Cardiovascular Complications of Diabetes Mellitus

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This thesis is submitted to the Faculty of Medicine at the Royal College of Surgeons for the degree of Doctor in Medicine.

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Declaration

I declare that this thesis is entirely my own work and has not previously been submitted for a degree to this or any other institution.

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I guarantee that there is no commercial association that might arise or create a conflict of interest and that no benefit in any form has been or will be received from a commercial party related directly or indirectly to the subject matter of this thesis.

Dr. Fuad Khuder Faraj
Dedication

I would like to dedicate this work to my parents especially to my father whom I lost before I finished this work. I believe he is very happy that I did it. Also to my wife (Heba), brothers, sisters, Salwa and to my friends.
Abbreviations

MS: Metabolic Syndrome.
DM: Diabetes Mellitus.
T1DM: Type 1 Diabetes Mellitus.
T2DM: Type 2 Diabetes Mellitus.
ATP: Adult Treatment Panel.
NCEP: National Cholesterol Education Program.
BMI: body mass index.
DDC: diabetes day centre.
TC: total cholesterol.
TG: triglyceride.
LDL: low density lipoproteins.
HDL: high density lipoprotein cholesterol.
OGTT: oral glucose tolerance test.
FPG: fasting plasma glucose.
IFG: impaired fasting glucose.
IGT: impaired glucose tolerance.
IR: insulin resistance.
WCM: waist circumference.
WHR: waist-hip ratio.
HC: hip circumference.
SBP: systolic blood pressure.
DBP: diastolic blood pressure.
MI: myocardial infarction.
CVD: cardiovascular disease.
CHD: coronary heart disease.
CRP: C-reactive protein.
Fib: fibrinogen.
HOMA-IR: homeostasis model assessment insulin resistance.
AA: aminoacids.
PI: isoelectric point.
CAPN10 gene: Calpain 10 gene.
SNP: single nucleotide polymorphism.
ins/del: insertion/deletion.
LD: Linkage disequilibrium analysis.
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Aims of the Thesis.

The aims of this thesis are to attempt to identify subjects at increased risk of diabetes-associated cardiovascular disease (CVD) at various stages of the condition, focusing predominantly on the most common form of the condition, type 2 diabetes mellitus (T2DM). This includes attempting to identify a subset of the population that already has diabetes who may be at particularly increased risk of CVD, to determine if measuring some of the novel markers of CVD risk in subjects with diabetes may be useful in assessing cardiovascular risk, assessing risk of CVD in newly diagnosed diabetes and attempting to identify prevalence of cardiovascular complications in newly diagnosed patients. Finally I will determine whether genetic heterogeneity in the Calpain 10 (CAPN10) gene might be a useful indicator of diabetes risk in Irish subjects and as such be a way of identifying subjects at future risk of CVD prior to the onset of DM when interventions may be more effective in reducing CVD risk.

The specific aims are:

1. To determine the prevalence of the metabolic syndrome (MS) in patients with diabetes. To estimate and compare the prevalence of the MS in both T1DM and T2DM patients.

2. To evaluate the medium and long term cardiovascular risk utilizing the United Kingdom Prospective Diabetes Study (UKPDS) risk engine in patients who are newly diagnosed with T2DM.

3. To evaluate patients with newly diagnosed diabetes for the presence of significant subclinical macrovascular disease.

4. To study the association of novel risk of cardiovascular factors, fibrinogen and C-reactive protein (CRP) and albuminuria as markers of CVD risk in newly diagnosed patients with T2DM.

5. To estimate the prevalence and the relationship of three polymorphisms (43, 63 and 2037) and insertion-deletion 19 of the CAPN10 gene in a cohort of Irish patients with T2DM.
Conclusions

Despite major improvements in the management of T2DM, subjects with the condition remain at considerable risk of cardiovascular disease. In this work a number of strategies have been discussed whereby subjects with increased CVD risk could be identified earlier and targeted for particularly aggressive interventions. We suggest that an integral component of the annual assessment of patients with diabetes should be the calculation of an MS score and patients with particularly high cardiovascular risk can be targeted for especially aggressive risk factor management, with particular attention to hypertension and obesity, which are the most prevalent components of the MS. The Steno-2 study highlighted the importance of treatment of hyperglycaemia, dyslipidaemia and hypertension in patients with T2DM as measures to reduce the risk of life-threatening complications, particularly CVD which is the most common cause of death in population. Using an MS score could help identify those who require particularly aggressive, steno-2 style interventions.

The risk of vascular complications in patients with T2DM can also be calculated using the UKPDS risk engine. In newly diagnosed T2DM this risk is considerable in both genders at the time of the diagnosis. The determination of the risk of vascular events using this risk engine should be estimated at diagnosis and recalculated during subsequent years in order to ensure that optimized treatment of vascular risk factors is achieved.

Patients with newly diagnosed T2DM may have subclinical vascular complications that mainly involve the coronary and peripheral circulation. Screening for these asymptomatic or subclinical vascular complications at the time of the diagnosis in high risk patients may identify those who may most benefit from these interventions. Significant abnormalities may be identified in some subjects as was found in this study. Monitoring of inflammatory markers and/or WCM, BMI may also be helpful to identify high CVD risk patients.

Finally, modification of risk factors for developing T2DM and thus CVD, prior to the development of any dysglycaemia may be the most effective way of reducing the cardiovascular burden of the disease. Genetic screening of high risk subjects may be
worthwhile to identify highly susceptible subjects. CAPN10 gene study suggests associations between genetic variability in this gene and T2DM in the Irish population and further exploration of this and other genes may lead to earlier identification of subjects for interventions to reduce the risk of development of the disease.
Chapter One

1.1 General introduction of vascular complications of Diabetes Mellitus.

Diabetes Mellitus (DM) is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia).

Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease (CVD) have reached epidemic proportions globally (Zimmet 2002). The escalation in global diabetes prevalence is accompanied by significant increases in frequency of CVD (Zimmet 1997). Multinational Study of Vascular Disease in Diabetes indicates that CVD is the most common cause of mortality in patients with T2DM, accounting for 52% of all deaths (Morrish 2001). In addition to the significant morbidity and mortality associated with diabetes related CVD, these CVD conditions also impose substantial global economic burden (Zimmet et al 2001).

1.2 Epidemiology of Diabetes Mellitus.

By the year 2025, the prevalence of DM in adults is predicted to rise to 5.4% worldwide and will affect about 300 million people (King 1998). Most new cases of T2DM are forecast to occur in developing countries a 170% increase (from 84 million to 228 million) compared with a 42% increase (from 51 million to 72 million) in developed countries. By the year 2025, 75% of all people with DM will be from the developing countries, largely concentrated in urban areas. India, the Middle Eastern crescent, and sub-Saharan Africa will see the biggest increases in prevalence of DM, and the countries with the highest prevalence of DM will be India, China and the USA. The number of women with DM will continue to outnumber the number of men with the disease. Moreover DM is already appearing at an earlier age in developing countries, where most cases occur in people aged 45-64 years. In comparison, the average age of people with DM in developed countries is ≥ 65 years. This trend towards DM developing in younger age groups is likely to increase the risk of atherosclerotic CVD at young age.

The Diabetes Federation of Ireland has estimated that there are 200,000 people with diabetes in Ireland and a further 200,000 people who have diabetes but are unaware that they have the condition. Many of these people will only be diagnosed
through an acute medical event due to complications of long term untreated hyperglycaemia. A further 250,000 people many have impaired glucose tolerance (IGT) or "pre diabetes" of whom 5-8% per year will develop DM, if lifestyle changes are not made. About 6% of the health budget is spent on managing patients with DM, and most of this expenditure is spend on treating the complications of this condition.

1.3 Metabolic syndrome and Diabetes Mellitus.
Metabolic syndrome (MS) is a major risk factor for cardiovascular and metabolic diseases. Visceral obesity plays a central role in the development of MS and in its clinical consequences. Adipose tissue is now considered to be an active endocrine organ that secretes various humoral factors (adipokines), and its shift to production of proinflammatory cytokines in obesity likely contributes to the low-level systemic inflammation that is seen in MS associated chronic pathologies such as atherosclerosis (Nishimura S 2009). The circulating mediators of inflammation participate in the mechanisms of vascular insult and atheromatous change, and many of these inflammatory proteins are secreted directly from adipocytes and adipose tissue-derived macrophages. These highlight the role of adipose tissue in the development of a systemic inflammatory state that contributes to obesity-associated vasculopathy and cardiovascular risk (Berg 2005). The development of therapeutic strategies that target both adipose inflammation and IR may help to prevent T2DM and CVD in the emerging epidemic of obesity (Shah 2008).

The MS in European populations, however defined, is associated with an approximate 2-fold increased risk of incident cardiovascular morbidity and mortality (Dekker 2005). Several other studies, including a large population-based Italian study, the Framingham Offspring Study, the Botnia Study, the Kuopio Ischemic Heart Disease Study, the National Health and Nutrition Examination Survey II Mortality Study, the San Antonio Heart Study, and the DECODE study have shown the presence of MS using different definitions is associated with a significantly increased risk of total mortality and cardiovascular morbidity and mortality (Hu 2005) and this highlights the importance of identifying subjects with the MS.

There are abundant data linking CVD risk in patients with T2DM and MS. Carotid intimal media thickness (IMT) and intra-renal resistances are elevated at an early stage in T2DM patients with MS and are associated with a dysregulated production of fat-derived hormones and cytokines (Buscemi et al 2009). The clustering of visceral
obesity with at least 2 of the 3 components of MS, and diabetes are independently associated with increased carotid IMT. This suggests that the components of MS and T2DM interact to affect vascular thickness synergistically (Kawamoto 2007). T2DM is associated with a 0.13 mm increase in IMT compared with normal subjects (Broholl 2006). Patients with hypertension have an increased risk of developing MS which, in turn, increases the cardiovascular risk associated with increased blood pressure (Olsen 2009). MS even with the absence of frank diabetes significantly increased the risk of ischemic stroke or transient ischemic attack, in patients with atherosclerotic heart diseases (Koraen-morag 2005). MS score (number of MS components) provides a clinical useful index of MS severity and the associated atherosclerotic risk factor profile. It is also correlated with the angiographic severity of coronary artery disease and its clinical complications (Solymus BC et al 2004). A considerable proportion of T2DM patients have silent coronary heart disease (CHD). Patients with incipient or overt diabetes nephropathy should be examined for the presence of CHD, therefore the definition of MS may be modified for early detection of CHD in patients with T2DM (Tsai 2004). The risk of CHD and stroke increased three fold in subjects with the syndrome. (Isomaa et al 2001) and the African- Americans have the highest prevalence of MS and the highest CVD mortality of any ethnic group (Hall et al 2003).

Although MS is typically thought to be associated with T2DM, anecdotal reports identify an MS-type phenotype in patients with type 1 diabetes mellitus (T1DM) also. The results of major intervention studies in T1DM have led to greater use of intensive insulin regimens which can cause excessive weight gain in some subjects. Since abdominal obesity and hyperglycaemia are two components of the MS, an increase in the percentage of MS in patients with T1DM is not surprising, though its prevalence does not reach that reported for T2DM. For insulin sensitivity quantification in patients with T1DM the estimated glucose disposal rate consists of calculating a score based on clinical factors of the patient, which shows an inverse relationship with the development of micro- and macrovascular complications. Moreover, IR identification in T1DM may have therapeutic implications (Chillarón 2008). The benefits of improved glycaemia appear to outweigh the risks related to development of the MS. The Diabetes Control and Complications Trial (DCCT) showed that higher IR at base line, estimated by glucose disposal rate, was associated with increased subsequent risk of both micro- and macrovascular complications. The estimated glucose disposal rate
at baseline was significantly strongly predicted the development of retinopathy, nephropathy and CVD. Through mainly weight gain, the prevalence of the MS according to IDF criteria (see appendix) was increased steadily from baseline to year 9 in conventionally treated (from 15.5 to 27.2%) and especially in the intensively treated (from 13.7 to 45.4%) patients (Kilpatrick 2007).

Pambianco (2007) described the participating T1DM patients in the Pittsburgh Epidemiology of Diabetes Complications Study with complete 12-year follow-up. Clinical data were classified by baseline MS status according to three definitions: those of the National Cholesterol Education Program Adult Treatment Panel III (modified by the American Heart Association), the International Diabetes Federation (IDF), and the World Health Organization (WHO). The outcomes included coronary artery disease, renal failure, diabetes-related death, and the aggregate of these three major outcomes of diabetes. The MS prevalence was ranged from 8% (IDF) to 21% (WHO). All definitions showed reasonable specificity (> or = 83%) for each outcome. In patients with T1DM, there may be value in documenting the prevalence of the MS in order to improve the prediction of major complications.

The existence of multiple definitions for the MS has caused confusion and has resulted in many studies and research papers comparing the merits of each definition. Clinicians, researchers and a number of organizations have formulated many definitions of the MS. These are concordant on the essential components, the glucose intolerance, obesity, hypertension, and dyslipidaemia, but all differ in the detail and definitions. (See appendices). Microalbuminuria is a component of WHO definition of MS but not in other definitions. Although there is some doubt as to whether the MS actually constitutes a true syndrome, and the International Diabetes Federation and the American Diabetes Associated have cast some doubt on its usefulness, the above data suggest that it is still helpful to think of the conglomeration of CVD risk factors in this way as it clearly identifies subjects at increased risk of metabolic and cardiovascular disease.

The diagnostic criteria proposed by the Adult Treatment Program III of the National Cholesterol Education Program (ATPIII /NCEP) have led to greater awareness of the components and treatment strategies (table 1). Three out of five ATPIII/NCEP diagnostic criteria are required to diagnose MS. (Wilson 2003).
Table 1. National Cholesterol Education Program criteria for MS (ref 51).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Waist Circumference</td>
<td>&gt;102 cm</td>
<td>&gt;88 cm</td>
</tr>
<tr>
<td></td>
<td>&gt;40 inches</td>
<td>&gt;35 inches</td>
</tr>
<tr>
<td>Serum HDL</td>
<td>&lt;1.02 mmol/l</td>
<td>&lt;1.28 mmol/l</td>
</tr>
<tr>
<td></td>
<td>&lt;40 mg/dl</td>
<td>&lt;50 mg/dl</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>&gt; 6.1 mmol/l</td>
<td>&gt; 6.1 mmol/l</td>
</tr>
<tr>
<td>Hypertension</td>
<td>&gt;130/85 mmHg</td>
<td>&gt;130/85 mmHg</td>
</tr>
<tr>
<td>Serum triglyceride</td>
<td>&gt;1.7 mmol/l</td>
<td>&gt;1.7 mmol/l</td>
</tr>
<tr>
<td></td>
<td>&gt;150 mg/dl</td>
<td>&gt;150 mg/dl</td>
</tr>
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</table>
1.4 Epidemiology and clinical manifestations of cardiovascular diseases in Diabetes Mellitus.

As alluded to above, T2DM has reached epidemic proportions in many parts of the world, largely related to the explosion in obesity rates that has occurred in many countries in recent decades. Many of these patients will die prematurely from the cardiovascular (large blood vessel or macrovascular) complications of the disease. Diabetes complications can also affect small vessels (microvascular). Microvascular complications can lead to nephropathy, neuropathy and retinopathy. Diabetes nephropathy is the commonest cause of end stage renal disease in most western countries and retinopathy is the most common cause of blindness in working age population. Diabetes vascular and neuropathic complications make diabetes the leading cause of amputation. However it is well recognized that it is the macrovascular disease that underlies most of the excess mortality in patients with T2DM, causing up to 75% or 2-4 folds increase in the prevalence of all deaths, principally as a result of myocardial infarction and stroke (KFPI 1996, Feskens 1992). Elevated plasma glucose levels in the long-term contribute in particular to microvascular complications, they are also associated with increased risk of macrovascular disease. Plasma glucose should probably be considered as a continuous CVS risk factor, similar to BP and TC according to the meta-regression analysis of 20 studies including 95,783 individuals without DM who were followed for 12.4 years and experienced a total of 3707 CVS events (Coutinho 1999). This meta-analysis showed that increasing plasma glucose level is a risk factor for CVS events across a broad range of glycaemia and even within a range that is below the DM threshold.

Premature aggressive atherogenesis is the hallmark of diabetes macrovascular disease. Histologically identical in the non-diabetic population, atheromatous lesions are more severe and widespread in patients with diabetes and result in the clinical presentation of vascular disease at an earlier age that is of greater severity than in non-diabetic patients. T2DM can be viewed as a collection of risk factors for CVD, closely linked with IR, hyperinsulinaemia, hypertension, visceral obesity, dyslipidaemia, inflammation and alterations in fibrinolytic system. The management of all factors is essential to reduce the morbidity and premature mortality associated with T2DM.
The clinical manifestations of atherosclerosis occur primarily in 3 vascular beds: coronary arteries, lower extremities, and extra-cranial carotid arteries. In one population-based study (Haffner 1998), the 7 year incidence of first MI or death for patients with diabetes was 20% but was only 3.5% for non cardiac patients. History of MI increased the rate of recurrent MI or cardiovascular death for both groups 18.8% in non diabetes persons and 45% in those with diabetes.

In patients with unstable angina pectoris or non-Q-wave MI compared with control, the presence of diabetes increases the risk of in-hospital MI, complications of MI, and mortality. Data from the OASIS registry, a 6-nation study of unstable angina and non-Q-wave MI, show that diabetes independently increased risk of death by 57% (Malmberg et al 2000). The age-adjusted relative risk of mortality for patients with diabetes in the GISSI-2 trial of fibrinolytic therapy was 1.4 for men and 1.9 for women, regardless of intervention assignment (Zuanetti 1993). Patients with diabetes also have an adverse long-term outcome after MI, increased rates of reinfarction, congestive heart failure, and death (Malmberg et al 2000).

In a Finnish study of patients with acute MI, diabetes increased 28 day mortality by 58% in male and 60% in women. In fact, the 5 year mortality rate may be as high as 50% for patients with DM more than double that for non diabetes patients (Herlitz 1998).

There are also considerable data to suggest that diabetes increases the risk of heart failure. The Framingham Heart Study demonstrated over years, those patients with DM has a high incidence of heart failure which contributes significantly to their increased cardiovascular morbidity and mortality (Garcia 1973, Kannel 1974). The age-adjusted risk of developing heart failure was 2.4 times higher in men with diabetes than men without diabetes. In women, the impact of diabetes was even more striking, with risk of heart failure being 5.1 times greater in the presence of DM. The incidence of heart failure in older patients was substantial 22-27/1000 patient year over the 18 years after study initiation. Although Framingham and many other studies did not distinguish between patients with T1DM and T2DM, most patients probably had T2DM. However, in the era before treatment of T2DM with combined oral agents, many patients were treated with insulin; those patients had a four- to five fold increased risk of heart failure compared with non diabetic patients.
Diabetes is often present in older patients and is associated with hypertension, CHD and obesity (Ho 1993) which also predispose to the development of heart failure. An important issue is whether diabetes independently increases the risk of heart failure in these patients. In the Framingham study, the diabetes-associated risk of heart failure persisted after adjustment not only for the age, hypertension, hypercholesterolemia and obesity, but also for clinically evident CHD (Chen 1999).

1.5 Peripheral arterial disease.
Epidemiological evidence also confirms an association between diabetes and increased prevalence of PAD. Individuals with diabetes have increased rates of PAD (Newman et al 1993), both in males 11.9% and females 16% (Meijer 1998) and they have absent femoral bruits and pulses more than non diabetic subjects (Abbott 1990). The duration and severity of diabetes correlates with incidence of PAD (Jude 2001). Diabetes changes the nature of PAD. Patients with DM more commonly have infrapoplitical arterial occlusive disease and vascular calcification than non diabetic cohorts (Becks et al 1995). The Hoon study (Uusitupa 1990) examined the rates of PAD among groups ranging from patients with normal glucose tolerance to those with diabetes requiring multiple medications. The 7% prevalence of abnormal ankle-brachial indices in individuals with normal glucose tolerance increased to 20.9% in those requiring multiple hypoglycaemic medications. Patients with diabetes more commonly develop the symptomatic form of PAD - intermittent claudication - and other requires amputation. In the Framingham cohort (Kannel 1985), DM increased the risk of claudication by 3.5 fold in men and 8.6 fold in women and results in most non traumatic lower extremity amputations in the United States.

1.6 Cerebrovascular disease.
Diabetes adversely affects the cerebrovascular arterial circulation. The risk of stroke is increased 15% to 40% for patients with diabetes (Jamrozik 2000 and the frequency of diabetes among patients presenting with stroke is 3 times more than matched controls (Himmelmann 1998).). Age, plasma TC and diabetes were positively related to the prevalence of extracranial carotid artery atherosclerosis and to the degree of vascular narrowing (Fabris et al 1994). In patients undergoing dental panoramic radiographs, patients with DM were found to have a 5 fold excess prevalence of calcified carotid atheromas (Friedlander 2000).
In the Multiple Risk Factors Intervention Trial (MRFIT) (Stamler 1993) of 347,978 men, subjects taking medications for diabetes were 3 times as likely to develop a stroke. Diabetes particularly affects the risk of stroke among younger patients (Jorgensen 1994). In the population younger than 55 years, diabetes increases the risk of stroke in white and black men and women. The Baltimore-Washington Cooperative Young Stroke Study (Rohr et al 1996) examined 296 cases of incident ischemic stroke among white subjects aged from 44 to 81 years. The presence of diabetes markedly increased the risk of stroke, ranging from 3.3 for black women to as high as 23.1 for men. Additionally DM affects stroke outcomes. It increases the risk of stroke-related dementia more than 3-fold (Luchinger 2001), doubles the risk of recurrence, and increases total and related mortality (Tuomilehto et al 1996).

1.7 UKPDS 56 and cardiovascular diseases in Diabetes Mellitus.
Aggressive treatment of cardiovascular risk factors can produce dramatic reductions in cardiovascular mortality and morbidity (Haffner 2000). Many studies have demonstrated the benefits of intensive treatment in reducing cardiovascular mortality and morbidity in patients with diabetes.

The Diabetes Control and Complications Trial (DCCT 1993) in patients with TIDM and the UK Prospective Diabetes Study (UKPDS 1998) in T2DM found that diabetes-related microvascular complications can be eased with good glycaemic control. The DCCT cohort with good glycaemic control had reduced CVD events many years later.

The UKPDS 56 risk engine (Stevens 2001) has confirmed that other vascular risk factors are more important determinants of CHD than FPG measures, and there is presently a controversy as to whether pre-prandial, post-prandial or integrated glycaemic measures are more predictive of CVD in DM. This new UKPDS risk engine can be utilized to assess cardiovascular risk and is specific for T2DM. It incorporates the HbA1c, systolic blood pressure (SBP), TC/HDL-C ratio, age, sex, ethnic group, smoking and the duration of the DM, but interestingly it does not include microalbuminuria. The risk engine is used to predict CHD risk, defined as fatal or non fatal MI or sudden death, but could be extended to report morbidity and mortality and to recognize different levels of risk for CHD, PVD and cerebrovascular disease.
1.8 Screening for Diabetes Mellitus and cardiovascular complications.

Because diabetes is such a major epidemic and because it becomes more challenging to prevent in particular the macrovascular complications when the condition has developed, there is a need to focus the efforts on prevention of the disease. This is especially true for T2DM since there are now many studies showing that onset of this condition can be delayed or prevented (Mauricio 2008). Ideally screening should be based at the population level, concentrating on the groups with increased risk of diabetes (early prevention). Currently, it seems that screening studies can be conducted only in the groups with high risk of T2DM (late primary prevention). In Ireland screening for the DM can be easily done at the primary health care by a FPG. This allows for relatively early detection of disturbances in carbohydrate metabolism. It has been suggested that screening for diabetes should be performed in subjects aged > 35 years who are overweight or obese and have at least one additional risk factor of arteriosclerosis (hypertension, dyslipidaemia, smoking and family history of T2DM). That was suggested by a study of the prevalence of undiagnosed diabetes in a healthy population (2700 subjects, aged 35-65 years) of professionally active inhabitants in Pleszew. Clinically latent diabetes or impaired glucose tolerance (IGT) was found in 5.3% cases. 92.8% patients with IGT or diabetes were obese or overweight (Wierusz-Wysocka 2001).

Little is known as to what is the most appropriate screening for CHD in patients with DM with no clear evidence of CHD. The American Diabetes Association (ADA)/American College of Cardiology (ACC) have developed a consensus regarding which patients are at increased risk for cardiac events, which patients should be screened, and what is the appropriate follow-up for a positive screening test result. Once patients with DM diagnosed with CHD further management should be established to prevent complications. While no evidence exists for risk intervention in patients with DM and asymptomatic CHD, aggressive therapy has been shown to reduce cardiovascular mortality in individuals with diabetes and known CHD. Thus the identification of CHD in asymptomatic patients may be important in order to institute aggressive secondary preventative measures. While stress testing clearly indicated in patients with DM who also have established CHD, it is not known what test would be appropriate in patients who do not have known CHD.
The followings are indications set out by the ADA/ACC consensus panel for screening for CHD in subjects with diabetes:

- Typical or typical cardiac symptoms.
- Resting ECG suggestive of ischemia or infarction.
- Peripheral or carotid occlusive arterial disease.
- Sedentary lifestyle, age ≥35 years, and plans to begin a vigorous exercise program.
- Two or more of the risk factors listed below in addition to diabetes:
  - Serum TC ≥ 6.18 mmol/L, LDL -C ≥ 4.12 mmol/L, or HDL-C < 0.9 mmol/L.
  - BP >140/90 mmHg.
  - Smoking.
  - Family history of CHD.
  - Positive micro/macrolubiminuria test.

Cardiac stress testing is indicated or warranted in patients with:

1. Atypical or typical cardiac symptoms.
2. A resting ECG showing evidence of infarction or ischemia and patients with PAD.
3. In patients with peripheral and carotid arterial diseases patients, beginning a vigorous exercise program, a minor ST-T wave changes on ECG or 2 or more vascular risk factors.
4. Multiple risk factors in the same patient with diabetes increase the possibility of identifying significant CHD.
5. Microalbuminuria is also an important risk factor for cardiovascular mortality.

Stress test is not indicated in asymptomatic diabetes patients with one or fewer factors with normal ECG and in patients with autonomic neuropathy. The later is strongly associated with cardiac morbidity and mortality but there is insufficient data as to whether this is an independent risk factor sufficient to warrant this test.

The newly T2DM patients exhibit a higher and greater degree of early atherosclerosis of the carotid intimal media thickness (CIMT) than normal glucose tolerant subjects.
matched for age and sex (Temelkova-Kurktschiev 1999, Güvener 2000). Güvener (2000) found the age; BMI and the presence of CHD have a strong influence on the atherosclerotic process of the carotid arteries in patients with DM. A study by Butt (2009) described in patients with T2DM, both sides of the CIMT with risk factors of atherosclerosis are correlated with duration of the disease and BMI and had an inverse correlation with HDL cholesterol. CIMT has no significant correlation with other variables; age, gender, history of ischemic heart disease, hypertension, smoking, HbA1c, serum TC and TG levels.

In healthy individuals measurement of the ABI may improve the accuracy of cardiovascular risk prediction of cardiovascular events and mortality independently of the Framingham risk score. (Fowkes et al 2008). In patients with DM there is a close correlation between low ABI and various cardiovascular risk factors and it is a useful noninvasive tool for predicting CHD severity (Chang 2009). Systolic blood pressure (SBP), duration of diabetes and serum HDL cholesterol with blood urea are independent risk factors influence the development of Peripheral and carotid artery diseases in T2DM patients (Bosevski 2009). Polenova (2009) described a low ABI in overweight patients with T2DM and prediabetes besides other factors as age, smoking and white blood cells count but not lipids.

1.9 Environmental factors influence the pathogenesis of Diabetes Mellitus.

1.9.1 Obesity and Diabetes Mellitus.

Obesity and T2DM are linked to complex multifactorial chronic diseases that develop from an interaction of genotype and environment. The World Health Organization (WHO 1999) has declared overweight as one of the ten top risk conditions in the world and one of the top five in developed nations. World wide, more than one billion adults are overweight and over 300 million are obese. Most countries are experiencing dramatic increases in obesity as in China in whom, the prevalence of overweight individuals doubled in women and tripled in men from 1989 to 1997 (Wolf 1998). The National Health and Nutrition Examination survey (NHANES) data of 1999-2000, showed that almost 65% of adult populations in the US are overweight compared to 56% seen in the data between 1988 and 1994 and there is an increase in
the prevalence of obesity form 23 to 31% over the same period of time. The prevalence of obesity in children and adolescents also increased by 36% during this time. Assuming continued increase at this rate, in 2008 the prevalence of obesity will be 39% (Hill 2003). The prevalence of obesity in the US is increased from 12% in 1991 to 17.9% in 1998 in all states, in both sexes, across age groups, races, educational levels and occurred regardless of smoking status (Mokdad 1999).

The North/South Ireland Food Consumption Survey in 2000 (NSIFC), showed that only 42% of the population was in the normal weight range. A total of 18% were obese (20% of men and 16% of women) and (39%) were over weight (46% of men and 33% of women). Since 1990, the prevalence of obesity had increased by (67%) overall, up 1.25 fold in women (from 13%) and up 2.4 fold in men (from 8%). The prevalence of serious obesity also doubled in Britain between 1980-1991 (Prentice 1995).

Although most of these studies used BMI as the marker of obesity, there is now a greater appreciation of the closer relationship between waist circumference (WCM) as a marker of obesity and clustering of other risk factors for CVD (Zhao 2003). There is a considerable association between measures of abdominal obesity, BP and plasma lipid levels. WCM is the measure of abdominal obesity most highly correlated with these CVD risk factors (Reeder et al 1997).

The risk of developing T2DM increases with increasing obesity in both men and women. This risk is increasing in overweight and obese adults by approximately 4% and 11-20% respectively (Field et al 2001). Similar correlation has been observed between increasing grades of obesity and IR, a key underlying defect of both T2DM and MS (Duman 2003). Overweight individuals have an increased relative risk and a population-attributable risk for the development of CVD as a clinical consequence of MS (Wilson 2002). Obesity is an independent risk factor for death from CHD (Xavier Pi-Sunyer 1993, Kuth et al 2002) and is strongly associated with several major health risk factors (Mokdad et al 2003) including stroke for which BMI is considered a continuous variable and a modifiable risk factor. Each unit increase of BMI was associated with a significant 6% increase in the adjusted relative risks of total ischemic and hemorrhagic stroke (Kuth et al 2002). Women with male pattern obesity have less favourable lipoprotein levels and at greater risk of CHD.
Subphenotypic components of human obesity such as percent body fat are continuously varying traits reflecting contribution from numerous quantitative trait loci (Chagnon 2003).

1.9.2 Obesity-insulin resistance and inflammatory markers.

Intra-abdominal or visceral adipose tissue has been proposed as the major site of fat deposition associated with the adverse metabolic sequences of obesity (Wajchenberg 2000). Increased intra abdominal adiposity determined by a simple waist/hip ratio (WHR) is a strong independent predictor of vascular endothelial dysfunction even in healthy overweight adults (Brook 2001). Intra-abdominal fat is not the only location of stored fat linked with MS. The increased amount of fat in the liver and muscle are also associated with metabolic abnormalities (Westerbacka et al 2004, Forojiangi et al 1999). Several recent studies have found increased IR associated with non alcoholic fatty liver disease (Marchesini et al 2001, Marceau et al 1999). It has been suggested that fat accumulation in the liver or muscle is more closely related to features of IR than the amount of intra-abdominal fat (Antony 1997). However there is a clear association between visceral adiposity and cardiometabolic risk and it is thought that the abdominal adiposity results in IR, by increasing fatty acid flux in the portal and systemic circulations. Intra-abdominal adipose tissue may also contribute to other mechanisms of increased atherosclerotic risk being a source of inflammatory, prothrombotic, and fibrinolytic factors. The most frequently mentioned atherosclerotic factors include nonesterified fatty acids, cytokines, plasminogen activator inhibitor (PAI-1), adiponectin, leptin and resistin (Gueree - Millo 2002). Recent studies have suggested that some of these factors (most notably adipocytokines like tumour necrosis alpha and interleukin-6) may not actually be produce by adipocytes, but are released by macrophages that have migrated to adipose tissue (Curat et al 2004). Whereas intra-abdominal, liver and muscle fat stores, which are associated with increased CVD risk, subcutaneous fat might have favourable effects on CVS risk.

Inflammation has been implicated as an important aetiological factor in the development of both IR and T2DM. This is demonstrated by the associations between elevated (but ‘normal range’) levels of circulating acute phase inflammatory markers, typified by C-reactive protein (CRP), and indices of IR and the development of T2DM. There is a strong association between CRP and fat mass. This interaction of
Adiposity and IR may interact to raise CRP (Kriketos et al 2004). The body fat has been suggested to be the primary determinant of circulating inflammatory marker levels in the basal state and marginally elevated levels of circulating interleukin-6 and CRP in obesity are a consequence rather than a cause of IR. Clearly, CRP is one of the strongest inflammatory markers associated with increased cardiometabolic risk and, in addition, may participate directly in the arterial cell wall mechanisms leading to atherosclerotic lesions and cardiac events (Burke et al 2002). CRP is an acute phase B-globulin synthesized in the liver and is normally present as a trace constituent in serum. CRP levels may rise during infectious and non-infectious inflammatory conditions such as rheumatoid arthritis, cardiovascular and peripheral vascular diseases. Within 24-48 hours following acute tissue damage, serum CRP level dramatically rises to approximately 1,000 times the constituent level. CRP may remain elevated for several days before returning to normal. Increased CRP is the most recognized marker of IL-6 action. However there are numerous other IL-6-dependent factors which may contribute to cardiovascular risk. Increases of fibrinogen, another acute-phase reactant, are mediated by IL-6, as are increases in both platelet number and platelet activity, all of which would contribute to the risk of clot formation (Burstein et al 1996). Circulating TNF-α concentration, in turn, also rises with increasing obesity and correlates with IR (Tsigos et al 1999). Moreover, endothelial cells and vascular smooth muscle cells are targets of IL-6 action, resulting in increased expression of adhesion molecules and activation of local rennin-angiotensin pathways, both modifications that favour vascular wall inflammation and damage (Wassmann et al 2004). The high levels of the above inflammatory markers like leptin, Endothelin-1, rennin angiotensin system, free fatty acids, TNF-α, CRP as well as other circulating inflammatory markers increasing the evidence to suggest a role of inflammation in the pathogenesis of IR and atherosclerosis (Wheatcroft 2003).

In summary, visceral obesity is associated with a chronic, low-grade inflammatory state, suggesting that inflammation may be a potential mechanism whereby obesity leads to IR. This is followed by the consequences of vascular complications due to associated vascular risk factors, inflammatory markers and atherosclerosis.
1.9.3 Genes associated with T2DM.

Genetic factors contribute to the development of diabetes. It is apparent that diabetes mellitus is not a single polygenic disease, but consists of numerous distinct genetically heterogeneous entities. The delineation of genetic heterogeneity has important implications for genetic counselling, treatment, and prognosis, and is essential in determining the pathophysiology and aetiology of a disorder. Unless each of the distinct component diseases of a symptom complex has been identified and separated, the basic pathophysiological abnormalities cannot be identified, and a common biochemical abnormality will not be found in all affected individuals. In approaching the genetics of any common disease disorder in man, heterogeneity must first be unmasked, and genetic and pathogenetic studies must be separately performed in each of the components, etiologically distinct disorders producing the aberrant phenotype. T2DM is a heterogeneous metabolic disorder, and is characterized by defects of both insulin secretion and insulin action. Both T2DM and IR are complex traits in which multiple gene effects and metabolic and environmental factors combine to contribute to the overall pathogenesis of the conditions. This complexity has complicated the search for susceptibility genes and has led to different but complementary approaches being used for the detection of gene effects. These include the study of monogenic cases of insulin resistance and T2DM, association studies of candidate genes and genome-wide scans. The peroxisome proliferator-activated receptor gamma (PPARγ) and calpain-10 (CAPN10) genes have been identified as T2DM susceptibility genes (McIntyre 2002).

Very little is known about the specific genetic susceptibility variants that contribute to the clinical and metabolic abnormalities in T2DM. There are several reasons for this:

- Unlike single gene disorders, there is evidence that multiple gene defects influence overall susceptibility to T2DM.
- There is variable expression of the genetic predisposition, which is influenced by lifestyle and environmental factors such as dietary intake and exercise.
- The metabolic consequences of the impaired insulin secretion and insulin action in T2DM in turn feedback and influence the key metabolic defects. Thus, hyperglycaemia and elevated blood nonesterified fatty acid levels
exert adverse effects on both insulin secretion and peripheral insulin action (Unger 1995).

This overall complexity has made it extremely difficult to establish direct links between the metabolic changes and the underlying genetic susceptibility variants. As a consequence, several alternative approaches have been pursued to define the genetic susceptibility to IR and T2DM (McIntyre 2002).

There is much evidence for the clear genetic variation in the pathogenesis of T2DM and IR. First, concordance rates for T2DM are higher in monozygotic twins, who share 100% of their genes, than in dizygotic twins, who share only 50% (Barnett 1981, Newman 1987). A second line of evidence comes from segregation analyses of T2DM transmission through families. However, no consistent inheritance pattern has emerged, with some studies suggesting a major gene effect while others are more in keeping with polygenic inheritance (Cook 1993). Nonetheless, non diabetes first-degree relatives of T2DM patients have an almost three fold increased lifetime risk of T2DM in comparison to the background population (Kobberling 1982).

IR is an early metabolic feature of nondiabetic first-degree relatives of T2DM patients (Eriksson et al 1989, Humphriss et al 1997) and also shows familial clustering in keeping with an underlying genetic predisposition (Lillioja et al 1987, Martin 1992). The defects of insulin action are retained in cultured skeletal muscle cells from IR subjects and T2DM patients (Henry 1995, Jackson 2000) suggesting that genetic variation contributes to decreased insulin action. While IR is a common feature of T2DM, the severity and clinical importance varies considerably across the T2DM population (Defronzo 1991).

1.9.3. A. Maturity onset diabetes of the young (MODY).

The genetics of certain forms of diabetes are now well described and particularly so in monogenic forms of diabetes typical of maturity onset diabetes of the young (MODY). MODY is a comparatively rare type of DM, but the early age of onset and clear autosomal dominant inheritance pattern generates multigenerational pedigrees ideal for classical linkage studies. MODY is characterized by β-cell dysfunction and a young age of diagnosis, usually before 25 years old. There are at least six genes
(table2) implicated in the pathogenesis of MODY (Frayling et al 2001, Pearson et al 2001). A study looking at the contribution of various genes to the onset of diabetes in 90 MODY families found that 63% had mutations in hepatocyte nuclear factor-1α (HNF-1α; MODY3), 20% had glucokinase mutations (MODY2), 2% had HNF-4α mutations (MODY1), and 1% had HNF-1β mutations. No mutations of the insulin promoter factor 1 (IPF-1) or NeuroD1/BETA2 were detected in this cohort although they have been shown to be causes of MODY (Stoffers 1997, Malecki et al 1999). The study of MODY families has been instrumental in defining the transcription factor cascade involved in normal β-cell function, and the pivotal role of glucokinase as the β-cell glucose sensor. For the more common forms of MODY, genotype phenotype correlations have emerged. Individuals with HNF-1α mutations show a fairly rapid decrease in β-cell function and are at risk of microvascular complications. Conversely, individuals with glucokinase mutations characteristically have only moderately raised blood glucose levels that tend to be stable throughout life, and as a consequence the risk of complications is very low (Hattersley 1998). Despite the importance of glucokinase and the hepatic nuclear transcription factors in the regulation of normal β-cell dysfunction, none of the variants identified to date has been shown to play a role in the pathogenesis of the classical form of T2DM (Elbein 1993, Frayling et al 1999, Frayling et al 2000) However, point mutations of IPF-1 have, on the contrary, been shown to increase the relative risk of T2DM (Hani et al 1999, Macfarlane et al 1999). These mutations, D76N, Q59L, C18R and R197N, cause reduced glucose-stimulated insulin secretion and might have a collective prevalence as high as 6% in the classical T2DM population (Hani et al 1999). Furthermore, an insertion mutation, InsCCG243, has been shown in one family to result in a late-onset autosomal dominant T2DM. The following table describes the various forms of MODY.
<table>
<thead>
<tr>
<th>Criteria</th>
<th>HNF-4α MODY1</th>
<th>Glucokinase MODY2</th>
<th>HNF-1α MODY3</th>
<th>IPF-1 MODY4</th>
<th>HNF-1β MODY5</th>
<th>Unknown MODY-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency % in UK</td>
<td>2</td>
<td>20</td>
<td>64</td>
<td>&lt;1</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Penetrance % of Mutation at age 40 years</td>
<td>&gt;80</td>
<td>45</td>
<td>95</td>
<td>80</td>
<td>95</td>
<td>unknown</td>
</tr>
<tr>
<td>Onset of Hyperglycaemia</td>
<td>Adolescence</td>
<td>Early childhood</td>
<td>Adolescence</td>
<td>Early Adulthood</td>
<td>Similar to HNF-1α</td>
<td>uncertain</td>
</tr>
<tr>
<td>Severity of Hyperglycaemia</td>
<td>Progressive</td>
<td>Mild</td>
<td>Progressive</td>
<td>Similar to HNF-4α</td>
<td>Similar to HNF-1α</td>
<td>Variable</td>
</tr>
<tr>
<td>Microvascular Complications</td>
<td>Frequent</td>
<td>Rare</td>
<td>Frequent</td>
<td>Few data</td>
<td>Frequent</td>
<td>Variable</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>β- cell dysfunction</td>
<td>β- cell dysfunction</td>
<td>β- cell dysfunction</td>
<td>β- cell dysfunction</td>
<td>β- cell dysfunction</td>
<td>β- cell dysfunction</td>
</tr>
<tr>
<td>Other features</td>
<td>Reduced birth weight</td>
<td>Low renal threshold, Sensitivity to Sulphonylurea</td>
<td>Pancreatic agenesis in homozygous</td>
<td>Renal cyst Proteinuria renal failure, uterine &amp; genital abnormalities</td>
<td>-------</td>
<td></td>
</tr>
</tbody>
</table>
1.9.3. B. Monogenic DM.

- Mitochondrial DM.

Mutations in mitochondrial DNA (mtDNA) can lead to impaired oxidative phosphorylation, cellular dysfunction and ultimately disease, including DM. As the mitochondrial genome is passed exclusively down the maternal line, associated diseases show a maternal pattern of inheritance. The commonest mutation is an A to G transition at position 3243 of the tRNA_{Leu(UUR)} gene. Originally this mutation was described in relation to the MELAS syndrome (myopathy, encephalopathy, lactic acidosis and stroke-like episodes, which is sometimes associated with diabetes) (Goto 1990). Subsequently, however, the same mutation has been shown to cause maternally inherited diabetes and deafness (MIDD) (Van den Ouweland et al 1992, Maassen 1996). This is characterized by bilateral sensori - neural deafness and diabetes that usually presents in middle age. Impaired insulin secretion is the primary metabolic defect and as a consequence affected patients usually proceed to insulin therapy. There is some evidence that in general T2DM there is an excess transmission via the maternal line compared to the paternal line. It was therefore thought that mitochondrial defects might play an important role in the pathogenesis of T2DM. However, this has not been borne out by studies to date, with the frequency of the A to G mutation affecting only 0.5–2.8% of the general diabetic population (Vionnet 1993, Newkirk et al 1997) as well as the 3243 mutation, other mitochondrial gene defects have been linked to the development of T2DM (Daly 1996).

- Monogenic forms of insulin resistance.

Mutations described to date causing insulin resistance tend to generate cases of extreme IR. In excess of 60 mutations have been described in the insulin receptor gene and as a result a clear genotype–phenotype association is emerging (Krook 1996, Whitehead et al 1998, Whitehead 1998). The most abundant mutations are heterozygous leading to decreased tyrosine phosphorylation of the β-subunit of the insulin receptor.

- Type A insulin resistance syndrome is the most common phenotype, which includes acanthosis nigricans and hyperandrogenism without obesity or lipoatrophy.
• Homozygous or compound heterozygous mutations lead to the Rabson–Mendelhall syndrome with severe impairment of insulin receptor function (Krook 1996).

• Dunnigan type familial partial lipodystrophy is an autosomal dominant form of IR. The LMNA gene encodes laminin types A and C and mutations of the gene are associated with lipodystrophy and IR (Hegele 2000, Shackelton et al 2000). Fat distribution is normal in affected individuals during childhood, but from puberty there is progressive loss of subcutaneous fat from the limbs giving a muscular appearance. There is also an increased prevalence of T2DM, hypertension and dyslipidaemia, i.e. features of the MS, and of premature CHD (Hegele 2001).

• The peroxisome proliferator-activated receptors (PPARs) are a group of three nuclear receptor isoforms, PPARalpha, PPARgamma and PPARdelta, encoded by different genes, and they form a subfamily of the nuclear receptor superfamily. The clinical interest in PPARs originates with fibrates and thiazolidinediones, which, respectively, act on PPARalpha and PPARgamma and are used to ameliorate hyperlipidaemia and hyperglycaemia in patients with T2DM. PPARs play a central role in these patients due to their ability to regulate the expression of numerous genes involved in glycemic control, lipid metabolism, vascular tone and inflammation. PPARs are ligand-regulated transcription factors that control gene expression by binding to specific response elements (PPREs) within promoters. PPARs bind as heterodimers with a retinoid X receptor and, upon binding agonist, interact with cofactors increasing the rate of transcription initiation (Berger 2002, Biscetti 2009). Two families with loss of function mutations (P476L and V290M) in the ligand-binding domain of PPARγ have been described. The individuals with these mutations were found to have severe IR with early-onset hypertension, T2DM, dyslipidaemia, low levels of high density lipoprotein (HDL)-cholesterol and high triglyceride levels (Barroso et al 1999). Phenotypically they have high a waist-to-hip ratio, but normal body mass index (BMI) and no evidence of lipodystrophy. Females with the
mutation have primary oligo - or amenorrhea and are subfertile (Barroso et al 1999). Studies to determine if mutations in the PPARγ gene predispose to diabetes in the general population show a complex relationship.

- **Insulin gene.**

In a study of 155 European T2DM parent–offspring trios, variation in the variable number tandem repeat (VNTR) regulatory polymorphism 5′ to the insulin gene (INS) has been shown to influence susceptibility to T2DM, and this effect is mediated exclusively by the paternal derived allele (Huxtable et al 2000).

- **SUR-1 gene**

The SUR-1 gene encodes the sulphonylurea receptor-1 that plays a key role in glucose-stimulated insulin secretion. Two polymorphisms in SUR-1 have been identified, in exon 16 (the 3c → t variant in the splice acceptor site) and in exon 18 (Thr759Thr, ACC → ACT) are associated with diabetes.

- **IRS-1 gene.**

Insulin receptor substrate-1 (IRS-1) is a major docking protein linking the tyrosine-phosphorylated insulin receptor to the downstream elements of the insulin signalling pathway (White 1997). The Gly972Arg polymorphism in the IRS-1 gene was associated with decreased fasting insulin levels in T2DM patients (Almind 1993), and a subsequent study of human islets showed that those with the Gly972Arg variant had impaired insulin secretion (Porzio et al 1999).

- **PC-1 gene.**

PC-1 is a membrane glycoprotein that inhibits autophosphorylation of the insulin receptor and might therefore play a role in modulating insulin action. A single nucleotide polymorphism in exon 4 of the PC-1 gene generates Q (Gln121) allele and the more common K (Lys121) allele. The Q allele was associated with markers of IR in non diabetes subjects, although the frequency of the allele was not increased in T2DM patients (Gu et al 2000).
- **Glycogen synthase gene.**

An XbaI polymorphism has been described in the glycogen synthase gene and associated with IR (Groop et al 1993). Subsequently, the discordant sib-pair analysis approach has shown that the sibling with the rare A2 allele had more features of the MS and increased risk of myocardial infraction (Orho-Melander 1999). The essence of this approach is that families with the condition of interest are genotyped using a set of polymorphic microsatellite markers that span the genome (Lander 1994, Gloyn 2001).

- **TCF7L2**

The discovery of TCF7L2 as a diabetes gene illustrates foundation of novel true diabetes genes. A common variant of TCF7L2 gene, when present in two copies, is associated with an approximate 2-fold higher risk of T2DM (Weedon 2007). These variants have been consistently associated with T2DM in populations of different ethnic descent (Cauchi 2007, Hattersley 2007) and at individual level, carrying the TCF7L2 risk allele increases T2DM risk 50% (Cauchi 2008). A meta-analysis demonstrated that four variants of TCF7L2 gene containing rs7903146 (C/T), rs7901695 (T/C), a rs12255372 (G/T), and rs11196205 (G/C) polymorphisms are all associated with T2DM, and indicates a multiplicative genetic model for all the four polymorphisms, as well as suggests the TCF7L2 gene involved in near 1/5 of all T2DM (Tong et al 2009).

- **Calpain 10 (CAPN10) gene.**

Calpain is cytoplasmic cysteine protease requiring calcium ions for activity. Although its’ physiological function is still not fully understood, it is implicated in variety of calcium-regulated cellular process such as signal transduction, cell progression, differentiation, apoptosis, membrane fusion and platelets activation (Sadó 1994, Sorimachi 1997, Carafoli 1998, Sorimachi 2001, Huang 2001, Sreenan et al 2001). The molecular biological studies have shown that calpains constitute a superfamily, which exists ubiquitously in organisms ranging from humans to microorganism. CAPN has attracted much attention because of recent discovery of correlations between CAPN gene mutations and human diseases, together with the elucidation of
its three-dimensional structure (Hosfield 1999, Strobl et al 2000) and calcium-induced activation mechanisms (Moldaveanu 2002, Reverter 2001). The specific functions of calpain-10 (CAPN10) gene remain to be determined but it is expressed in many tissues including those involved with the pathogenesis of T2DM pancreatic islets, muscle and fat. Studies have a previously shown that genetic variation in this gene is associated with increased risk of T2DM in some populations but not in others (Stephanie et al 2002).

1.10 Association Studies of CAPN10 gene with Type 2DM.

CAPN10 gene was proposed as susceptibility locus of T2DM based on the initial report of association between the T2DM and CAPN10 gene (Horikawa et al 2000). A combination of two haplotypes generated by variation at three polymorphisms, designated SNPs 43 (G/A; rs3792267), 19 (ins/del at position 241182968) and 63 (C/T; rs5030952), accounts for increased risk to T2DM. All the three variants are located in intronic regions of CAPN10 gene. Preliminary evidence suggests that variation at SNP43 affects gene transcription (Horikawa et al 2000).

This was supported by the observation of decreased CAPN10 gene mRNA levels in skeletal muscle from insulin-resistant subjects homozygous for the G allele at SNP43 (Baier et al 2000). Importantly, homozygosity for the G allele is part of the high-risk haplotype combination, which is comparatively rare in populations of North European extraction and increases the overall risk of T2DM by about 4%. Further, in cross-sectional studies, (Lynn et al 2002) and (Evans et al 2001) showed that nor diabetes subjects with high-risk haplotype combination had evidence of impaired insulin secretion and a tendency to insulin resistance (IR) when compared to subjects with the other haplotype combinations. In addition, a variant at the locus SNP44 (rs7607759, closely mapped to the SNP43) was also reported to associate with increased susceptibility to T2DM (Evans et al 2001).

Several subsequent studies provide further support for the association of polymorphisms and/or haplotypes combinations of CAPN10 gene with T2DM or related quantitative phenotypes. In Pima Indians (Baier et al 2000), and in African Americans (Garant et al 2002) significant association was reported with one of the
key polymorphisms (SNP43) and T2DM. Specifically, the association was reported with the homozygosity for the G allele and increased risk of T2DM.

The greatest risk of T2DM in Mexican Americans was associated with the CAPN10 gene haplotype combination 112/121 (G-del-T/G-ins-C) made up of the markers 43, 19 and 63 (del Bosque-Plata et al 2004). This was also the case in the Botnia area of Finland and German T2DM (Fingerlin 2002). The haplotype was found to increase the susceptibility to the disease by 2.55 and 4.95 fold in the Finnish and the German T2DM populations respectively (Horikawa et al 2000).

In a population of South Indians, Cassell et al (2002) reported that the high-risk haplotype combination 112/121 (G-del-T/G-ins-C) was significantly associated with T2DM. Lynn et al (2002) observed the variation in the CAPN10 gene affects blood glucose levels in the British population. Subjects with G/G or homozygosity for the G allele at the marker or SNP43 (rs3702267) was associated with increased 2-hours plasma glucose. The haplotype combination 112/121 (G-del-T/G-ins-C) was significantly associated with increased fasting and 2 hours plasma glucose and decreased insulin secretory response adjusted for the level of insulin resistance. This haplotype was associated with an approximately threefold increased risk of diabetes in the British population.

Studies in Pima Indians (Baier et al 2000) are also consistent with a role for SNP43 (rs3702267) variation at CAPN10 gene in insulin sensitivity. Non diabetes individuals homozygous for the G allele at SNP43 (rs3702267) were found to have decreased rates of postabsorptive and insulin-stimulated glucose turnover that apparently result from decreased rates of glucose oxidation. The results of molecular association studies between T2DM and CAPN10 gene are not always consistent.

In contrast to the above supportive findings, several reports failed to replicate the association of the G allele at SNP43 and/or the high risk haplotype involving this variant. No association of any CAPN10 variant with T2DM was reported in a Finnish population of T2DM (Fingerlin et al 2002). In addition, Evans et al 2001 examined 153 parents-offspring trios from the Diabetes UK Warren 2 ascertained in the UK. They reported that none of the originally identified polymorphisms or haplotypes was over transmitted from heterozygous parents to affected offspring. Furthermore, (Horikawa et al 2003) also found no evidence for association of the CAPN10 gene
variations defining the high-risk haplotype 112/121 (G-del-T/G-ins-C) with T2DM in a Japanese T2DM sample.

In summary therefore, DM and in particular T2DM is now reaching epidemic proportion around the globe with increasing incidence at a younger age and this is likely to lead to an epidemic of cardiovascular disease in a younger population over the next few decades. This highlights the need to identify those subjects with diabetes who may have a particularly increased risk of CVD events, to begin to evaluate CVD risk and the presence of CVD at the time of diagnosis of T2DM and to develop novel ways to identify subjects at risk of diabetes before they develop abnormalities in glucose tolerance (either DM or prediabetes). This should facilitate more aggressive cardiovascular risk reduction in subjects who already have DM and CVD or a substantial risk of CVD in this setting and allow identification of subjects potentially at increased CVD risk prior to development of dysglycaemia. Together these strategies would be expected to help reduce the potential burden of dysglycaemia-associated CVD.
Chapter two

2.1 Patients and Methods.

The protocols of these studies in this thesis were approved by the local Ethics Committee in Connolly Hospital. A written consent was obtained from each patient and subject who participated in these three studies. Each patient was consented to participate in one study. The demography and characteristics of each group are different from others. The patients were attending the Diabetes Day Centre (DDC) for their annual review and were diagnosed with T2DM according to the World Health Organization criteria (1999). Descriptive statistics of the Minitab statistic programme was used in this thesis.

2.2 Metabolic syndrome study.

Two hundred consecutive patients with either T1DM or T2DM were evaluated for the metabolic syndrome (MS). The patients were classified as having T1DM or T2DM at presentation and the diagnosis was recorded from their medical records. Patients with T1DM had the typical presentation of T1DM i.e. usually before the age of 40, required insulin from the onset of their condition, presented with diabetic ketoacidosis and in some cases (where checked) had positive autoantibodies. A diagnosis of T2DM was also made at presentation to the hospital or referred from the local general practitioner according to WHO criteria. There are some disproportions in patients gender and age in this study and that were related to the actual number of patients who were accepted in participate in this study.

Waist circumference (WCM) from the midpoint between the superior anterior iliac spine and the lower costophrenic angle was measured. Blood was drawn for measurement of fasting plasma glucose (FPG) and fasting lipid profile. These patients were evaluated for MS according to the criteria of Adult Treatment Panel of the National Cholesterol Education Program (ATP-III) criteria for MS. By definition, 3 or more criteria (table 1, chapter 1) are required to make the diagnosis.
HbA1c was measured by Adams-Menarini - 8160 method which employs two kinds of high pressure liquid chromatography elution techniques. The first is reversed phase partition chromatography, which will elute fractions up to stable HbA1c. The second method is ion exchange chromatography, which then elutes the remaining fractions. The peak of the labile was measure by using a substance composed of labile Hb, acetylated and carbamylated Hb in a range of 0.1-10.0%.

Glucose was measured by the standard VITROS GLU Slide method by using the VITROS Chemistry (Glucose Oxidase) products calibrator kit 1 on VITROS Chemistry system.

Cholesterol was measured by the standard VITROS CHOL Slide method by using the VITROS Cholesterol Slides and the VITROS Chemistry products calibrator kit 2 on VITROS Chemistry system. The Friederic equation enables plasma LDL-cholesterol concentration to be calculated and is often used in clinical laboratories.

$$\text{LDL-C} = \text{TC} - \text{HDL} - \frac{\text{TG}}{2.2}.$$  

This equation makes certain assumptions, namely that the patient is fasting and the plasma TG concentration does not exceed 5.0mmol/L (otherwise the chylomicrons make the equation inaccurate).

Two analyses were performed. For the purposes of estimating the probable overall prevalence of MS in the group (EMS), the glycaemic criterion was considered to be satisfied in all patients since they were diagnosed as having DM. The blood pressure criterion was deemed to be satisfied if the patient was on anti-hypertensive medications or if the blood pressure (BP) measurement was measured above 130/85 mmHg in patients not on any anti-hypertensive medication, on at least 2 occasions. One physician made BP measurements after at least 10 minutes resting. Lipid lowering therapy was not taken as a reflection of satisfying the lipid criteria for MS since the primary lipid risk factor targeted in patients with diabetes is the low-density lipoproteins, which are not used in the definition of MS. Secondly the prevalence of MS based on the actual measurement of FPG and BP made on the day of evaluation was calculated (Actual Metabolic Syndrome prevalence, AMS).

Data are presented as mean (standard deviation) for different variables (age, sex, FPG, HbA1c, lipid profile, WCM and BP). Values for these variables were compared
between subjects with T2DM and T1DM, and between patients with and without MS using unpaired t-tests. Chi-square was used to determine whether there was a difference in prevalence of MS in males compared to females and in T1 compared to T2 DM.

2.3 Subclinical vascular complications study.

One hundred patients were all of them recently diagnosed T2DM at the Diabetes Day Centre were investigated for subclinical vascular complications of DM. Patients agreed and consented to take part in this study were 72 males and 28 females. The mean age was 54.2 years (range 26-79) and the mean diabetes duration from the time of diagnosis was 4.4 months (range 0-13). Dietary regime was in 35% and the rest of the group were on the same with one or two oral hypoglycaemic agents. Patients were asked to fast overnight before the day of the blood test. Fasting blood samples were obtained for FPG and lipid profile. Further blood sample was taken for HbA1c, CRP and fibrinogen (Fib) levels. On physical examination, BP, WCM, and BMI were measured. Height and weight of each patient were measured without shoes, hat or heavy clothes. Obesity and overweight were defined according to WHO definitions-underweight (BMI<18 kg/M²), normal (BMI 18-24.9kg/M²), overweight (BMI 25-29.9kg/M²), or obese (BMI>30 kg/M²). Obese patients were further subdivided into grade I (BMI 30-34.9 kg/M²), grade II (BMI 35-39.9 kg/M²), or grade III (BMI >40 kg/M²) obesity. Urine sample for microalbuminuria was obtained. The patients were classified to four quartiles according to the WCM and comparison of the CRP and Fib levels between the two quartiles, the highest WCM ≥112cm and lowest WCM ≤ 94 cm was performed.

The principle procedure of the CRP measurement was by solid phase, chemiluminescent, and immunometric assay (table1) and Fibrinogen Determination by-Claus Fibrinogen Methods.

2.3.1 The high sensitivity CRP (hsCRP) analysis.

High sensitivity serum CRP levels were analysed on the Immulite 2000® (Diagnostic Product Corporation, L.A., USA). This instrument uses an immunometric chemiluminescent assay using ligand-labelled anti-CRP murine monoclonal
antibodies and anti-ligand coated beads (solid phase) to determine the hsCRP in serum samples.

The analytical sensitivity of this test is 0.1mg/L and shows good linearity up to 150mg/L. Serum samples are stable for up to 2 months when separated and stored at -20°C. Samples were thawed at 37°C in a water bath and re-spun at 4000 rpm for 5 minutes immediately prior to testing the supernatant.
<table>
<thead>
<tr>
<th>hsCRP mg/L</th>
<th>Relative cardiovascular risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td>Low risk</td>
</tr>
<tr>
<td>1.0-3.0</td>
<td>Average risk</td>
</tr>
<tr>
<td>3.1-10.0</td>
<td>High risk</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>Persistent levels result from non cardiovascular inflammation.</td>
</tr>
</tbody>
</table>
2.3.2 Fibriogen determination- Clauss Fibrinogen method.

Test principle:
The British Committee for standards in Haematology guidelines recommend that fibrinogen measurements are best achieved using the Clauss method. Fib levels were measured on the ACL9000® (Instrumentation Laboratory, Milan, Italy) using the MemosIL™ Fibrinogen -C kit which uses an excess of thrombin to convert fibrinogen to fibrin in diluted plasma. At high thrombin and low fibrinogen concentration, the rate of the reaction is a function of fibrinogen concentration. All patient samples were centrifuged for 10 minutes at 4000rpm, 12°C, separated into poly tubes and frozen at -20°C within 4 hours of phlebotomy. Frozen plasma samples were then thawed in a 37°C water bath and mixed well immediately before analysis. Results are expressed in g/L and the laboratory normal range is 2-4 g/L.

2.3.3 UKPDS risk engine (Diagram 1).

The UKPDS risk engine was applied to determine the risk of the vascular events at 3, 5 and 10 years. The vascular risks which can be obtained by this formula are the percentages risks of CHD (fatal and non fatal) and stroke (fatal and non fatal). The formula components are age, sex, ethnic group, duration of DM, smoking, HbA1c, atrial fibrillation, systolic BP, serum TC and HDL as shown in diagram (UKPDS 56 by Stevens 2001). Descriptive statistics were performed using Pearson and Spearman correlation coefficient as appropriate and two sample T- tests were performed. P value <0.05 was considered significant.
Diagram 1. The UKPDS risk engine.
2.3.4 Measurement of the ankle-brachial pressure index (ABI).

The ABI was performed according to the Protocols of the Vascular Technology in the Great Britain and Ireland, using the pencil probe of Microlite Flow-lab at a frequency of 8MHz. Accurate pressure measurements can only be achieved if all cuffs are placed correctly on the limbs. To do this, the procedure was carried out as follows:

1. The edges of the cuff were aligned so that they were parallel, rather than skewed, even though angling the cuff may appear to produce better conformation of the cuff to the limb.

2. Cuffs were wrapped firmly, to allow inflation of the cuff to transmit the pressure in the bladder immediately into tissue, rather than into a space between the cuff and the limb (which would produce falsely elevated pressure readings).

Lines were drawn on the cuff to indicate the position of the bladder, and a range that indicates the correct limits for wrapping the cuff. If the wrapping range requirements were not met, so that less than two-thirds of the limb was encased, either the cuff was wrapped more firmly or a larger sized cuff was used.

2.3.5. A. Measurement of the resting brachial pressures.

Measurement of the brachial systolic pressure (BSP) is relatively easy compared with the ankle and it is recommended to make the arm measurement before the ankle pressure measurements are attempted because of the following:

a) It provides the operator with some idea of the pressure to be expected at the ankle. For example, if the patient has a BSP of 140 mm Hg, then the operator can forewarn the patient of how tight the cuff will feel around the ankle and may achieve better compliance.

b) Auscultation of the heart for arrhythmia is important if the pedal pulses are irregular. This can be very helpful in patient with difficult to find signals of the pedal artery.

Step 1
The cuff was wrapped firmly around the upper arm, as high as possible, with the bladder of the cuff over the brachial artery.
Step 2
The tip of the Doppler probe was covered with ultrasonic gel. The probe was held between the thumb and the second and third fingers (like a pencil) and the lateral edge of the hand rested against the patient’s bare skin. This helped to keep the probe absolutely still. The signal was located from the brachial artery.

Step 3
Keeping the probe absolutely still, the cuff was inflated until the artery was occluded and the Doppler signal disappeared. As the artery occluded, the pressure on the dial was noted. In order to be sure of complete cessation of flow, the cuff was inflated at least 20mmHg above the pressure at which the last Doppler arterial signal was appreciated.

Step 4
The cuff was slowly deflated. The arterial Doppler signal returns suddenly and sharply as the SBP equals and then exceeds the pressure in the cuff. At this point, the pressure reading was recorded. (The pressure at which the Doppler signal returns on deflation of the cuff is often lower than the pressure at which the Doppler signal disappears on inflation. It is the former that is the true measurement of the SBP- only in the deflation mode is cuff pressure truly representative of the arterial pressure within the limb).

Step 5
Without moving the probe, the cuff was rapidly and completely deflated and note taken that the Doppler signal remained which indicates that the probe had not moved from over the artery.

Step 6
The probe and cuff were removed and a written record of the pressure measurement made.

Step 7
Steps 1-6 were repeated on the other arm. The highest reading of the two was used to calculate the ankle/brachial pressure index of both legs.
2.3.5. B. Measurement of the ankle pressure.

This method by repeated measurements at different sites around each ankle in order to evaluate quantitatively the highest ankle pressure. It was obtained by comparing the pressures at different sites on the same foot and the reactive hyperaemic response produced by respected pressure measurements was negligible.

Step 1
The cuff was placed around the right ankle, just above, but not covering, the malleolus.

Step 2
The flow in the posterior tibial artery (PTA) was located behind or along the posterior edge of the medial malleolus on a line between the medial malleolus and the heel. The probe was adjusted on the bare skin to achieve the best Doppler signal.

Step 3
The probe was held absolutely still and the cuff was inflated until the artery was occluded (and the Doppler signal disappeared). A mental note was made of the pressure at which this occurred. Then the cuff was further inflated at least 20mmHg above the pressure at which the Doppler arterial signal disappeared in order to ensure cessation of flow.

Step 4
The cuff was slowly deflated, making sure not to move the Doppler probe, and noting the pressure reading when the Doppler signal returned.

Step 5
Then the cuff was rapidly deflated and a written record of the Doppler pressure was made.

Step 6
The dorsalis pedis artery (DPA) usually in the soft spot between the base of the halluc and the second toe, on the top of the arch of the foot or the tibial artery (ATA) usually
found on the crease line between the foot and the leg were then located. The pressure readings were taken from one of these best sites.

Step 7
The location of the peroneal artery (PERA) should be found at the lower leg just above the lateral malleolus or on the foot arch in case of absence of signals from the PTA or DPA/ATA sites.

The ankle pressure readings were taken from the PERA was as described previously for the other arteries. For the ankle brachial pressure index calculation the highest pressure reading obtained at the ankle (whether it comes from the PTA, DPA/ATA or PERA) was used in order to quantify objectively the optimal source of blood flow to the foot.

Step 8
Steps 1-7 were repeated on the left leg.

ABI on each side was calculated as the highest each ankle pressure divided by highest brachial pressure. PVD was defined by ABI less than 0.9 in either leg (Table2).
<table>
<thead>
<tr>
<th>Degree of ABI index</th>
<th>Type of the disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1.31</td>
<td>Non compressible.</td>
</tr>
<tr>
<td>≥ 0.91 ≤ 1.30</td>
<td>Normal.</td>
</tr>
<tr>
<td>0.71-0.90</td>
<td>Mild Peripheral Arterial Disease.</td>
</tr>
<tr>
<td>0.40-0.70</td>
<td>Moderate Peripheral Arterial Disease.</td>
</tr>
<tr>
<td>≤ 0.40</td>
<td>Severe Peripheral Arterial Disease.</td>
</tr>
</tbody>
</table>
2.3.6 Measurement of the carotid Doppler's.

DSC was performed according to the Protocols of the Vascular Technology in the Great Britain and Ireland, by using the Logic 9 General Electric machine duplex ultrasound scanner with high definition imaging of a linear transducer of high frequency at 7MHZ. The normal carotid intimal media thickness (CIMT) is less than 0.5mm (Pignoli 1986). A pulse wave Doppler is essential as carotid arteries can not be assessed adequately by B-mode imaging alone. The colour Doppler display is very helpful but not essential test.

B-Mode imaging

The B-mode examination helped to achieve an accurate picture of the anatomy and the best visualization of atheroma for characterisation. Doppler and colour evaluation were performed to determine the flow characteristics and severity of the disease. The transverse and longitudinal planes were used to measure the type, site, extent and severity of plaque thickness, intimal thickness and the flow and the severity (degree) of the stenoses.

2.3.7 Stress test.

The Bruce technique or protocol was selected for the evaluation of the subclinical cardiovascular disease. Each of the three stages was continued for 3 minutes.

2.3.8 Blood pressure monitor.

This was done by using the cuff to the arm of the patients and keeping the recorder or the box at the belt for 24 hours.

2.3.9 Microalbuminuria.

Microalbuminuria was defined by the presence of urinary albumin excretion rate of >20 g min⁻¹ and < 200 g min⁻¹ (30 to 300 mg/day). Correspond to albumin/creatinine ratio of approximately 2.5–25 (mg/ mmol) in males and 3.5–35 in females. Urine was collected in patients at rest (an outpatient procedure). Incipient diabetic nephropathy was suspected when microalbuminuria was found in two of three urine samples collected consecutively, preferably within 6 months and minimally 1 month. Other causes for increased urinary albumin excretion rate were excluded (Mogensen 2003).
2.4 Calpain 10 gene study.
A total of 236 patients with T2DM (129 males / 107 females) with a mean age at the
time of the study of (57.9±15.3years: Mean ± SD) and at the time of the diagnosis of
(47.5±9.5years: Mean ± SD) were recruited. A group of 120 control subjects were
also recruited (54 males and 66 females) with a mean age of (42.5± 17.5 years: Mean
± SD). The patients and the non-diabetic controls were all of Irish descent as
determined by knowing up four grandparents of Irish descent. Controls had no family
history of T2DM in first (parents, brothers, sisters, sons and daughters) second
(uncles, aunts and cousins from both parents side) or third degree (grandfathers and
grand mothers) family relatives and all were from Dublin. This entire group had an
oral glucose tolerance test (OGTT) performed to ensure that they had normal glucose
tolerance. The classification of dysglycaemia was made according to the recent
American Diabetes Association Criteria (Genuth et al 2003). EDTA blood sample
was obtained from each patient and control subject for DNA extraction.

2.4.1 Preparation of high molecular weight genomic DNA.
A rigorous two day extraction protocol was used to obtain high molecular weight
DNA with a minimum of contamination. The protocol used in this study was that
described by Maniatis (1989).

Day 1
Ten ml blood samples were slowly thawed on ice (3-4 hours), and transferred to 50
ml Falcon tubes. The volume was made up to 25 ml with sterilised distilled water.
Twenty-five ml of 1 X lysis buffer was added to the contents of each tube and mixtures
were placed on ice for 30 minutes with continuous shaking. The buffer was a salt
solution which is slightly hypo-osmolar compound to the red blood cells (RBC). RBCs
absorbed water from the solution by osmosis, swelled and ruptured while the white
blood cells remained intact. The mixture was centrifuged at 3500 rpm at 4C for 15min.
The supernatant containing the plasma and soluble contents of RBCs were removed
by aspiration, leaving about 8ml in the bottom of the tube. The tubes were refilled
with 1X lysis buffer and paced on ice for10 minutes with an occasional inversion to
ensure that any remaining RBCs were lysed. Samples were centrifuged at 3500 rpm at
4C for 15 minutes and supernatants were discarded. The pellet was re-suspended in
3ml of 1X suspension buffer and transferred to sterile 15ml solvent resistant Sarstedt tubes.

Sodium dodecyl sulphates (SDS) at a final concentration of 1% (0.3ml of 10% stock solution) and 120 μl proteinase K (50 μg/ml) were added. Proteinase K is a powerful proteolytic enzyme that ensures the degradation of nucleoproteins. Thus a DNA preparation of high molecular weight was obtained. The tubes were placed on a rotary shaker platform at 37 °C overnight.

Day 2

After overnight incubation, 0.45ml of 6M sodium perchlorate was added to the contents of each tube and the tubes were placed for 2 hours at 37 °C on a rotary platform to denature any remaining proteins. Following this step, phenol-chloroform extraction was carried out in order to remove any remaining proteins. DNA remains in the aqueous layer (Sambrook 1989). Four ml of phenol was added to each tube; the tubes were then vigorously shaken for 10 minutes and centrifuged at 6000 rpm for 10 minutes at room temperature. The upper white layers containing the DNA were carefully aspirated using sterile pipette, and transferred to a fresh sterile Sarstedt tubes (care being taken not to remove any of the interface between the two layers). Further extractions were performed with 2 ml of phenol and 2 ml of chloroform (chloroform/isoamyl alcohol, 24/1, v/v). After a final extraction with chloroform only, the aqueous layers were carefully transferred to a sterile 50ml universal tube and the DNA was precipitated with 0.1ml of 3M sodium acetate and two volumes of ethanol (stored at -20°C). To remove excess salt, each DNA pellet was washed with 1 ml of 70% cold ethanol (-20°C). The 70% ethanol wash was then repeated and the DNA pellets dried in a vacuum desiccator for 15 minutes. The DNA pellet was dissolved in 0.25-0.5 ml of TE buffer and placed at 4°C for 2-4 days.

DNA quantification

Two methods were used to measure the concentration of the prepared DNA (Manitis 1989). The first method was spectrophotometric; the absorbance of 1/100 dilution of the DNA sample was measured at wavelengths of 260 nm, 280 nm and 300 nm, corresponding to DNA, RNA and protein, respectively. The DNA concentration was calculated on the basis that an optical density (OD) of 1 corresponds to 50μg/ml of DNA at 260 nm. To confirm the spectrophotometric measurement and to assess the
physical condition of the extracted DNA, 5µl samples were electrophoresed on 1% agarose gels. The DNA was stained with ethidium bromide (1µg/ml) and visualised under UV.

The fluorescence of the genomic DNA was compared with that of unknown concentrations of λ DNA digested with Hind III. The DNA preparation showed a major band of molecular weight greater than 20-30kb and sheared DNA during the extraction process, was visible under UV light.

Agarose gel electrophoresis
Electrophoresis buffer (1 x Tris-acetate; TAE) was prepared by dilution (1x TAE) of 100ml of a 10 x TAE with 900 ml of distilled water. Agarose gels were prepared by dissolving the required amount of agarose in 1 x electrophoresis buffer in a microwave for about 4 minutes. While the agarose was dissolving, the end of the gel tray was sealed with autoclave tape and proper comb was placed at one end of the tray. The agarose was cooled to approximately 45-50°C, poured into the gel tray and left to set for about 30 minutes. The comb was removed and the gel was placed in the electrophoresis tank filled with 1 x TAE electrophoresis buffer. DNA samples were loaded on the gel after adding 2µl of 10 x Ficoll Orange G. Agarose gel concentration and the electrophoresis time were dependent upon the molecular weight of the DNA and the required DNA resolution. However, 1% agarose gels were used as checking gel after DNA extraction from blood. For the detection of the polymerase at the variant 19 (at position 241182968) ins/del 3% agarose gels were used. DNA electrophoresis was performed at 50V for 3 hours to separate the resulting fragments.

2.4.2 Genotyping
Three single nucleotide polymorphisms (SNPs) 43, 2037, 63 and insertion/deletion 19 (ins/del) variants at the CAPN10 gene were analysed in this investigation. Genotyping of the three SNPs were commercially conducted at the UK Gene Service (genotyping@geneservice.co.uk). The ins/del variant at position 19 at position was genotyped at the Genetics Department of Trinity College/Dublin using polymerase chain reaction (PCR) and size fractionation on agarose gels (Photo 1)
Gel electrophoresis and DNA visualisation

Agarose Gels

Ten microlitres of PCR amplified samples were mixed with 3µl loading dye prior to being loaded on a gel along with appropriate size standards. The samples were then electrophoresed on 3% (w/v) Agarose gels containing 1µg/ml ethidium bromide. The gels were run in TAE buffer (X1), visualised on an ultra violet illuminator and photographed using a Polaroid camera.

2.4.3 Buffers and solutions.

TAE Buffer:
10mM Tris base 50g X 242g Glacial acetic acid 57.1 ml.
0.1mM sodium EDTA (Ethylenediaminetetraacetic acid) of pH 8.0.

DNA Extraction Reagents:
10x lysis buffer: 50mM Tris-HCl (pH 7.5), 25mM MgCl2,
6H2O, 0.6M sucrose, 5% (v/v) Triton X100.
10x suspension buffer: 0.1M Tris-HCl (pH 7.5),
0.1M NaCl, 0.1M sodium EDTA (pH 8.0)

Electrophoresis Buffer: 10x TAE, 0.4 M Tris- Base, 10mM 0.5 M EDTA
The Buffer’s pH was brought up to 7.5 with glacial acetic acid.
10x PCR Buffer: 500mM KCl, 100mM Tris-HCl (pH 8.9),
15mM MgCl2, 1% Triton x100,
0.1 Gelatin.
Photograph 1:
The agarose gel showing insertion deletion genotypes. Sample1 homozygous for the deletion variant. Samples, 2,4,7,8, and 12 are homozygous for insertion while samples 3, 5,6,9,20,11 are heterozygous.
2.4.4 Statistical Analysis

Hardy Weinberg Equilibrium

Genotypic data from control individuals were examined to ensure that they conformed to Hardy Weinberg equilibrium. The Hardy Weinberg principle assumes that allele frequencies should conform to the following mathematical equation:

\[ p^2 + 2pq + q^2 = 1 \]

Where \( p^2 = P (AA) \), the probability of being an AA homozygote
\( 2pq = P (AB) \), the probability of being an AB heterozygote
\( q^2 = P (BB) \), the probability of being a BB homozygote

This equilibrium can be tested for using the \( \chi^2 \) statistic according to the equation:

\[ \chi^2 = \Sigma (O-E)^2 / E \]

Where \( O \) = the observed frequency of the allele
\( E \) = the expected frequency of the genotype

A significant \( \chi^2 \) value is indicative of deviation from the Hardy Weinberg principle. This may result from either genotyping errors or from a lack of conforming to the Hardy Weinberg assumptions, which are as follows: 1) an infinite population size, 2) discrete generations, 3) random mating, 4) no selection, 5) no migration, 6) no mutation.

Odds Ratio (OR)

The OR is also a measure of the strength of association between a marker and disease. It measures the probability that disease is present compared with the probability that it is absent. It is calculated according to the following formula

\[ OR = \frac{a}{b} \]

\( a \) = Number of allele or haplotypes in the affected disease population
\( b \) = Number of allele or haplotypes in the control population.
Linkage Disequilibrium measurement and Haplotype analysis

Haplotype analysis in this thesis was conducted using the program Haploview which is available at the website http://www.broad.mit.edu/mpg/haploview/. It is designed to simplify and expedite the process of haplotype analysis by providing a common interface to several tasks relating to such analyses. Haploview currently supports the following functionalities:

1. Linkage disequilibrium (LD) between markers estimated as $D'$ (ranging between 0 and 1) and haplotype block analysis.
2. Haplotype population frequency estimation.
3. Single nucleotide polymorphism (SNP) and haplotype association tests.
4. Permutation testing for association significance measured as Chi-square and p value.
5. Automatic downloads of phased genotype data from HapMap.
Chapter Three

Result of the metabolic syndrome study.

Two hundred patients were well known to our service with a previous certified diagnosis with type 1 and 2 DM according to the WHO criteria (1999). Thirty six patients (18%) had T1DM and 164 (82%) had T2DM. The majority of the patients were male (127, 63.5%). The average age was 59.9 years (SD ± 14.98). Of patients with type-2 diabetes, 61% on oral hypoglycaemic agents, 23.2% were on diet control, 6.7% were on insulin only and 9.1% were on oral agents together with insulin. Two T2DM patients and 2 T1DM patients were on angiotensin converting enzyme inhibitors because of microalbuminuria or proteinuria rather than for hypertension. However, none of these patients satisfied the criteria for the MS. Anti-hypertensive medications were prescribed in 70% of patients with T2DM and new hypertension was in 7.9%. In T1DM the anti-hypertensive medications were prescribed in 36.1% and in 5% in patients with proteinuria. Lipid lowering therapy was not taken as a reflection of satisfying the lipid criteria for MS since the primary lipid risk factor targeted in patients with diabetes are the low-density lipoproteins, which are not used in the definition of MS. Anti-lipid medications were prescribed in 50.6% patients with T2DM and newly was in 23.7%. In T1DM the anti-lipid medications were prescribed in 33.3% and 8.3% were newly described with dyslipidaemia.

The mean FPG was 8.2 mmol/L (SD ± 3.3), mean blood pressure 135.2/81.8, (SD±22.7/11.0) mean WCM 104 cm (SD±13) for males and 96.7 (SD±15.1) for females, mean HDL cholesterol 1.23 mmol/L (SD±0.36) for males and 1.46 mmol/L (SD ± 0.44) for females and mean fasting plasma TG 1.63 mmol/L (SD±1.04).

The FPG and HDL were higher in patients with T1DM (P<0.024 and P <0.001, respectively) while the blood pressure (systolic and diastolic), WCM and fasting TG were higher in patients with T2DM (table 1).

The estimated metabolic syndrome (EMS) prevalence was 61% (122 of 200). The EMS prevalence was greater in T2DM (69.5%) than in T1DM (22%) (P< 0.001). Three criteria were met in 55 patients (45%), four criteria in 48 patients (39.4%) and five criteria were met in 19 patients (15.6%). On the day of study, despite treatment for DM (100%), hypertension (69.5%) and dyslipidaemia (48.3%), the actual
metabolic syndrome (AMS) prevalence was 57% (114 patients, of whom 109 (95.6%) had T2DM). Female patients with diabetes were more likely to have MS (71% vs. 55%, p<0.05).

Of patients with EMS, 88% had the blood pressure criterion, 86.8% met the WCM criterion, 49.1% met the HDL criterion and 43.4% met the TG criterion. The proportion meeting the blood pressure criterion was higher in T2DM patients (90% vs. 62% in T1DM p<0.001). Similarly the proportion meeting the WCM criterion was higher in T2DM (89.4% vs. 50% p<0.001). The proportion meeting the TG criterion tended to be higher in the T1DM group (75.5% vs. 41.2 in T2DM) while the proportion meeting the HDL criteria was similar (50% T1DM vs. 49.1% T2DM).

In patients with MS based on parameters measured on the day of the study, all patients with T1DM met the glycaemic criterion on the day of measurement compared to 81.6% of T2DM group. Of T2DM patients, 85.3% and 89.9% met the blood pressure and WCM criteria respectively compared to 40% and 60% in T1DM respectively. In contrast, all T1DM patients in this group met the TG criterion compared to 43.1% of T2DM patients while 60% of T1DM and 49% of T2DM patients met the HDL criterion.

There was no significant difference in FPG levels between patients with and without MS. However, blood pressure, WCM and TG were all higher in patients with MS compared to those without and the HDL was lower (table 2, diagrams 1-6). Similar trends were seen both in T1DM and T2DM with the exception of WCM which was similar in patients with T1DM with and without the MS.

HbA1c it was significantly higher (table 1) in patients with T1DM (7.13 ±1.44, vs. 6.48 ± 1.35 T2DM p<0.01). In both genders, there was no significant difference in HbA1c levels between patients with and without MS (table 2).
Table 1. Demographic data and cardiovascular risk factors of all patients with Type 1 and 2 Diabetes Mellitus (The mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Type 1 DM</th>
<th>Type 2 DM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>36 (18%)</td>
<td>164 (82%)</td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td>44.8 ± 15.7</td>
<td>63.2 ± 12.6</td>
<td>*0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>16</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>9.78 ± 4.9</td>
<td>7.80 ± 2.71</td>
<td>*0.024</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114.6 ± 15.8</td>
<td>139.7 ± 21.6</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.5 ± 8.3</td>
<td>83.0 ± 11.2</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>88.9 ± 13.7</td>
<td>104.1 ± 12.8</td>
<td>*0.001</td>
</tr>
<tr>
<td>Female</td>
<td>92.0 ± 11.3</td>
<td>106.4 ± 12.0</td>
<td>*0.001</td>
</tr>
<tr>
<td></td>
<td>84.9 ± 15.7</td>
<td>100.0 ± 13.2</td>
<td>*0.002</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.58 ± 0.45</td>
<td>1.26 ± 0.37</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>1.45 ± 0.45</td>
<td>1.19 ± 0.32</td>
<td>*0.022</td>
</tr>
<tr>
<td></td>
<td>1.75 ± 0.41</td>
<td>1.38 ± 0.42</td>
<td>*0.005</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.24 ± 0.92</td>
<td>1.72 ± 1.05</td>
<td>*0.008</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>7.13 ±1.44</td>
<td>6.48 ± 1.35</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*: Significant p value.
Table 2. The estimated metabolic syndrome prevalence in the whole group and in subgroups of patients with Type 1 and Type 2 diabetes mellitus according to metabolic syndrome criteria (The mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>With MS</th>
<th>Without MS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>8.0 (± 2.7)</td>
<td>8.4 (± 4.0)</td>
<td>0.450</td>
</tr>
<tr>
<td>T1DM</td>
<td>9.9 (± 3.6)</td>
<td>9.7 (± 5.3)</td>
<td>0.903</td>
</tr>
<tr>
<td>T2DM</td>
<td>7.9 (± 2.6)</td>
<td>7.7 (± 2.9)</td>
<td>0.648</td>
</tr>
<tr>
<td>SBP reading (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>143.6 (± 20.2)</td>
<td>121.9 (± 20.2)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>T1DM</td>
<td>128.6 (± 14.9)</td>
<td>110.6 (± 13.9)</td>
<td>*0.012</td>
</tr>
<tr>
<td>T2DM</td>
<td>144.7 (± 20.2)</td>
<td>128.2 (± 20.5)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>DBP reading (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>84.6 (± 11.8)</td>
<td>77.5 (± 8.0)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>T1DM</td>
<td>82.1 (± 10.9)</td>
<td>74.9 (± 6.9)</td>
<td>0.114</td>
</tr>
<tr>
<td>T2DM</td>
<td>84.8 (± 11.9)</td>
<td>78.9 (± 8.4)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>107.2 (± 12.4)</td>
<td>92.2 (± 11.1)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>111.0 (± 11.0)</td>
<td>95.4 (± 9.6)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>102.1 (± 12.5)</td>
<td>83.5 (± 12.7)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>T1DM</td>
<td>93.7 (± 9.3)</td>
<td>87.5 (± 14.5)</td>
<td>0.160</td>
</tr>
<tr>
<td>Male</td>
<td>100.0 (± 1.0)</td>
<td>90.6 (± 11.7)</td>
<td>*0.005</td>
</tr>
<tr>
<td>Female</td>
<td>90.0 (± 10.2)</td>
<td>82.6 (± 17.6)</td>
<td>0.312</td>
</tr>
<tr>
<td>T2DM</td>
<td>108.2 (± 12.1)</td>
<td>94.8 (± 8.9)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>111.5 (± 11.0)</td>
<td>97.5 (± 7.8)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>103.4 (± 12.1)</td>
<td>84.4 (± 3.5)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1.20 (± 0.36)</td>
<td>1.49 (± 0.41)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1.1 (± 0.32)</td>
<td>1.39 (± 0.33)</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>1.34 (± 0.37)</td>
<td>1.76 (± 0.48)</td>
<td>*0.001</td>
</tr>
<tr>
<td>T1DM</td>
<td>1.25 (± 0.33)</td>
<td>1.67 (± 0.44)</td>
<td>*0.011</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>T2DM</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>0.94 (± 0.04)</td>
<td>1.43 (± 0.29)</td>
<td>1.20 (± 0.37)</td>
</tr>
<tr>
<td>T2DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1.97 (± 1.14)</td>
<td>1.11 (± 0.56)</td>
<td></td>
</tr>
<tr>
<td>T1DM</td>
<td>2.37 (± 1.28)</td>
<td>0.91 (± 0.45)</td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td>1.94 (± 1.13)</td>
<td>1.22 (± 0.59)</td>
<td></td>
</tr>
<tr>
<td>HbA1c%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>6.46 (± 1.32)</td>
<td>6.69 (± 1.46)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.73 (± 1.42)</td>
<td>6.47 (± 1.29)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6.46 (± 1.36)</td>
<td>7.0 (± 1.86)</td>
<td></td>
</tr>
<tr>
<td>T1DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7.19 (±1.41)</td>
<td>7.0 (± 1.47)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6.9 (± 0.86)</td>
<td>7.0 (± 1.32)</td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.4 (± 1.3)</td>
<td>6.6 (±1.5)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6.6 (± 1.3)</td>
<td>6.5 (± 1.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.4 (± 1.3)</td>
<td>6.7 (± 2.0)</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant p value.
Diagram 1. Fasting plasma glucose (FPG) in patients with and without MS in TIDM and T2DM (The mean ± SD).
Diagram 2. Systolic Blood Pressure (SBP) in patients with and without MS in TIDM and T2DM (The mean ± SD).
Diagram 3. Diastolic Blood Pressure (DBP) in patients with and without MS in T1DM and T2DM (The mean ± SD).
Diagram 5. Serum HDL in patients with and without MS in TIDM and T2DM for M: males and F: females (The mean ± SD).
Diagram 6. Serum TG in patients with and without MS in TIDM and T2DM (The mean ± SD).
Chapter Four
4.1 Results of the subclinical vascular complications study.
In this study 100 patients were consented (72 males and 28 females) with a mean age of 54.4 ± 12.4 years. A statistical gender difference was seen in HbA1c (p=0.035) and urinary albuminuria (p=0.017). The demographic details of the patients in table 1.
Table 1. The demographic data of all patients (The mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>100</td>
<td>72</td>
<td>28</td>
<td>--------</td>
</tr>
<tr>
<td>Age/ years (Mean ± SD)</td>
<td>54±12.4</td>
<td>53.2±12.5</td>
<td>56.7±12.2</td>
<td>0.217</td>
</tr>
<tr>
<td>Duration of DM. (means by months)(range)</td>
<td>4.4±3.9 (0-13)</td>
<td>4.5±4.1 (0-13)</td>
<td>4.3±3.8 (0-12)</td>
<td>--------</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>10.7±4.2</td>
<td>10.9±4.1</td>
<td>10.2±4.2</td>
<td>0.457</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>8.6±2.2</td>
<td>8.9±2.2</td>
<td>7.8±2.0</td>
<td>*0.035</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.2±1.3</td>
<td>5.1±1.3</td>
<td>5.4±1.2</td>
<td>0.434</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.7±2.5</td>
<td>2.9±2.8</td>
<td>2.2±1.2</td>
<td>0.104</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±3.0</td>
<td>1.4±3.5</td>
<td>1.2±0.3</td>
<td>0.550</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.9±1.2</td>
<td>2.8±1.2</td>
<td>3.1±1.0</td>
<td>0.231</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135.6±18.5</td>
<td>135.5±18.3</td>
<td>135.8±19.5</td>
<td>0.953</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.5±8.8</td>
<td>82.7±9.8</td>
<td>81.9±5.5</td>
<td>0.611</td>
</tr>
<tr>
<td>Albuminuria (mg/d)</td>
<td>36.2±72.9</td>
<td>43.7±83.4</td>
<td>16.8±24.7</td>
<td>*0.017</td>
</tr>
<tr>
<td>BMI%</td>
<td>30.7±9.0</td>
<td>30.0±7.1</td>
<td>32.4±12.6</td>
<td>0.369</td>
</tr>
<tr>
<td>WCM (cm)</td>
<td>102.4±12.9</td>
<td>102.9±11.0</td>
<td>101.1±16.9</td>
<td>0.609</td>
</tr>
<tr>
<td>SBP monitor (mmHg)</td>
<td>133.8±12.3</td>
<td>134.1±10.7</td>
<td>133.2±15.1</td>
<td>0.791</td>
</tr>
<tr>
<td>DBP monitor (mmHg)</td>
<td>81.3±7.9</td>
<td>82.2±7.8</td>
<td>79.7±8.4</td>
<td>0.257</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>5.2 (±6.0)</td>
<td>5.86 (±0.79)</td>
<td>5.86 (±6.6)</td>
<td>0.564</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.66 (±0.9)</td>
<td>3.6 (±0.8)</td>
<td>3.78 (±1.0)</td>
<td>0.480</td>
</tr>
<tr>
<td>Smoker</td>
<td>18 (18%)</td>
<td>14 (19%)</td>
<td>4 (14%)</td>
<td>--------</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>11 (11%)</td>
<td>9 (13%)</td>
<td>2 (7%)</td>
<td>--------</td>
</tr>
<tr>
<td>Non</td>
<td>71 (71%)</td>
<td>49 (68%)</td>
<td>22 (78%)</td>
<td>--------</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------</td>
<td>--------------------------</td>
<td>--------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>92 (92%)</td>
<td>64 (88.9%)</td>
<td>28 (100%)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>7 (7%)</td>
<td>7 (9.7%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>African</td>
<td>1 (1%)</td>
<td>1 (1.4%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>History of Dyslipidaemia.</td>
<td>28 (28%)</td>
<td>19 (26.4%)</td>
<td>9 (32.2%)</td>
</tr>
<tr>
<td></td>
<td>Newly Dx. Dyslipidaemia.</td>
<td>20 (20%)</td>
<td>15 (20.8%)</td>
<td>5 (17.8%)</td>
</tr>
<tr>
<td></td>
<td>Normal lipid profiles.</td>
<td>52 (52%)</td>
<td>38 (52.8%)</td>
<td>14 (50%)</td>
</tr>
<tr>
<td></td>
<td>History of HTN.</td>
<td>40 (40%)</td>
<td>27 (37.5%)</td>
<td>13 (46.4%)</td>
</tr>
<tr>
<td></td>
<td>Newly Dx. HTN.</td>
<td>10 (10%)</td>
<td>8 (11%)</td>
<td>2 (7.2%)</td>
</tr>
<tr>
<td></td>
<td>Normal BP.</td>
<td>50 (50%)</td>
<td>37 (51.3%)</td>
<td>13 (46.4%)</td>
</tr>
<tr>
<td></td>
<td>Atrial Fibrillation</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* A significant p value.
4.2 UKPDS risk engine.
The UKPDS risk engine formula showed significant increases in risk of fatal and non-fatal CHD% in males compared with females. There was no significant difference in projected stroke rates. The risk of fatal and non-fatal CHD% vascular events was greater than fatal and non-fatal stroke vascular events in 3, 5 and 10 years duration. *table 2*. These represent considerable risk.
Table 2. The risk of the vascular events in males and females (The mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P value</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3y</td>
<td>8.7±7.9</td>
<td>4.3±5.4</td>
<td>*&lt;0.002</td>
<td>7.5±7.5</td>
</tr>
<tr>
<td>5y</td>
<td>14.5±12.6</td>
<td>7.5±8.9</td>
<td>*&lt;0.003</td>
<td>12.5±12.1</td>
</tr>
<tr>
<td>10y</td>
<td>29.3±21.3</td>
<td>16.1±16.8</td>
<td>*&lt;0.002</td>
<td>25.6±20.9</td>
</tr>
<tr>
<td>Fatal CHD%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3y</td>
<td>4.9±5.9</td>
<td>2.7±4.7</td>
<td>*&lt;0.05</td>
<td>4.3±5.7</td>
</tr>
<tr>
<td>5y</td>
<td>8.5±9.7</td>
<td>4.8±7.8</td>
<td>*&lt;0.05</td>
<td>7.5±9.3</td>
</tr>
<tr>
<td>10y</td>
<td>18.6±18.0</td>
<td>10.9±15.0</td>
<td>*&lt;0.03</td>
<td>16.4±17.5</td>
</tr>
<tr>
<td>Stroke%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3y</td>
<td>1.2±1.7</td>
<td>1.6±3.9</td>
<td>&lt;0.59</td>
<td>1.3±2.5</td>
</tr>
<tr>
<td>5y</td>
<td>2.3±3.1</td>
<td>3.0±6.9</td>
<td>&lt;0.56</td>
<td>2.5±4.4</td>
</tr>
<tr>
<td>10y</td>
<td>6.3±7.8</td>
<td>7.6±14.3</td>
<td>&lt;0.64</td>
<td>6.7±10.0</td>
</tr>
<tr>
<td>Fatal stroke%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3y</td>
<td>0.2±0.5</td>
<td>0.3±0.6</td>
<td>&lt;0.62</td>
<td>0.2±0.5</td>
</tr>
<tr>
<td>5y</td>
<td>0.4±1.0</td>
<td>0.5±1.1</td>
<td>&lt;0.61</td>
<td>0.4±1.0</td>
</tr>
<tr>
<td>10y</td>
<td>1.1±2.6</td>
<td>1.4±2.4</td>
<td>&lt;0.69</td>
<td>1.2±2.5</td>
</tr>
</tbody>
</table>

* A significant value.
4.3 The absolute values of the inflammatory markers to the waist circumference (WCM).

In the total group, mean CRP was 5.2 ± 6 mg/L and mean Fib was 3.6 ± 0.9 g/L respectively. In the whole group there was a statistically significant correlation between CRP and WCM (figure 1, r=0.35 and p<0.01) but not between WCM and Fib levels (figure 2, r=0.02 and p=0.24). The mean CRP (mg/L) was higher 7.2 ± 1.6 in the highest quartile of the WCM compared to the lowest quartile 2.6± 1.9 (p<0.01) but a similar difference was not found for Fib (3.8 ± 0.2 in the highest quartile compared with 3.43 ± 0.7 in the lowest p=0.114) which is most likely due to the small sample size (table 3, diagram 1 and figures 1 and 2).
Table 3. CRP and Fib subjects with recently diagnosed diabetes separated into quartiles of WCM (The mean ± SD).

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Mean WCM ± SD</th>
<th>Mean CRP ± SD</th>
<th>Mean Fib ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85.9 (±6.0)</td>
<td>2.6 (±1.9)</td>
<td>3.43 (± 0.74)</td>
</tr>
<tr>
<td>2</td>
<td>97.5 (±2.3)</td>
<td>4.76 (±4.49)</td>
<td>3.56 (±1.0)</td>
</tr>
<tr>
<td>3</td>
<td>106 (± 3.13)</td>
<td>6.33 (± 7.1)</td>
<td>3.83 (±0.82)</td>
</tr>
<tr>
<td>4</td>
<td>118.5 (±1.26)</td>
<td>7.2 (± 1.6)</td>
<td>3.8 (±0.2)</td>
</tr>
</tbody>
</table>
Diagram 1. The absolute values of the inflammatory markers to fourth quartiles of WCM (The mean ± SD).
Figure 1. The absolute values between CRP and WCM (The mean ± SD).
Figure 2. The absolute values between Fib and WCM (The mean ± SD).
4.4 The absolute values of the inflammatory markers to BMI.

The mean BMI, CRP and Fib for the whole group were 31.8±6.5, 5.2±6.0 and 3.6±0.9 respectively. Of the whole group, the absolute value of the CRP was a statistically significant to BMI (p<0.02), but the similar was not seen with Fib (p=0.92). There were no significant values identified between these inflammatory markers and normal, overweight and obese patients. (table 4, figures 3 and 4, diagram 2).
Table 4. The values of the inflammatory markers (CRP and Fib) to different BMI (The mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>CRP</th>
<th>P value BMI/CRP</th>
<th>Fib</th>
<th>P value BMI/Fib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (100 patients)</td>
<td>31.8±6.5</td>
<td>5.2±6.0</td>
<td>*&lt;0.02</td>
<td>3.5±0.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Normal (10 patients)</td>
<td>22.8±1.3</td>
<td>2.9±2.1</td>
<td>0.84</td>
<td>3.7±1.0</td>
<td>0.54</td>
</tr>
<tr>
<td>Overweight (35 patients)</td>
<td>27.8±1.2</td>
<td>4.0±4.5</td>
<td>0.76</td>
<td>3.5±0.76</td>
<td>0.75</td>
</tr>
<tr>
<td>Obese (55 patients)</td>
<td>36.0±5.9</td>
<td>6.4±7.0</td>
<td>0.24</td>
<td>3.7±0.9</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* A significant value.
Figure 3. The absolute value of CRP to BMI (The mean ± SD).
Figure 4. The absolute value of the Fib to BMI (The mean ± SD).
Diagram 2. The absolute values of the BMI to CRP and Fib (The mean ± SD).
4.5 Vascular tests.

Carotid Doppler study was performed to measure carotid intimal media thickness (CIMT) and to determine the presence or absence of carotid plaque in 90 patients (62 males and 28 females). In this study we observed no significant difference between the mean of the CIMTs in males and females in right side (0.91±1.47, 0.55±0.25, p=0.14) and left side (0.88±1.77, 0.6±0.23, p=0.23) (table 5). In male patients, 22 (35.5%) had normal tests and 40 (64.5%) had measurable plaque with different plaque characteristics (24 simple, 4 heterogeneous, 3 complex and 9 mixed). In females 12 (43%) had normal test and 16 (57%) had abnormal plaques (13 simple, one with a complex and two had mixed). (See Photos 1-4). In both gender the abnormal CIMT was in 56 (62%) patients. Significant carotid stenosis (>50%) was recorded in one female with complex plaque and 2 males with simple plaque.

ABI was performed in 62 males and 27 females. A significant abnormal test with ABI less than 0.60 was recorded in one male patient who also had a positive stress and smoking habits.

This study showed a significant difference between the mean of ABI test in males and females in both right side (1.1±0.11, 1.06±0.11, p=0.035) and left side (1.12±0.13, 1.06±0.11, p=0.036) (table 5).

Stress test was done in 68 patients (46 males and 22 females). Four male patients (5%) had a positive test and underwent coronary angiography which showed generalized vascular disease. Those patients were asymptomatic and one had coronary stenting.

In this small number of male patients with positive stress test (table 6) the mean of CIMT of both sides was (right 0.76±0.57, left. 0.76±0.12) and the mean of CIMT negative test patients was (right 0.92±0.20, left 0.89±1.83). The mean of ABI in patients with positive stress test was (right 1.11±0.33, left 1.08±0.30) and (right 1.11±0.09, left 1.12 ± 0.11) in patients with negative test. All UKPDS vascular scores events were higher in patients with positive test than patients with negative stress test. It is difficult to calculate the significant between these variables in patients with positive and negative stress test because of small number of patients (5%) with positive stress test.

Further more none significant correlations were observed between 10 years CHD and stroke vascular events with the mean of CIMTs and ABI tests in males and female’s patients’ (table 7 and 8).
CRP but not Fib was more significantly correlated to ABI (right \( p=0.06 \) and left \( p<0.01 \)) than CIMT (table 9). This correlation was more in males than females (table 10 and 11). On classifications of patients with normal CRP (1-3) and abnormal high CRP more than 3, the means of the UKPDS scores were higher but not significant in patients with high CRP than patients with normal level. The following risks of the CHD\%, fatal CHD\%, stroke and fatal stroke in patients with higher CRP were 26.7±23.0, 16.3±17.5, 8.57±14.0, 1.65±3.6 higher than patients with normal CRP 26.2±18.47, 17.9±17.5, 5.7±5.2, 1.05±1.38 with \( p<0.89, 0.67, 0.20 \) and 0.31 respectively.

The mean of albuminuria (36.2±7) was not significantly correlated to the inflammatory markers (CRP, \( p=0.28 \) and Fib, \( p=0.64 \)). Similar correlations of the same were observed with ABI (right, \( p=0.86 \) and left, \( p=0.41 \)) study and CIMT (right, \( p=0.45 \) and left, \( p=0.64 \)).

The inflammatory marker CRP also was not correlated to CIMT (right, \( P=0.64 \) and left, \( p=0.82 \)) and ABI (right, \( p=0.35 \) and left, \( p=0.16 \)) as well as similar correlation of Fib with ABI (right, \( p=0.22 \) and left, \( p=0.20 \)) and CIMT (right, \( p=0.96 \) and left, \( p=0.88 \)).
Table 5. The carotid Doppler and ABI study of males and females patients (The mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right carotid</td>
<td>0.8±1.24</td>
<td>0.91±1.47</td>
<td>0.55±0.25</td>
<td>0.14</td>
</tr>
<tr>
<td>left carotid</td>
<td>0.8±1.48</td>
<td>0.88±1.77</td>
<td>0.6±0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Right ABI</td>
<td>1.1±0.11</td>
<td>1.11±0.11</td>
<td>1.06±0.11</td>
<td>*0.035</td>
</tr>
<tr>
<td>Left ABI</td>
<td>1.1±0.12</td>
<td>1.12±0.13</td>
<td>1.06±0.11</td>
<td>*0.036</td>
</tr>
</tbody>
</table>

* A significant value.
Table 6. The variables in male patients with positive and negative stress test (The mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males with positive test</th>
<th>Males with Negative test</th>
<th>R</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT. Carotid</td>
<td>0.76±0.57</td>
<td>0.92±0.20</td>
<td>0.80</td>
<td>0 to 0</td>
</tr>
<tr>
<td>LT. carotid</td>
<td>0.76±0.15</td>
<td>0.89±1.8</td>
<td>0.65</td>
<td>0 to 0</td>
</tr>
<tr>
<td>RT. ABI</td>
<td>1.11±0.33</td>
<td>1.11±0.09</td>
<td>-0.11</td>
<td>-0.97 to 0.95</td>
</tr>
<tr>
<td>LT. ABI</td>
<td>1.08±0.30</td>
<td>1.12±0.0.11</td>
<td>-0.30</td>
<td>-0.98 to 0.93</td>
</tr>
<tr>
<td>10Y %CHD</td>
<td>34.7±17.7</td>
<td>29.0±21.6</td>
<td>0.96</td>
<td>-0.40 to 1.0</td>
</tr>
<tr>
<td>10Y fatal %CHD</td>
<td>31.4±25.6</td>
<td>17.8±17.4</td>
<td>0.99</td>
<td>0.65 to 1.0</td>
</tr>
<tr>
<td>10Y %Stroke</td>
<td>8.8±3.16</td>
<td>6.21±8.0</td>
<td>0.54</td>
<td>-0.99 to 0.88</td>
</tr>
<tr>
<td>10Y%fatal %Stroke</td>
<td>1.42±7.9</td>
<td>1.17±2.72</td>
<td>0.37</td>
<td>0.92 to 0.98</td>
</tr>
</tbody>
</table>
Table 7. The absolute values of the males 10 years vascular risk to the mean of CIMT and ABI tests (The mean ± SD).

<table>
<thead>
<tr>
<th>Male vascular risk 10 Y</th>
<th>Variable</th>
<th>Males</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD% 29.3±21.3</td>
<td>Right CIMT</td>
<td>0.91±1.47</td>
<td>r -0.09 \ p&lt;0.49</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.88±1.77</td>
<td>r -0.03 \ p&lt;0.82</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.11±0.11</td>
<td>r -0.15 \ p&lt;0.24</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.12±0.13</td>
<td>r -0.20 \ p&lt;0.11</td>
</tr>
<tr>
<td>Stroke% 6.3±7.8</td>
<td>Right CIMT</td>
<td>0.91±1.47</td>
<td>r -0.09 \ p&lt;0.49</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.88±1.77</td>
<td>r -0.05 \ p&lt;0.68</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.11±0.11</td>
<td>r -0.07 \ p&lt;0.60</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.12±0.13</td>
<td>r -0.09 \ p&lt;0.48</td>
</tr>
</tbody>
</table>

* A significant value.
Table 8. The absolute values of females 10 years vascular risk to the mean of CIMT and ABI tests (The mean ± SD).

<table>
<thead>
<tr>
<th>Female vascular Risk 10Y</th>
<th>Variable</th>
<th>Females</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD% (16.1±16.8)</td>
<td>Right CIMT</td>
<td>0.55±0.25</td>
<td>r 0.22, p&lt;0.29</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.6±0.23</td>
<td>r 0.13, p&lt;0.52</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.06±0.11</td>
<td>r -0.15, p&lt;0.44</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.06±0.11</td>
<td>r -0.22, p&lt;0.26</td>
</tr>
<tr>
<td>Stroke% (7.6±14.3)</td>
<td>Right CIMT</td>
<td>0.55±0.25</td>
<td>r 0.15, p&lt;0.47</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.6±0.23</td>
<td>r 0.09, p&lt;0.67</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.06±0.11</td>
<td>r -0.33, p&lt;0.098</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.06±0.11</td>
<td>r -0.37, p&lt;0.06</td>
</tr>
</tbody>
</table>

* A significant value.
Table 9. The absolute values of the inflammatory markers of all patients to the mean of CIMT and ABI tests (The mean ± SD).

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>Variable</th>
<th>All Patients.</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (5.2±6.0 mg/L)</td>
<td>Right CIMT</td>
<td>0.8±1.24</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.8±1.5</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.11±0.12</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.1±0.13</td>
<td>*&lt;0.01</td>
</tr>
<tr>
<td>Fib (3.6±0.9 g/L)</td>
<td>Right CIMT</td>
<td>0.8±1.24</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.8±1.5</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.11±0.12</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.1±0.13</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* A significant value.
Table 10. The absolute values of the inflammatory markers in male patients to the mean of CIMT and ABI tests (The mean ± SD).

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>Variable</th>
<th>Males</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (5.0±5.7 mg/L)</td>
<td>Right CIMT</td>
<td>0.91±1.47</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.88±1.77</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.11±0.11</td>
<td>*&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.12±0.13</td>
<td>*&lt;0.01</td>
</tr>
<tr>
<td>Fib (3.6±0.86 g/L)</td>
<td>Right CIMT</td>
<td>0.91±1.47</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.88±1.77</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.11±0.11</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.12±0.13</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* A significant value.
Table 11. The absolute values of the inflammatory markers in female patients to the mean of CIMT and ABI tests (The mean ± SD).

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>Variable</th>
<th>Females</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (5.8±6.6 mg/L)</td>
<td>Right CIMT</td>
<td>0.55±0.25</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.6±0.23</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.06±0.11</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.06±0.11</td>
<td>0.45</td>
</tr>
<tr>
<td>Fib (3.7±1.0 g/L)</td>
<td>Right CIMT</td>
<td>0.55±0.25</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Left CIMT</td>
<td>0.6±0.23</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Right ABI</td>
<td>1.06±0.11</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Left ABI</td>
<td>1.06±0.11</td>
<td>0.51</td>
</tr>
</tbody>
</table>

* A significant value.
Photo 1. Scanning position.

Photo 2. Normal Bifurcation of the carotid artery.
Photo 3. Normal Intima of the arteries.

Photo 4. Plaque Types of the carotid artery.
Chapter Five

Results of the CAPN10 gene study.

A total of 236 patients with T2DM (129 males / 107 females) with a mean age at the time of the study of (57.9±SD 15.3 years) and at the time of the diagnosis of (47.5±SD 9.5 years) were recruited. A group of 120 control subjects were also recruited (54 males and 66 females) with a mean age of (42.5±SD 17.5 years). The patients and the controls were all of purely Irish descent as determined by asking that their grandparents were of Irish descent.

The observed genotype frequencies from the control sample did not significantly differ from those expected according to Hardy-Weinberg equilibrium for all examined markers. This indicates that the current control is relevant to use as a comparative group against the T2DM. Three single nucleotide polymorphisms (SNPs) and the insertion/deletion variants were analysed in this study. The markers SNP43 (rs3792267), ins/del 19 (at position 241182968) and SNP63 (rs5030952) are intronic while the marker SNP2037 (rs3749166) is exonic. Marker name, genomic position, genotyping success rate and allele frequencies are presented in table 1. Allelic numbers and frequencies of all markers of patients and controls are presented in table 2. No significant differences were observed between the two groups. However, a trend to an increased frequency of the deletion marker (marker19) was observed in the patients group but was not statistically significant ($\chi^2=3.2$, p=0.07).

Similar to the allelic, genotypic numbers and frequencies (table 3) did not show significant deviations between the two groups. However, as in the allelic, increased del/del genotype was seen in the patient group but did not attain significance at the 0.05 level ($\chi^2=2$, p=0.16).

Linkage disequilibrium analysis (LD) was conducted using the program Haploview. Significant LD (measured as D') ranges between (0.71-0.9) was detected between all markers (Diagram 2). Haplotype analysis (using two, three and four sliding windows) was also conducted using the same program. The two marker haplotype analyses (tables 4, 5 and 6) showed an under representation of a haplotype made up of the G allele of the marker rs3792267 (SNP 43) and the ins allele of the ins/del marker in the patient group.
In contrast, a significant overrepresentation of a haplotype made of the ins allele and the G allele of the marker (SNP 2037) rs3749166 ($\chi^2= 5.76, p=0.016$) was seen in the patient group.

The three windows haplotypes (G-del-G) made of the markers rs3792267 (SNP43), (ins/del 19) and rs3749166 (SNP2037) showed a significant association with T2DM ($\chi^2=5.3, P=0.021$) (*table 7)*.

A protective ($\chi^2=6.7, P=0.0097$) but rare (4%) haplotype made up of the alleles G-ins-G of the above markers was also detected (*table 7*). Similarly a protective ($\chi^2=8.4$, $P=0.0036$) but rare (4%) haplotype made up of the alleles ins-GC of the above markers was also detected (*table 8*).

The four window haplotype (*table 9*) of the examined markers showed a strong trend toward association of a haplotype constructed of the allele G-del-GT of the markers rs3792267 (SNP 43), ins/del 19 (position 241182968), rs3749166 (SNP 2037) and rs5030952 (SNP63) respectively and T2DM. ($\chi^2= 3.32, p=0.068$). The frequency of this haplotype is 7% which is less effective than three markers haplotype made up of G-del-G of 31% frequency in the sample.
Table 1. General information of the four CAPN10 gene examined markers.

<table>
<thead>
<tr>
<th>Name</th>
<th>Old Name</th>
<th>Position</th>
<th>Obs Het</th>
<th>Pred Het</th>
<th>HWI</th>
<th>Genotype</th>
<th>MAF</th>
<th>M.A</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3792267</td>
<td>43</td>
<td>241179847</td>
<td>0.405</td>
<td>0.387</td>
<td>0.4902</td>
<td>99.4</td>
<td>0.262</td>
<td>A</td>
</tr>
<tr>
<td>Ins/del</td>
<td>19</td>
<td>241182968</td>
<td>0.475</td>
<td>0.468</td>
<td>0.8889</td>
<td>97.7</td>
<td>0.374</td>
<td>del</td>
</tr>
<tr>
<td>rs3749166</td>
<td>2037</td>
<td>241186094</td>
<td>0.474</td>
<td>0.465</td>
<td>0.8181</td>
<td>93.8</td>
<td>0.367</td>
<td>G</td>
</tr>
<tr>
<td>rs5030952</td>
<td>63</td>
<td>241191376</td>
<td>0.147</td>
<td>0.161</td>
<td>0.2637</td>
<td>75.4</td>
<td>0.088</td>
<td>T</td>
</tr>
</tbody>
</table>

Obs= Observed, Pre= predicted, HET= heterozygosity, HW= Hardy Weinberg equilibrium, MAF= Minor allele Frequency, M.A= Minor allele.
Table 2. Allele numbers and frequencies of patients with T2DM and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Marker</th>
<th>Alleles</th>
<th>$\chi^2$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs3792267</td>
<td>A</td>
<td>1.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Controls</td>
<td>(SNP43)</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>56(24%)</td>
<td>180(76%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>128(27%)</td>
<td>338(73%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Position</td>
<td>ins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>241182968</td>
<td>del</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ins/del 19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>156(67%)</td>
<td>76(33%)</td>
<td>3.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Patients</td>
<td>276(60%)</td>
<td>182(40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs3749166</td>
<td>A</td>
<td>0.59</td>
<td>0.44</td>
</tr>
<tr>
<td>(SNP 2037)</td>
<td>(SNP 2037)</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>145(56%)</td>
<td>77(35%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>274(62%)</td>
<td>166(38%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs5030952</td>
<td>C</td>
<td>2.3</td>
<td>0.13</td>
</tr>
<tr>
<td>(SNP 63)</td>
<td>(SNP 63)</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>167(94%)</td>
<td>11(6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>318(90%)</td>
<td>36(10%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: value in parenthesis represent allele frequencies.
Table 3. Genotype numbers and frequencies in patients with T2DM and controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Marker</th>
<th>Genotype</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs3792267</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(SNP 43)</td>
<td>5(4.1%)</td>
<td>46(38.3%)</td>
<td>69(57.6%)</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Patients</td>
<td>15(6.4%)</td>
<td>99(42.5%)</td>
<td>119(51%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\chi^2)</td>
<td>0.83</td>
<td>0.78</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.36</td>
<td>0.38</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Position</td>
<td>(ins/ins)</td>
<td>(ins/del)</td>
<td>(del/del)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>241182968</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ins/del 19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Patients</td>
<td>51(42.5%)</td>
<td>57(47.5%)</td>
<td>12(10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs3749166</td>
<td>(AA)</td>
<td>(AG)</td>
<td>(GG)</td>
<td></td>
</tr>
<tr>
<td>(SNP 2037)</td>
<td></td>
<td>83(36.5%)</td>
<td>109(48%)</td>
<td>35(15.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\chi^2)</td>
<td>1.17</td>
<td>0.01</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.28</td>
<td>0.92</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs5030952</td>
<td>(CC)</td>
<td>(CT)</td>
<td>(TT)</td>
<td></td>
</tr>
<tr>
<td>(SNP63)</td>
<td></td>
<td>45(37.5%)</td>
<td>64(53.4%)</td>
<td>11(9.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>86(38.9%)</td>
<td>103(46.6%)</td>
<td>32(14.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\chi^2)</td>
<td>0.07</td>
<td>1.41</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.79</td>
<td>0.23</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs5030952</td>
<td>(CC)</td>
<td>(CT)</td>
<td>(TT)</td>
<td></td>
</tr>
<tr>
<td>(SNP63)</td>
<td></td>
<td>79(87.7%)</td>
<td>11(12.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>145(81.9%)</td>
<td>28(15.8%)</td>
<td>4(2.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\chi^2)</td>
<td>1.51</td>
<td>0.62</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.22</td>
<td>0.43</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values in parenthesis represent genotype frequencies.
Diagram (1) of Linkage disequilibrium (LD) relations (measured as D') in CAPN10 gene examined markers.
Table 4. Two markers haplotype analysis of the (SNP43) and ins/del 19) in patients and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Case, Control Ratios</th>
<th>Chi-Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-ins</td>
<td>0.394</td>
<td>170.4 : 299.6, 107.8 : 128.2</td>
<td>5.84</td>
<td>0.0157</td>
</tr>
<tr>
<td>G-del</td>
<td>0.344</td>
<td>170.4 : 299.6, 72.2 : 163.8</td>
<td>2.233</td>
<td>0.1351</td>
</tr>
<tr>
<td>A-ins</td>
<td>0.234</td>
<td>114.5 : 355.5, 50.7 : 185.3</td>
<td>0.728</td>
<td>0.3935</td>
</tr>
<tr>
<td>A-del</td>
<td>0.028</td>
<td>14.7 : 455.3, 5.3 : 230.7</td>
<td>0.439</td>
<td>0.5074</td>
</tr>
</tbody>
</table>

Table 5. Two markers haplotype analysis of the (ins/del 19 and SNP2037) in patients and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Case, Control Ratios</th>
<th>Chi-Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ins-A</td>
<td>0.579</td>
<td>267.0 : 199.0, 139.3 : 96.7</td>
<td>0.195</td>
<td>0.6589</td>
</tr>
<tr>
<td>Ins-G</td>
<td>0.317</td>
<td>161.8 : 304.2, 60.9 : 175.1</td>
<td>5.768</td>
<td>0.0163</td>
</tr>
<tr>
<td>del-A</td>
<td>0.056</td>
<td>23.3 : 442.7, 16.3 : 219.7</td>
<td>1.067</td>
<td>0.3016</td>
</tr>
<tr>
<td>del-G</td>
<td>0.048</td>
<td>13.9 : 452.1, 19.5 : 216.5</td>
<td>9.637</td>
<td>0.0019</td>
</tr>
</tbody>
</table>
Table 6. Two markers haplotype analysis of the two SNPs (2037 and 63) in patients and controls.

<table>
<thead>
<tr>
<th>Haplotype Frequency</th>
<th>Case, Control Ratios</th>
<th>Chi-Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>0.626</td>
<td>284.9: 177.1, 152.2: 83.8</td>
<td>0.538</td>
</tr>
<tr>
<td>GC</td>
<td>0.289</td>
<td>133.0: 329.0, 68.8: 167.2</td>
<td>0.01</td>
</tr>
<tr>
<td>GT</td>
<td>0.079</td>
<td>41.8: 420.2, 13.3: 222.7</td>
<td>2.525</td>
</tr>
</tbody>
</table>

Note: combination of A-T is rare and did not appear in this sample.

Table 7. Three markers haplotype analysis of the (SNP43, ins/del 19 and SNP2037) in patients and control groups.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Case, Control Ratios</th>
<th>Chi Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-ins-A</td>
<td>0.349</td>
<td>154.9: 315.1, 91.3: 144.7</td>
<td>2.261</td>
<td>0.1326</td>
</tr>
<tr>
<td>G-del-G</td>
<td>0.315</td>
<td>161.3: 308.7, 60.8: 175.2</td>
<td>5.323</td>
<td>0.021</td>
</tr>
<tr>
<td>A-ins-A</td>
<td>0.231</td>
<td>114.9: 355.1, 48.0: 188.0</td>
<td>1.502</td>
<td>0.2203</td>
</tr>
<tr>
<td>G-ins-G</td>
<td>0.043</td>
<td>13.7: 456.3, 16.8: 219.2</td>
<td>6.698</td>
<td>0.0097</td>
</tr>
<tr>
<td>G-del-A</td>
<td>0.031</td>
<td>10.9: 459.1, 11.1: 224.9</td>
<td>2.977</td>
<td>0.0844</td>
</tr>
<tr>
<td>A-del-A</td>
<td>0.025</td>
<td>12.5: 457.5, 5.0: 231.0</td>
<td>0.176</td>
<td>0.6745</td>
</tr>
</tbody>
</table>
Table 8. Three markers haplotype analysis of the (ins/del 19 and SNP2037 and SNP63) in patients and control groups.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Case, Control Ratios</th>
<th>Chi Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ins-AC</td>
<td>0.576</td>
<td>267.4: 202.6, 138.9: 97.1</td>
<td>0.253</td>
<td>0.6149</td>
</tr>
<tr>
<td>del-GC</td>
<td>0.245</td>
<td>122.8: 347.2, 49.9: 186.1</td>
<td>2.105</td>
<td>0.1468</td>
</tr>
<tr>
<td>del-GT</td>
<td>0.073</td>
<td>40.6: 429.4, 11.0: 225.0</td>
<td>3.69</td>
<td>0.0547</td>
</tr>
<tr>
<td>del-AC</td>
<td>0.052</td>
<td>22.0: 448.0, 14.8: 221.2</td>
<td>0.807</td>
<td>0.3691</td>
</tr>
<tr>
<td>ins-GC</td>
<td>0.044</td>
<td>13.4: 456.6, 18.0: 218.0</td>
<td>8.479</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

Table 9. Four window haplotype of all examined markers (SNP43, ins/del 19, SNP2037 and SNP63) in patients and control groups.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Case, Control Ratios</th>
<th>Chi Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-ins-AC</td>
<td>0.346</td>
<td>153.0: 317.0, 91.3: 144.7</td>
<td>2.593</td>
<td>0.1074</td>
</tr>
<tr>
<td>G-del-GC</td>
<td>0.245</td>
<td>122.9: 347.1, 50.0: 186.0</td>
<td>2.073</td>
<td>0.1499</td>
</tr>
<tr>
<td>A-ins-AC</td>
<td>0.230</td>
<td>115.0: 355.0, 47.6: 188.4</td>
<td>1.641</td>
<td>0.2002</td>
</tr>
<tr>
<td>G-del-GT</td>
<td>0.070</td>
<td>38.9: 431.1, 10.7: 225.3</td>
<td>3.319</td>
<td>0.0685</td>
</tr>
<tr>
<td>G-ins-GC</td>
<td>0.040</td>
<td>13.0: 457.0, 15.5: 220.5</td>
<td>5.882</td>
<td>0.0155</td>
</tr>
<tr>
<td>G-del-AC</td>
<td>0.031</td>
<td>10.8: 459.2, 10.7: 225.3</td>
<td>2.677</td>
<td>0.1018</td>
</tr>
<tr>
<td>A-del-AC</td>
<td>0.019</td>
<td>9.7: 460.3, 3.8: 232.2</td>
<td>0.164</td>
<td>0.6852</td>
</tr>
</tbody>
</table>

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Chapter Six
Discussion
Type 2 Diabetes is associated with 2-4-fold increased mortality, which is largely due to cardiovascular disease. Due to the failure to demonstrate reduction in this risk of mortality in studies targeting aggressive control of glycaemia (Demissie 2009), it is commonly thought that by the time that T2DM develops, it may be too late to prevent the cardiovascular complications. Strategies that could be employed to attempt to reduce the cardiovascular events could include prevention of the development of the condition. It is also possible that earlier identification of subjects at particularly increased risk of cardiovascular disease might allow more aggressive interventions to be initiated in these subjects from an early stage in the disease process and thus prevent development of CVD complications. In this thesis a number of strategies that might provide such an opportunity have been explored: using the presence of the MS to identify subjects at particularly increased CVD risk; use of the UKPDS risk engine at the time of diagnosis and during follow up to identify subjects at particularly increased cardiovascular risk and their response to treatment; measurement of non-"traditional" cardiovascular risk markers; use of genetic screening as a means of identifying subjects at risk of T2DM prior to onset of the condition.

MS results from a complex interaction of genetic predisposition and environmental factors: obesity; physical inactivity and excess calorie intake (Timer 2000, McQueen 2003). The prevalence of MS in various populations has already been reported (Villegas 2003, Al-Lawati et al 2003, Ford 2002). Here, in the first study to document the prevalence of the MS in patients with DM in Ireland, we have demonstrated a high prevalence (61%) of MS in patients with diabetes. Our data found that the prevalence of the MS in T2DM patients (69.5%) was higher than in patients with T1DM (22%) as expected because of association of T2DM with insulin resistance (IR). However, many clinicians have observed that some patients with T1DM develop an MS phenotype and speculate that it develops as a result of insulin-induced weight gain. My data suggested that the prevalence of MS in T1DM is similar to that previously reported in the non-diabetic Irish population (Villegas 2003).
Of note is that the prevalence of MS in females with diabetes appears to be greater than in males but this may be related to small number of women recruited in this study. This is in contrast to the prevalence of obesity in Irish adults (IUNA.NSIFCS).

Reports of the prevalence of MS in patients with diabetes emerged some years ago. Analysis of the NHANES data suggested a substantially higher prevalence of MS in patients with diabetes - 86% (Alexander 2003). The higher prevalence rate noted in that study compared to the current data may be accounted for by the facts that the analysis was confined to subjects with diabetes over the age of 50 and that the population was heterogeneous with certain ethnic groups being intentionally oversampled. More recent reports from Australia have shown similar results to our study, with a prevalence of MS in T2DM of 72.3% (Wong 2006) and in T1DM of 15% (McGill 2008) using WHO criteria to diagnose MS.

There is considerable evidence that the MS confers increased cardiovascular risk. A retrospective analysis of subjects involved in WOSCOPS trial (Sattar et al 2003), showed that the 26% who had MS by ATP III criteria had a hazard ratio of 1.3 for cardiovascular events after correction of other risk factors. In the Framingham volunteers (Najarian 2006), during 10 years of follow-up, men with the MS had a 78% greater risk of strokes than those without, and for women the risk was more than double. In addition it has been shown that increasing MS score correlates with increased frequency of cardiovascular events, interventions and the angiographic severity of coronary artery disease (Solymoss et al 2004).

In patients with diabetes it has also been shown that the presence of the MS is associated with a 2-3-fold increase in coronary heart disease prevalence compared to patients with diabetes but without MS (Alexander 2003). Therefore it is important for those caring for people with diabetes to be aware of whether or not their patients also have MS. In this regard it is of particular concern that two thirds of patients with T2DM in this study actually met the criteria of the MS despite most being treated for diabetes, hypertension and dyslipidaemia with most subjects satisfying the WCM, BP and glycaemic criteria. This study demonstrated in all patients with T2DM including those with and without MS, the WCM is more likely the potential for abnormal lipids (TG and HDL) than HbA1c. However the glycaemia control was satisfactory in those
patients. In T1DM patients, high serum TC, TG, LDL and TG/HDL are significantly correlated with HbA1c (Ladeia 2006). In this study the lipid profile of the patients with T1DM was within the target but that was lost in the subgroups of patients with and without MS. This is likely also related to increase WCM in patients with MS as their HbA1c was also satisfactory.

Our data, acquired from a homogeneous cohort attending a single centre, are probably representative of patients attending specialist diabetes centres in Ireland and show that even in a quite aggressively treated group of patients, there is a significant subgroup that still meets the criteria for the MS and which therefore remains at significantly increased cardiovascular risk (table 1. chapter 3). This emphasizes the need for a Steno-2 style approach for the management of cardiovascular risk in subjects with T2DM (Gaede 2003). The prevalence of MS in T1DM is a lot lower than in T2DM but somewhat higher in this sample of the Irish population compared to the Australian sample (McGill 2008). However, it appears to be similar to that reported in the non-diabetic Irish population as cited above. As the MS in T1DM is associated with greater risk of complications, it is likely to be as important to identify patients with T1DM who have the MS phenotype as it is in T2DM.

We suggest that an integral component of the annual assessment of patients with diabetes should be the calculation of an MS score so that those who have particularly high cardiovascular risk can be targeted for especially aggressive risk factor management. This would be especially important in recently diagnosed patients as the time of diagnosis probably represents the best opportunity to risk stratify patients with T2DM and intervene to reduce risk. For example, while there remains controversy regarding the use of aspirin for primary prevention of CVD in patients with T2DM (Sirois 2008, Price 2009), in subjects at particular cardiovascular risk, such as those with co-existent T2DM and MS, this may be a more appropriate intervention. We would also advise that particular attention to hypertension and obesity, which are the most prevalent components of the MS in our patients with diabetes, is required.

Another strategy that could be used to identify T2DM subjects at particularly increased CVD risk at the time of diagnosis would be to use the recently proposed UKPDS risk engine. This risk engine could be reviewed annually in patients, of both genders in whom the vascular risk is expected to be high. The risk engine is used to
predict CHD risk, defined as fatal or non fatal MI or sudden death and to recognize different levels of risk for CHD and cerebrovascular disease. The new risk engine which is specific for T2DM incorporates the HbA1c, systolic BP, total cholesterol: HDL cholesterol ratio, age, sex, ethnic group, smoking and the duration of the DM, but interestingly it does not include microalbuminuria and hypertriglyceridaemia.

The projected risk, using the risk engine, of the cardiovascular events (fatal and non fatal) at 3, 5 and 10 years was substantial in this cohort. The risks for cardiovascular events were higher in males than females, but interestingly the cerebrovascular risk events (fatal and non fatal) were higher in females than males. Therefore both males and females who have T2DM would be considered to be at higher risk of different vascular events which are cumulative throughout the course of the disease provided there were no changes in the modifiable risk factors of the risk engine formula (Hypertension, Dyslipidaemia, smoking habit, atrial fibrillation with controlled DM). However, in real life in patients with T2DM interventions will be made which may change the proposed above vascular event risk. The increased risk of cerebrovascular risk events in females with recently diagnosed diabetes may suggest that further investigation (eg carotid Doppler) might be useful to target high risk patients for primary preventative strategies. In keeping with this suggestion, there was a trend to a positive correlation between carotid intima media thickness and 10 year stroke risk calculated from the UKPDS risk engine in women (table 8).

Although there were a small number of subjects in this study, surprisingly, the HbA1c in males was significantly higher than females. The reason for this is not immediately clear, but it could suggest that women came to attention earlier than men. Not surprisingly, the reverse was true for the HDL. These factors would have contributed to the higher calculated cardiovascular risk in male patients. Atrial fibrillation was not reported in either gender. Although the risks in both genders are high, the risk of vascular events as calculated from the risk engine would be substantially reduced by aggressive control of the modifiable components of the formula such as hypertension, dyslipidaemia and glycaemic control with cessation of smoking.

There are data to support interventions for these risk factors in diabetes. Although the impact of smoking was not studied specifically in the UKPDS, 78% of men and 56%
of women were smokers or ex-smokers. In the present study smoking would have contributed less to calculated risk as only 32% of males and 21% females were smokers. Those who smoke have a higher mortality from cardiovascular disease than those with diabetes who do not smoke. Reduction in smoking would potentially produce the single most important reduction in cardiovascular risk factors. The loss of life expectancy associated with smoking and hypertension is greater than that occurring from hypercholesterolemia, but stopping smoking would prolong life by a mean of around four years in a 45 year old non-diabetic man and three years in a diabetic man, whereas aspirin and antihypertensive treatment would provide approximately one year of additional life expectancy in both categories (Yudkin 1993).

The Hypertension Optimal Treatment (HOT) study showed a 50% reduction in major cardiovascular events in patients with T2DM with more aggressive blood pressure targets (diastolic BP 80mmHg versus 90mmHg, Hansson et al 1998). There are also ample data to support the use of statins for primary prevention in patients with diabetes (Steiner 2000). All patients with T2DM with highest coronary risk of >30% risk over one year or >30% over 10 years should unequivocally be treated with a statin (DAIISI 2001). In the UKPDS risk engine, the HbA1c was a risk factor in estimating the vascular risk formula. It has confirmed as a more important determinant of CHD than FPG measures, and there is a controversy as to whether pre-prandial, post-prandial or integrated glyceamic measures are more predictive of CVD in DM. In our study the HbA1c was greater in males than females and probably influences the CHD% risk in male’s patients. The data supporting interventions targeting tighter glyceamic control in T2DM are a little less clear. However, there is plenty of evidence to support aggressive risk factor control in patients with diabetes who are at high cardiovascular risk with a multifaceted approach being very effective (steno 2 study).

In this study the UKPDS risk engine has helped us identify subjects with diabetes who are at particularly high risk. The risk of vascular complications in newly diagnosed T2DM is considerable in both genders at the time of the diagnosis and on the following years. The 10 years CHD% risk was more than 30% in 29 (40.2%) males and 4 females (14.2%) patients. For the same period of time the risk of the stroke was
more than 15% in 7 males (9.7%) and 2 females (7.1%) patients. The 10-years CHD% risk was > 50% in 11 males (15.2%) and 2 females 7.1%). This group of patients in particular should be targeted for a Steno-2 style approach. The risk of vascular events should be estimated and annually to ensure that interventions targeting CVD risk factors are effectively reducing projected risk.

Patients with T2DM resident in two Caribbean Islands of Tobago and Trinidad were evaluated for vascular risk event by using the UKPDS risk engine. The 10-year CHD and stroke risks were statistically stratified into <15%, 15-30% and >30% CHD risk levels and differences between patients of African and Asian-Indian origin were compared. The patients in Tobago (Trinidad) irrespective of gender, had higher proportion of 10-year CHD risk (10.4 vs. 23.6%, P<0.001) whereas the overall 10-year stroke risk prediction was higher in patients resident in Tobago (16.9 vs. 11.4%, P<0.001). Ethnicity-based analysis revealed that irrespective of gender, higher proportion of patients of Indian origin scored >30% of absolute 10-year coronary heart disease risk compared with patients of African descent (3.2 vs. 28.2%, P<0.001) (Ezenkawa CE et al 2009) The estimation of vascular risk factors by using the UKPDS risk engine was performed on Mediterranean patients with T2DM and without established CHD. The reduction risk was 37% for CHD, 44% for fatal CHD, 10% for stroke and 25% for fatal stroke.

Although the UKPDS risk engine can be utilised to estimate risk of vascular events in subjects with diabetes, if atheromatous macrovascular changes are present in newly diagnosed T2DM this may be of more significance. Although there are data on the presence of microvascular complications of diabetes at diagnosis, there are few studies which have attempted to document the presence of macrovascular complications at the time of diagnosis of T2DM.

In this study it was found that males were more likely to have vascular complications at diagnosis of T2DM however the ABI test was significantly lower in females than males (Right ABI p<0.035, Left ABI p<0.036. Table 5). The ABI test was reduced below normal (<0.90) in one patient of each gender. The male patient had positive treadmill exercise test (TMET) and CHD was confirmed by coronary angiography, but the female patient did not attend for the stress test. There were no correlation of the ABI test to the albuminuria and inflammatory markers in either gender. TMET
was positive in four male patients who had abnormal CIMT and one had coronary angiography and stenting. There were no significant correlations between inflammatory markers (CRP and Fib) and albuminuria to ABI or CIMT tests which are likely related to small number of patients in this study.

In male patients the ABI was significantly lower in positive TMET than in those with a normal test but there was no difference between the findings on the carotid Doppler study in these two groups. These data would need to be interpreted cautiously however as there were only 4 patients with a positive TMET. The ABI and TMET tests were positive in the absence of symptoms.

Consideration could be given to recommend measuring the ABI in all new patients with T2DM regardless the presence or absence of the PVD symptoms. Most of the patients with positive TMET have abnormal ABI study. The ABI test is easy, cheap and available to be performed to identify asymptomatic and subclinical PVD which may be associated with CHD in newly diagnosed T2DM patients. In this situation, early diagnosis could prevent or delay serious late complications. The ABI test is the recommended tool for the screening PVD in both, patients with DM and subjects without DM (Schmidt 1999). In this study (table 6) male patients with positive stress test and lower CIMT have higher UKPDS scores compared with patients with negative test. This suggests further essential investigations should be performed like stress test and carotid Doppler study in new patients with T2DM with higher UKPDS scores. ABI may be also required however there was only one male patient with low abnormal results. In this group of patients who were asymptomatic, it is difficult to suggest the best test for screening of subclinical vascular complications. These tests are strongly indicated in symptomatic and high risk patients and consideration for screening in asymptomatic depends on the availability of the tests in that particular service. We think more studies are required to find the best diagnostic tool to diagnose vascular diseases in asymptomatic newly diagnosed patients with DM. However, as the ABI is simpler to perform than carotid Dopplers or TMET, and the yield of additional positive tests for CVD is likely to be high when the ABI is abnormal, it is probably the best test for screening for subclinical vascular disease. The yield in new patients would be expected to be low based on the current data however.
Hypertrophy of arterial wall is an early sign of atherosclerosis. Increased CIMT is a non invasive marker of arterial wall alteration. This has previously been shown to correlate with other risk factors such as SBP, serum lipids and smoking, as well as being positively linked to the presence and extent of CHD in both genders. There is a correlation between the CIMT and CVD events such as stroke and MI (Ludwig 2003, Crouse et al 1996). CHD is associated with increased CIMT and arterial stiffness and low HDL concentration is an independent risk factor for increased CIMT and subclinical marker of increased atherosclerosis in DM (Alagona et al 2003, Moussavi 2004). In this study despite most of the patients having abnormal carotid Doppler test there was no significant difference in either gender. Our findings showed most (87%) of the patients had abnormalities on carotid Doppler. However there was no correlation between the abnormal CIMT and the inflammatory markers and albuminuria. CIMT has been proposed as a marker for early atherosclerotic disease (Poli 1988) and has been shown to predict MI and stroke in older adults (O’Leary 1999). There was no significant difference in CIMT between the 2 genders although CIMT tended to be lower in females than males therefore this study has shown that recently diagnosed T2DM patients have subclinical vascular complications, mainly in the coronary and peripheral circulations and more commonly in men. The data demonstrate that screening at the time of the diagnosis can detect abnormalities in 56 (62%) of patients and this may be advantageous to patients by identifying those at particular CVD risk for aggressive risk factor interventions. This study showed atherosclerosis is a major subclinical disease in newly T2DM in both genders which should be investigated. The DSC is the most likely abnormal and patients with reduced ABI and abnormal DSC are at higher risk of subclinical CHD. There is a particular strong association between disease in the carotid arteries and the lower extremities (Sutton 1987). TEMT should be considered in these particular patients.

The UKPDS risk score is dependent mostly on traditional risk factors for CVD such as hypertension, dyslipidaemia and hyperglycaemia. Other risk factors or risk markers could potentially highlight increased cardiac risk including visceral obesity which can be inferred from increased waist circumference. Visceral obesity has been shown to be accompanied by a constellation of metabolic derangements, including insulin resistance (IR), low HDL, elevated TG , and raised BP (Reaven 1988) and to be associated with increased CVD risk (Lakka et al 2002). It is thought to play a role in
endothelial dysfunction and vascular inflammation that promote atherosclerosis and waist circumference (WCM) had independently shown to be a risk factor for atherosclerosis. It is also associated with a chronic, low-grade inflammatory state, suggesting that inflammation may be a potential mechanism whereby visceral obesity leads to IR (Wisse 2004). C-reactive protein (CRP), which may be a marker of this inflammatory response, can be bound to oxidized low density lipoprotein (LDL), implicating the interaction of CRP and oxidized LDL as a potential trigger for the cascade of events leading to atherosclerosis. CRP has been shown to be associated with increased cardiovascular risk and diabetes. One study demonstrated that elevated baseline CRP levels were associated with the development of DM over 3-5 year period (Barzilay et al 2001).

In this study, we described a correlation between WCM and BMI and markers of inflammation (CRP and Fibrinogen). CRP is stronger marker metabolic risk than Fib related directly or indirectly to the arterial cell wall mechanisms leading to atherosclerotic lesions and cardiac events. CRP is an independent predictor of cardiovascular events in populations with and without established CVD (Munk 2009). Elevated baseline levels are strong independent predictor of future myocardial infarction, stroke, peripheral vascular disease and cardiovascular death amongst apparently healthy populations (Blake 2001, 2002). In patients with T2DM, multiple markers of chronic inflammation are low in the early stages of the disease and become elevated with disease progression and development of vascular complications (Hwang et al 2008). CRP is an independent risk factor associated with peripheral vascular disease (PVD) in patients with T2DM (Yu 2004) from the first day of diagnosis regardless of the duration of the disease and/or the presence of other vascular risk factors. In this study the inflammatory markers were higher than normal levels. CRP but not fibrinogen levels were higher in the highest quartile of WCM compared to the lowest quartile. The same values of (CRP but not Fibrinogen) were correlated to BMI of the group but they were not correlated to BMI subgroups (normal, overweight and obesity). These data suggest that these inflammatory markers and WCM through its relationship with traditional and non-traditional risk factors could also be considered as screening tools to identify those patients with recently diagnosed diabetes at high risk of CVD complications.
Both the SBP and DBP were slightly lower in females (133.2±15.1, 79.7±8.4), than males (134.1±10.7, 82.2±7.8) and were closer to the target (<130/80 mmHg). In neither gender was a correlation found between BP monitor readings and albuminuria or inflammatory markers. Hypertension was not a major vascular risk factor in this cohort but in Suurkula (1994), the CIMT was significantly larger in hypertensive group than in control group. Lumen diameter and mean cross-sectional area of the intima-media complex were larger both for hypertensive patients with a positive history of manifest clinical CVD and for hypertensive patients with no such history than in control group.

Microalbuminuria is a marker closely correlated with the increased cardiovascular risk and the presence of persistent microalbuminuria in IDDM is strongly predictive of the future development of end stage renal failure and of CVD to a lesser extent. It predicts early mortality both in T1DM and T2DM. It is an important marker for more pronounced diabetic vascular disease in general as well as for nephropathy (McKenna 1997, Jerums 2002, Bramlage et al 2003, Mogensen 2003). In this study, despite a small sample of patients, the mean microalbuminuria was significantly higher in males than females but it was not significantly correlated to the inflammatory markers (CRP and Fib) or to the macrovascular findings of both carotid and ABI tests. However, the UKPDS risk engine score suggested that the male patients were at higher risk of CVD than females and the higher microalbuminuria may be in keeping with this increased risk.

DM is a vascular and metabolic disease which is multifactorial in origin and due to interaction of genetic predisposition and environmental factors. Metabolic Syndrome (MS) results from a complex interaction of genetic predisposition and environmental factors such as obesity; physical inactivity and caloric excess (Timar 2000, McQueen 2003). The data discussed above have all been derived from patients who already have DM, but as alluded to above, data from studies such as the UKPDS which showed how difficult it can be to prevent macrovascular complications once T2DM has already developed could be taken to indicate that the best approach to prevention of these complications in patients with the condition is to prevent its development in the first instance. Although it is well documented that T2DM can be prevented by a variety of strategies including lifestyle interventions and several medications (Pisunyer 2007, Knowler et al 2002), DM can still develop in those with prediabetes.
Despite these measures. An ability to identify subjects at risk of developing T2DM prior to their manifesting dysglycaemia could confer an advantage in terms of facilitating interventions to reduce the risk of the disease. In this context, identifying the causative genes related to DM could lead to reduced incidence of the disease in the community and thus less likelihood of macrovascular disease complications. CAPN10 gene is one of genes related to DM in other populations which have not been studied before in Irish people.

The result of CAPN10 gene investigation shows a trend to a slight increase in the frequency of allele G at the SNP43 (rs3702267, \( \chi^2 = 1.13, p = 0.28 \)) in the patients with diabetes. However, genotypic frequencies of both patients and control groups did not show significant differences. Similar to SNP43, an increased frequency (trend toward association) of the deletion allele was seen in the patient group but was not statistically significant (\( \chi^2 = 3.2, p = 0.07 \)). The genotypic frequencies of individuals homozygous for the deletion/deletion were similarly increased but did not attain statistical significance at 0.05 (Table 2 and 3. Chapter 6). Therefore the relationship of several haplotypes made of the above alleles and T2DM were studied (Tables 4, 5, 6, 7, 8 and 9. Chapter 6).

The frequency of the examined markers are comparable to those reported in several Caucasian population such as the Polish (Malecki et al 2002), Finnish (Fingerlin et al 2002) and the Hap Map Caucasian sample. It is generally accepted (with some exceptions) that the so far examined allelic/genotypic frequencies of CAPN10 gene markers in this study and several others did not show significant difference between T2DM cases and comparative controls. However, haplotypes made up of CAPN10 gene markers were reported to be more consistently associated with T2DM. Recent genomic findings (Daly 2001) showed that the human genome is segmented in to haploblocks with size ranging between 50-100kb in average. Individual markers within these blocks tend to have strong LD relations. These haploblocks are usually inherited together and recombination events within these blocks are rare.

The original findings by Horikawa et al (2000) reported the absence of association between any of three examined individual markers (SNP43/ rs3792267, ins/del 19/ position 241182968, and SNP63/ rs5030952) and T2DM. In contrast, they reported
that the combination of the haplotypes 121/112 confers high risk for T2DM in Mexican as well as some Caucasian populations. Two other haplotypes (table 2, chapter 5) 111/111 and 121/221 were also associated with T2DM in German and Botnia Finish T2DM populations (Horikawa et al, 2000).

Haplotype analysis in the current sample shows a trend to increased frequency of the haplotype 112 of the same markers in T2DM patients compared to controls but did not attain significance \( (\chi^2 = 2.8 \ p=0.094) \). In contrast, a significant but protective 111 haplotype was observed in the T2DM sample \( (\chi^2 = 5.5 \ p=0.018) \). Combination of the two haplotype 121/121 (A-del-C/A-del-C) was not statistically significant \( (\chi^2=1.5 \ p=0.22) \). As in the German and Botnia T2DM populations, the combination of the haplotypes 112/221 (A-ins-T/G-del-C) tended to be increased in the Irish T2DM sample but was not statistically significant \( (\chi^2=2.2 \ p=0.14, \ OR=1.27) \).

The lack of a clear relationship between these haplotypes and DM in the Irish sample raises the possibility that the so far examined markers of CAPN10 gene (SNP43, ins/del 19 and SNP63) are not the causative variants for T2DM. Other functional variants within the CAPN10 gene or closely mapped genes (which are in significant LD) may predispose to the development of the disease.

A recent study by Yan (2002) identified functional A/G variant (rs3749166, SNP2037) at exon 13 of the of the CAPN10 gene. This substitution was found to change the amino acid (AA) alanine to valine. Both amino acids are neutral, non-polar and hydrophobic and the net charge of these AA is slightly different. Individuals with the G variant of the rs3749166 were found to have higher expression of Calpain 10 compared to those with A variant (expression ratios of A: G was 1:7). However, Allelic and genotypic frequencies of this variant were not significantly different between patients and control groups in this study (tables 2 and 5, chapter 5).

In contrast, haplotype analysis showed a significant association between T2DM and a haplotype made up of the Ins and G alleles of the markers 19 and rs3749166 respectively \( (\chi^2=5.8 \ p=0.015) \) (table 4, chapter 5). Another T2DM significantly associated haplotype made of the G-del-G alleles of the markers 43, ins/del and SNP2037) and T2DM \( (\chi^2=5.3 \ p=0.021) \) (table 7, chapter 5) was also observed.
Furthermore, a trend to increased frequency (though not significant) of four sliding window haplotype comprising of the G-del-GT alleles (of the markers 43, 19, rs3749166 and 63 respectively) was also observed in the T2DM sample (table 9, chapter 5).

It is important to note that the G allele of markers (SNP43/rs3792257 and SNP 2037/ rs3749166) respectively are in significant LD with each other (Diagram 1, chapter 5) and part of a haplotype combination that showed increased frequency in the T2DM population compared to controls (table 9). This may indicate a functional relationship between T2DM development and decreased CAPN10 gene mRNA levels in skeletal muscle from insulin-resistant subjects homozygous for the G allele at SNP43 (Baier et al, 2000).

Furthermore, The G allele of (SNP 2037) rs3749166 was also functional and confers 7 fold increase in the expression of the gene compared to the A allele (Yan, 2002). This may influence the development of T2DM independently or in combination with other CAPN10 gene variants. However, the mechanism by which these variants influence the development of T2DM has not been explored.

The three previously examined markers (SNP 43, del/ins19 and SNP 63) are intronic which are spliced out during RNA processing. Nevertheless, several recent reports demonstrated that intronic variants could have significant effect on gene expression particularly those situated in or near exon- intron boundaries (Martin et al, 2007). It is possible therefore that so far examined CAPN10 gene intronic variants are functional but of minor effect.

The inconsistent pattern of allelic and haplotypic associations reported by different groups including this investigation can be attributed to one/combination of the following reasons.

1. Sample size: Most of the so far published findings of T2DM and CAPN10 gene were based on fairly small/moderate samples which in many cases have insufficient power to detect genes of minor effect. To address the question of
limited detection power, meta-analysis should be attempted to overcome this problem. This can be done by combining results from all available studies (in the absence of heterogeneity) which will either confirm or reject the role of CAPN10 gene in T2DM.

2. Heterogeneity due to ethnic origin of the source populations. The associated haplotype reported in different studies may indicate population heterogeneity reflected in alleles and consequently linkage disequilibrium relations. This can be seen from the allele frequencies in allele and haplotype combinations in the Mexicans, Europeans and Asian populations. For example the haplotype (made of alleles G of 43, deletion of 19 and T of 63 which is associated with T2DM in our sample is at frequency of 38% compared to 23% in the Mexicans.

3. There may be complex interactions between different genes that confer slightly increased risk for T2DM.

A new study by the Wellcome Trust Case Control Consortium in UK (2007) found a cluster of SNPs on chromosome 10, with TCF7L2 represented by rs4506565 generating the strongest association signal for T2DM. The SNP r 4506565 is in tight Linkage Disequilibrium with rs7903146, the variant with the strongest aetiological claims. TCF7L2 scan reveals two signals for association with T2DM. The first within FTO (fat-mass and obesity associated) gene on chromosome 16q with several adjacent SNPs (ra9939609, rs7193144 and rs8050136). The other association was described on chromosome6p22 with a feature of clustering of highly associated SNPs including rs9465871 with a risk of allele frequency between 18 and 35% mapping to introns 5 of the CDKAL1. Finally this study observed a modest cluster association of SNPs on chromosome 10 including rs10758582 and rs7923866. None of the new chromosome 10 SNPs associated with T2DM is located near the CAPN10 gene indicating an independent association of CAPN10 gene variants with diabetes. It is therefore obviously clear that the T2DM is associated with several risk genes (each of minor/moderate effect) contributing to the pathogenesis of the disease.

Yet, the exact causative variants at the above susceptibility loci and the mechanism by which they contribute to the development of T2DM have not been established. Further genetic work is required to define the exact DNA variant and how it functions
in creating risk for T2DM. The identification of the functional variants is essential to understand the biological processes underlying the pathogenesis of T2DM, and how these variants interact with each other and with environmental factors to bring about the condition. It is also essential for future work to be extended to pharmacogenetics as it is clinically important and economically essential to predicted drug response based on genetic make up of individuals.

However, these kinds of studies could be used to identify subjects at risk for development of T2DM and allow targeting of preventative measures towards those at risk for development of the condition in the future. This may prove to be the most effective method for reduction in the cardiovascular burden of T2DM.
Chapter Seven

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Appendices

Definitions of Metabolic Syndrome

The existence of multiple definitions for the MS has caused confusion and has resulted in many studies and research papers comparing the merits of each definition. Clinicians, researchers and a number of organizations have formulated many definitions of the MS. These are concordant on the essential components, the glucose intolerance, obesity, hypertension, and dyslipidaemia, but all differ in the detail and definitions.

WHO

The World Health Organization criteria (WHO1999) requires the presence of Diabetes Mellitus, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) or IR and two of the followings:

1. Blood pressure ≥ 140/90 mmHg (or treated hypertension);
2. Dyslipidaemia: TG ≥ 1.69 mmol/L and or HDL ≤ 0.9 mmol/L (male), ≤ 1.0 mmol/L (female).
3. Central obesity: waist-hip ratio >0.90 (male), >0.85 (female) and/or body mass index (BMI) ≥30 kg/m².
4. Microalbuminuria: urinary albumin excretion ratio ≥ 20mg/min or albumin: creatinine ratio ≥ 30mg/g.

EGIR

The European Group for the Study of Insulin Resistance (EGIR) by (Balkau 1999). It requires IR defines as the top 25% of the fasting insulin values among non-diabetic individuals and two or more of the followings:

1. Blood pressure ≥ 140/90 mmHg or antihypertensive medications.
2. Dyslipidaemia: TG ≥ 2.0mmol/L and or HDL ≤ 1.0 mmol/L or treated for dyslipidaemia.
3. Central obesity: WCM ≥94cm in males and ≥ 80cm in females.
4. FPG ≥ 6.1mmol/L.
The World Health Organization (WHO) definition and the European Group for the Study of Insulin Resistance (EGIR) require glucose intolerance or insulin resistance IR as an essential component in the definitions.

**NCEP**


1. High WCM for male >40 inches (102 cm) and females >35 inches (88 cm).
2. HDL for males <1.02 mmol/L (<40 mg) and females <1.28 mmol/L (<50 mg/dL).
3. Elevated FBG ≥6.1 mmol/L.
4. High blood pressure ≥130/85 mmHg.
5. Elevated TG ≥1.7 mmol/L (>150 mg/dL).

ATP III avoids the implication that insulin resistance is the primary or only cause of associated risk factors. Although this criteria was able to identify CVD as the primary clinical outcome of the MS, most people with this syndrome have IR, which confers increased risk for T2DM. The CVD risk will significantly increased when T2DM becomes a clinical disease.

Oral glucose tolerance test (OGTT) should performed to detect dysglycaemia according to the WHO and AACE criteria. But it is not the case in ATP III. We think the test is costly and not available in most of the primary health care practice. However the OGTT is helpful in identifying the subclinical cases of dysglycaemia (IGT not the IFG) which might change to frank T2DM in next years and also it certainly will help to identify more cases with MS. Nevertheless it will be appropriate to be requested in high risk subjects.
AACE
The American Association of Clinical Endocrinologists (AACE) clinical criteria for

Risk factor components
1. Overweight/obesity
2. Elevated triglyceride
3. Low HDL-C
4. Elevated Blood pressure
5. 2-Hour post glucose challenge.
6. Fasting Plasma Glucose
7. Other risk factors.

Cut points for abnormality
BMI ≥25 kg/m²
≥150 mg/dL (1.69 mmol/L).
For males <40 mg/dL (1.04mmol/L)
and females <50 mg/dL (1.29 mmol/L).
≥130/85 mm Hg.
>140mg/d L.(7.8mmol/L)
Between 110 and 126 mg/dL.(6.1-7.0mmol/L)

AHA
The American Heart Association (AHA) National Heart, Lung and blood institute in
2005 criteria of MS. Blood pressure ≥ 130/85 mmHg or use of antihypertensive
medications. (Grundy 2004)
1. Elevated TG ≥150mg/dL or ≥1.69mmo/L.
2. Reduced HDL≤ 1.0 mmol/L (<40mg/d L) in males and women <1.27mmol/L
(<50mg/d L).
3. Elevated FPG ≥ 100 mg/dL (5.6mmo/L) or use medication for
hyperglycaemia.
4. Elevated WCM in males ≥40inches (102cm) and females ≥ 35inches (88cm).
Note: The American College of Endocrinology, argued that obesity should not be
included in the syndrome, stating that obesity is a cause of, not a consequence of IR.
IDF

The International Diabetes Federation (IDF) definition (Zimmet 2005) of MS
Central obesity (WCM ≥ 94 cm for Europid men and ≥ 80 cm for Europid women with
ethnicity specific values for other groups (South Asian and South-East Asian men ≥ 90 cm, women ≥ 80 cm: Japanese men ≥ 85 cm, women ≥ 90 cm).

Plus any of the followings:

1. Raised blood pressure (systolic ≥ 130 or diastolic ≥ 85 mmHg) or treatment of
   previously diagnosed hypertension.

5. Elevated TG ≥ 1.7 mmol/L.

6. Reduced HDL ≤ 1.03 mmol/L in males and women < 1.29 mmol/L or specific
treatment for these lipids abnormalities.

7. Elevated FPG ≥ 5.6 mmol/L or previously diagnosed DM.

This new definition considered the treatment of hypertension and dyslipidaemia
criteria and also there is lower cut down of the WCM and FPG level. More cases of
MS expected to be diagnosed with applying this criterion.