RXR to specific DNA response elements in target gene promoters. PPAR-\(\gamma_2\) gene contains 9 exons and spans more than 100 kilo bases of genomic DNA (Figure 2.11) (Fajas et al., 1997).

**Figure 2.11:** Genomic organization of the human PPAR-\(\gamma\) gene and positions of various PPAR-\(\gamma\) polymorphisms (Meirhaeghe et al., 2004).

Mutations in PPAR-\(\gamma\) gene have been of great interest in helping to study and unravel the complex and multiple biological actions of PPAR-\(\gamma\) in vivo. Many genetic variations in the PPAR-\(\gamma\) gene have been studied in different populations. A more common structural
polymorphism in the PPARγ2 gene (Pro12Ala), a CCA-to-GCA missense mutation in codon 12 of exon B of the PPAR-γ gene was studied in several populations (Yen et al., 1997). Many studies have found an association of P12A polymorphism with type 2 diabetes. Recently significant association of Pro12Ala polymorphism with type 2 diabetes is reported in Spanish and UK population (Zeggini et al., 2005; Soriguer et al., 2006). Altshuler et al., (2000) reported that the Ala allele was indeed protective against type 2 diabetes with an OR = 0.8 and that, conversely, the Pro allele was a risk factor for type 2 diabetes (OR ~ 1.25). It was first reported that the frequency of the Ala allele was lower in Japanese American type 2 diabetic patients (2%) than in individuals with impaired glucose tolerance (4%) and in non-diabetic subjects (9%) (Deeb et al., 1998), suggesting that the Ala allele play a protective against type 2 diabetes. Since then, the protective effect of the Ala allele against type 2 diabetes has been replicated in Danish (Johansen et al., 2006), Korean (Moon et al., 2005) Japanese (Hara et al., 2000; Mori et al., 2001), Caucasian American (Li et al., 2000), Finnish (Douglas et al., 2001), and Danish populations (Poulsen et al., 2003). Conversely, a deleterious effect of the Ala allele against type 2 diabetes has been demonstrated in Canadian Oji-Cree individuals (Hegele et al., 2000), Germans (Evans et al., 2001), and obese IGT Finns (Lindi et al., 2002). Other studies did not find an association with type 2 diabetes in Italians (Mancini et al., 1999), Germans (Ringel et al., 1999; Zietz et al., 2002), French (Clement et al., 2000), Polish (Malecki et al., 2003), Tunisian (Meirhaeghe et al., 1998; Zouari-Bouassida et al., 2005) Koreans (Oh et al., 2000) or Pima Indians (Muller et al., 2003).

Insulin resistance is a common finding in patients with T2DM. It can precede and predict the development of type 2 diabetes. Inadequate stimulation of muscle glucose disposal and reduced suppression of lipid oxidation by insulin are characteristic of skeletal muscle insulin resistance and type 2 diabetes (Kelley et al., 2000). Many studies have reported an association between the Ala allele and improved insulin sensitivity. They include studies undertaken in Brazilian Caucasians (Tavares et al., 2005), White Americans (Chen et al., 2003), Danish monozygotic twins (Poulsen et al., 2003), and non-diabetic Pima Indians (Muller et al., 2003), Japanese and Chinese families (Hara et al., 2000; 2002; Chuang et al., 2001), Spanish women (Gonzalez Sanchez et al., 2002) and Finns (Deeb et al., 1998). In keeping with greater insulin sensitivity, Ala homozygotes had a lower risk of
developing the metabolic syndrome (OR = 0.24) compared with Pro-Pro or Pro-Ala subjects in the Danish MONICA cohort (Frederiksen et al., 2002). Recently, Stefanski et al. (2006) reported the lack of association between the Pro12Ala polymorphism and body weight changes, insulin resistance and chronic diabetic complications in obese patients with type 2 diabetes. No association was found in Pro12Ala polymorphism of PPARγ gene with metabolic syndrome in Korean females (Rhee et al., 2006), Hispanic and non-Hispanic white women (Moffett et al., 2005) and French population (Meirhaeghe et al., 2005). Adamo et al., (2005) reported that Pro12Ala polymorphism may influence the glycaemic response to exercise in type 2 diabetes.

Studies in healthy non-obese dizygotic twin subjects showed that the PPARγ gene locus could be linked to HDL, LDL, and BMI as quantitative traits (Knoblauch et al., 1999). Numerous studies have attempted to find an association between the Pro12Ala polymorphism and obesity markers in lean, obese and type 2 diabetic patients, but unfortunately the results were inconsistent. Some studies reported a higher BMI in Ala carriers (Beamer et al., 1998; Ek et al., 1999; Cole et al., 2000; Meirhaeghe et al., 2000) while others described a lower BMI in Ala carriers (Deeb et al., 1998; Ek et al., 1999; Pihlajamaki et al., 2000; Doney et al., 2002; Barbieri et al., 2005). Finally, others could not find any association (Mori et al., 1998; Ringel et al., 1999; Clement et al., 2000; Swarbrick et al., 2001; Frederiksen et al., 2002; Danawati et al., 2005). Some studies reported a higher risk (2-fold) of developing obesity (BMI > 30 kg/m²) for carriers of the Ala allele in Caucasian women (Li et al., 2000) or analysis showed that in subjects with a BMI above 27 kg/m², Ala allele carriers had a significantly higher BMI than non-carriers (Masud et al., 2003). Such an association was not present in lean subjects. These results support the notion that the Pro12Ala polymorphism only has an effect on BMI (being higher) in markedly obese individuals (Ek et al., 1999), probably because the impact of this polymorphism is modified by environmental and/or genetic factors in Spanish men (Gonzalez -Sanchez et al., 2002) but this result was not replicated in Germans (Hamann et al., 1999) French (Clement et al., 2000) or Koreans (Oh et al., 2000).

PPARγ have been the focus of intense academic and pharmaceutical research and play important roles in lipid metabolism, glucose homeostasis, and adipocyte differentiation (Tontonoz et al., 1994; Spiegelman et al., 1996; Beamer et al., 1997). (Figure 2.12). The
thiazolidinedione (TZD) antidiabetic compounds such as rosiglitazone and pioglitazone are specific PPARγ ligands that are effective in lowering hyperglycemia, hyperinsulinemia and hypertriglyceridemia in type 2 diabetic subjects (Berger et al., 2005). They modulate the expression of numerous genes in adipocytes, which results in improved insulin sensitivity, increased fatty acid uptake and decreased lipolysis. As a result, circulating FFA levels are diminished.

Figure 2.12: The beneficial metabolic effects of PPAR ligands (Berger et al., 2005).

Activation of PPAR-γ also results in changes in adipokine production, remodeling of adipose tissue, and the concurrent repartitioning of lipids from lipolytic visceral fat into subcutaneous fat that contains newly generated, small insulin-sensitive adipocytes. 

PPARγ agonists also decrease the inflammation of adipose tissue that is induced by obesity and contributes to increased insulin resistance. Because of these multiple adipocentric actions, PPARγ activation improves insulin sensitivity in skeletal muscle and liver, and reduces hyperglycemia (Picard and Auwerx, 2002; Berger et al., 2005)
2.14.2.3 Ecto-Nucleotide Pyrophosphatase/Phosphodiesterase-1 (ENPP1) Gene: ENPP1, also known as plasma cell membrane glycoprotein-1, or PC-1) is a membrane glycoprotein that adversely influences insulin sensitivity by inhibiting insulin receptor signaling (Maddux et al., 1995; 2000; Dong et al., 2005). A functional missense polymorphism (K121Q) of the ENPP1/PC-1 gene has been described (Pizzuti et al., 1999). ENPP1/PC-1 protein binds to the insulin receptor molecule, causing inhibition of the tyrosine kinase domain (Maddux et al., 2000). A Lys121Gln (K121Q) variant in exon 4 of the PC-1 gene (chromosome 6q22–23) has been associated with insulin resistance, type 2 diabetes and features of the metabolic syndrome (Pizzuti et al., 1999; Gu et al., 2000). This is in concert with the findings in genotype discordant sibling pairs; siblings with the Gln-allele had higher fasting glucose and impaired insulin action to glucose during an oral glucose tolerance test (Gu et al., 2000). Similarly, carriers of the Gln121 allele had impaired insulin-stimulated glucose uptake compared with carriers of the Lys121 allele (Rasmussen et al., 2000). The 121Q variant is associated with a progressive deterioration of the IR-atherogenic phenotype; among diabetic individuals, it is associated with earlier onset of type 2 diabetes and MI (Bacci et al., 2005). The 121Q variant binds insulin receptor more strongly than the 121K variant (Costanzo et al., 2001). It is therefore, a stronger inhibitor of insulin signaling and is associated with IR and related abnormalities in the vast majority of the studied populations (Pizzuti et al., 1999; Gu et al., 2000; Rasmussen et al., 2000; Frittitta et al., 2001; Abate et al., 2003; Kubaszek et al., 2003). In contrast, conflicting results have been reported about the effect of the ENPP1/PC-1 121Q variant on risk for type 2 diabetes (Pizzuti et al., 1999; Gu et al., 2000; Rasmussen et al., 2000; Hamaguchi et al., 2004; Kubaszek et al., 2004; Abate et al., 2005). A recent report shows no association with type 2 diabetes, obesity and quantitative metabolic traits in Danish white subjects (Gonzalez-Sanchez et al., 2003; Grarup et al., 2006).

2.14.2.4 Angiotensin-I Converting Enzyme (ACE) Gene: Angiotensin-I converting enzyme (EC 3.4.15.1, dipeptidyl carboxypeptidase) is a zinc metallopeptidase which cleaves C terminal dipeptide (His-Leu) from angiotensin I (Ang-I) and generates a vasoconstrictor angiotensin II (Ang-II) (Davis et al., 1997) which is associated with the regulation of blood pressure and maintenance of salt and water homeostasis in the body.
ACE is both pro-inflammatory and pro-oxidant, which leads to cellular toxicity and apoptosis (Phillips et al., 2002; Seshiah et al., 2002). Some studies have shown that systemic inflammation predicts the future risk of impaired glucose tolerance and type 2 diabetes (Freeman et al., 2002). There is also some evidence from pharmacogenetic studies that ACE inhibitors reduce the risk of developing diabetes (Rigat et al., 1990). It has been proposed that inflammation, possibly mediated by the increased Ang-II may contribute to the development of type 2 diabetes.

The ACE gene consists of 26 exons and spans 21 kb on chromosome 17. It is associated with the regulation of blood pressure and maintenance of salt and water homeostasis in the body (Ward, 1995). The ACE polymorphism identified by Rigat and co-workers (1990) is one of the best-researched polymorphisms. The ACE gene has been described in detail at genetic polymorphism level and contains many novel polymorphisms. This polymorphism of the ACE gene is based on the presence or absence of a 287-bp element on intron 16 on chromosome 17. Various studies have demonstrated that ACE insertion/deletion (I/D) polymorphism is associated with type 2 diabetes (Hsieh et al., 2000; Feng et al., 2002; Daimon et al., 2003; Stephens et al., 2005; Singh et al., 2006), hypertension (Pujia et al., 1994; Mastana and Nunn, 1997), coronary heart disease (Tiret et al., 1993; Ruiz et al., 1994), and diabetic nephropathy (Navis et al., 1999; Hsieh et al., 2000; Viswanathan et al., 2001). In a study of Pima Indians, Nagi et al., (1998) revealed that plasma ACE concentrations were associated with plasma triglyceride and total cholesterol levels. Recently, several components of the renin-angiotensin system (RAS) were detected in adipose tissue, and local RAS may be involved in the regulation of adipose tissue physiology and possibly in the pathophysiology of obesity and obesity-associated hypertension (Engeli et al., 2000). Therefore, ACE might be a good candidate gene for metabolic syndrome. It has been proposed that there is an association between the D allele and higher plasma levels of ACE, which further cumulates the diabetic complications (Marre et al., 1994; Stephens et al., 2005).

2.14.2.5 Paraoxonase 1 (PON1) Gene: Paraoxonase is a high-density lipoprotein (HDL)-associated enzyme, which protects lipoproteins from oxidation (Durrington et al., 2001). In vitro studies indicate that PON can significantly reduce lipid peroxide generation during LDL oxidation and thus may provide HDL-associated protection.
against atherosclerosis. Furthermore, serum PON activity has been found to be lower in patients with diabetes and familial hypercholesterolemia (Mackness et al., 1991), myocardial infarction (McElveen et al., 1986), fish-eye disease (Mackness et al., 1987), and Tangier disease (Mackness et al., 1989). PON has largely been studied for its role in hydrolyzing a large number of organophosphate compounds used in pesticides, insecticides, and nerve gases (La Du 1992; Davies et al., 1996). Serum PON levels and activity vary widely among populations of different ethnic backgrounds (Diepgen et al., 1986; Roy et al., 1991). Lipoprotein oxidation is potentially central to the development of microvascular disease in diabetes, as evidenced by the toxicity of oxidised lipoproteins for endothelial cells and pericytes in retinal capillaries (Lyons et al., 1994). Paraoxonase activity is known to vary widely between individuals. There is evidence that serum PON activity is reduced in patients with diabetes compared with healthy control subjects (Mackness et al., 1991; Abbott et al., 1995; Ikeda et al., 1998; Boemi et al., 2001). Lower paraoxonase activity was found in type 2 diabetes patients with neuropathy (Abbott et al., 1995) and retinopathy (Ikeda et al., 1998; Mackness et al., 1999; Mackness et al., 2000). Besides its protection against lipid oxidation, paraoxonase’s candidacy for an antiatherogenic role is further supported by the increased risk of atherosclerosis associated with polymorphisms of the paraoxonase gene (Kordonouri et al., 1996; Schmidt et al., 1998; Shih et al., 1998; Leev et al., 2001; Durrington et al., 2001).

The PON gene cluster maps to human chromosome 7q21–22, and several polymorphisms in the promoter and coding regions have been identified (Brophy et al., 2001a; Leev et al., 2000; Suehiro et al., 2000). Previous studies suggest that genetic polymorphisms influence gene expression levels and therefore PON activity (Brophy et al., 2001b). PON1 is genetically polymorphic, because of the occurrence of two common isoforms that differ by substitution of an amino acid (Gln–Arg) at codon 192 (Adkins et al., 1993; Humbert et al., 1993). Individuals homozygous for Gln (the Q allele) have lower PON activity than is seen in individuals homozygous for Arg (the R allele), the latter having eight times more activity than has Gln in hydrolyzing paraoxon (Davies et al., 1996; La Du 1996). PON activity is substrate dependent and may be quite opposite for different substrates (Furlong et al., 1988; La Du et al., 1988; Davies et al., 1996).
Notably, compared with the PON1 Q variant, the PON1 R variant is more efficient in hydrolyzing paraoxon but is less efficient in hydrolyzing three additional organophosphates: diazoxon, sarin, and soman (Davies et al., 1996). The R allele of the PON1 codon 192 polymorphism has been found to be associated with an increased risk of CHD (Ruiz et al., 1995; Serrato and Marian, 1995). Recently a study conducted in Asian Indians and Chinese populations has indicated that the R allele is a significant risk factor for CHD in Asian Indians but not in Chinese (Sanghera et al., 1997). However two additional studies carried out in Caucasians reported lack of association of the R allele with CHD (Herrman et al., 1996).