GENETIC CONTRIBUTION TO THE RISK FOR METABOLIC SYNDROME: AN INVESTIGATION OF CANDIDATE GENE POLYMORPHISMS RELATED TO LIPID AND CARBOHYDRATE METABOLISM

by

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DOCTOR OF PHILOSOPHY (PhD)

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Declaration

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To my parents

Presentations and Publications

- University of KwaZulu Natal (2010): *Oral presentation* on the "Genetic Contribution for the risk of Metabolic Syndrome"
- University of Stellenbosch- South African Centre for Epidemiological Modelling and Analysis (SACEMA): Research Day (2011): *Oral presentation* on the "Genetic Contribution for the risk of metabolic Syndrome"
- University of Stellenbosch- South African Centre for Epidemiological Modelling and Analysis (SACEMA): Research Day (2012): *Oral presentation* on the "Genetic Contribution for the risk of metabolic Syndrome: An investigation for Candidate Polymorphisms related to Lipid and Carbohydrate Metabolism"
- University of Stellenbosch- South African Centre for Epidemiological Modelling and Analysis (SACEMA): Research Day (2013): *Oral presentation* on the "Genetic Contribution for the risk of metabolic Syndrome: An investigation for Candidate Polymorphisms related to Lipid and Carbohydrate Metabolism"
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Abbreviations

AACE	American Association of Clinical Endocrinologists
ADP	Adiponectin
ANA/NHLBI	American Heart Association for National Heart, Lung and Blood
APOA5	Apolipoprotein A5
AUC	Area Under the Curve
BMI	Body Mass Index
bp	Base Pair
CAD	Coronary Artery Disease
CE	Cholesterol Ester
CETP	Cholesterol Ester Transfer Protein
CI	Confidence Interval
CV	Cardiovascular
CVD	Cardiovascular Disease
DEPC	Diethylpyrocarbonate
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetra-Acidic Acid
EGIR	European Group of Study of Insulin Resistance
F	Forward
FFA	Free Fatty Acids
GC	Percentage of Nitrogenous Bases on a DNA Molecule
HDL-C	High Density Lipoprotein Cholesterol
HOMA IR	Homeostatic Model of Insulin Resistance
HWE	Hardy Weinberg Equilibrium
IDF	International Diabetes Federation
IGT	Impaired Glucose Tolerance
	Institute
IR	Insulin Resistance
IRS	Insulin Receptor Substrate
LDL	Low Density Lipoprotein

LEP	Leptin
LPL	Lipoprotein Lipase
MCP	Monocyte Chemoattractant Protein 1
MGP	Magnetic Glass Particles
mRNA	Messenger RNA
MS	Metabolic Syndrome
NCEPATPIII	National Education Cholestrol Education Program of Adult
No MS	No Metabolic Syndrome
P1	Probe 1
P2	Probe 2
PCR	Polymerase Chain Reaction
PON1	Paraoxonase 1
R	Reverse
ROC	Receiver Operator Curves
ROS	Reactive Oxygen Species
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
T2D	Type 2 Diabetes
TG	Triglycerides
Tm	Temperature
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization
X^2	Chi-Square

Abstract

Background: The development of the metabolic syndrome (MS) is complex, involving multiple etiological factors which include lifestyle, environmental factors and genetics. Obesity and insulin resistance (IR) are considered driving factors for susceptibility, occurring mainly due to common underlying mechanisms. The aim of the study was to establish the genetic profiles of participants in the Indian community of Phoenix and to determine genetic patterns associated with MS.

Methodology: This study was a cross sectional investigation of 1000 participants who consented to genetic screening in the larger Phoenix Lifestyle Project. All demographics were recorded in the STEPS Instrument for Non-communicable Disease (NCD) Risk Factors (Version 1.3a). Clinical measurements were obtained from each participant and blood samples for the genetic analysis were collected in ethylenediaminetetra-acidic acid (EDTA) tubes. The diagnosis of the MS was in accordance with the NCEP ATPIII, IDF and harmonized definitions, and IR was measured using the homeostatic model of insulin resistance (HOMA-IR). Gene polymorphisms related to lipid and carbohydrate metabolism (apolipoprotein A5 Q139X), insulin resistance (lipoprotein lipase Hinf I, human paraoxonase 1 192Arg/Gln, cholesteryl ester transfer protein Taq1B) and obesity (adiponectin 45T>G, leptin 25CAG) in participants with MS (according to the IDF and harmonized definitions) were genotyped on the LightCycler 480 using specific probes for the mutation. For quality control, 5% of randomly selected samples were sequenced by the Sanger method.

Results: A high prevalence of the MS was recorded in this population [44.3% (95%CI: 41.2-47.4): NCEP ATPIII definition, 51.6% (95%CI: 48.4-54.7): IDF criteria, 49.0% (95%CI: 45.8-52.1): harmonized criteria], with an increased risk of the MS in females as compared to than males [NCEP ATPIII (48.1% vs 33.2%), IDF (53.7% vs 45.2%), harmonized (51.0% vs 42.8%)]. IR was high in participants diagnosed with the MS (76.5%: IDF criteria; 76.3%: harmonized criteria), with more females being IR than males [IDF (78.4% vs 69.9%), harmonized (78.3% vs 69.2%)]. Receiver operator curves indicated blood glucose levels (AUC: 0.7573) and waist circumference measurements (AUC: 0.7507) to be associated with IR and the MS. Genotypes of the studied polymorphisms in MS participants varied, with no significant differences being detected. Analysis of the individual components of the MS between genders demonstrated male participants with the adiponectin 45T>G (diagnosed with the MS) to be more inclined to have reduced HDL-C levels when the IDF (P=0.004) and harmonized (P=0.001) definitions were applied. The harmonized definition additionally demonstrated male participants with the human paraoxonase 1 192Arg/Gln to be more inclined to higher systolic blood pressure (P=0.02). The lipoprotein lipase HinfI (IDF: P=0.04) and human paraoxonase 1 192Arg/Gln (IDF: P=0.03; harmonized: P=0.04) polymorphisms appeared to be protective against the risk for MS in non-smokers; and the human paraoxonase 1 192Arg/Gln (IDF: P=0.04; harmonized: P=0.04) and leptin 25CAG (IDF: P=0.01; harmonized: P=0.01) in non-alcohol consumers. IR participants with the MS (HOMA IR values >2.6) carrying the human paraoxonase 1 192Arg/Gln were more likely to have high systolic blood pressure when the harmonized definition was applied (P=0.04). When gender was compared, IR males with the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln were more likely to have higher systolic and diastolic blood pressure (IDF

and harmonized definitions); and IR males with the MS carrying the adiponectin 45T>G polymorphism were more inclined to have reduced HDL-C (harmonized: P=0.00). IR participants with the adiponectin 45T>G and leptin 25CAG polymorphisms who performed physical activities (IDF: P=0.04; harmonized: P=0.03) and who did not consume alcohol (IDF: P=0.00; harmonized: P=0.00) respectively were protected against the risk for MS.

Conclusion: Our results show a high number of Indians from the Phoenix community with the MS. Age, gender, risk factor clustering and the definition used for diagnosis contribute to the development of the MS. None of the studied polymorphisms increased the risk for the MS, but the adiponectin 45T>G and the human paraoxonase 1 192Arg/Gln polymorphisms were found to be genetic markers that may assist in identifying participants who are susceptible to hypocholesterolemia (in males with the MS and with IR) and hypertension (in males with the MS), respectively. The lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms associated with IR may also serve as genetic markers that may assist in identifying males with MS who are susceptible to hypertension. Gene-environmental associations exerted a degree of protection against the risk for the MS and IR. Hence, the genetic risk for MS may lie in its components (which includes environmental factors) rather than the MS as an entity. We propose that genetic studies on larger sample sizes with advanced laboratory techniques such as genome wide association studies and bioinformatics may produce more meaningful results, allowing for effective interventions that will reduce the prevalence of the MS and CVD in this community.

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CHAPTER 1

INTRODUCTION

Chapter 1: Introduction

The etiology of the metabolic syndrome (MS) also known as syndrome X, the insulin resistance syndrome and the deadly quartet, is complex and comprises interrelated phenotypes i.e. central obesity, insulin resistance, clustering of hepatic, vascular and immunologic molecules, proinflammatory state, aging and hormonal changes (Grundy *et al*., 2004). Over the last decade the definition of the MS has been simplified and modified by health organizations such as the World Heath Organization, the European Group for study of Insulin Resistance, the National Cholesterol Education Program of the Adult treatment Panel III, the American Association of Clinical Endocrinologists and the International Diabetes Federation (Alberti *et al*., 2009). In this chapter we provide an overview of the MS and justify the validity for this study.

1.1 Definition of the metabolic syndrome

The MS is defined as the clustering of several metabolic risk factors including abdominal obesity, elevated triglycerides, reduced high density lipoproteins cholesterol (HDL-C), elevated blood pressure and elevated blood glucose serum levels (Park *et al*., 2003; Alberti *et al*., 2005). Evidence suggests that insulin resistance (IR) and obesity (Hanley *et al*., 2003; Reavan *et al*., 2005; Hennes *et al*., 2007) are driving factors for the development of the MS and type 2 diabetes (T2D) (Zimmet, 2000). These metabolic risk factors collectively contribute to the development of cardiovascular disease (CVD) (Reaven, 1988; Ferrannin *et al*., 1991). However, the exact relationship and/or mechanism between CVD with the MS is not clearly demonstrated in the literature (Marroquin *et al*., 2004; Iribarren *et al*., 2006).

Metabolic risk factors play an integral part in the high prevalence of the MS in both developed and developing countries (Byrne *et al*., 2006). It is suggested that increasing age, gender (Ardern *et al*., 2007), ethnicity (Grep *et al*., 1996; Ford *et al*., 2002; Grundy *et al*., 2004), lifestyle (Wannamethee *et al*., 2006) and genetic factors all could contribute to the development of the MS (Groop, 2000; Corella *et al*., 2005; Elder *et al*., 2009). It is not clear however, how genetics influences the susceptibility to these metabolic risk factors among different populations (Das, 2009). As a result, population based genetic research has become an area of intense scientific endeavor, and data obtained may allow for the implementation and development of medical interventions that could counteract susceptibility to the MS, thereby reducing the risk for CVD.

1.2 Aims and rationale

The primary aim of the study was to establish the epidemiology and genetic profile (of a few polymorphisms) in a randomized sample of participants in the South African Indian community of Phoenix and to determine genetic patterns that may be associated with the MS. Within this primary aim, secondary aims occur which fall into two categories i.e. *epidemiological and genetics*.

<u>*Epidemiology*</u>

- Identify the prevalence of the MS in this community as determined by the latest descriptors of this condition (Alberti *et al*., 2009).
- Determine the prevalence of IR as defined by the HOMA model using serum insulin and fasting glucose levels in participants with the MS.

- Define the pattern of risk factor clustering that is associated with IR (as defined by the HOMA model).

Results of these aims are given in chapter 4.

<u>*Genetic*</u>

- Identify genetic patterns in participants with the MS focusing on genes related to lipid and carbohydrate metabolism i.e. Apolipoprotein A5
- Identify genetic patterns in participants with the MS focusing on genes related to insulin resistance i.e. Lipoprotein Lipase, Human Paraoxonase I and Cholesteryl Ester Transfer Protein
- Identify genetic patterns in participants with MS focusing on genes related to obesity i.e. Adiponectin and Leptin

Results of these aims are given in chapter 5

We provide an overview of the MS in Figure 1 illustrating the complexity of the MS and its association with the selected candidate gene polymorphisms, in relation to IR and obesity.

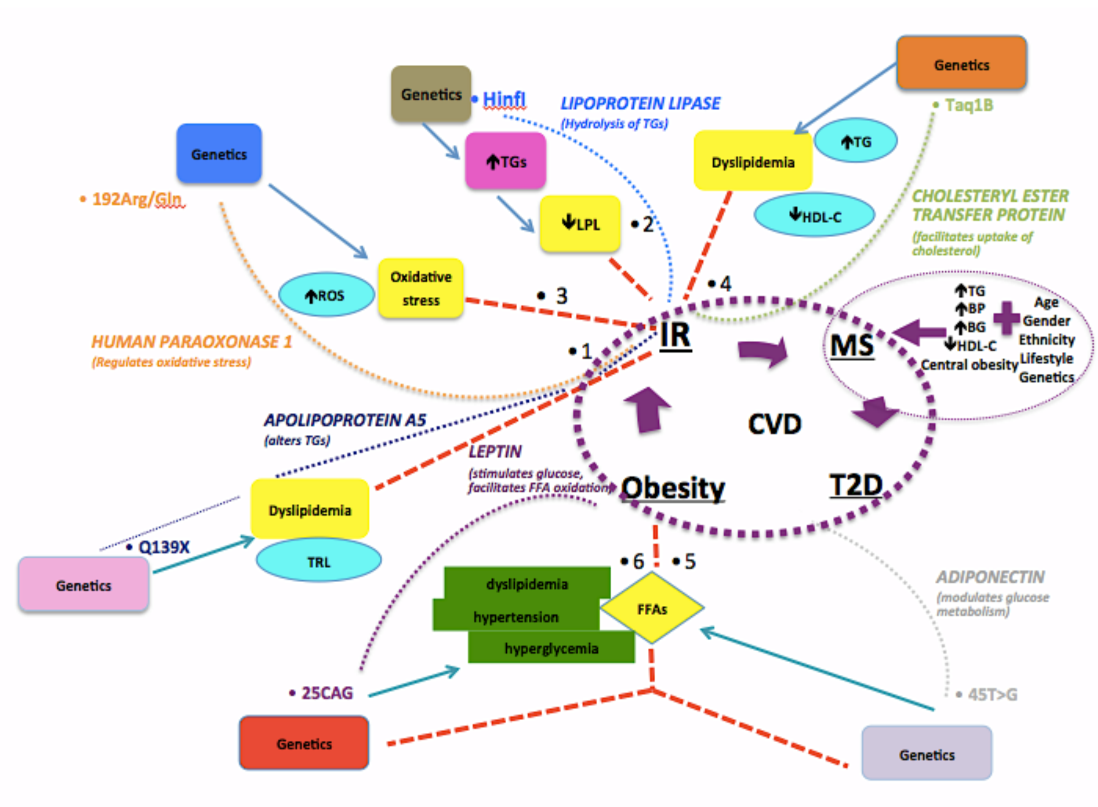


Figure 1: *Overview of the Metabolic Syndrome*

Key: ROS- reactive oxygen species; **IR**- insulin resistance; **TGs**- triglycerides; **HDL-C**- high-density lipoprotein cholesterol; **MS**- metabolic syndrome; **BP**- blood pressure; **BG**- blood glucose; **CVD**- cardiovascular disease; **T2D**- type 2 diabetes; **FFAs**- free fatty acids; **LPL**- lipoprotein lipase; **TRL**- triglyceride rich lipoprotein

(Legend for Figure 1: The MS involves interrelated factors (IR, obesity, elevated TGs, elevated BP, elevated BG, reduced HDL-C, central obesity, age, gender, ethnicity, lifestyle and genetics) that contribute to T2D and CVD (depicted in purple circle). IR and obesity are the driving factors for the development of the MS (depicted in purple circle) and their proposed pathophysiological mechanisms (depicted in yellow), contributing to the various metabolic risk factors (depicted in light blue, green, and dark pink) is demonstrated .The metabolic risk factors is further linked with genetics (depicted in red, grey, light pink, blue, brown and orange); and selected candidate gene polymorphisms is depicted viz. **1**- apoliporpotein A5 is associated with IR/ lipid and carbohydrate metabolism; **2-4**- lipoprotein lipase, human paraoxonase 1 and cholesteryl ester transfer protein gene polymorphisms is associated with IR; and **5,6**- adiponectin and leptin is associated with obesity. Motivation for selection of candidate gene polymorphisms for this study is briefly described in text to follow)

The genetic associations underlying the pathogenesis of the MS in South African Indians who present with high CV risk profiles, is limited in the literature. Our hypothesis assumes that common genetic variants may predispose the South African Indian community to the MS with support being obtained from several genetic epidemiological studies which have identified gene polymorphisms that confer increased susceptibility to CVD (Ranjith *et al*., 2009; Ranjith *et al*., 2011). Few studies have examined these polymorphisms in communities prior to the development of CVD. Based on the findings from the literature, we selected candidate genes related to IR, obesity, lipid and carbohydrate metabolism which have been implicated in an increased risk for the MS and thus CVD.

1.3 Epidemiological Factors

1.3.1 Prevalence of the metabolic syndrome

The prevalence of the MS is escalating worldwide (Mozumdar *et al*., 2011). The United States has demonstrated an increase from 24% (Ford *et al*., 2002) to 34.2% (Mozumdar *et al*., 2011). Italians (22.2%) (Bo *et al*., 2005), the Portuguese (23.9%) (Santos *et al*., 2003) and the Greeks (24.5%) (Athyros *et al*., 2005) also show a high current prevalence for the MS. This increasing prevalence has been linked to lifestyle factors, particularly abdominal obesity (Azadbakht *et al*., 2005; Orchard *et al*., 2005; Andreassi *et al*., 2009).

Abdominal obesity is more prevalent in Asians than Whites (Lear *et al*., 2007). Therefore investigating the MS in South Africans Asians, in particular participants of Indian descent, will prove informative when assessing and diagnosing the MS in this cohort.

1.3.2 Prevalence of insulin resistance (IR) as defined by the HOMA model

The homeostasis model assessment of IR (HOMA-IR) estimates IR (occurring due to obesity) and is obtained by measuring fasting blood glucose and fasting insulin levels. Higher HOMA-IR levels, dependent on defining cutoff values, indicate a greater degree of IR (Lee *et al*., 2006). For example, HOMA-IR>4 in a French study showed a 35.8% prevalence of IR (Tresaco *et al*., 2003) and HOMA-IR>4.39 in an American study revealed a 52.1% prevalence (Lee *et al*., 2006). Similarly, HOMA-IR≥3.8 revealed a 45.4% prevalence in the Spanish (Tapia-Ceballos *et al*., 2007) whilst HOMA-IR>2.5 in an Italian study revealed a 40.8% prevalence in children and HOMA-IR>4.0 in adolescents showed a 41.2% prevalence (Valerio *et al*., 2006). Similarly, in Asian Indians there is also a rising prevalence for IR (Ramachandran *et al.,* 2003). Since IR is predictive for Type 2 Diabetes (T2D) (Lillioja *et al*., 1993; Pyorala *et al*., 2000; Shulman, 2000; Sharma *et al*., 2006) and the South African Indian population records the highest prevalence for T2D in South Africa (Levitt *et al*., 1999), assessing the prevalence of IR in this cohort will prove informative for the predisposition of IR and T2D.

1.3.3 Risk factor clustering associated with IR

IR correlates with blood pressure (Cruz *et al*., 2004; Sinaiko *et al*., 2006), HDL-C (Ramachandran *et al*., 2003; Dixon *et al*., 2006), triglyceride levels (Snehalatha *et al*., 2005) and blood glucose levels which develop as a result of increased BMI (Ramachandran *et al*., 2007). There is a rising prevalence of increased BMI in Asian Indians (McKeigue *et al*., 1993; Ramachandran *et al*., 2004) and data obtained from

our study cohort will contribute towards prevention strategies for IR and its associated metabolic risk factors.

1.4 Genetic Factors

1.4.1 Genes affecting lipid and carbohydrate metabolism

One candidate gene polymorphism related to lipid and carbohydrate metabolism will be addressed in this study.

Apolipoprotein A5 gene polymorphism related to lipid and carbohydrate metabolism

Dyslipidemia contributes to CHD (Hokanson *et al*., 1997; Wood, 2007), familial hyperlipidemia, the MS and atherogenic lipoprotein profiles (Durrington *et al*., 2003). It is believed that interaction of triglycerides with reactive oxygen species (ROS) (Gianturco *et al*., 1994), reduced high density lipoprotein (HDL-C) (Brewer *et al*., 2004) and or atherogenic low density lipoprotein (LDL) (Hokanson *et al*., 1997) contributes significantly to CV events (Figure 1).

Several single nucleotide polymorphisms (SNP) have associated dyslipidemia with CV events. Apolipoprotein A5 in particular, is a triglyceride associated enzyme (Tai *et al*., 2008; Garelnabi *et al*., 2013) which is encoded by the apolipoprotein A5 gene (Laurila *et al*., 2010) and has significant anti-atherogenic properties (Tai *et al*., 2008; Garelnabi *et al*., 2013). Apolipoprotein A5 activity has been shown to elevate triglyceride expression (Pennacchio *et al*., 2001; Van de Vilet *et al*., 2001) which contributes to CV

events. It is established that the rare apolipoprotein A5 gene polymorphism, Q139X, is involved in the alteration and activity of this enzyme (Marcais *et al*., 2005) (Figure 1). Since the MS is associated with triglyceride rich lipoproteins and the MS (Ruotolo *et al*., 2002), we believe that the association between the MS and the apolipoprotein A5 Q139X polymorphism are mechanisms for CHD in populations that have an increased risk for CHD.

There is presently limited data on the CV risk factors and genetic variations contributing to the MS in the South African Asian population (Ranjith *et al*., 2009), which presents with an increased risk factors for CVD. We assessed this community for associations between the Q139X polymorphism in the apolipoprotein A5 gene and CV risk factors in order to establish if risk factors for the MS was conferred by the presence of the Q139X polymorphism.

1.4.2 Genes affecting insulin resistance

Three candidate gene polymorphisms related to IR will be addressed.

Lipoprotein lipase gene polymorphism associated with IR

IR is considered to be the underlying or driving factor for the MS (Reaven, 1988) that contributes to obesity (Quatanani *et al*., 2007) and T2D (Lillioja *et al*., 1993; Pyorala *et al*., 2000; Shulman, 2000; Sharma *et al*., 2006). It is suggested that IR occurs by impaired insulin signaling at the different signaling pathways (Saltiel *et al*., 2001; Hribal *et al*., 2002; Song *et al*., 2012). During IR, lipoprotein lipase expression from parenchymal cells is inhibited (Mead *et al*., 2002) by hydrolysis of triglycerides in lipoproteins, such as very

low density lipoproteins (VLDL) and chylomicrons (Braun *et al*., 1992; Santamarina-Fojo *et al*., 1994; Mead *et al*., 2002). This contributes to down regulation of lipoprotein lipase (Pollare *et al*., 1991) which in turn leads to hypertriglyceridemia, a characteristic feature for the MS. Studies further indicate a possible link between hypertriglyceridemia and genetic determinants (Brunzell *et al*., 1995) (Figure 1).

Candidate gene polymorphisms, particularly the HinfI, is reported to stimulate the hydrolysis of triglycerides (TG) and to mediate the removal of remnant lipoproteins from the plasma (Beiisiegel *et al*., 1991) (Figure 1). Therefore, knowledge of the molecular mechanisms underlying IR and lipoprotein lipase activity that predispose patients to the MS could prove useful in understanding the susceptibility to the MS in this cohort.

Human paraoxonase 1 gene polymorphism associated with IR

IR is further associated with oxidative stress (Henriksen *et al*., 2000; 2006) by the overproduction of ROS (Solinas *et al*., 2010) (Figure 1). This association activates mitochondrial H_20_2 or mitochondrial NADPH oxidase that leads to reduced glucose transport activity and impaired insulin signaling (Henriksen *et al*., 2011), contributing to obesity, T2D and the MS (Meigs *et al*., 2007).

Several genetic factors, in particular the human paraoxonase 1 (Durrington *et al*., 2001; Li *et al*., 2003) has been associated with mitochondrial dysfunction culminating in IR and other metabolic risk factors (Kim *et al*., 2008). This atheroprotective candidate gene (Durrington *et al*., 2001; Li *et al*., 2003) is

exclusively associated with HDL (Shih *et al*., 1998; Sorenson *et al*., 1999) and is known to regulate oxidative stress which contributes to the development of T2D (Bhattacharya *et al*., 2008) (Figure 1).

There is further evidence associating the human paraoxonase-1 gene polymorphism (192 Arg/Gln) to human paraoxonase-1 activity, coronary heart disease (CHD) (Senti *et al*., 2001) and reduced protection from lipid peroxidation (Martinelli *et al*., 2005). Since human paraoxonase-1 is associated with antioxidant protection, and the MS with increased oxidative stress and CHD, we find it essential to explore the relationship between the human paraoxonase-1 gene polymorphism (192Arg/Gln) and the MS in South African Indians who are at a higher risk of developing CHD.

Cholesteryl ester transfer protein gene polymorphism associated with IR

There is long standing evidence that IR leads to atherogenic dyslipidemia, a component of MS, that is characterized by high levels of triglycerides and reduced levels of HDL-C (Reaven, 1988; Wood, 2007) (Figure 1). These lipoproteins are metabolized by a hydrophobic glycoprotein, known as cholesteryl ester transfer protein (CETP) (Tall, 1993), which facilitates the uptake of cholesterol from peripheral tissues to the liver (Mohrschladt *et al*., 2005) (Figure 1).

CETP is a key regulator of lipoprotein metabolism and has been shown to facilitate cholesterol ester transfer from HDL-C to APoB, thereby allowing for the counter flux of triglycerides, which forms triglyceride rich HDL-C that is

hydrolyzed and excreted from circulation resulting in reduced HDL-C (Barter *et al*., 2006). Therefore, high levels of CETP may result in reduced HDL-C, which has been shown in a study that implicates CETP in the MS (Sandhofer *et al*., 2006).

Evidence suggests that CETP gene polymorphisms may influence lipid metabolism (Bernard *et al*., 1998) and since dyslipidemia is a component of the MS (Grundy, 2006), and CETP activity is influenced by lipid metabolism and genetics (Bernard *et al*., 1998), we believe it is significant to study SNPs (namely, the CETP Taq1B) that participate in lipid metabolism and to determine whether these polymorphisms (Taq1B) confer susceptibility to the MS.

1.4.3 Genes affecting obesity

Two candidate gene polymorphisms related to obesity will be addressed in this study.

Leptin gene polymorphism associated with obesity

Obesity is considered to be a major problem in many populations (WHO, 2011) and has been linked to IR and the MS. It occurs due to insulin signaling defects (Kim *et al*., 2008) at many levels, particularly in the liver, adipose tissue and heart (Ouwens *et al*., 2005) and lipolysis of adipose tissue triglycerides, which produces free fatty acids (FFAs) in the portal vein that are transported to the liver (Despres *et al*., 1990) (Figure 1). It is believed that the transportation of increased FFAs to the liver impairs the ability of insulin to suppress hepatic glucose output and restricts insulin-mediated glucose

removal in skeletal muscle (Boden *et al*., 1997).

This hypothesis provides explanations for the physiological mechanisms linking obesity with health outcomes such as dyslipidemia, hyperglycemia and hypertension (Figure 1). With ensuing IR, the expression of leptin, an adipocyte-derived hormone stimulates glucose uptake, facilitates FFA oxidation (Wauters *et al*., 2000; Minokoshi *et al*., 2002) and inhibits insulin secretion (Seufert *et al*., 1999). It is regarded as a key modulator for energy homeostasis (Elmquist *et al*., 1998; Friedman *et al*., 1998; Bates *et al*., 2003). Leptin activity is hence considered to correlate with the susceptibility to the metabolic disorders (Frederich *et al*., 1995; Considine *et al*., 1996; Harigaya *et al*., 1997; Kennedy *et al*., 1997) of obesity ((Ragin *et al*., 2009), IR and diabetes mellitus (DM) (Zhang *et al*., 1994). It has been suggested that energy homeostasis associated with the leptin hormone is influenced by genetic mutations on the leptin gene (Considine *et al*., 1995; Hager *et al*., 1998; Li *et al*., 1999). Thus, studying the molecular mechanisms of the leptin polymorphisms, namely the rare 25 CAG, is considered relevant in the predisposition of T2D and CVD, which are said to be fueled by obesity associated IR.

Adiponectin gene polymorphism associated with obesity

Adipokines such as adiponectin, which is secreted in abundance from adipose tissue (Maeda *et al*., 1996) have been associated with obesity. An inverse relationship between obesity and circulatory adiponectin levels has been observed in the literature (Arita *et al*., 1999; Yang *et al*., 2001). Adiponectin

has been shown to modulate glucose metabolism by improving insulin sensitivity (Berg *et al*., 2001; Yamauchi *et al*., 2002; Wu *et al*., 2003), which occurs by reducing FFAs and triglycerides by oxidation via the AMP-activated protein kinase (Fruebis *et al*., 2001; Wu *et al*., 2003) (Figure 1).

Adiponectin possesses both anti-inflammatory and anti-atherogenic properties (Yung *et al*., 2006; Knudson *et al*., 2007) and is considered a possible pathway between the MS and CV related events. However, the effects of adiponectin are further determined by genetics (Vasseur *et al*., 2002; Chuang *et al*., 2004; Menzaghi *et al*., 2007). It is well established that adiponectin SNPs (45T>G, -12823, -11426G>A, -11377G>C etc.) alter both the level and activity of the adiponectin enzyme (Vasseur *et al*., 2002; Mackevics *et al*., 2006; Li *et al*., 2007) leading to obesity (Arita *et al*., 1999; Weyer *et al*., 2001).

Since the MS increases the risk for CVD and is associated with a higher degree of obesity and adiponectin is a key determinant for obesity, we felt it was important to investigate the interaction between the MS and the adiponectin polymorphism, namely the, 45T>G. There are few studies on the adiponectin 45T>G polymorphism in communities with high risk factor profiles, thus the objective was to study this polymorphism in South African Indians who are susceptible to obesity, IR, T2D and CVD, and to determine whether this polymorphism increases the risk for the development of the MS

Overall, these polymorphisms *(Section 1.4.1-1.4.3)* employed random sampling and comprised of smaller samples in previous studies. For example, the apolipoprotein A5 Q139X polymorphism was evaluated only in 9 White participants (Marcais *et al*., 2005), the lipoprotein lipase HinfI in 99 Caucasians (Garenc *et al*., 2000), the human paraoxonase 1 192Arg/Gln in 434 French (Ruiz *et al*., 1995), the cholesteryl ester transfer protein Taq1B in 365 Taiwanese (Hsieh *et al*., 2007), the adiponectin 45T>G in 151 Uygur (Li *et al*., 2007) and the leptin 25CAG in 30 Thai participants (Komgmacheep *et al*., 2009). Population stratification could have confounded the genetic findings in these studies since false-positive findings were observed. Although our study was performed on 1000 Indian subjects the larger sample made population stratification less likely and increased the robustness of our findings which show no significant relationship between the polymorphisms studied the MS.

Furthermore, genotype/ allele frequencies for polymorphisms vary according to ethnicity (Ioannidis *et al*., 2004) and this variation accounts for differences in gene expression (Spielman *et al*., 2007) and overall in the prevalence of metabolic disorders (Porto *et al*., 2004; Mattei *et al*., 2009). These observations strongly suggest that Indians may carry disease associated genotypes/alleles at frequencies different from other populations, and that such differences could explain the high cardiovascular mortality rate within this community. Given the large sample size of our study, more robust conclusions regarding the influence of genetics on the pathogenesis of the MS and the influence of environmental factors in subjects with certain genotypes may be drawn.

The summary provided represents the basis for this study which will be explored in depth in the following chapter.

CHAPTER 2

LITERATURE REVIEW

Chapter 2: Literature Review

This chapter provides recent controversies surrounding the definition of the MS. Its prevalence worldwide, particularly in Asian Indians is highlighted and we provide support for selecting the South African Indian population group for evaluation. The physiological mechanisms associated with its development are briefly explored. This is followed by the genetic associations of the MS (primary focus), which motivates and supports selection of candidate genes polymorphisms for investigation. Also, the genetic patterns for selected polymorphisms among different population groups are provided. Overall, an in-depth discussion for all studied polymorphisms is provided.

2.1 The Metabolic Syndrome

The MS refers to a cluster of several metabolic risk factors (abdominal obesity, elevated triglycerides, reduced HDL-C, elevated blood pressure and elevated blood glucose serum levels) (Park *et al.*, 2003), which increase the risk for CVD (Reaven, 1988; Ferrannin *et al.*, 1991). However, the exact mechanism associating these risk factors to CVD remains unclear (Marroquin *et al.*, 2004; Iribarren *et al.*, 2006).

Originally, the recognition of MS dates back as early as 1921 when Joslin (1921) associated it with DM and obesity, and Kylin (1923) with hypertension, hyperglycemia and hyperuricaemia. This was later followed by Vague's (1947) findings, which associated abdominal obesity and fat distribution with diabetes and other metabolic disorders. In 1988, Reaven identified risk factors for diabetes and CVD (IR being the predominant contributor) and termed the syndrome "Syndrome X". Later, Kaplan (1989) renamed the syndrome "The Deadly Quartet" and Haffner

(1992) “The Insulin Resistant Syndrome”. Their findings shared similar associations with IR as originally proposed by Reaven (1988). Presently, the “Metabolic Syndrome” is the most widely accepted term for clustering of several metabolic abnormalities.

With the evolution of the definition of the MS, several Health Organizations have simplified and modified it. The first attempt was made by the World Health Organization (WHO) in 1999 by a team of diabetologists who stated that IR is a predominant factor for the diagnosis of the MS, with impaired glucose regulation (diabetes, IR and impaired glucose tolerance (IGT)) as the core factor, and two other risk factors (increased triglycerides, obesity and hypertension) required for the diagnosis (Alberti *et al*., 1998) (Table I).

In response, the European Group for Study of Insulin Resistance (EGIR) modified the WHO definition in 1999 and proposed that IR is a predominant factor for the diagnosis of the MS, but they substituted IGT with insulin levels (to determine IR and impaired fasting glucose: IFG) (Table I).

In 2001, the National Cholesterol Education Program of the Adult Treatment Panel III (NCEP ATP III) released its definition and stated that the MS included three of five risk factors (abdominal obesity/ increased triglycerides/ reduced HDL-C/ elevated arterial blood pressure/ increased fasting plasma glucose), with no measure of IR (as stated by WHO and EGIR) (Cleeman *et al*., 2001) (Table I). The American Association of Clinical Endocrinologists (AACE) in 2003 (Einhorn *et al*., 2003) supported the NCEP ATP III (2001) definition for the MS; however, no specific

number of risk factors was highlighted for diagnosis (solely dependent on the judgment of the clinical practitioner) (Table I).

The development of these definitions by the WHO, EGIR, NCEP ATPIII and AACE, suggested that a single unifying definition was needed (Ford, 2004). In the hope of achieving this, the International Diabetes Federation (IDF) in 2005 replaced IR with waist circumference (ethnic/racial specific) as one of the five factors for the diagnosis of the MS (three of five factors diagnoses the MS) (Alberti *et al*., 2009) *(demonstrated in Materials and Methods, Chapter 3, of this dissertation)*. This definition was later adopted by the American Heart Association/National Heart, Lung and Blood Institute (ANA/NHLBI) (2005), but their cutoffs for waist circumference among Europeans differed.

Later, in a statement resulting from inputs from several societies (International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity) it was agreed that abdominal obesity should not be a prerequisite for the diagnosis of the MS, but should be included as one of the five risk factors, which included ethnic/race specific cutoffs. As a result the "harmonizing (2011) definition" was formed, which is identical to the NCEP/ATP III (2001), but includes the IDF (2005) ethnic specific waist circumference cutoffs and requires three of five factors to diagnose the MS in an individual (Alberti *et al*., 2009)

Each of the proposed definitions of the MS represents similar features, but their cutoffs differ, thus influencing the application and diagnosis of the MS. For example, the WHO (1999), EGIR (1999) and AACE (2003) definitions focus primarily on IR by glucose tolerance testing and by the hyperinsulinemic-euglyemic clamp, which is labor intensive and time consuming (frequently used in research settings) (Ritchie *et al*., 2007). In contrast, the NCEP ATP III (2001) uses biochemical and clinical measurements for the diagnosis of the MS, which is a speedy approach and hence has been the foundation for subsequent definitions, namely, the IDF (2005) (Ritchie *et al*., 2007) and the "harmonizing (2011) definition".

On the other hand, the WHO (1999) and NCEP ATPIII (2001) demonstrated problems when diagnosing the MS, as application of obesity cutoffs among different ethnicities and population groups were not considered. This problem was encountered in Asians, for example, who present with reduced cutoffs for obesity but have an increased risk for T2D, as compared to Europeans, indicating the need for ethnic specific cutoffs when diagnosing the MS (Kaur, 2014). In an attempt to rectify these problems, the IDF (2005) and "harmonized (2011)" definitions unified obesity cutoffs for the MS based on ethnic specificity (Alberti *et al*., 2009); and this accounts for the fact that different ethnic and population groups present with different measurements for abdominal obesity and body weight, indicating an association between the risk for T2D and CVD (Kaur, 2014).

***TABLE I**: DEFINITIONS FOR THE METABOLIC SYNDROME AS PROPOSED BY HEALTH ORGANIZATIONS*

CLINICAL MEASURE	WHO (1998) *(WHO, 1999)*	EGIR (1999) *(Alberti, 2005)*	NCEP ATP III (2001) *(NECP, 2001)*	AACE (2003) *(Einhorn, 2003)*
Insulin Resistance	T2D or IR and or IGT, glucose intolerance, plus two or more of the following:	Plasma insulin >75th percentile, plus two or more of the following:	None but any 3 of the following 5 factors:	IFG or IGT including either one of the following (as per clinicians judgment)
Body weight	body mass index ≥ $30kgm^2$ or waist to hip ratio >0.90 for males and >0.85 for females	waist circumference >94cm in males or >80cm in females	waist circumference >102cm/>40 inches in males or >88cm/>35 inches in females	waist circumference >102cm/>40 inches in males or >88cm/>35 inches in females
Lipids	Triglycerides: ≥ 1.7 mmol/l or 150mg/dl; HDL-C: <0.9mmol/l or 35mg/dl in males and <1.0 mmol/l or 39mg/dl in females	Triglycerides: ≥ 2.0 mmol/l; HDL-C: <1.0 mm/l or treatment	Triglycerides: ≥ 150mg/dl/1.7 mmol/l; HDL-C: <40mg/dl/1.03 mm/l in males or <50mg/dl/1.29mmol/l in females	Triglycerides: ≥ 1.7 mmol/l or 150mg/dl; HDL-C: <40mg/dl or 1.03mmol/l in males;<50mg/dl or 1.3mmol/l in females
Blood Pressure	≥ 140/90 mmHg	≥ 140/90 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg
Glucose	IGT, IFG or T2D	Fasting plasma glucose: ≥ 6.1/7.8mmol/l but <7.0/11.1 mmol/l	Fasting plasma glucose: ≥ 6.1 mmol/l/ 110mg/dl	Fasting plasma glucose: 110mg/dl
Other	Microalbuminuria: urinary albumin excretion rate >20mg/min or albumin creatinine ratio ≥ 30mg/g	-	-	Features of IR such as family history of T2D, polycystic ovary syndrome, sedentary lifestyle, advancing age, ethic susceptibility to T2D

Key: IR- insulin resistance; **IGT**- impaired glucose tolerance**; IFG-** impaired fasting glucose; **T2D-** type 2 diabetes; **HDL-C**- high-density lipoprotein cholesterol

2.2 Prevalence of the metabolic syndrome

The prevalence of the MS is increasing internationally (Mozumdar *et al*., 2011) and is strongly dependent on age (Okafor, 2012), gender (Lim *et al*., 2011), the definition applied (Isomaa *et al*., 2001) and ethnicity (Emanuela *et al*., 2012). In the United States, the prevalence of the MS in the adult population was estimated to be 24% (Ford *et al*., 2002). However, recent data from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999-2006 show that the prevalence has increased to 34.2% in the US (Mozumdar *et al*., 2011). Similar findings were documented in Italians (22.2%) (Bo *et al*., 2005), the Portuguese (23.9%) (Santos *et al*., 2003) and in the Greeks (24.5%) (Athyros *et al*., 2005).

The prevalence of the MS has been linked to lifestyle factors. Obesity, in particular, has contributed to an increase in prevalence (Azadbakht *et al*., 2005; Orchard *et al*., 2005; Andreassi *et al*., 2009), and Asians are more likely to have abdominal obesity than Caucasians (Lear *et al*., 2007). South Asians have a 20%–25% prevalence of the MS (Corti *et al*., 2004; Fuster et al., 2005), whereas Asian Indians have a much higher prevalence (43%) (Prasad *et al*., 2012).

2.2.1 Metabolic syndrome in Asian Indians

It has been shown that non-communicable diseases are reaching epidemic proportions worldwide (Enas *et al*., 2007), and has been linked to westernization, the area of residence and behavioral and cultural factors. Asian Indians have an unusually high tendency to develop DM and CVD (Misra *et*

al., 2009). A third of the urban population in the major cities of India have the MS (Misra *et al*., 2009). Southern India for example, has shown a prevalence of 26.9% of the MS in males and 18.4% in females (Chow *et al*., 2008), whilst a study in Western India, in the city of Mumbai, revealed a 19.5% prevalence of the MS (using the NCEP-ATP III definition) (Sawant *et al*., 2011); and the CURES study in Chennai showed a further 18.3% prevalence (using the NCEP-ATP III definition) (Deepa *et al*., 2011). The reasoning surrounding the high prevalence of the MS among Asian Indians has not been clearly described. However, physiological mechanisms such as impaired insulin signaling (addressed under section 2.3.2) have linked the high prevalence with this ethnic group.

IR and CV risk factors (Misra *et al*., 2007) such as hypertension, dyslipidemia and obesity, are considered predominant contributors to the risk for the MS (Defronzo *et al*., 1991). These contributors have been significantly associated with abdominal obesity (Misra *et al*., 2008) but the exact mechanism underlying this association remains unclear. It has been hypothesized that changes in TNF-α levels (Stephens *et al*., 1997) and chronic low-grade inflammation could explain the obesity related pathologies such as IR and T2D (Hotamisligil *et al*., 1994; Hu *et al*., 2004; Boura-Halfon *et al*., 2009) which occurs by down-regulation of GLUT4 that reduces the kinase functioning of insulin receptors (Stephens *et al*., 1997). What this translates into is that Asian Indians with abdominal obesity (associated with elevated TNF-α) have decreased activity of GLUT4 in adipocytes (Stephens *et al*.,

1997), which subsequently influences the insulin-signaling pathway (*the pathway is addressed under sections 2.3.2, 2.3.3, 2.3.4 and 2.3.5*); and this factor possibly contributes to the high prevalence of the MS in Asian Indians.

Evidence suggests that the MS in Asian Indians is initiated during the pediatric and adolescent years as a result of advancing BMI. Obesity and overweight in Indian urban children have increased from 16% in 2002 to 24% in 2006 and to 29% in 2007 (Bhardwaj *et al*., 2008). The prevalence of pediatric MS in Asian Indians varies from 3.1% to 12.7%; whilst in adolescents an overall prevalence of 4.2% was demonstrated (Singh *et al*., 2007). In pediatrics a 6.5% prevalence was observed. What emerges is that Asian Indian children and adolescents who are obese and overweight in the early stages of life have an increased risk for the MS during adulthood, and hence, obesity could be the trigger for the MS in Asian Indians (Kapil *et al*., 2010).

Migrant Asian Indians residing in other countries have shown a high risk for non-communicable diseases such as DM, CVD and the MS (Omar *et al*., 1985; Samanta *et al*., 1991; McKeigue *et al*., 1992, 1993; Ramaiya *et al*., 1995; Enas *et al*., 1996; Hughes *et al*., 1997; Anand *et al*., 2000; Chandie Shaw *et al*., 2002; Mohanty *et al*., 2005), with IR contributing to the increased risk (McKeigue *et al*., 1992; McKeigue, 1996). Overall, study findings suggest that there are similar cultural heterogeneity and lifestyle factors between Asian Indians from the Indian subcontinent and migrant Indians residing in other

countries. These similarities could possibly be the reason for the high prevalence of the MS in migrants of Indian origin. Therefore, investigating the MS in South Africans of Indian descent, will prove informative when assessing and diagnosing the MS in this cohort.

2.2.2 *Metabolic syndrome in South Africans*

South African Indians have the second highest prevalence of DM after the Indian population in India (Levitt *et al*., 1999). Studies have shown a prevalence of 13% for DM in South African Indians, followed by 5% to 8% in South African Blacks and 4% in the South African White population (Huddle, 1994). DM is considered a complication of the MS (Goldberg, 2012) and patients with pre-diabetes or undiagnosed diabetes have a higher prevalence for the MS (Motala *et al*., 2011; Kengne *et al*., 2012), however limited data in this regard is available in the South African population. Data that has been collected shows a crude prevalence of 18.5% (using the joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity) in the Black South African community who are of Zulu descent and where there are higher frequencies present in females than males (Motala *et al*., 2011). A much higher prevalence of 31% in a group of Black and White South African corporate executives has been observed (Ker *et al*., 2007). There is only one study (Ranjith *et al*., 2011) in local South African Indians with acute

myocardial infarction, which shows a 61% prevalence of the MS using the NCEP ATPIII definition and 60% when the IDF definition was applied (with proportionally more females being diagnosed with MS than males).

The increasing prevalence of the MS in South African Indians contributes to an increased risk of CVD (Ranjith *et al*., 2011) and a large part of this risk is attributed to obesity, which is considered a driving factor in the development of IR, T2D and the MS (Reaven, 2011). As a result, there has been much interest in obesity and the MS over the past decade and physiological mechanisms surrounding their development have since been proposed. Impaired insulin signaling has been shown to be the central factor for the development of the MS through abnormal glucose and lipid metabolism (as a result of obesity), thus contributing to CVD (Carr *et al*., 2004; Grundy *et al*., 2004; Hu *et al*., 2004).

2.3 Physiological Mechanisms

Below we summarize the physiological mechanisms of insulin signaling under normal physiological conditions, followed by impaired insulin signaling due to obesity and the MS.

2.3.1 Insulin Signaling under normal physiological conditions

Insulin lowers blood glucose levels by facilitating the uptake of glucose from target tissues through glucose transporters (GLU) (Shulman, 2000) (Figure 2).

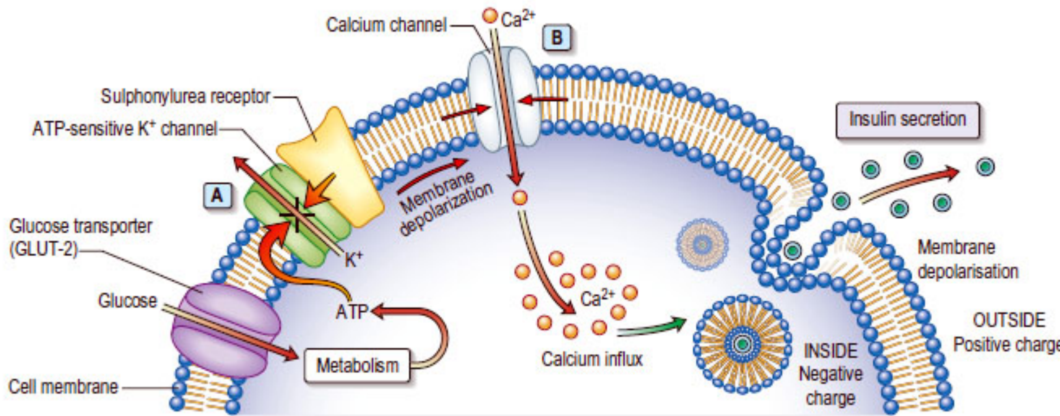


Figure 2*: The pathway for insulin secretion regulated by blood glucose levels (Adapted from http://medicinexplained.blogspot.com/2011/08/insulin-secretion-local-regulation.html)*

Key: GLUT2- glucose transporter; **ATP**- adenosine tri-phosphate; $\mathbf{K^+}$- potassium

(Detailed legend of Figure 2: Elevated blood glucose levels contribute to an uptake of beta cells by GLUT2, which is oxidized by glycolysis to form pyruvate. Subsequently, pyruvate is oxidized by PDHc by the TCA cycle to form acetyl-coA. The resulting NADH and FADH2 are oxidized by oxidative phosphorylation to form elevated ATP levels, which inhibit the K+ ATP channel, contributing to an influx of Ca2+ that leads to the transportation of the insulin vesicle to the plasma membrane, hence releasing insulin into the blood (Lowe *et al*., 1998))

Once insulin is released into systemic circulation, it binds to its receptors and stimulates tyrosine kinase which leads to phosphorylation of insulin receptor substrates (IRS) by activating phosphatidylinositol-3'-kinase (PI3-Kinase) (Figure 3). PI3-Kinase relocates GLUT4 to the cell surface and this translocation leads to metabolic effects such as glucose and insulin homeostasis, and mitogenesis in muscle and adipose tissue (Saltiel *et al*., 2002) (Figure 3) However, activation of other insulin signaling pathways (not illustrated in Figure 3) such as the protein kinase mammalian target of rapamycin (mTOR), has been shown to contribute to both protein synthesis (Proud, 2002) and glucose transport (by lipogenesis) (Laplantea *et al*., 2010), whilst the Akt/PKB pathway is known to regulate glycogen synthesis (Hooper, 1998) (Figure 3). Defects along this insulin signaling pathway have been linked to IR (Saltiel *et al*., 2002).

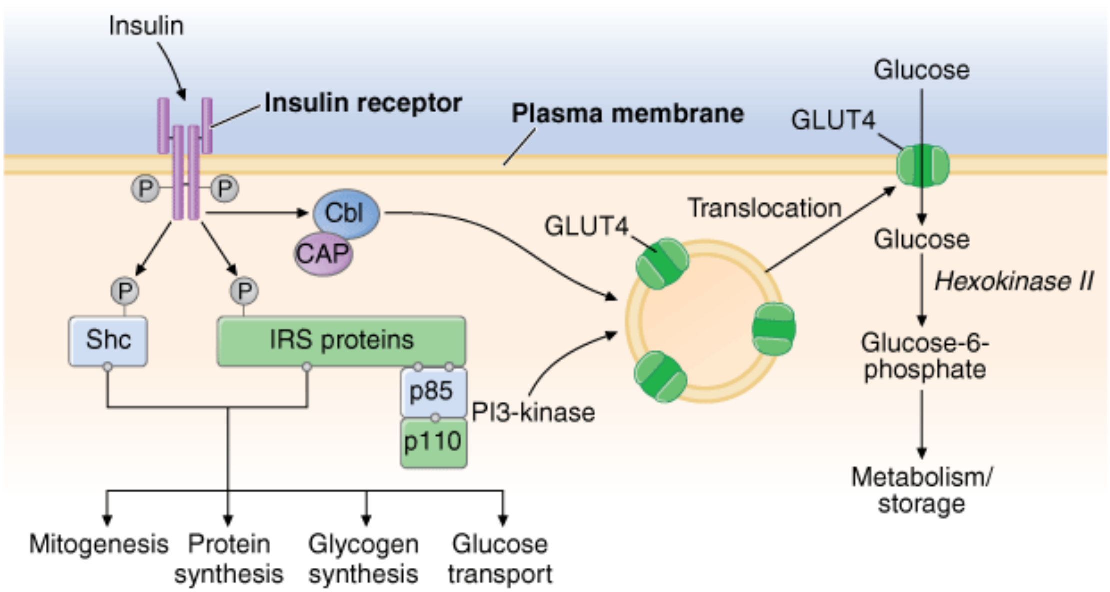


Figure 3*: The pathway for insulin synthesis (Adapted from Lowe et al., 1998)*

Key: GLUT4- glucose transporter; **p85, p110, PI3-kinase**- cellular proteins; **IRS, Shc-** insulin receptor substrate proteins

(Detailed legend of Figure 3: Insulin receptor is activated by tyrosine kinase and interacts with insulin receptor substrate proteins such as IRS and Shc; and subsequently binds to cellular proteins such as GrB-2, SOS, SHP-2, p65, p110, and PI3-kinase, which contribute to the effects brought about by insulin. Insulin transports glucose through the PI3-kinase and Cbl pathways to GLUT4 that transports the glucose to the plasma membrane)

2.3.2 Impaired insulin signaling

In the IR state, the insulin signaling pathway described above is compromised, leading to inadequate glucose uptake by tissues (Figure 4) and insufficient hepatic glucose output (Saltiel *et al*., 2002). This dysfunction occurs by increased phosphorylation of IRS-1, which reduces IRS-1 tyrosine phosphorylation, inhibits PI3-kinase activation, and limits phosphorylation and stimulation of the Akt protein kinase (Samuel *et al*., 2010) (Figure 4), hence contributing to the development of dyslipidemia (Figure 5), hyperglycemia (Figure 6) and hypertension (figure 7) (Soumaya *et al*., 2012); all of which are characteristic features associated with the MS.

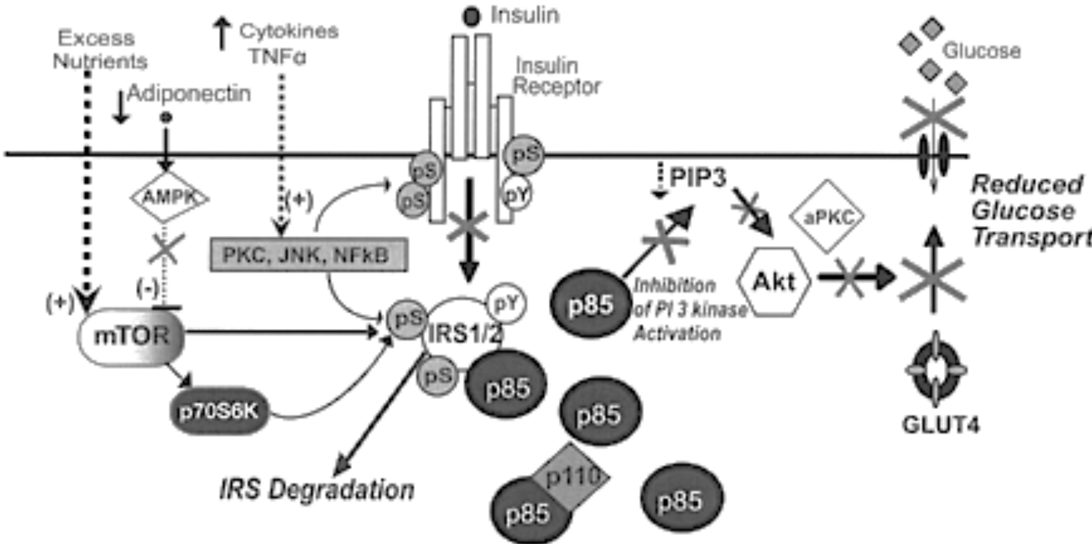


Figure 4*: The pathway for insulin resistance in skeletal muscle (Adapted from Barbour et al., 2007)*

Key: GLUT4- glucose transporter; **p85, p110** - regulatory subunits of PI3-Kinase; **pY-** tyrosine residues; **PI3-Kinase**-phosphatidylinositol-3'-kinase; **PIP_3** - phosphoinositol-3,4,5-phosphate; **pS-** serine phosphorylation;
Akt- protein kinase; **PKC**- protein kinase C; **TNF-α –** inflammatory cytokine

(Detailed legend of Figure 4: Impaired phosphorylation of IRS-1 tyrosine inhibits serine phosphorylation and increased serine phosphorylation of IRS1, which stimulates JNK and PKC (enzymes stimulated by TNF-α); and activates the mTOR-p70S6K pathway. AMPK is a target for the signaling of adiponectin and negatively regulates mTOR. Inhibition of the PI3-Kinase activation due to increased serine phosphorylation of IRS-1 and increased p85 contributes to limited transportation of the GLUT4 vesicle to the cell membrane, hence resulting in reduced glucose transport (stimulated by insulin) to skeletal muscle)

2.3.3 Dyslipidemia in insulin signaling

Impaired insulin signaling in adipose tissue contributes to abnormal metabolism of lipids (Semenkovich, 2000) (Figure 5). The increased FFAs (by impaired lipolysis) (Villena *et al*., 2004) are transported to the liver where posttranslational modification of apolipoprotein B (apoB) occurs, leading to the activation of very low-density lipoproteins (VLDL) (Ginsberg, 2006). This translates into a combination of elevated FFAs and reduced modification of apoB may explain the characteristic feature of hypertriglyceridemia associated with IR. Meanwhile elevated levels of triglyceride rich VLDL occur due to the

presence of cholesteryl ester transfer proteins (CETP) which may explain impaired HDL metabolism in IR participants (Ginsberg, 2006).

CETP regulates the exchange of cholesteryl esters in HDL for triglycerides in VLDL, forming triglyceride enriched HDL and cholesteryl ester enriched VLDL particles (Ginsberg, 2006). This suggests that hypertriglyceridemia contributes to triglyceride enriched HDL particles that is suitable for hepatic lipase, which is significantly elevated in the IR state, and HDL is reduced as a result of increased metabolism (Semenkovich, 2000) (Figure 5). Therefore, hypertriglyceridemia and reduced HDL are characteristic features of atherosclerosis (Szapary *et al*., 2004), and their progression may occur due to the presence of VLDL particles, or due to reduced HDL particles in the vasculature that participate in "reverse cholesterol transport". Hence, the liver becomes IR which leads to dyslipidemia (Bergman *et al*., 2007) (Figure 5).

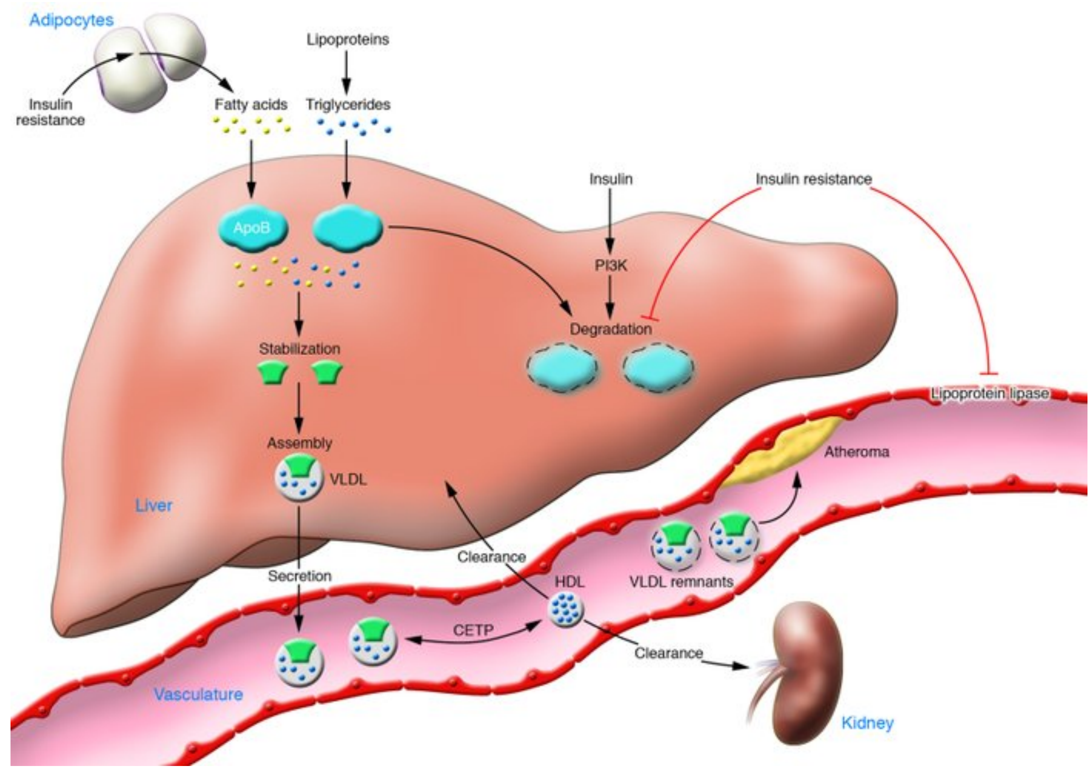


Figure 5: *The pathway associating impaired insulin signaling with dyslipidemia (Adapted from Semenkovich, 2006)*

Key: VLDL- very low-density lipoprotein; **CETP**- cholesteryl ester transfer protein; **HDL**- high-density lipoprotein

(Detailed legend of Figure 5: IR increases FFAs due to impaired effects of insulin on lipolysis located in adipocytes. The FFAs are transported to the liver, and regulates apoB production. Impaired insulin signaling degrades the apoB pathway that leads to elevated VLDL production. Lipoprotein lipase, located in the endothelium limits the removal of triglyceride rich lipoproteins during IR, leading to hypertriglyceridemia (as a result of elevated VLDL production and reduced VLDL removal). Metabolism of VLDL to "remnant lipoproteins" is significant for atheroma development and VLDL further influences HDL metabolism. The triglycerides in VLDL is relocated to HDL by CETP, resulting in triglyceride enriched HDL, which is rapidly removed from circulation, and leaves less HDL to collect cholesterol from the vasculature)

2.3.4 Hyperglycemia in insulin signaling

Impaired insulin signaling due to mitochondrial dysfunction (Lowell *et al*., 2005) contributes to reduced transportation of glucose into muscle and adipocytes, contributing to hyperglycemia by increased acyl CoA and diacylglycerol (DAG) (Gonzalez-Franquesa *et al*., 2012). This in turn stimulates the serine/threonine pathway through serine/threonine phosphorylation of IRS-1 (Marco *et al*., 2013) (Figure 6). Subsequently,

hyperglycemia stimulates the β cells of the pancreas and further contributes to hyperinsulinemia. As insulin is stimulated, β cell hypertrophy occurs and with time, β cell failure occurs, followed by prolonged β cell failure that contributes to T2D (Cavaghan *et al*., 2000). This occurs due to increased NADH and FADH2 that increases the flux of the oxidative respiratory system (OXPHOS) to the inner membrane of the mitochondria, leading to oxidative stress and hyperglycemia (Gonzalez-Franquesa *et al*., 2012) (Figure 6).

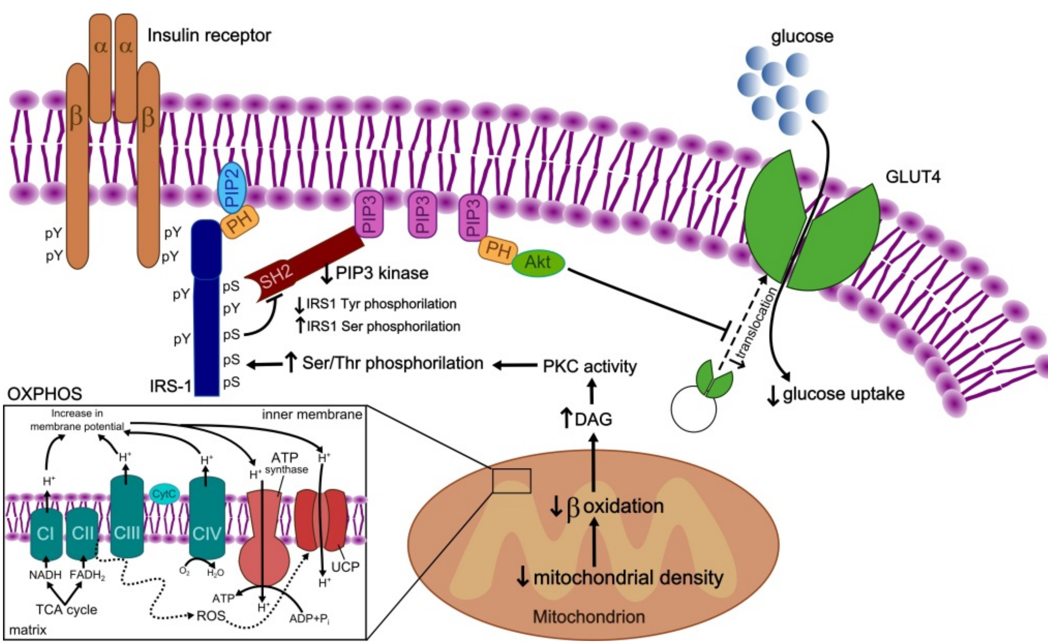


Figure 6*: The pathway associating impaired insulin signaling with hyperglycemia and T2D (Adapted from Gonzalez-Franquesa et al., 2012)*

Key: NADH- nicotinamide adenine dinucleotide; **FADH2**- flavin adenine dinucleotide (hydroquinone form); **ATP**- adenosine triphosphate; **ADP**- adenosine diphosphate; **Pi-** inorganic phosphate; **DAG**- diacylglycerol; **Thr**- threonine; **Ser, S**- serine; **Tyr, Y**- tyrosine; **IRS-1**- insulin receptor substrate 1; **PIP2**- phosphatidyil inositol 2; **PH**- pleckstrin homology domain of the IRS-1; **PIP3**- phosphatidyl inositol 3; **SH2**- Src homology 2 domain; **Akt**- protein kinase; **GLUT4**- glucose transported type 4; **TCA**- tricarboxylic acid cycle; **CytC**- cytocrome C; **OXPHOS**- oxidative phosphorylation; **CI-CII-CIII-CIV**- complexes I, II, III and IV within the oxidative phosphorylation system

(Detailed legend of Figure 6: When increased ATP is absent, reactive oxidative species increases, leading to oxidative stress, thereby reducing mitochondrial density, contributing to mitochondrial dysfunction and a decrease in β oxidation, which increases the concentration of diacylglycerol (DAG). Subsequently, DAG activates serine/threonine phosphorylation of IRS-1 by protein kinase C (PKC). PKC stimulates the serine kinase pathway and increases IRS-1 serine (Ser, S) phosphorylation of IRS-1, which prevents the phosphorylation of IRS-1 tyrosines (Tyr, Y) by IRS-1; and this inhibits phosphatidyl inositol 3-kinase activity (PI3-kinase), which leads to reduced Akt activity (stimulated by insulin) that restricts the activation and transportation of GLUT4 to the membrane, hence inhibiting insulin induced glucose uptake and thus the removal of glucose from circulation (Gonzalez-Franquesa *et al*., 2012))

2.3.5 *Hypertension in insulin signaling*

Impaired insulin signaling caused by obesity contributes to endothelial dysfunction and hypertension (Grekin *et al*., 1995), mainly by inhibition of IRS-1 phosphorylation and impairment of the PI3-Kinase pathway (Shulman, 2004) which interferes with endothelium vasodilation (Jonk *et al*., 2007) (Figure 7). The MAPK pathway is thus activated during vasoconstriction and produces endothelin-1 (ET-1) that mediates (Kim *et al*., 2006) and contributes to increased ROS production (De Keulenaer *et al*., 1998; Laight *et al*., 1998), limited nitric oxide (NO) availability in the endothelium (Landmesser *et al*., 2006), and in the muscle and kidneys (by eNOS expression) (Li *et al*., 2005; Hickner *et al*., 2006) (Figure 7).

ET-1 is stimulated by TNF-α, AngII and FFAs, all of which are derived from adipose tissue, which impairs the insulin signaling pathway of the endothelial vasculature through inhibition of IRS-1 phosphorylation (Hotamisligil *et al*., 1993). Firstly, the proinflammatory cytokine, TNF-α contributes to vasoconstriction/hypertension (Hotamisligil *et al*., 1993) by down regulating eNOS expression (Rask-Madsen *et al*., 2007), up regulating ET-1 expression (Mohamed *et al*., 1995) and by activating and increasing NADPH oxidase and ROS production respectively (Inoguchi *et al*., 2000).

Secondly, expression of AngII (of the renin angiotensin system) impairs the insulin signaling pathway and leads to hypertension (Schling *et al*., 1999) through several mechanisms viz.

(i) it stimulates IRS-1 phosphorylation (Velloso *et al*., 1996) resulting in the inhibition of glucose uptake and NO synthesis (Sowers *et al*., 2001),

(ii) it stimulates the production of ROS that increases NO degradation (Fujita, 2006),

(iii) it stimulates ET-1 production in the endothelium (Paul *et al*., 2006); and

(iv) AngII secretes and increases inflammatory cytokines levels (Horiuchi *et al*., 2006) such as TNF-α (Togashi *et al*., 2000).

Thirdly, obesity hormones such as leptin and adiponectin (secreted in response to FFAs) (Singhal, 2005) influence vasoconstriction of the endothelium through elevated leptin activating PI3-kinase (Dyck *et al*., 2006), leading to impaired insulin signaling and increased ROS production in the endothelium (Singhal, 2005). Similarly, adiponectin contributes to glucose uptake in the endothelium by increased IRS-1 phosphorylation in the insulin signaling pathway (Chandran *et al*., 2003).

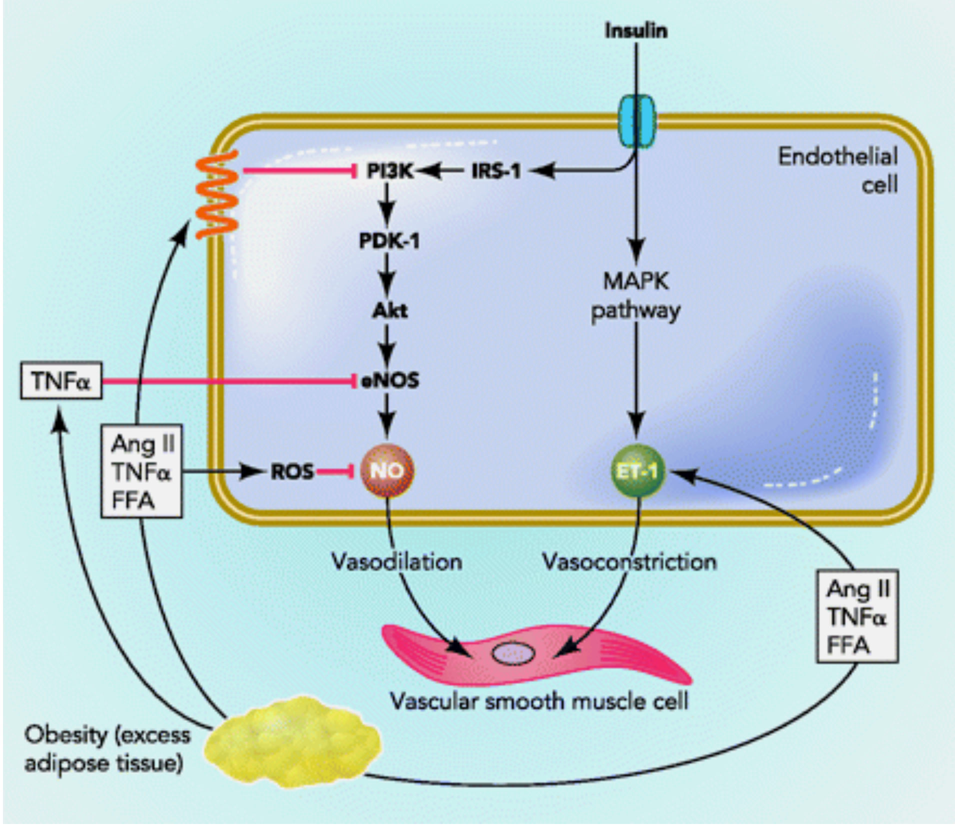


Figure 7: *The mechanism associating NO and ET-1 with vasodilation and vasoconstrictor (Adapted from Jonk et al., 2007)*

Key: AngII- Angiotensin II; **TNF-α**- tumor necrosis factor α; **FFA**- free fatty acids; **PI3K**- PI3-kinase. **IRS-1**- insulin receptor substrate 1; **PDK-1**, phosphoinositide-dependent kinase 1; **Akt-** protein kinase B; **eNOS**- endothelial nitric oxide synthase; **NO**- nitric oxide; **ET-1**- Endothelin 1; **ROS**- reactive oxygen species

(Legend of Figure 7: AngII, TNF-α and FFA inhibit the PI3-Kinase pathway and stimulates the MAPK pathway producing ET-1 that leads to vasoconstriction and hypertension)

These insulin mediated mechanisms link obesity to the MS (Song *et al*., 2012), and many of the risk factors i.e. diabetes, hypertension and obesity cluster in families, suggesting that a genetic component may underlie their development (Gallagher *et al*., 2010).

2.4 Genetics of the metabolic syndrome

There is evidence linking genes with the MS (Pollex *et al*., 2006; Povel *et al*., 2011). Studies show that 90% of heritability has been associated with the components of the MS and ~30% with the MS entity (Teran-Garcia *et al*., 2007; Joy *et al*., 2008; Monda *et al*., 2010), which is thought to be mediated by unidentified candidate genes (Carmelli *et al*., 1994; Liese *et al*., 1997; Hong *et al*., 1998). Candidate genes have been associated with common underlying mechanisms (Aizawa *et al*., 2006; Benyamin *et al*., 2007; Sjogren *et al*., 2008), contributing to CVD (Tillman *et al*., 2009). This indicates that disturbances in the biochemical traits, due to defects in the signaling pathways, contribute to most common diseases, which are predominantly influenced by genetics rather than their associated metabolic risk factors.

There is a large body of evidence that links ethnicity and lifestyle with the MS and the risk for CVD (Schwandt *et al*., 2010), with a genetic influence among different population groups being observed. Therefore, investigating the role of genetic factors and mechanisms underlying the pathogenesis of the MS in South African Indians, with high CV risk profiles, could explain the high CV mortality rate within this community.

Given that obesity is a hallmark for the development of IR, which contributes to the MS and T2D (which collectively lead to CVD), we propose that there is an association between genes related to lipid and carbohydrate metabolism, IR and the MS by dysregulation in the insulin signaling pathways. These associations might have an impact on the individual components of the MS. Therefore, we chose to explore

candidate genes related to lipid and carbohydrate metabolism (Apolipoprotein A5), IR (Lipoprotein Lipase, Human Paraoxonase 1, Cholesteryl Ester Transfer Protein) and obesity (Adiponectin and Leptin) and their polymorphisms in relation to the MS. The rationale for the selecting these candidate gene polymorphisms is demonstrated below:

2.4.1 Genes related to lipid and carbohydrate metabolism

We looked at one relevant gene which we describe below.

Apolipoprotein A5

Hypertriglyceridemia has been associated with CHD (Hokanson *et al.*, 1997) familial hyperlipidemia, the MS and atherogenic lipoprotein profiles (Durrington *et al.*, 2003). More than one mechanism has been proposed, which associates the CV events with increased triglycerides. Firstly, triglyceride rich lipoprotein is directly involved in atherosclerosis due to its interaction with oxygen free radicals (Gianturco *et al.*, 1994). Secondly, triglycerides probably reduce HDL-C by activating cholesterol ester (CE) from HDL which is mediated by cholesteryl ester transfer protein. CE has been shown to attach to hepatic scavenger B1 receptors, enabling the re-routing of the reverse cholesterol transport to VLDL and chylomicrons by cholesteryl ester transfer protein. This is followed by the attachment of triglycerides in chylomicrons remnants (derived from chylomicrons) and LDL (derived from VLDL), which have atherogenic properties (Brewer *et al.*, 2004). Lastly, triglyceride rich lipoproteins are associated with small, dense atherogenic LDL (Hokanson *et*

al., 1997), which contributes to elevated ApoB levels and is a major risk factor in atherogenesis (Lamarche *et al*., 1997).

Apolipoprotein A5 is the key enzyme responsible for modulating triglycerides, hence limiting atherogenic effects. It is synthesized predominantly by the liver (Tai *et al*., 2008; Garelnabi *et al*., 2013) as a 366 amino acid pre-protein and is secreted into circulation once the signal peptide from the 343rd residue is removed (Pennacchio *et al*., 2001; Van de Vilet *et al*., 2001). In circulation, elevated levels of apolipoprotein A5 contribute to reduced triglyceride concentrations (Pennacchio *et al*., 2001), and this occurs by restricting VLDL-triglyceride production and by activating LPL-mediated VLDL-triglyceride hydrolysis (Elosua *et al*., 2006). On the other hand, reduced apolipoprotein A5 levels correlate with elevated triglyceride concentrations (Pennacchio *et al*., 2001; Van de Vilet *et al*., 2001), increasing the susceptibility to CHD (the mechanisms are discussed above). These observations suggest that the apolipoprotein A5 gene could be a significant candidate gene for dyslipidemia, due to its associations with triglyceride expression (Laurila *et al*., 2010). South African Indians present with a high prevalence of dyslipidemia (Bradshaw *et al*., 2003) and investigating the apolipoprotein A5 gene polymorphisms could determine whether South African Indians are at an increased risk for developing CV related disorders.

Polymorphisms in the Apolipoprotein A5 gene

The apolipoprotein A5 gene is located ~27kb distal to the APOA1/C3/A4 gene cluster on chromosome 11q23 and consists of 4 exons encoding 366 amino acids (Pennacchio *et al.*, 2001) and 2 apolipoprotein A5 haplotypes viz. APOA5*2 [defined by 3 polymorphisms (1259T>C, IVS3+476G>A and −1131T>C)] and APOA5*3 (defined by 56C>G), associated with elevated triglycerides (Pennacchio *et al.*, 2002). Few polymorphisms in the promoter region of the apolipoprotein A5 locus, namely the 1131T>C, -3A>G, S19W, IVS3+476G>A, 1259T>C and G185C polymorphisms have been similarly associated with elevated triglycerides levels (Pennacchio *et al.*, 2002; Aouizerat *et al.*, 2003; Martin *et al.*, 2003; Austin *et al.*, 2004), occurring due to down-regulation of apolipoprotein A5 mRNA during translation (Kathiresan *et al.*, 2008). However, one family study showed a rare Q139X (alternate name 415C>T) polymorphism to contribute severely to hyperchylomicronemia as observed in five of nine participants, one being a homozygote carrier. This involves defective lipoprotein lipase expression that leads to impaired lipolysis and dyslipidemia (Marcais *et al.*, 2005).

Since dyslipidemia is associated with triglyceride rich lipoproteins and the MS (Ruotolo *et al.*, 2002) and the rare apolipoprotein A5 Q139X polymorphism confirmed an association with triglyceride rich lipoproteins (Marcais *et al.*, 2005), we investigate the association between the Q139X polymorphism with the MS and its risk factors in this sample. This sample presents with a high prevalence of dyslipidemia (Seedat *et al.*, 1990) and the MS (Ranjith *et al.*,

2011). Therefore associations between the Q139X polymorphism, dyslipidemia and the MS may indicate if risk factors for the MS are conferred by the presence of the Q139X polymorphism.

2.4.2 Genes related to IR

We looked at three relevant genes namely lipoprotein lipase, human paraoxonase 1 and cholesteryl ester transfer protein.

Lipoprotein lipase

Lipoprotein lipase is an enzyme that plays a significant role in the regulation and transportation of lipids. It is localized to the luminal surface of capillary endothelial cells (Goldberg, 1996) by heparin sulphate proteoglycans (Braun *et al*., 1992; Enerback *et al*., 1993), and facilitates the hydrolysis of triglycerides in circulation, which is demonstrated in Figure 8.

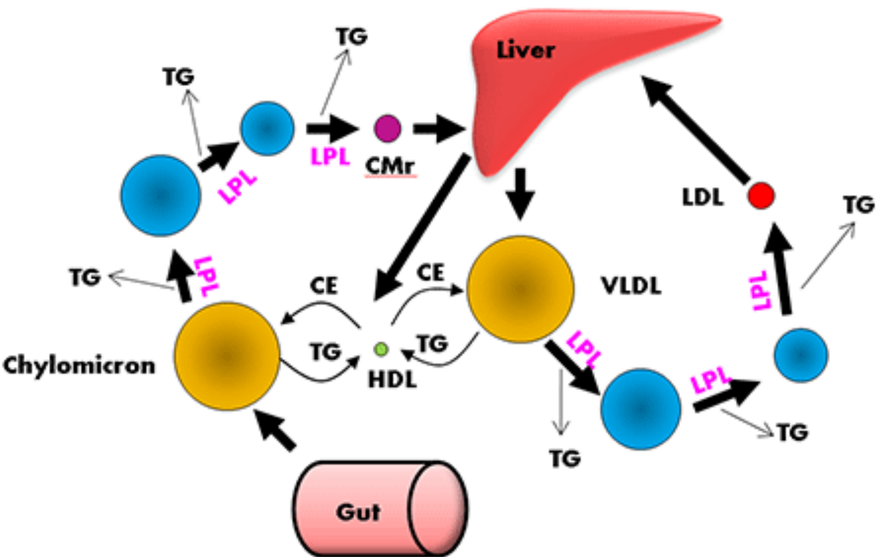


Figure 8: *Illustration showing the hydrolysis of triglycerides by the LPL enzyme (Adapted from http://www.gbhealthwatch.com/GND-Triglycerides-LPL.php)*

Key: VLDL- very low-density lipoprotein; **LPL**- lipoprotein lipase; **HDL**- high-density lipoprotein; **TG**- triglycerides; **CMr**-chylomicron remnants; **LDL**- low- density lipoproteins; **CE**-cholesterol ester

(Detailed legend for Figure 8: Hydrolysis of triglycerides occurs by transporting triglycerides in lipoproteins such as very low-density lipoproteins (VLDLs) (synthesized from the liver) and chylomicrons (synthesized in the gut from dietary fat), which is then hydrolyzed by the lipoprotein lipase enzyme in circulation to produce FFAs that are released to the target cells. The triglyceride content in VLDL and chylomicrons decrease producing low-density lipoproteins (LDL) and chylomicron remnants, respectively, which is absorbed and recycled by the liver to produce new VLDL and high- density lipoproteins (HDL) (Eckel, 1989; Goldberg *et al*., 2001)).

Besides its hydrolytic role, lipoprotein lipase is utilized by tissues but is processed differently. Hence, it acts as a bridging protein that stimulates the uptake of lipoproteins by

(i) attaching to the vessel walls (Panzenboeck *et al*., 1997; Rinninger *et al*., 1998; Seo *et al*., 2000),

(ii) facilitating the exchange of lipids between lipoproteins (Medh *et al*., 2000; Strauss *et al*., 2001; Long *et al*., 2006), and

(iii) by functioning as ligands for lipoprotein receptors (Takahashi *et al*., 1995).

During these processes, lipoprotein lipase regulates the production of FFAs and monoacylglycerols in various tissues (such as, adipose tissue, cardiac and skeletal muscle and mammary glands) (Fielding *et al*., 1998) for either storage or oxidation (Wang *et al*., 2009) which contributes to lipoprotein lipase deficiency symptoms associated with central obesity, insulin sensitivity, foam cell formation (Mead, 2002), cardiovascular risk factors (Mead, 2002) and the MS (Aguilera *et al*., 2008). This tissue specific lipoprotein lipase regulation influences lipoprotein lipase deficiency symptoms and their effects, but studies indicate that the expression of lipoprotein lipase is further linked to genetic determinants (Brunzell *et al*., 1995).

Lipoprotein lipase Gene and Polymorphisms

The lipoprotein lipase enzyme is encoded by the lipoprotein lipase gene that resides on chromosome 8p22 (Sparkes *et al*., 1987) and comprises 10 exons and 9 introns, spanning a ~30kb region (Kirchgessner *et al*., 1989). Along this region, several lipoprotein lipase haplotypes (Goodarzi *et al*., 2007) and SNPs (Cho *et al*., 2008) have been identified and linked to clinical phenotypes such as lipid metabolism, hypertension, dyslipidemia, obesity and IR. For example, the lipoprotein lipase haplotype 19-4 in the 3^1 UTR has been associated with the MS (Goodarzi *et al*., 2005) and lipoprotein lipase haplotypes 1 and 4 with insulin sensitivity and IR, respectively (Goodarzi *et al*., 2007). Similarly, lipoprotein lipase SNPs have been associated with lipid metabolism. This was observed in homozygote deficiencies that correlated with hypertriglyceridemia and reduced HDL-C concentration, whilst heterozygote deficiencies were

linked to normal to elevated triglyceride levels and reduced HDL-C concentrations (Marcais *et al*., 2000; Ma *et al*., 2003; Sorquard *et al*., 2006; Cho *et al*., 2008). In this regard, lipoprotein lipase SNPs such as D9N, Gly188Glu and Asn291Ser, have been associated with reduced HDL-C levels and S447X and HindIII with elevated HDL-C levels (Kontush *et al*., 2011). Besides its association with lipid metabolism, lipoprotein lipase SNPs such as –G53C and –T93G have been linked to obesity (Jemaa *et al*., 1995), IR (Ahn *et al*., 1993) and T2D (Wang *et al*., 2009). However, their effects have been shown to differ according to ethnicity and race (Radha *et al*., 2007). For example, the –G53C SNP was shown to be a rare variant in the Black, Chinese and Dutch population groups (Ehrenborg *et al*., 1997), but in Asian Indians it was associated predominantly with a reduced risk of obesity and T2D (Radha *et al*., 2007). The promotor –T93G has been associated with an increased risk of obesity in Asian Indians (Radha *et al*., 2007), but no such association was identified in individuals of African descent (Oliveira *et al*., 2012).

Most of the listed lipoprotein lipase SNPs above have been associated with enzymatic dysfunction, resulting in predisposition to elevated triglyceride and reduced HDL-C levels (Rip *et al*., 2006). In contrast, the common Hinf I SNP (alternate names: S447X, Ser447Stop, Ser447Ter), a "restriction site" in exon 9 (Salah *et al*., 2009), with varying frequencies in different ethnicities (Table II), and which occurs by substituting a serine amino acid with a stop codon (Salah *et al*., 2009), is an intriguing polymorphism as the variant alleles have

been shown to encode the premature truncated lipoprotein lipase enzyme, resulting in increased lipolytic activity (Ross *et al.*, 2005).

TABLE II: *THE LIPOPROTEIN LIPASE HINFI ALLELE FREQUENCIES IN THE VARIOUS POPULATIONS*

POPULATION	**HinfI SNP**		**REFERENCE**
	C allele (%)	**G allele (%)**	
Chinese	88.0	12.0	Huang *et al* (2011)
Caucasians (London)	89.1	10.9	Stocks *et al* (1992)
Caucasians (Welsh)	91.0	9.0	Mattu *et al* (1994)
Asian South Africans	89.0	11.0	Ranjith *et al* (2009)

- The frequency of the C allele of the HinfI SNP among the different population groups showed a relatively high frequency as compared to the G allele

The main function of the HinfI polymorphism is to stimulate the hydrolysis of triglycerides and to mediate the removal of remnant lipoproteins from the plasma (Beiisiegel *et al.*, 1991). This has been emphasized in many candidate gene studies, where consistent associations between the G allele (447X) of the HinfI SNP with reduced triglyceride, elevated HDL-C levels (Jemaa *et al.*, 1995; Groenemeijer *et al.*, 1997; Gagne *et al.*, 1999; Wittrup *et al.*, 1999; Garenc *et al.*, 2000; Huang *et al.*, 2006) and a greater degree of protection against the MS was shown (Goodarzi *et al.*, 2004; Komurcu-Bayrak *et al.*, 2007; Jensen *et al.*, 2009). Additionally, the GOLDN study (American Whites of European descent) further demonstrated consistent associations between the G allele (447X) with increased IR, increased waist circumference, elevated triglyceride and elevated blood glucose levels for the MS (Wood *et al.*, 2011), which collectively contributed to an increased risk of CHD. However, inconsistencies surrounding the HinfI SNP with CHD have been demonstrated

that has been linked to population and or environmental factors (Jemaa *et al*., 1995; Gagne *et al*., 1999; Sing *et al*., 1999; Arca *et al*., 2000; Clee *et al*., 2001; Wittrup *et al*., 2002; Yang *et al*., 2004).

Smoking (Lee *et al*., 2004; Komurcu-Bayrak *et al*., 2007) and alcohol intake (Lee *et al*., 2004) have been significantly associated with the HinfI polymorphism due to their opposing effects with HDL-C levels. Smoking is associated with reduced HDL-C levels and alcohol intake with elevated HDL-C levels (Van Tol, 2001). This has been shown to alter plasma lipoprotein lipase activity thereby influencing their genotypic effects (Freeman *et al*., 1998). Besides smoking and alcohol intake, adiposity has been shown to influence the genetic effects of the HinfI SNP. However, their effects are not consistent as portrayed in the literature (Arca *et al*., 2000; Garenc *et al*., 2000; Lee *et al*., 2004). For example, Garenc *et al* (2000) associated the G allele (447X) with reduced triglyceride serum levels in obese participants (BMI≥ 30) but not in lean participants (BMI<25). Arca *et al's* (2000) study, on the other hand, showed favorable effects of triglyceride levels in G allele (447X) carriers specifically in lean participants (BMI ≤ 25). One study, however, highlighted no significant interaction between the HinfI polymorphism with obesity in terms of lipid parameters (Lee *et al*., 2004). It is known that adiposity increases the efflux of FFAs from adipose tissue to the liver, resulting in an increase of VLDL, which is a predominant factor driving obesity (Jensen *et al*., 2009). Thus, it is possible that HinfI could be of significance in participants where the normal lipid transport system is under

stress to maintain the normal lipid parameters, and this makes the polymorphism a promising target for the development of drugs for lipoprotein lipase regulation (Jensen *et al*., 2009).

Studies provide evidence that the C allele of the HinfI SNP is associated with hypertension (Clee *et al*., 2001; Talmud *et al*., 2007), whilst the G allele, is protective against hypertension, possibly due to its influence with vascular tone (independent of lipid parameters) which occurs by anchoring lipoprotein lipase to the vascular endothelium by heparin sulphate proteoglycans, thereby altering cell signaling due to the production of nitric oxide (Clee *et al*., 2001). Later, Talmud *et al* (2007) confirmed and associated the C allele of the HinfI SNP with hypertension and the G allele with a 50% decrease in CVD.

A study of 30 Mexican families showed the CC genotype of HinfI polymorphism to confer susceptibility to the development of T2D (Munoz-Barrios *et al*., 2012). The notion for this association is based on the mechanism that elevates FFAs and transports it to the β cells of the pancreas thereby increasing lipoprotein lipase activity, resulting in impairment of β cell functioning which contributes to apoptosis in participants with hyperinsulinemia and T2D (Cruz *et al*., 2001). These findings may partly contribute to the association between the HinfI SNP and T2D. However, it remains possible that differences between study populations may further provide an explanation for the genetic variations of the HinfI SNP.

The HinfI SNP contributes to differing phenotypes as presented in this survey, and this makes it an attractive polymorphism for investigation. We intend on investigating its association with metabolic risk factors as insight on the molecular mechanisms behind this association is significant for our understanding of lipid parameters in our randomized sample of South African Indians. Additionally, we observed that Asian Indians have a higher prevalence of lipid profiles and T2D (Seedat *et al*., 1990), and this emphasizes the need to document the genetic associations of the HinfI polymorphism in our cohort of South African Indians who are of Indian origin.

Human Paraoxonase 1

There is increasing evidence that oxidative stress and inflammation contribute to CV related disorders (Hubert *et al*., 1983; Hulthe *et al*., 2002; Ceriello *et al*., 2004; Holvoet *et al*., 2006; Vincent *et al*., 2006), T2D (Ceriello *et al*., 2004), obesity (Couillard *et al*., 2005; Weinbrenner *et al*., 2006) and the MS (Dandona *et al*., 2005). A common marker for oxidative stress, known as oxidized LDL has been shown to be elevated in participants with metabolic disorders (Holvoet *et al*., 2001; Holvoet *et al*., 2003, Holvoet *et al*., 2004; Hoogeveen *et al*., 2007; Njajou *et al*., 2009), suggesting that regulation of oxidized LDL is vital for the progression of metabolic changes (Rosen *et al*., 2001).

Oxidized LDL has been shown to stimulate monocytes (Miller *et al*., 2010) and promotes infiltration through the vascular wall. This is a contributory

factor for the development of atherosclerosis (Cipolletta *et al*., 2005). However, increased oxidative stress is dependent on the enzyme HDL-C, as it possesses anti-inflammatory and antioxidant characteristics (Mackness *et al*., 2004) that are essential for inhibiting the oxidation of LDL-C (Mackness *et al*., 1995). In fact, the HDL-C enzyme is coupled with a protein, known as the human paraoxonase 1, which facilitates the metabolism of lipoprotein phospholipids and prevents LDL peroxidation and atherosclerosis (Eckersen *et al*., 1989) (Figure 9).

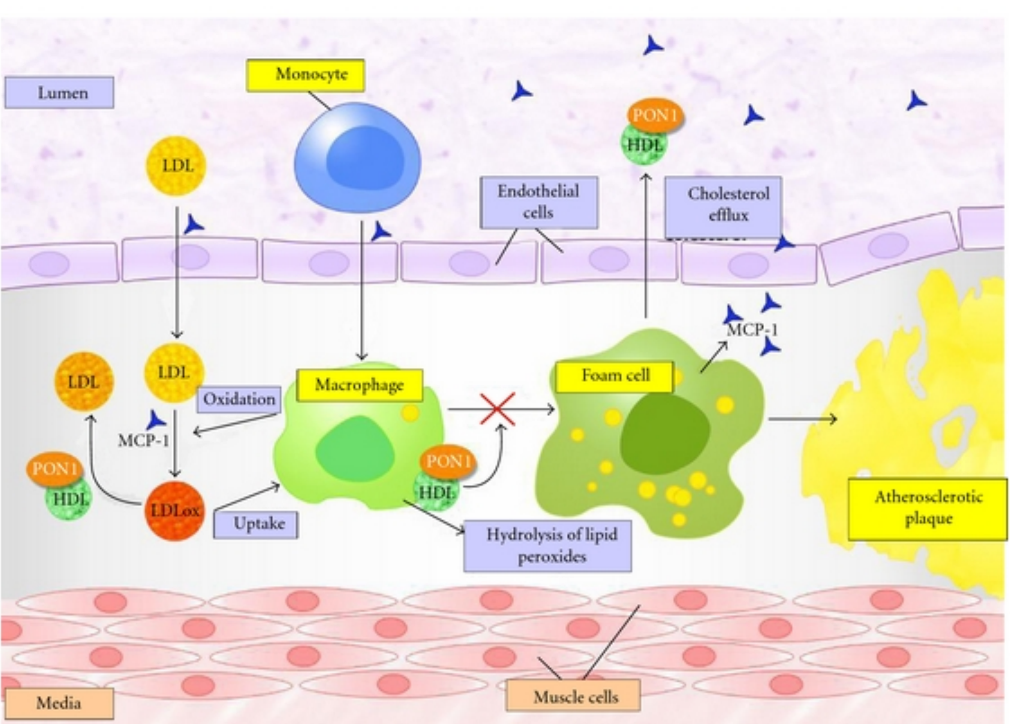


Figure 9*: Illustration of LDLs role in oxidative stress (Adapted from Camps et al., 2012)*

Key: LDL- low-density lipoprotein; **PON1-** human paraoxonase 1; **HDL-** high-density lipoprotein; **MCP-1-** monocyte chemoattractant protein-1

(Detailed legend for Figure 9: LDL particles (in the absence of PON1) are oxidized and are internalized into macrophages, becoming foam cells that contribute to the development of atherosclerosis. PON1 is key for the prevention of atherosclerosis as it hydrolyzes oxidized LDL and reverses it back to its natural state; and also prevents MCP-1 (that coordinates inflammation in damages tissue) production, which enables cholesterol efflux from the macrophages).

The human paraoxonase 1 enzyme is a calcium dependent esterase (La *et al*., 1993; Kelso *et al*., 1994), which belongs to the family of paraoxonases (PON1, PON2 and PON3) (Getz *et al*., 2004). It consists of 354 amino acids with a molecular weight of 43kDA (Primo-Parma *et al*., 1996; Mackness *et al*., 1996), and is synthesized and secreted from the liver (Costa *et al*., 2005). Reduced paraoxonase 1 activity has been shown to contribute to greater LDL oxidation and an increased risk of atherosclerosis, whilst overexpression of paraoxonase 1 reduces aortic lesions (Shih *et al*., 1998; Shih *et al*., 2000; Tward *et al*., 2002; Rozenberg *et al*., 2003). Thus, paraoxonase 1 activity is considered to be a predictor for CVD (Hegele *et al*., 1999; Durrington *et al*., 2001; Reddy *et al*., 2001; Watzinger *et al*., 2002; Li *et al*., 2003; Robertson *et al*., 2003; Lawlor *et al*., 2004; Mackness *et al*., 2004; Nguyen *et al*., 2004), along with the simultaneous association of SNPs present in the promoter and coding regions of the human paraoxonase 1 gene (Mackness *et al*., 1997; Leviev *et al*., 2000).

Human paraoxonase 1 gene and polymorphisms

The human paraoxonase 1 gene is located on chromosome 7 between q21.3 and q21.1 (Glendenning *et al*., 1996; Hegele *et al*., 1999) and contains nine exons and eight introns (Mackness *et al*., 1998). This gene does not contain haplotypes suggesting that its polymorphisms are independently associated with metabolic disorders (Jarvik *et al*., 2003). In this regard, 200 polymorphisms have been identified on the human paraoxonase 1 locus, with seven polymorphisms in the promoter region, 171 in the introns, five in the

exons and 15 polymorphisms in the 3[1]untranslated region (La *et al*., 2003). The most common missense polymorphism investigated includes the 192Arg/Gln (alternate name: Q192R) due to its association with paraoxonase 1 activity (Adkins *et al*., 1993; Humbert *et al*., 1993; Mackness *et al*., 1998).

The 192Arg/Gln polymorphism occurs at codon 192 and substitutes the amino acid arginine with glutamine, resulting in two alleles viz. 192Arg and 192Gln (Aubo *et al*., 2000). These alleles have shown varying associations with paraoxonase 1 activity. For example, the 192Arg has been associated with higher paraoxonase 1 activity and the 192Gln with reduced (Serrato *et al*., 1995), suggesting that the 192Arg allele prevents LDL oxidation as compared to the 192Gln allele (Mackness *et al*., 1997).

Oxidation of LDL is inhibited by HDL (Mertens *et al*., 2001) and HDL binding is influenced by the human paraoxonase1 192Arg/Gln polymorphism (Gaidukov *et al*., 2006). The 192Arg allele attaches to HDL with a 3-fold reduced affinity as compared to the 192Gln allele, and further modulates macrophages by cholesterol efflux. What this translates into is that carriers of the high activity 192Arg allele have reduced oxidative stress, corresponding with reduced risk of CV related disorders (Bhattacharyya *et al*., 2008).

The 192Arg/Gln polymorphism associated with CAD has been inconsistently reported on in the literature. The North American general population (Serrato *et al*., 1995), the Japanese (Odawara *et al*., 1997) and French (Ruiz *et al*.,

1995) with T2D carrying the 192Arg allele have demonstrated an increased risk for CAD, whilst the Finnish (Antikainen *et al*., 1996) and Japanese (Suehiro *et al*., 1996) with CAD and no T2D showed no risk with CAD. Similarly, Europeans (Herrmann *et al*., 1996) carrying the 192Arg allele with myocardial infarction demonstrated no risk with CAD. It is conceivable that participants with T2D are dependent on paraoxonase 1 activity than non-diabetics. What this translates into is that the effect of paraoxonase 1 against peroxidation is more significant in diabetic participants and this provides a clearer effect of the 192Arg/Gln polymorphism on CAD in T2D individuals than in the general population (Pfohl *et al*., 1999).

The 192Arg allele in smokers has shown a greater risk for CAD. This is due to the production of excess free radicals in individuals who smoke (Church *et al*., 1985). Thus, oxidized LDL in smokers produces more lipid peroxidation than non-smokers (Scheffler *et al*., 1992), suggesting that paraoxonase 1 activity is more significant in smokers than non-smokers (Pfohl *et al*., 1999). Hence, gene-environment interaction contributes significantly to the susceptibility of CAD (Osei-Hyiaman *et al*., 2001).

There has been limited information on the association of the human paraoxonase1 192Arg/Gln polymorphism in communities presenting with high CV risk factors. Therefore, we intend demonstrating the genetic pattern of this polymorphism and its association with the development of the MS in our sample.

Cholesteryl Ester Transfer Protein (CETP)

Cholesteryl ester transfer protein (CETP) is a 74kDa hydrophobic glycoprotein that metabolizes lipids and lipoproteins (Tall, 1993), by promoting the uptake of cholesterol from peripheral tissues to the liver, a process referred to as reverse cholesterol transport (Mohrschladt *et al*., 2005). This occurs by CETPs role in catalyzing the transfer of esterified cholesterol from HDL-C to very low-density lipoproteins (VLDL) and LDL-C in exchange for triglycerides. During this process, CETP lowers HDL-C levels and produces small sized LDL (Mann *et al*., 1991; Lagrost *et al*., 1994). As a result CETP expression is dependent on the development of atherosclerosis (Austin *et al*., 1988; Sharrett *et al*., 2001; Harder *et al*., 2007).

CETP deficiency has been shown to contribute significantly to anti-atherogenic lipid profiles that are characterized by reduced LDL-C and elevated HDL-C levels (Miller *et al*., 2003; Plengpanich *et al*., 2009). Many mechanisms, in this regard has been proposed for CETP deficiency viz.

(i) CE transfer is inhibited resulting in triglyceride rich and cholesterol poor LDL;

(ii) removal of cholesterol poor LDL by the liver results in up-regulation of LDL receptors, contributing to reduced ApoB levels; and

(iii) the transfer of HDL to LDL inhibits the modification of LDL oxidation, thereby limiting lipid peroxidation (Parthasarathy *et al*., 1990).

However, evidence suggests that the CETP levels and plasma lipid concentrations are further influenced by the CETP gene polymorphisms (Bernard *et al*., 1998).

CETP gene and polymorphisms

The CETP gene (encoding the CETP protein) resides on chromosome 16q21 (Thompson *et al*., 2008; Ridker *et al*., 2009) and comprises 16 exons and 15 introns spanning a 25kb region (Drayna *et al*., 1987; Ordovas *et al*., 2000). In the first intron of the CETP gene, haplotype analysis of 4 polymorphisms i.e. -2568 C/A, -1700 C/T, -998 A/G and Taq1B revealed associations with HDL-C levels i.e. ATAA haplotypes with elevated HDL-C levels and CTAG haplotypes with reduced HDL-C levels (Schierer *et al*., 2012). The common Taq1B polymorphism [occurs due to a base change from guanine (G) to adenine (A) at the 277th nucleotide of the first intron of the CETP gene and disrupts a Taq1 restriction site (Dixit *et al*., 2005; Kashari *et al*., 2010)], in particular, has been independently associated with CETP activity and HDL-C levels (Kondo *et al*., 1989; Freeman *et al*., 1990; Freeman *et al*., 1994; Hannuksela *et al*., 1994; Mitchell *et al*., 1994; Fumeron *et al*., 1995).

For example, low CETP activity contributes to elevated HDL-C levels and is determined by the B2 allele of the Taq1B polymorphism (Dixit *et al*., 2005; Kashari *et al*., 2010), whereas high CETP activity influences reduced HDL-C levels and is determined by the B1 allele (Noone *et al*., 2000; Boekholdt *et al*., 2004). However, the exact mechanism associating CETP activity and HDL-C

levels with the Taq1B polymorphism is not known, but studies assume that the B2 allele (associated with increased CETP activity) is in linkage disequilibrium with an unknown functional mutation located on the CETP locus, which contributes to varying degrees of CETP and HDL-C levels (Ordovas *et al*., 2000). Interestingly, Le Goff *et al* (2002) suggested that the unknown functional mutation is closely linked with CETP polymorphisms such as the CETP-971, the CETP-629 and the popular Taq1B, which significantly influences the modulation of HDL-C and CETP levels.

The Taq1B polymorphism has been linked to CAD through its association with HDL-C modification (Kawasaki *et al*., 2002; Chaaba *et al*., 2005; Relvas *et al*., 2005; Hsieh *et al*., 2007), with the B1 allele (increased CETP activity and reduced HDL-C levels) being effective in its development (Padmaja *et al*., 2009). A Chinese study showed individuals without the B2 allele to have a two times higher risk for CAD, but no such association with CAD was seen in Malays and Indians without the B2 allele. This suggests that the association between the Taq1B genotypes with CAD is ethnic specific (Lu *et al*., 2013). One study, however, has shown the risk of CAD to be independent of HDL-C levels (Rahimi *et al*., 2012). What this translates into is that association between the polymorphism with HDL-C could be independent on CETP activity, suggesting that these associations are probably not modulated through CETP (Fumeron *et al*., 1995; Corbex *et al*., 2000; Ordovas *et al*., 2000), but are probably related to genetic variations in all proteins involved in lipid metabolism (Von Eckardstein *et al*., 2001). Additionally, environmental

factors have exerted some influence on HDL-C levels, which influences the susceptibility of CAD (Klerkx *et al*., 2003),

Environmental factors significantly influence HDL-C levels of the Taq1B polymorphism by the transfer of lipid proteins. For example, smoking has been shown to influence the activity of CETP by restricting the increase of HDL-C as observed in participants carrying the B2 allele (Freeman *et al*., 1993; Freeman *et al*., 1998; Mero *et al*., 1998). This is due to the release of catecholamine and the flux of FFAs that elevates VLDL and LDL levels and reduces HDL-C levels (Campbell *et al*., 2008). Smoking has been shown to increase the risk for obesity (Ruan *et al*., 2010), which is further enhanced by the B1 allele due to increased CETP activity that contributes to reduced delivery of CE to the liver by means of the HDL pathway (Sattar *et al*., 1998). B1 allele carriers who consume alcohol have shown CETP levels to vary (Fumeron *et al*., 1995). For example, in participants with hypertriglyceridemia who consume alcohol, elevated CETP levels have been shown to contribute to an increased transfer of triglycerides to LDL and HDL (Miller *et al*., 1998), suggesting that the reverse cholesterol transport to the liver by HDL and LDL pathways are reduced (Fielding *et al*., 1995), thereby contributing to a decreased hepatic output of ApoB (Sniderman *et al*., 1993). However, in healthy participants, elevated CETP levels are linked with elevated ApoB (Fumeron *et al*., 1995). What this translates into is that the function of the B1 allele on ApoB metabolism associated with alcohol intake significantly correlates with progression of CAD (Kuivenhoven *et al*., 1998).

There is evidence which shows B1 allele carriers to have a higher degree of IR (Lopez-Rios *et al*., 2009), T2D (Lopez-Rios *et al*., 2011) and the MS (Oszait *et al*., 2008). These findings have been linked to increased CETP activity and elevated triglycerides in HDL-C particles, leading to increased flux of FFAs to the liver from HDL. This physiological process contributes to decreased hepatic sensitivity to insulin, and disorders like T2D and the MS (Lopez-Rios *et al*., 2009). Therefore, the influence of this polymorphism on the MS and on lipid profiles in a community-based cohort like the Phoenix community in South Africa may prove beneficial as participants are predominantly of Asian origin with a high prevalence of T2D and adverse lipid profiles (Seedat *et al*., 1990).

Overall, Asian Indians demonstrate a high propensity for DM and dyslipidemia and studying these associations in the presence of the Taq1B polymorphism in our sample could determine whether CV risk is actually conferred by Taq1B.

2.4.3 Genes related to obesity

We looked at two relevant genes namely adiponectin and leptin.

Adiponectin

Adiponectin belongs to the adipokine family and is secreted in abundance from adipose tissue (Maeda *et al*., 1996). It possesses both anti-inflammatory and anti-atherogenic properties (Yung *et al*., 2006; Knudson *et al*., 2007) that

inhibit transcription of the adiponectin gene (Brunn *et al*., 2003; Hajer *et al*., 2008), resulting in reduced adiponectin levels in obesity (Arita *et al*., 1999; Weyer *et al*., 2001). This process occurs in skeletal muscle and in the liver by stimulation of PPARαand AMPK by AdipoR1 and AdipoR2 receptors (Yamauchi *et al*., 2002; Yamauchi *et al*., 2007; Iwabu *et al*., 2010). The mechanism is illustrated in Figure 10.

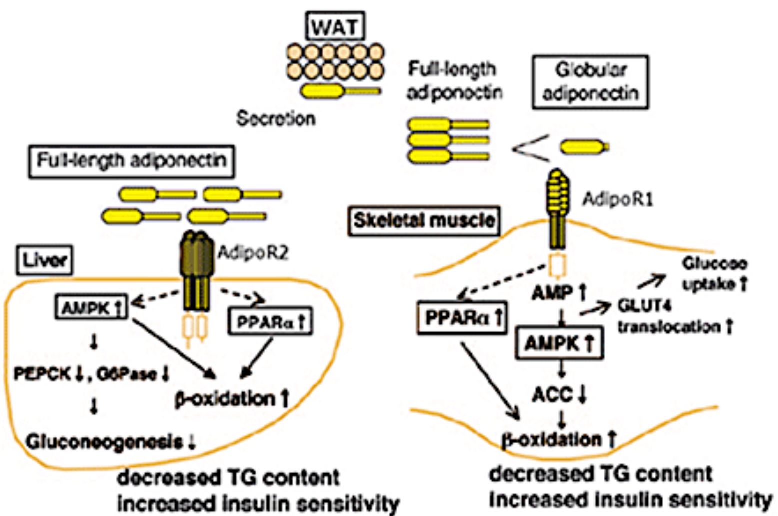


Figure 10: *Process for fat burning (β oxidation) and insulin sensitivity in the liver and skeletal muscle in response to upregulation of adiponectin (Adapted from Kodowaki et al., 2005)*

Key: WAT- white adipose tissue; **PEPCK**- phosphoenolpyruvate carboxykinase; **G6Pase**- glucose-6-phosphatase; **TG**-triglycerides; **ACC**- acyl-coenzyme A oxidase; **AMP**- Adenosine monophosphate; **AMPK**- AMP kinase

(Detailed legend of Figure 10: In skeletal muscle, high and low molecular weight adiponectin upregulates PPARα and stimulates AMPK via the AdipoR1 receptors resulting in elevated FFA β oxidation and elevated glucose uptake, respectively. In the liver, only high molecular adiponectin can act via. the AdipoR2 and this leads to upregulation of PPARα and stimulation of AMPK. The PPARα elevates FFA β oxidation, but AMPK stimulation limits the activities of genes that are involves in gluconeogenesis viz. PEPCK and G6Pase (Kodowaki *et al*., 2005))

Findings from other studies show adiponectin's involvement in the risk of T2D (Hotta *et al*., 2000; Lindsay *et al*., 2002; Spranger *et al*., 2003; Li *et al*.,

2009) and insulin sensitivity (Snijder *et al*., 2006). This association triggers the inhibition of gluconeogenesis, stimulation of FFA oxidation in the liver, activation of insulin synthesis, and stimulation of FFA oxidation and uptake of glucose by skeletal muscle. However, evidence indicates that the effects of PPARα and AMP on the signaling cascade may further modulate the progression of T2D (Kadowaki *et al*., 2006; Rabe *et al*., 2008) and insulin sensitivity (Leonardini *et al*., 2009).

Adiponectin is associated with elevated triglycerides and reduced HDL-C levels (Ryo *et al*., 2004), which collectively are critical features of dyslipidemia in the MS (Schneider *et al*., 2005). Studies indicate that such an association occurs as a result of hepatic lipase activity present in the liver (Laakso *et al*., 1987; Deeb *et al*., 2013; Sibley *et al*., 2003). However, it has been further hypothesized that this association could be mediated by PPARα (Lewis *et al*., 2004), but the exact mechanism for such an association has not yet been elucidated (Schneider *et al*., 2005).

Additionally, adiponectin has been correlated with hypertension (Adamczak *et al*., 2003; Ouchi *et al*., 2003; Iwashima *et al*., 2004), which occurs under conditions of stress, by stimulation of vascular endothelial cells by eNOS-dependent and COX-2 dependent regulatory mechanisms, and regulation of macrophage functioning (Ohashi *et al*., 2010)

Overall, adiponectin is an insulin-sensitizing protein that possesses anti-diabetic, anti-inflammatory and anti-atherogenic properties. Therefore, adiponectin is associated with varying degrees of cardiovascular risk factors and is a key determinant for metabolic disorders, which has been attributed to environmental and or lifestyle factors (Nishizawa *et al*., 2002; Combs *et al*., 2003). Additionally, the salutary properties of adiponectin are further determined by genetic functions (Vasseur *et al*., 2002; Chuang *et al*., 2004; Menzaghi *et al*., 2007).

Adiponectin Gene and Polymorphisms

The adiponectin protein is encoded by the adiponectin gene, which resides on chromosome 3p27 (Kissebah *et al*., 2000; Vionnet *et al*., 2000; Francke *et al*., 2001) and comprises three exons and two introns, spanning a 16kb region (Heid *et al*., 2006), with several polymorphisms being identified in the last decade (Table III). The common +45T>G and +276G>T polymorphisms studied revealed a haplotype i.e. T/T at 45 and G/G at 276 which was associated with elevated fasting blood glucose and insulin levels (Menzaghi *et al*., 2002). For this study, one polymorphism, (+45T>G) was analyzed in depth and is the focus of this review.

TABLE III: *ADIPONECTIN POLYMORPHISMS IDENTIFIED IN THE ADIPONECTIN LOCUS*

SNPs	**METABOLIC DISORDER**	**POPULATION**	**REFERENCE**
−12823	No association with T2D, BMI or insulin sensitivity	Pima Indians	Courten *et al* (2005)
−11426G>A	Associated with increased risk for weight gain in diabetics Associated with fasting plasma glucose in T2D patients and in those with IGT G allele moderately associated with T2D	Chinese Swedish Caucasians French Caucasians	Yang *et al* (2008) Gu *et al* (2004) Gibson *et al* (2004)
−1391G>A	A allele associated with elevated adiponectin levels, elevated BMI, and obesity A allele carriers have reduced weight, waist and hip circumferences and BMI GA carriers increased risk for hyperglycaemia A allele associated with elevated adiponectin levels A allele associated with elevated adiponectin levels A allele associated with elevated adiponectin levels A allele associated with elevated adiponectin levels A allele associated with elevated adiponectin levels in obese children A allele associated with elevated adiponectin levels A allele associated with elevated adiponectin levels A associated with reduced adiponectin levels, reduced insulin sensitivity and increased risk of T2D in the obese	Europeans French Caucasians French Caucasians Hispanic Americans and African Americans Caucasians Caucasian women French Caucasians Caucasian and African American Caucasians French Caucasians	Morandi *et al* (2010) Menzaghi *et al* (2005) Fumeron *et al* (2004) Vasseur *et al* (2002) Guo *et al* (2006) Henneman *et al* (2010) Kyriakou *et al* (2008) Naji *et al* (2006) Woo *et al* (2006) Hivert *et al* (2008) Vasseur *et al* (2005)
−11377 G > C	C allele associated with elevated fasting plasma glucose levels in diabetics C allele associated with severe obesity G allele associated with reduced ADP levels, increased risk of hypertension SNP associated with increase in plasma oxidative stress markers G allele associated with reduced ADP levels, reduced insulin sensitivity, and higher risk of T2D in obese participants G allele associated with coronary stenoses and reduced adiponectin levels No association with adiponectin levels CC and CG genotypes had elevated BMI than GG	Chinese (T2DM) French Caucasians (obese/lean) Chinese (hypertensive) T2DM patients French Caucasians (lean/obese) European men with CVD Caucasian Italians Swedish Caucasians	Yang *et al* (2008) Naji *et al* (2006) Ong *et al* (2010) Prior *et al* (2010) Vasseur *et al* (2005) Hoefle *et al* (2007) Menzaghi *et al* (2005) Gu *et al* (2004)
−11365	SNP associated with reduced adiponectin levels No association with T2D, BMI, or insulin sensitivity	- Pima Indians	Qi *et al* (2006) Courten *et al* (2005)
−10677 C > T	SNP associated with reduced adiponectin levels	Chinese (hypertensive)	Ong *et al* (2010)
−10068 G > A	A allele associated with reduced adiponectin levels A allele associated with waist circumference G allele associated with elevated adiponectin levels	Hypertensive Chinese American Caucasian young adults Caucasian and African American adolescents	Ong *et al* (2010) Wassel *et al* (2010) Woo *et al* (2006)

−10066 G > A	G allele associated with elevated adiponectin levels	Caucasian women	Kyriakou *et al* (2008)
−7734 C > A	A allele associated with elevated adiponectin levels	Caucasian women	Kyriakou *et al* (2008)
−4041 A > C	No association with adiponectin levels	Caucasian Italians	Menzaghi *et al* (2005)
−4034	CC associated with CVD risk	-	Qi *et al* (2006)
−3971 G > A	A allele associated with glucose tolerance and insulin sensitivity, but not adiponectin levels	Caucasian Canadians (nondiabetic)	Ruchat *et al* (2008)
+276 G > T	T allele associated with obesity	African American men	Dimmer *et al* (2010)
	T allele associated with elevated adiponectin levels	Caucasian women	Kyriakou *et al* (2008)
	T allele associated with central obesity and hyperglycemia	Indigenous Taiwanese	Huang *et al* (2010)
	T allele associated with reduced adiponectin levels, diastolic blood pressure	Finnish men	Mousavinasab (2006)
	GG genotype associated with reduced adiponectin levels, IGT	Spanish	Sánchez *et al* (2005)
	T allele associated with severe obesity, but not adiponectin levels	French Caucasians (obese/lean)	Naji *et al* (2006)
	T allele associated with elevated adiponectin levels	Caucasian and African American adolescents	Woo *et al* (2006)
	No association with adiponectin levels or hypertension	Japanese men (hypertensive/ normotensive)	Iwashima *et al* (2004)
	No difference in allele frequencies between diabetic and nondiabetic, no difference in risk of T2D or IR	Korean (diabetic/ nondiabetic)	Lee *et al* (2005)
	No association with T2D, BMI or insulin sensitivity	Pima Indians	Courten *et al* (2005)
	TT genotype associated with reduced risk of CAD in T2D participants	Caucasian Italians (T2DM)	Bacci *et al* (2004)
	TT genotype associated with elevated adiponectin levels	Caucasian Italians	Menzaghi *et al* (2005)
	G allele, GT genotype associated with reduced ADP, no association with IR	Caucasians	Mackevics *et al* (2006)
	T allele associated with reduced BMI and HOMA-IR	Japanese (nondiabetic)	Nakatani *et al* (2005)
	T associated with elevated adiponectin levels	French Caucasians	Vasseur *et al* (2002)
+3228 C > T	T allele associated with elevated adiponectin levels	Caucasian women	Kyriakou *et al* (2008)
+3286	No association with BMI, T2D or insulin sensitivity	Pima Indians	Courten *et al* (2010)
+10211 T > G	G allele associated with a greater risk for diabetes, elevated BMI, and reduced adiponectin levels	Asian Indians	Vimaleswaran *et al* (2008)
G > T	T allele associated with reduced adiponectin levels	Chinese (hypertensive)	Ong *et al* (2010)
A > G	G allele associated with IR	African Americans	Speechia *et al* (2008)
T > C	C allele associated with elevated adiponectin levels	Hispanic Americans and African Americans	Guo *et al* (2006)

(Adapted from Enns et al., 2011)

Key: ADP- adiponectin; **IR**- insulin resistance; **BMI**- body mass index; **T2D**- type 2 diabetes; **CAD**- coronary artery disease; **IGT**- impaired glucose tolerance; **CVD**- cardiovascular disease

The +45T>G polymorphism on exon 2 is one of the most studied polymorphisms to date. Some studies demonstrate that the +45T>G polymorphism is a determinant for adiponectin levels (Li *et al.*, 2007; Zietz *et al.*, 2008; Zhang *et al.*, 2008). However, these findings are not consistent as other studies have shown differing associations (Mousavinasan *et al.*, 2006; Cesari *et al.*, 2007; Kim *et al.*, 2010; Suriyaprom *et al.*, 2010). For example, the +45G allele has been associated with elevated adiponectin levels as observed in French (Vasseur *et al.*, 2002), but others have shown reduced adiponectin levels in Whites with the +45T alleles (Mackevics *et al.*, 2006) and in the Chinese with the +45G alleles (Wang *et al.*, 2008). Additionally, Italians with the +45T>G showed no association with adiponectin levels (Menzaghi *et al.*, 2005; Melistas *et al.*, 2009).

A recent study demonstrates reduced adiponectin levels in DM participants with IR (Blaslov *et al.*, 2013). This was previously documented in a cross sectional study of 151 Uygur adults with T2D, which showed the +45G allele to be associated with reduced adiponectin concentrations and IR (Li *et al.*, 2007); and further with an increased risk of impaired glucose tolerance (IGT) (Gonzalez-Sanchez *et al.*, 2005), suggesting a fundamental association with insulin sensitivity (Li *et al.*, 2007). In addition, similar associations between the +45G allele and IR were observed in Wang *et al's* (2008) and in Melistas *et al's* (2009) findings. However, Yang *et al* (2003) and Zacharova *et al* (2005) findings showed the +45T allele to be associated with IR, whilst no association with IR was highlighted in Lee *et al's* (2005) study. For these reasons, the +45T>G polymorphism may influence IR related disorders, albeit

significantly dependent on the population background, and IR is known to be a driving factor for the MS (Reaven, 1995). Therefore, the genetic effects of the +45T>G possibly influence the MS (Li *et al*., 2012).

Previous studies suggest that the association between the +45T>G and the MS is unclear (Menzaghi *et al*., 2002; Heid *et al*., 2006), but a study by Li *et al* (2007) in the Chinese showed the +45G allele to be protective against the MS, while the +45T allele is a possible risk allele for the MS. Studies indicate that the MS develops primarily due to obesity (Alberti *et al*., 2005). The +45T allele therefore has been shown to be a risk factor for obesity in Italians (Menzaghi *et al*., 2002; Ronconi *et al*., 2010), the Swedish (Ukkola *et al*., 2003) and in the Taiwanese (Yang *et al*., 2003); but in the Chinese (Wang *et al*., 2008) and Japanese (Nakatani *et al*., 2005) population, the +45T correlated with reduced BMI. The +45G allele, on the other hand, was associated with increased BMI as observed in the Chinese (Li *et al*., 2007), Hispanic Americans (Sutton *et al*., 2005) and the French (Fumeron *et al*., 2004). The findings suggest that the +45T>G polymorphism is therefore a promising candidate for investigation as its genotypic effects may predispose to obesity related disorders and the MS among different ethnic groups (Li *et al*., 2011).

These discrepancies reflect differences between population groups in genetic background or environmental exposures. Despite these differences, the contribution of adiponectin activity and polymorphisms contributing to the insulin sensitivity, the MS and CAD is steadily growing in many population groups, but the data is still limited to draw final conclusions. Few studies on

the adiponectin 45T>G polymorphism on communities with high risk factor profiles have been investigated, thus the objective was to study the SNP in a sample that predisposes to T2D and CVD, and to determine whether the SNP increases the risk for the development of the MS in South African Indians.

Leptin

Leptin is a 16kDa (Masuzaki *et al*., 1997) hormone that is produced by adipocytes and is responsible for energy homeostasis (Elmquist *et al*., 1998; Friedman *et al*., 1998; Bates *et al*., 2003). It influences the metabolic pathways (Ruige *et al*., 1999) by stimulating glucose uptake, facilitating FFA oxidation (Wauters *et al*., 2000; Minokoshi *et al*., 2002) and by inhibiting insulin secretion (Seufert *et al*., 1999).

Circulatory leptin levels correlate with adverse pathophysiological conditions (Frederich *et al*., 1995; Considine *et al*., 1996; Harigaya *et al*., 1997; Kennedy *et al*., 1997). For example, increased leptin levels have been associated with reduced appetite and increased energy utilization (Martins *et al*., 2012), thereby contributing to obesity (Ragin *et al*., 2009), whilst leptin deficiency linked with weight loss is associated with increased appetite and reduced energy utilization (Martins *et al*., 2012), thereby contributing to IR and diabetes (Zhang *et al*., 1994).

The precise mechanism involved in leptin regulation is not fully understood, but it is believed that multiple chemicals and neurotransmitters are involved in its regulation, which subsequently correlates with obesity (Reaven *et al*., 1988;

Bandin *et al*., 2000; Sousa *et al*., 2009). For instance, neurons that contain neuropeptide Y, a key element for appetite secreted by adipocytes and involved in regulation of energy homeostasis, is inhibited due to leptin production. Neurons expressing α-melanocyte stimulating hormones produced by cells in the pituitary gland which are key elements for satiety, is inhibited due to down regulation of leptin (Yildiz, 2010). This phenomenon of leptin regulation linked with food intake and energy homeostasis, contributing to obesity related disorders (Coleman *et al*., 1978; Friedman *et al*., 1991; Zhang *et al*., 1994) has been related to genetic mutations on the leptin gene (Considine *et al*., 1995; Hager *et al*., 1998; Li *et al*., 1999).

Leptin Gene and Polymorphisms

The gene that encodes for leptin is located on chromosome 7q31.3 and contains three exons and two introns (Isse *et al*., 1995). There are several polymorphisms that have been identified on this locus, with two common polymorphisms (the G2548A (Gotada *et al*., 1997) and the A19G (Hager *et al*., 2002)) being repeatedly studied. A five polymorphism haplotype (H1328084, H1328083, H1328082, H1328081 and H1328080) revealed associations with leptin deficiency and obesity (Yiang *et al*., 2004). However, more recently a rare 25CAG polymorphism has been linked to morbid obesity in Thai children with obese parents (Kongmacheep *et al*., 2009).

The 25CAG polymorphism has been identified at the 3rd base of codon 25 on exon 2 in 6.7% of participants with parental obesity (Ohshiro *et al*., 2000; Kongmacheep *et al*., 2009). A frequency of 3% for the G allele was

demonstrated in the sample and a gender/genotype variation (AG vs AA) of 1:21 for boys and 1:7 for girls was shown in the Thai study by Kongmacheep *et al* (2009). What this translates into is that the 25CAG may possibly serve as marker for obesity, which is four times more prevalent in boys than girls. The study by Kongmacheep *et al* (2009) did not document leptin levels, which makes it difficult to analyze the opposing effects of leptin activity as determined by the alleles. Furthermore, their study looked at the association between eating behavior and the genetic variants, but no significant associations were reported. What emerges is that the polymorphism could possibly alter its signaling pathways followed by hunger sensations, but no conclusive evidence has been recorded due to the small sample size and limited studies.

Since obesity is the driving factor for the MS (Wabitschet *et al*., 1997) and is a key element for leptin regulation (Martins *et al*., 2012), the study selected the leptin gene for investigation. Furthermore, since there are limited effects of the leptin polymorphism on lipid profiles, the 25CAG polymorphism, which influences obesity susceptibility, was studied.

2.5 Summary

The MS is a complex entity with several definitions being proposed by health organizations. It involves clustering of several metabolic risk factors, namely, central abdominal obesity, elevated triglycerides, reduced HDL-C, elevated blood pressure and elevated blood glucose serum levels (Park *et al*., 2003; Alberti *et al*., 2005). Lifestyle, cultural, socioeconomic (Song *et al*., 2006) and genetic factors (Groop,

2000; Corella *et al*., 2005; Elder *et al*., 2009) further contribute to its susceptibility, thus, increasing the risk for CVD (Glazier *et al*., 2002; Eriksson, 2008). Few studies have assessed the genetic basis underlying the pathogenesis of the MS in South African Indians, who present with high CV risk profiles. Based on the findings, we selected candidate genes related to lipid and carbohydrate metabolism, which may possibly increase the risk for the MS and thus CVD.

CHAPTER 3

METHODOLOGY

Chapter 3: Materials and Methods

In this chapter we describe the study design, which includes the study population, study period and participant recruitment. We further demonstrate the sample size followed by exclusion criteria, how data (clinical measurements and biochemical profiles) was collected, and how IR and the MS were diagnosed. This is followed by a detailed methodology of all laboratory procedures performed including DNA extraction from whole blood, quantification of DNA, selection and genotyping of candidate gene polymorphisms and quality control of PCR products. Statistical and ethical considerations are demonstrated at the end of the chapter.

3.1 Study Design

Our study formed part of the larger Phoenix Lifestyle Project (PLP), which was conducted amongst South African Indians from the Phoenix community of KwaZulu-Natal. South African Indians are mostly Indian descendants of those brought to Natal between 1860 and 1911 to work as sugar cane laborers. Phoenix was originally a sugar cane estate but with time development of the area occurred, resulting in 23 areas/units, housing 176 989 people, of whom 85% are of Indian/Asian origin with varying socioeconomic statuses (Census, 2011).

3.1.1 Study population

This study was a cross sectional investigation of 1000 participants of the 1500 participants in the PLP who consented to genetic screening. These participants were categorized according to the presence or absence of the MS, and all genetic analysis were accordingly compared between these two groups. Owing to the high number of South African Indians in this community, we can

evaluate the prevalence of CAD in this cohort. Support for the high prevalence of CAD in this population group (Seedat *et al*., 1996) and in other Asian/Indian populations (Jha *et al*., 1993; Enas, 1998; Palaniappan *et al*., 2010) has been previously confirmed and it is believed that this association is due to their migration from the Indian subcontinent (Gundu *et al*., 2005). We believe that changes in socioeconomic statuses within Indian/Asians (of Indian origin) could have contributed to the high incidence of CAD worldwide. Therefore, evaluating the Phoenix community, due to its high prevalence of South Africans from Indian descent, could allow us to understand the principles surrounding the progression of atherosclerotic diseases.

3.1.2 Study period

The collection of data for the PLP began and ended in 2007 by trained personnel under the supervision of Professor DP Naidoo who was the principle investigator (Ethical Clearance Reference: E336/05). Cardiovascular risk factors and genetic materials from the 1000 consenting participants that were collected in E336/05 and used in this study were analyzed and performed by Ms. Tanya Maistry, which began in 2010.

3.1.3 Study recruitment

Participant's for the PLP were randomly selected from the previous population census and one participant from each of these households was selected using the Kish Method (WHO, 2008). All participants who enrolled in the PLP gave informed consent prior to participation (Ethical Clearance Reference:

E336/05). Permission was obtained from participants to store their genetic material for future use. Our further genetics study was reviewed and approved by the Biomedical Research Ethics Committee, University of KwaZulu-Natal in 2010 (Ethical Clearance Reference: BE232/010).

3.2 The study sample

3.2.1 Sample size

Although the PLP originally recruited 1500 participant, the genetics study investigated 1000 of the 1500 participants i.e. 750 females and 250 males under the direction of the biostatistician, Tonya Esterhuizen, from the University of KwaZulu-Natal. The high female to male ratio occurred due to random sampling. It should be noted that one female participant's records which was included as part of this study as a result of random sampling could not be analyzed due to errors observed in the data, which probably occurred during data capturing.

3.2.2 Exclusion criteria

The exclusion criteria for this study included conditions which alter peripheral blood cells and genetic patterns such as pregnancy, leukemia, chronic immunosuppressive therapy, history of solid organ transplant and anemia.

3.3 Data collection

Data collection for the PLP occurred as follows:

Where consent to participate was obtained, and prior to the test days, specially trained fieldworkers interviewed participants and all demographics were recorded in the STEPS Instrument for Non-communicable Disease (NCD) Risk Factors, a modified Version 1.3a (WHO, 2005) (Appendix I). The following demographics were recorded viz.

- Demographic profile and behavioral risk factors e.g. smoking, drinking, dietary, physical activity, psychological stress and sleeping habits for cardiovascular disease were obtained.
- History of diabetes and hypertension and family history of cardiovascular risk factors.

Participants were brought to the Lifestyle Centre, Inkosi Albert Luthuli Central Hospital where the following clinical measurements and biochemical profiles were obtained.

3.3.1 Clinical Measurements

Clinical measurements such as blood pressure, heart rate, waist circumference, height, weight and body mass index (BMI) were obtained as follows:

Blood pressure

Prior to the recording of blood pressure, participants were seated for at least five minutes. Fieldworkers used a mercury manometer connected to a standard 12.5 x 23 cm cuff with a mid-upper arm circumference below 33 cm and a larger cuff (15.5 x 32.5 cm) for those with a mid-upper arm circumference equal to and above 33 cm. The right arm was bare, unrestricted by clothing,

with the palm of the hand exposed upwards and the elbow flexed at the heart level. The inflatable rubber part of the cuff was placed over the inner upper-arm at least 2.5 cm above the cubital fossa. The blood pressure was taken on three occasions at 1-minute intervals. Both systolic and diastolic readings were recorded to the nearest mmHg. The mean diastolic with its matching systolic measurement was used for analyses.

Heart rate

The heart rate was obtained at the radial pulse for 15 seconds and the value multiplied by four to express the heart rate as beats per minute. Three readings were taken and the average of this was used for analyses.

Waist circumference

The waist circumference was measured with the participants standing comfortably. The smallest circumference located between the xiphisternum and the umbilicus during expiration was recorded as the waist circumference.

Height

The height of all participants was measured to the nearest 0.1 cm using a metal measuring tape that was attached to the wall and to a flat headboard at right angles to the wall in order to obtain correct readings. Participants were measured without shoes, with their heels against the wall and the angle of the eye at level with the external auditory meatus.

Weight

The weight of all participants was obtained using a balance scale set to the nearest 0.5 kg (standardized prior to study). Participants were in light clothing and no shoes during the recording.

Body mass index (BMI)

BMI of all participants was estimated as weight (kg)/ height $(m)^2$.

3.3.2 Biochemical Profile

Collection of whole blood samples

Blood samples for lipid and lipoprotein profiles were collected after an overnight fast whilst glucose and insulin estimations were taken at baseline, two hours after ingestion of 75 g glucose monohydrate dissolved in 250 ml water. Samples were transported within a maximum of one hour of bleeding to the Chemical Pathology Laboratory at Inkosi Albert Lutuli Central Hospital for the biochemical analysis.

Biochemical analysis

Total cholesterol, triglycerides and HDL-C were assayed from plasma by automated methods. The fasting blood glucose measurements were determined by spectrophotometry and plasma insulin by radioimmunoassay.

The measurements obtained from the PLP enabled us to diagnosis both IR and the MS within our cohort for this study as per the latest descriptors.

3.4. Insulin Resistance Diagnosis

Insulin resistance was measured using the Homeostatic model of Insulin Resistance (HOMA-IR) (Matthews *et al*., 1995) as follows:

$$\text{HOMA-IR} = \frac{\text{fasting insulin } (\mu u/ml) \times \text{fasting blood glucose } (mmol/l)}{22.5}$$

For diagnosis of IR, HOMA-IR values >2.6 were considered (Ascaso *et al*., 2003).

3.5 Diagnosing participants with the MS

The diagnosis of the MS in this population group as per biochemical and clinical measurements was performed using the following 3 definitions (Tables IV-VI):

- **NCEP ATP III (2001) Diagnosis**

TABLE IV: *NCEP ATP III (2001) DEFINITION FOR THE METABOLIC SYNDROME*

The NCEP ATP III (2001) cutoffs
Any 3 of the following 5 risk factors: • Central obesity: waist circumference≥102cm in males or ≥88cm in females • Triglycerides: ≥ 1.7 mmol/l • HDL-C: <1.03 mmol/l in males or <1.29mmol/l in females • Blood pressure: ≥ 130/85 mmHg • Fasting plasma glucose: ≥ 6.1 mmol/l

(Adapted from NCEP ATPIII, 2001)

- **IDF (2005) Diagnosis**

TABLE V: *IDF (2005) DEFINITION FOR THE METABOLIC SYNDROME*

The IDF (2005) cutoffs
Any 3 of the following 5 risk factors: • Central obesity: waist circumference (South Asians) ≥90cm in males or ≥80cm in females • Triglycerides: ≥1.7 mmol/l • HDL-C: <1.03 mmol/l in males or <1.29mmol/l in females • Blood pressure: ≥ 130/85 mmHg • Fasting plasma glucose: ≥ 5.6 mmol/l

(Adapted from Alberti et al., 2009)

- **Harmonized (2011) Diagnosis**

TABLE VI: *HARMONIZED (2011) DEFINITION FOR THE METABOLIC SYNDROME*

The Harmonized (2011) cutoffs
Any 3 of the following 5 risk factors: • Central obesity: waist circumference (Asians) ≥90cm in males or ≥80cm in females • Triglycerides: ≥ 1.7 mmol/l • HDL-C: <1.03 mmol/l in males or <1.29mmol/l in females • Blood pressure: ≥ 130/85 mmHg • Fasting plasma glucose: ≥ 6.1 mmol/l

(Adapted from Alberti et al., 2009)

3.6 Genotype Analysis

3.6.1 Collection of Samples

Samples were transported from the Lifestyle Centre, Chemical Pathology Laboratory at Inkosi Albert Lutuli Central Hospital (following biochemical analysis) to the Pfizer Molecular Biology Unit, Doris Duke Medical Research Institute (DDMRI), University of KwaZulu Natal, by road transportation in cold storage. The blood samples for the genetic analysis were then collected in

EDTA tubes and stored at -70 degrees until DNA extraction from whole blood occurred.

3.6.2 *DNA extraction from whole blood*

DNA was extracted from whole blood on the Magna Pure Instrument using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, South Africa). An amount of 200µl of whole blood was transferred to the sample cartridge and loaded onto the MagNA Pure LC workstation together with the necessary disposables and kit reagents. The MagNA Pure LC (Roche, South Africa) used magnetic bead technology and automatically performed the isolation and purification steps, binding of DNA, washing steps and elution of the nucleic acid (resulting in purified DNA) (Figure 10); and is described below:

- The samples were placed into the wells of the sample cartridge.
- To the samples, lysis or binding buffer was added, which contributed to cell lysis and the release of nucleic acids.
- Denaturing of the nucleases occurred and proteinase K was responsible for the digestion of proteins.
- The DNA attached to the silica surface of magnetic glass particles (MGPs) and was separated from the lysed sample.
- The DNA and MGPs were washed with the wash buffer to remove any unbound particles.
- The DNA and MGPs were magnetically separated from the wash buffer.
- The purified DNA was eluted at $+70^{0}$ C into the elution cartridge and the MGPs were transferred to reaction tips and discarded.

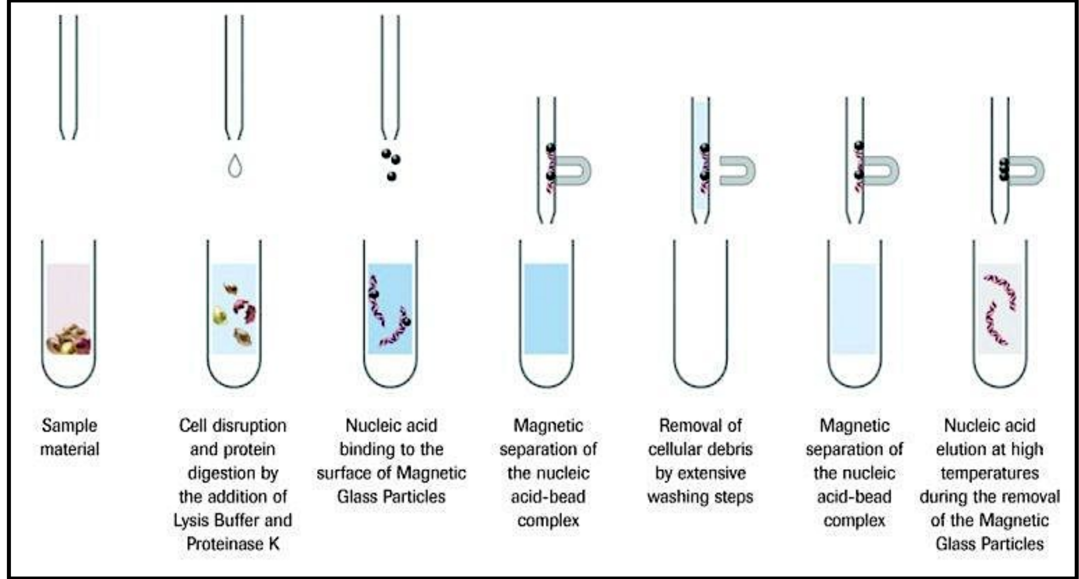


Figure 10*: The steps for DNA extraction using the MagNA Pure LC (Adapted from www.roche-applied-science.com)*

The nucleic acid was aliquoted and transferred to 1.5ml microtubes and stored at -70 ^{0} C until the quantification of DNA occurred.

3.6.3 Quantification of DNA

DNA concentrations were determined using the Nano-drop 1000 analyzer (Thermo Scientific). Once the DNA concentration was obtained, the DNA was diluted to 5ng/µL with diethylpyrocarbonate (DEPC) treated water and stored at -70^{0}C until the selection of single nucleotide polymorphisms (SNPs) and genotyping of these SNPs occurred.

3.6.4 Selection of candidate gene SNPs

Six candidate gene SNPs related to lipid and carbohydrate metabolism (apolipoprotein Q139X- rs121917821), insulin resistance (cholesterol ester

transfer protein Taq1B- rs708272; lipoprotein lipase Hinf I- rs328; paraoxonase1 192Arg/Gln- rs662) and obesity (leptin 25 CAG-rs104894023; adiponectin 45T>G- rs2241766) were selected by searching the single nucleotide polymorphism database (dbSNP) at NCBI *(http://www.ncbi.nlm.nih.gov/snp)*. All SNPs selected were assessed and based on their association with the MS and CVD. Support for this assessment came from the observed high frequencies for the MS and CVD within the South African Indian population (Ranjith *et al*., 2007).

3.6.5 Genotyping of SNPs

Genotyping of the SNPs was carried out using real time PCR (probe-specific) on the LightCycler 480 (Roche, South Africa). The probes had been shown to amplify the polymorphic site of the SNPs, thereby allowing for the identification of the different alleles (Cheng *et al*., 2004). Overall, this genotyping method was selected due to its association with precision, accuracy, speed and minimal risk for contamination during each PCR run (Reuter *et al*., 2005). The methodology for the genotyping is briefly discussed below:

- The mix for the PCR comprised a 13µL volume, which contained PCR grade water (Roche, South Africa), genotyping master mix (Roche, South Africa), forward and reverse primers, forward and reverse probes (designed by Roche, South Africa), which was pipetted into the 96 well plate (*The preparation for the PCR mixes is shown in Table VII and all primer and probe sequences are shown in Table VIII*).

TABLE VII: *PREPARATION OF PCR MIXES FOR STUDIED SNPs*

COMPONENT	**APOLIPOPROTEIN A5 Q139X** 1X RXN (µL)/Per well	**LIPOPROTEIN LIPASE HinfI** 1X RXN (µL)/Per well	**LEPTIN 25CAG** 1X RXN (µL)/Per well	**CETP Taq1B** 1X RXN (µL)/Per well	**PARAOXONASE 1 192Arg/Gln** 1X RXN (µL)/Per well	**ADIPONECTIN 45T>G** 1X RXN (µL)/Per well
PCR GRADE WATER	4.5	4.5	3.9	3.9	5	4.5
PROBE+PRIMER MIX	1.5	1.5	1.5	1.5	1	1.5
MASTER MIX	2	2	2	2	2	2
$MgCl_2$	0	0	0.6	0.6	0	0
DNA TEMPLATE	5	5	5	5	5	5
TOTAL VOLUME	**13**	**13**	**13**	**13**	**13**	**13**

Key: **CETP-** cholesteryl ester transfer protein

** The measurements per SNP varied as shown above. The measurements above produced the best melting curves and therefore best results*

** Probes were prepared in 20pmol/µl concentrations*

** Primers were prepared in 20pmol/µl concentrations*

TABLE VIII: *PRIMER AND PROBE SEQUENCES FOR STUDIED GENE POLYMORPHISMS*

GENE SNPs	**PRIMER SEQUENCE (5^{l} - 3^{l})**	**PROBE SEQUENCE (5^{l} - 3^{l})**
Apolipoprotein A5 *Q139X*	F: AgCCCTACATggCAgAg R: TgggCCTTggTgTCTTC	P1: LC640-CCTACTCCATCAgATCCATCgTgTAgg--PH P2: TCCTgCACgCgCAgggC--FL
CETP *Taq1B*	F: TggTgAgAAggTCCTAgC R: CCAAATATACACCAACCTCCTAAT	P1: CCCAgAATCACTggggTTCAAgTT--FL P2: LC640-ggTTCAgATCTgAgCCAggTTAgggg--PH
Lipoprotein lipase *Hinf I*	F: TTCTgTTCTAgggAgAAAgTgT R: CATgAAgCTgCCTCCCTTA	P1: LC640-ATTCAgAgACTTgTCATggCATTTCACAAATACCg--PH P2: AATgCTCACCAgCCTCACTTC--FL
Paraoxonase 1 ^{192}*Arg/Gln*	F: TATTgTTgCTgTgggACCT R: ACATACTTgCCATCggg	P1: LC640-CCCAAATACATCTCCCAggATCgTAAgTA--PH P2: CTTggACTATAgTAgACAACATACgACCACgCTA--FL
Leptin *25 CAG*	F: TTGTGGCTTTGGCCCTA R: GCTGGCTGCAGTTCTAC	P1: LC640-TTGGTGTCATCTTGGACTTTCTGGA--PH P2: GATCCTGGTGACAATTGTCTTGATGAGGG--FL
Adiponectin *45T>G*	F: gCTgggAgCTgTTCTAC R: gCCATCTCTgCCATCAC	P1: LC640-ggTTTCCTggTCATgCCg--PH P2: AggACTCCgggCCCTTgAgTC--FL

Key: **SNPs**- single nucleotide polymorphisms; **F**- forward primers; **R**- reverse primers;
P1- probe 1; **P2**- probe 2; **CETP-** cholesteryl ester transfer protein

This was followed by the addition of 5 μl of genomic DNA template to the PCR reaction plate.

- Two negative controls were used for each run, where the DNA template was replaced with PCR grade water in order to detect contamination (if any).
- The plate was thereafter sealed, centrifuged and transferred to the Lightcycler 480, and the real time PCR was initiated.
- After initiation, each PCR cycle was subjected to three temperature steps in order to facilitate amplification viz.
  - ✓ denaturation (95^0C for 10 seconds),
  - ✓ annealing (55^0C for 10 seconds), and
  - ✓ extension/elongation (72^0C for 10 seconds)
- On completion of the amplification, a melting curve step was observed by cooling (45^0C) and reheating (95^0C) the PCR mix
- Final cooling thereafter occurred for each PCR mix (40^0C for 30 seconds).

Each PCR run took 75 minutes.

3.6.6 Principle of melting curve analysis

The melting curve analysis is based on the principle that heat is required to dissociate base-base hydrogen bonds found between double stranded DNA (Ririe *et al*., 1997). There are three hydrogen bonds between guanine (G) and cytosine (C) (also known as GC content), whilst adenine (A) bonds specifically with thymine (T) by two hydrogen bonds. DNA with a higher GC content is presumed more stable than DNA with low GC content. Hence, a higher GC content will produce a higher melting temperature (Yakovchuk *et*

al., 2006). Melting points are reached when 50% of DNA is denatured by fluorescence measurements occurring mainly due to DNA-intercalating fluorophore (such as SYBR Green, EvaGreen and or probes [utilized in this study]) (Ririe *et al*., 1997).

This principle allows for the detection of the presence of SNPs by virtue of dissociation patterns that are illustrated as peaks. These patterns present as single early peaks (wild type homozygotes), single late peaks (mutant homozygotes) and or two distinct peaks (heterozygotes) as illustrated in Figure 11. The peaks correlate with the temperature where the corresponding probes melt away. Table IX demonstrates the melting curve temperatures for the determination of genotypes in this study.

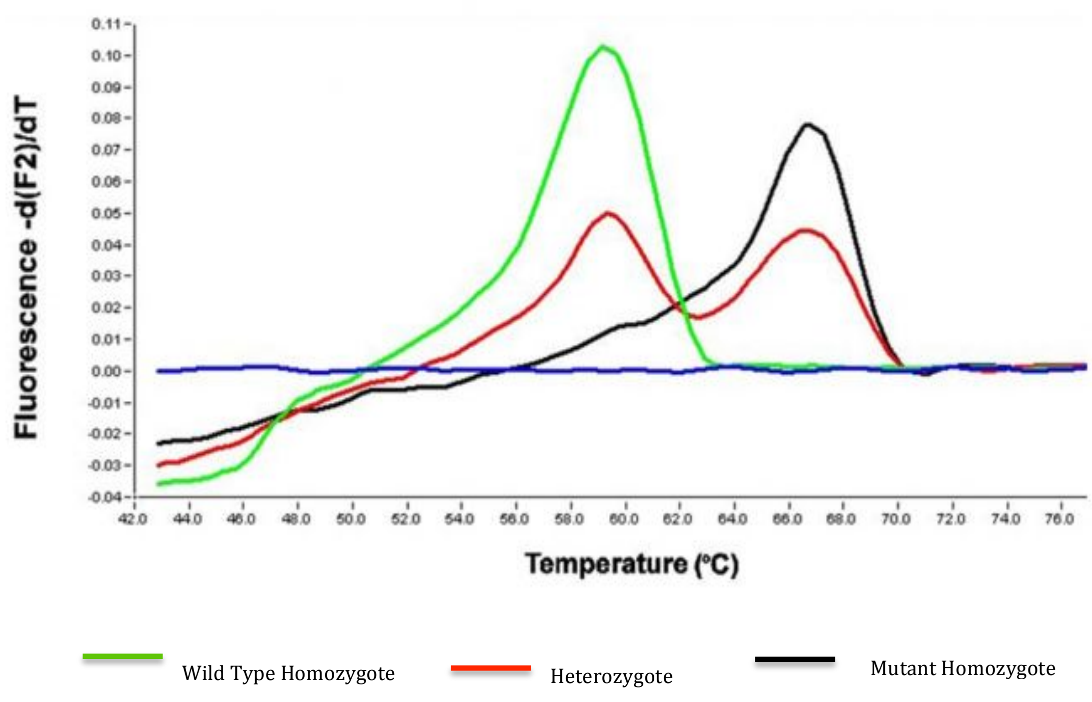


***Figure 11**: Illustration of the genotypes from a random PCR product determined from 3 melting points*

(Adapted from http://www.dkfz.de/en/tox/lung_cancer_genomics_epigenomics-neu.html)

TABLE IX: *MELTING CURVE TEMPERATURES FOR DETERMINATION OF GENOTYPES FOR THE STUDIED GENE POLYMORPHISMS*

COMPONENT	**APOLIPOPROTEIN A5 Q139X**	**LIPOPROTEIN LIPASE HinfI**	**LEPTIN 25CAG**	**CETP Taq1B**	**PARAOXONASE 1 192Arg/Gln**	**ADIPONECTIN 45T>G**
Amplicon Length (bp)	160	164	158	157	198	172
GC (%)	63.1	48.8	47.5	48.4	42.4	65.1
Mutation Position	1607	3694	116	454	2189	3080
Type of Mutation	C to T	C to G	A to G	G to A	A to G	T to G
Mutation Tm (^{0}C)	***62.2***	***58.7***	***59.2***	***59.9***	***59.0***	***55.4***
Wild Type Tm (^{0}C)	***65.6***	***65.1***	***64.9***	***65.0***	***64.7***	***64.9***

Key: **CETP-** cholesteryl ester transfer protein; **Tm**- temperature; **bp**- base pairs; **GC**- percentage of nitrogenous bases on a DNA molecule

**Non-shaded columns and rows provide additional information for the studied polymorphisms. Shaded columns and rows (in bold and italics) depict melting curve temperatures for the studied polymorphisms*

3.6.7 *Quality control of PCR products*

Purification of PCR products

The integrity of the PCR products was checked in 5% of randomly selected samples. The PCR products were purified using the Illustra GFX PCR DNA and gel band purification kit (GE Healthcare), which is described below:

- 500µl of capture buffer type 3 was added to the PCR product and was mixed thoroughly.
- The capture buffer type 3 sample mix was added to the GFX Microspin column and collecting tube which was spun for 30 seconds at 16000xg. The flow was discarded.
- The GFX Microspin column was placed inside the same collecting tube and 500µl wash buffer type 1 was added and spun for another 30 seconds at 16000xg. The collection tube was discarded.
- The GFX Microspin column was placed in a clean 1.5ml DNase-free microcentrifuging tube and 20µl of elution buffer type 6 was added and stood at room temperature for 60 seconds and thereafter spun for 60 seconds at 16000xg.
- The purified DNA was retained and stored at -20^0 C.

Purified PCR products were run on a 1.5% agarose gel.

<u>*Gel electrophoresis*</u>

The procedure for gel electrophoresis is briefly discussed below:

- The agarose gel was placed in an electrical gel electrophoresis apparatus containing 10x TBE buffer (Sigma, Life Science).
- The purified PCR samples and a DNA ladder were loaded on the gel and the electrical gel electrophoresis apparatus was set at 100 V, AMP 500 for 45 minutes.
- After electrophoresis, the gel was viewed under ultraviolet light using the GelVue UV Transilluminator (SynGene, London) and the images were captured and analyzed.
- Analysis of the migration of the DNA on the electrophoresis gel allowed for estimation of the base pairs/size of the DNA molecules (in accordance with the *DNA ladder massruler #R0491*) (Figure 12).

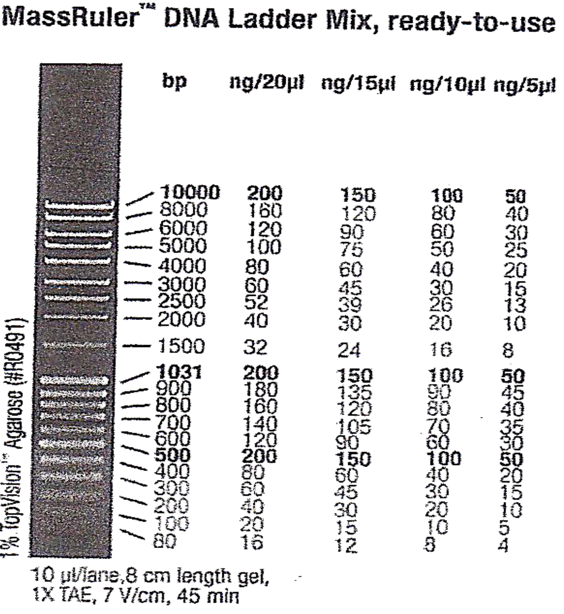


Figure 12*: Illustration of the DNA mass ladder*

(Adapted from www.thermoscientificbio.com)

The purified PCR product was diluted to 2ng/µL with DNASE/RNASE-Free water (Bioline), in accordance with the concentration levels as per DNA ladder massruler and stored at -20^0 C until the sequencing reaction occurred.

3.6.8 Sequencing reaction preparation

- The sequencing reaction mix comprised a 10µL volume for each SNP studied. The mix contained 0.4µL Big Dye v 3.1 Ready reaction mix (Applied Biosytems, South Africa), 2µL 5x sequencing buffer (Applied Biosytems, South Africa), 1µL primer (Roche, South Africa), 1.6µL PCR grade water (Roche, South Africa) and 5µL of the diluted PCR product (randomly selected per SNP). The mix was centrifuged and aliquoted into the 96 well plate and placed into the thermocycler (Perkinelmer 9700).
- The sequencing reaction according to the no of cycles, temperature and time was as follows:
  - ✓ cycle 1 (96^0C for 1 minute; 96^0C for 10 seconds), and
  - ✓ cycle 35 (50^0C for 5 seconds; 60^0C for 4 minutes; 4^0C and hold)

The sequencing products were then stored at 4^0C in the dark, which was followed by purification of the sequencing products.

<u>*Purification of sequencing products*</u>

Purification of the sequencing products occurred as follows:

- 1µl of 125mM EDTA pH 8.0 was added to each well of the sequencing plate and mixed thoroughly using the pipette tips.

- 26µl of combined 3M NaOAc (pH 5.2) and 100% ethanol was added to each well of the sequencing plate and mixed thoroughly using the pipette tips.
- Sealing tape was placed on the sequencing plate, sealed, vortexed lightly and centrifuged at 3000xg for 20 minutes.
- The sequencing plate was inverted on paper towel and centrifuged again for five minutes at 150xg. Immediately, 35µl of 70% cold ethanol was added to each well of the sequencing plate and centrifuged for another 5 minutes at 3000xg.
- The sequencing plate was inverted again on the paper towel and centrifuged for a further one minute at 150xg to dry.
- The selected samples were dried in the thermocycler (Perkinelmer 9700) for five minutes at 50ºC.
- The samples were sequenced using the Applied Biosystem 3130XL genetic analyzer (Life Technologies).
- Sequences were analyzed using sequencher 4.10.1 software package (Gene Codes).

3.7 Statistical analysis

The statistical analysis was performed using the statistical software program SPSS 17.0 for all epidemiological and genetic analysis viz:

Epidemiology

Categorical data was summarized as a percentage of the group total with corresponding means and standard deviations (where relevant). Box plots were used to determine significant relations between groups, in relation to age (where relevant), and means±SD for anthropometric, physiological and biochemical parameters of the sample were calculated by the Wilcoxon rank sum and bonferroni tests.

Participants were classified into those with and without MS based on the NCEP ATPIII (2001), IDF (2005) and Harmonized (2011) criteria. Their categorical data were summarized as a percentage of the group total with corresponding 95% confidence intervals (CI). Statistical differences between gender groups, in terms of the components of the MS associated with the latter definitions were calculated using the chi-square ($\chi 2$) and bonferroni tests. Similarly, IR between groups was assessed by the $\chi 2$ test and bonferroni tests. Receiver operator curves (ROC) were produced to demonstrate associations between clustering of the MS components with IR and the area under the curve (AUC) determined the best predictor for IR in the sample.

Genetics

Power calculations for single nucleotide polymorphisms

We detected a minimum f of 0.1 with a sample size of ˜1000 participants with 5% significance and 90% power based on the formula below:

Power for Testing HWE

$$X^2 = \sum_{genotypes} \frac{(O - E)^2}{E} = n\hat{f}^2$$

where

$$\hat{f} = 1 - \frac{2n \times n_{Aa}}{n_A n_a}$$

f is also the "inbreeding coefficient" of the population (more later).

When Hardy-Weinberg equilibrium (HWE) holds, x^2 has a chi-square distribution with 1 df. When HWE does not hold, x^2 has a non-central chi-square distribution with non-centrality parameter nf^2. The cut-off for significance at the 5% level of chi-square with 1 df is 3.84. That is, our p value will be less than 0.05 if we observe a test statistic greater than 3.84. In order to be at least 90% sure of rejecting HWE when HWE is false, the non-centrality parameter should be at least 10.51 based on the formula below:

Power for Testing HWE

$$nf^2 \geq 10.51$$

$$n \geq \frac{10.51}{f^2}$$

If f=0.01, then n has to be over 100,000.

Once we obtained the power calculations the genetic analysis was performed. The frequencies for the studied polymorphisms were calculated by gene counting, subsequently allowing for HWE to be determined using the $\chi 2$ and bonferroni tests. The parametric variables, expressed as mean±SD, associating the MS components with the studied polymorphisms in the sample were calculated by the ANOVA and bonferroni tests. Associations were constructed between genotypes in participants diagnosed with and with no MS (as per IDF and harmonized definitions), by using the $\chi 2$ and bonferroni tests. Median values for the components of the MS were compared between three groups i.e. homozygous wild type, mutant homozygous and heterozygous (in those with and with no MS) by one-way analysis of variant testing and or Kruskal Wallis and bonferroni testing. Finally, IR frequencies (by HOMA model) of polymorphisms between gender in those with and with no MS were calculated by the $\chi 2$ test and bonferroni; and median values for the components of the MS in IR participants with and with no MS were calculated by the ANOVA and bonferroni tests. A p value <0.05 was considered statistically significant.

3.8 Ethical considerations

The applicant in this study obtained full ethics approval (Ethical Clearance Reference: BE232/010) from the Bioethics Committee, University of KwaZulu Natal, for the genetic study. Ethics recertification for the Phoenix Lifestyle Project (Ethical Clearance Reference: E336/05) was obtained and granted by the Bioethics Committee, University of KwaZulu Natal.

CHAPTER 4

RESULTS

(EPIDEMIOLOGY)

Chapter 4 : Results for Epidemiological studies

In this chapter the epidemiological results are provided. These include, the demographics and the prevalence of the metabolic risk parameters including anthropometric parameters, physiological measurements and biochemical parameters (blood glucose, insulin and lipid parameters) in the sample. The prevalence of the MS, as defined by the latest descriptors and IR as per HOMA model, including clustering patterns of the metabolic risk factors associated with the MS and IR are also reported in this chapter.

4.1 The demographics of the sample

This study comprised 999 randomly selected South African Indians from Phoenix, KwaZulu Natal. The mean age for the total sample was 45.4±13.1. More females (75%) than males (25%) were present in this sample, with mean ages of 46.0±12.3 and 43.4 ±15.2 respectively. A greater proportion of subjects were in the 50-59 year age category (Table X).

TABLE X: DEMOGRAPHICS OF SOUTH AFRICAN INDIANS BY GENDER

AGE GROUPS	n (%)	FEMALES (n %)	MALES (n %)	P-VALUE	
				∞	‡
≤19 20-29 30-39 40-49 50-59 >60	60 (6.0) 80 (8.0) 148 (14.8) 266 (26.6) 330 (33.0) 115 (11.5)	33 (4.4) 52 (6.9) 112 (15.0) 214 (28.6) 256 (34.2) 82 (11.0)	27 (10.8) 28 (11.2) 36 (14.4) 52 (20.8) 74 (29.6) 33 (13.2)	***<0.001** [i]	**<0.001** [iii]
TOTAL	999	749 (75.0)	250 (25.0)		
MEAN AGE	45.4±13.1	46.0 ±12.3	43.4±15.2	***0.005** [ii]	0.007 [iii]

∞- unadjusted p value; ‡- adjusted p value; i: Chi-squared (χ^2) test; ii: t-test; iii: Bonferroni test; * Best p value chosen from different analysis

(Data are presented as prevalence rates with percentages in parenthesis.Variables were considered statistically significant if p<0.05- in bold)

(Highly significant differences between females and males in terms of their age profiles i.e. females were older of average compared to males)

4.2 The prevalence of metabolic risk factors for the sample

One hundred and eight subjects (10.8 %) in this randomly selected sample did not exhibit any of the five common metabolic risk factors (increased waist circumference, increased blood glucose and triglycerides, reduced HDL-C and increased blood pressure) (Table XI). The remaining 891 (89.2%) subjects presented with one or more metabolic risk factors. A higher proportion of females (92.5%) presented with at least one metabolic risk factor when compared to males (79.2%) (Table XI).

TABLE XI: *DISTRIBUTION OF METABOLIC RISK FACTORS BY GENDER*

METABOLIC RISK FACTORS	n (%)	FEMALES (n %)	MALES (n %)	P-VALUE	
				∞	‡
0	108 (10.8)	56 (7.5)	52 (20.8)	**<0.001***	**<0.001**
1	200 (20.0)	132 (17.6)	68 (27.2)		
2	248 (24.8)	201 (26.8)	47 (18.8)		
3	236 (23.6)	182 (24.3)	54 (21.6)		
4	155 (15.5)	130 (17.4)	25 (10)		
5	52 (5.2)	48 (6.4)	4 (1.6)		
TOTAL	999	749	250		
WITH METABOLIC RISK FACTORS	891 (89.2)	693 (92.5)	198 (79.2)	**<0.001***	**<0.001**

∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as prevalence rates with percentages in parenthesis. ∞ Comparison between groups was done using the Chi-squared ($\chi 2$) test. ‡ Comparison between groups was done using the Bonferroni test. Variables were considered statistically significant if p<0.05- in bold)

(Significant differences between females and males by number of risk factors and overall by gender for presence of any risk factor)

There was a significant relationship between the metabolic risk factors and age among females and males in the cohort (p<0.001). In the <19, 20-29 and 30-39 age groups, there was variation in the median distribution of the metabolic risk factors for both males and females, whilst in the 40-49, 50-59 and 60+ age category there was no variation in the median for the metabolic risk factors (Figure 13), suggesting that the metabolic risk factors manifests in the early age categories (≤19, 20-29 and 30-39) and not in the older age groups (40-49, 50-59 and +60).

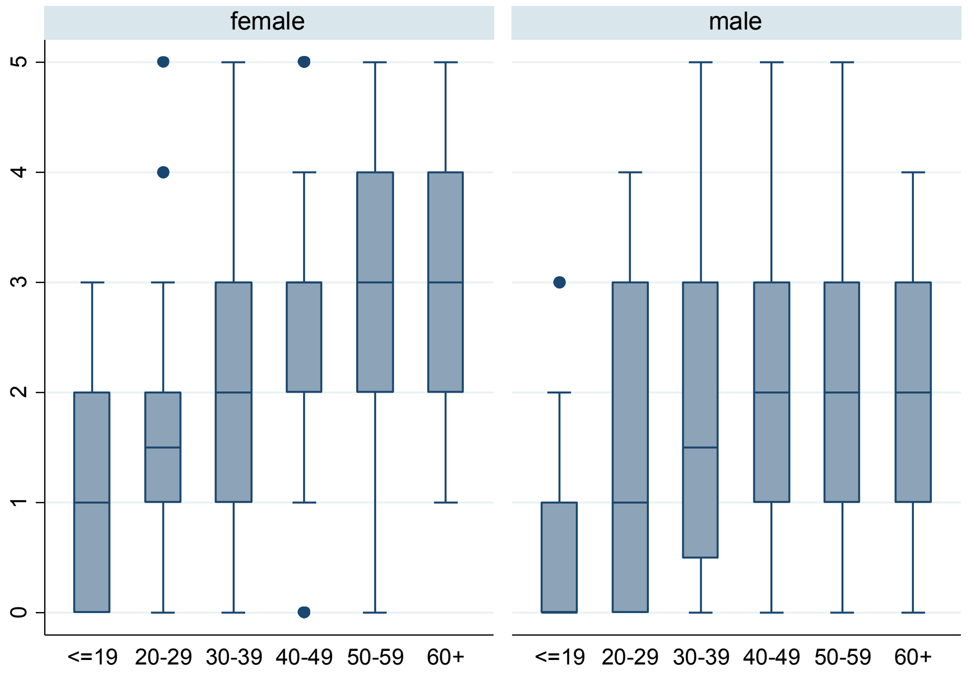


Figure 13*: Box plot representation showing a significant trend for the metabolic risk factors (vertical axis) with increasing age (horizontal axis) (p<0.001)*

(Each box represents values between the 25th and 75th percentiles with the bold horizontal line being the median value. The whiskers stretch from the 10th to 90th percentiles. Circles illustrate the outliers value)

4.3 Metabolic risk parameters of the sample

4.3.1 Anthropometric parameters of sample

The mean measurement for waist circumference in the sample was 95.5cm (94.6, 96.5). Females presented with a significantly larger waist circumference of 97.3cm (96.2, 98.4) than males 90.2cm (88.4, 92.0) ($p<0.001$) (Table XII).

4.3.2 Physiological measurement of sample

The mean systolic blood pressure was slightly higher in males [129.1mmHg (126.3, 131.9)] than in females [128.4mmHg (126.7, 130.2)], whilst the mean diastolic readings were slightly elevated in females [80.8mmHg (79.9, 81.7)]

than males [80.1mmHg (78.5, 81.7)]. Overall no significant differences were observed for systolic ($p=0.653$) and diastolic ($p=0.419$) blood pressure between gender groups (Table XII).

4.3.3 Biochemical Parameters in Sample

Blood Glucose and Insulin

The biochemical parameters were elevated across the sample. The mean fasting blood glucose in the female group was 6.2mmol/l (6.0, 6.4) and in males, a mean of 5.9mmol/l (5.6, 6.2) was observed, with no significant differences ($p=0.102$). Also, the mean serum insulin levels were significantly higher in females [16.5 μu/ml (15.4, 17.5) vs 12.2 μu/ml (10.8, 13.6)] than males, and the mean HOMA IR values were significantly higher in females than males [4.8 (4.4, 5.1) vs 3.5 (2.9, 4.1) $p<0.001$] (Table XII).

Lipid Parameters

Lipid profiles were also elevated in the sample with triglyceride levels being higher in males than females, but no significance was observed [1.9mmol/l (1.7, 2.0) vs 1.8mmol/l (1.7, 1.9) $p=0.179$]. Interestingly, HDL-C levels were significantly higher in females than males [1.5mmol/l (1.3, 1.7) vs 1.2mmol/l (1.1, 1.3) $p<0.001$] (Table XII).

Overall, these findings suggest that mostly high levels of cardiovascular risk were present in the sample (with the exception of HDL-C levels), and age clearly influenced the overall anthropometric, physiological and biochemical parameters of the sample (Figures 14 A-G).

TABLE XII: *PARAMETERS FOR ANTHROPOMETRIC, PHYSIOLOGICAL AND BIOCHEMICAL IN SOUTH AFRICAN INDIANS: (95% CI)*

PARAMETERS	TOTAL MEAN (95%CI)	FEMALES MEAN (95%CI)	MALES MEAN (95%CI)	P-VALUE	
				∞	‡
Waist circumference (cm)	95.5 (94.6, 96.5)	97.3 (96.2, 98.4)	90.2 (88.4, 92.0)	**<0.001***	**<0.001**
Systolic blood pressure (mmHg)	128.6 (127.1, 130.1)	128.4 (126.7, 130.2)	129.1 (126.3, 131.9)	0.653	0.653
Diastolic blood pressure (mmHg)	80.6 (79.8, 81.4)	80.8 (79.9, 81.7)	80.1 (78.5, 81.7)	0.419	0.501
Fasting blood glucose (mmol/l)	6.2 (6.0, 6.4)	6.2 (6.0, 6.4)	5.9 (5.6, 6.2)	0.102	0.119
Serum insulin (μu/ml)	15.4 (14.6, 16.3)	16.5 (15.4, 17.5)	12.2 (10.8, 13.6)	**<0.001***	**<0.001**
HOMA-IR	4.7 (4.1, 5.30)	4.8 (4.4, 5.1)	3.5 (2.9, 4.1)	**<0.001***	**<0.001**
Triglycerides (mmol/l)	1.8 (1.7, 1.9)	1.8 (1.7, 1.9)	1.9 (1.7, 2.0)	0.179	0.199
HDL-C (mmol/l)	1.4 (1.3, 1.6)	1.5 (1.3, 1.7)	1.2 (1.1, 1.3)	**<0.001***	**<0.001**

Key: **HDL-C**- high-density lipoprotein lipase cholesterol; **CI**- confidence interval; ∞- unadjusted p value; ‡- adjusted p value; * best p values chosen from different analysis

(Data are presented as mean values with 95% CI. ∞ Comparison between gender groups was done using the Wilcoxon rank-sum test. ‡ Comparison between gender groups was done using the Bonferroni test Variables were considered statistically significant if p<0.05- in bold)

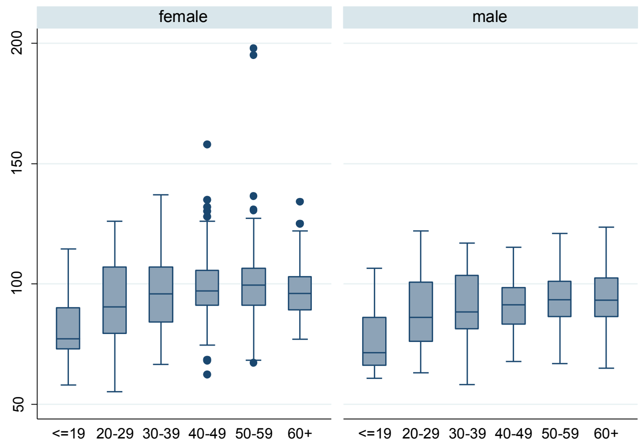


Figure 14a: *Box plot representation showing a significant trend for the waist circumference (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females with a larger waist circumference was in the 50-59 year category; whilst in males it was in the >50 year groups

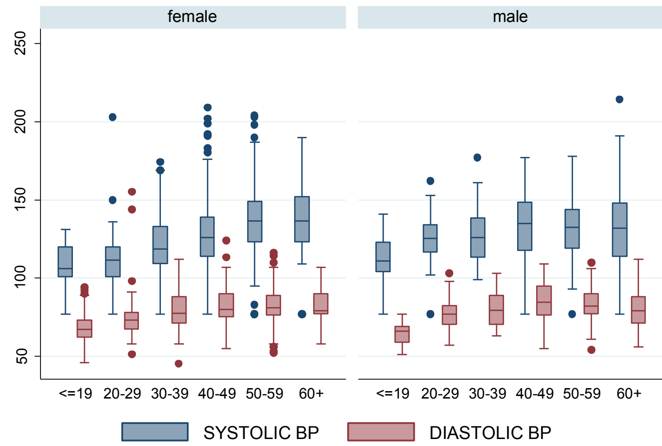


Figure 14b: *Box plot representation showing a significant trend for the blood pressure (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females with elevated systolic and diastolic blood pressure was in the >50 year groups and 50-59 year category, respectively; whilst in males it was in the 40-49 year category for both systolic and diastolic blood pressure

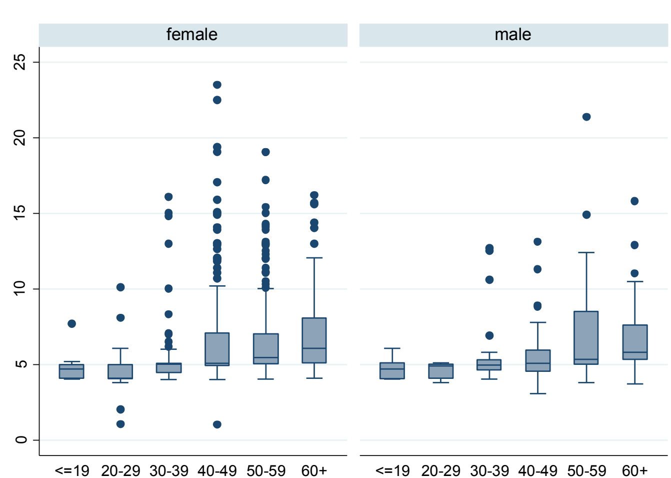


Figure 14c: *Box plot representation showing a significant trend for fasting blood glucose (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females and males with elevated fasting blood glucose was in the 60+ age category

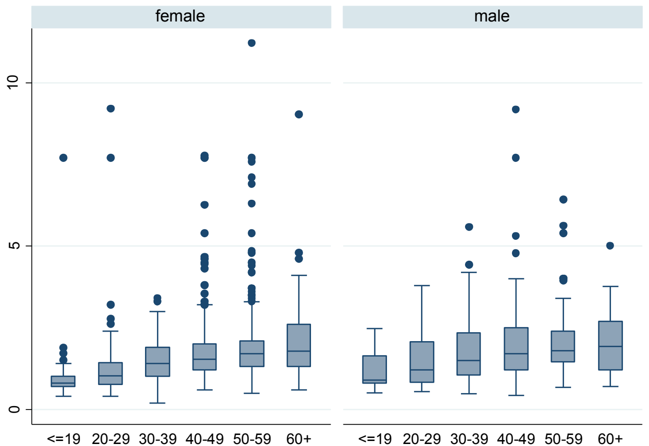


Figure 14d: *Box plot representation showing a significant trend for serum insulin (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females and males with elevated serum insulin was in the 60+ age category

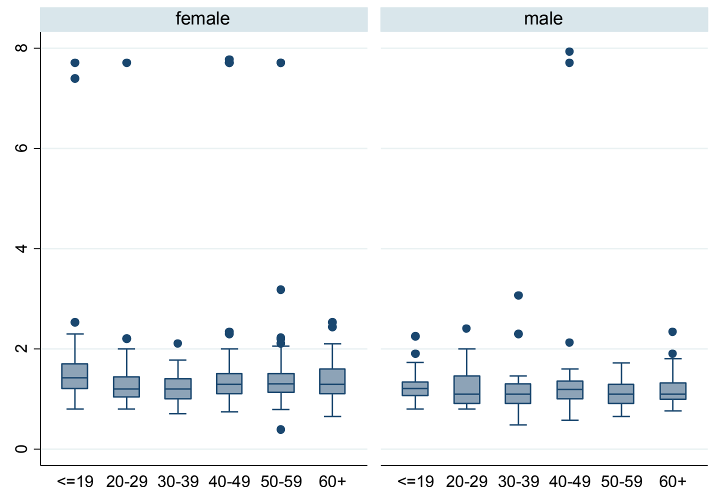


Figure 14e: *Box plot representation showing a significant trend for the HOMA IR (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females with increased HOMA IR was in the <19 age category; whilst in males it was in the <19 and 40-49 age group

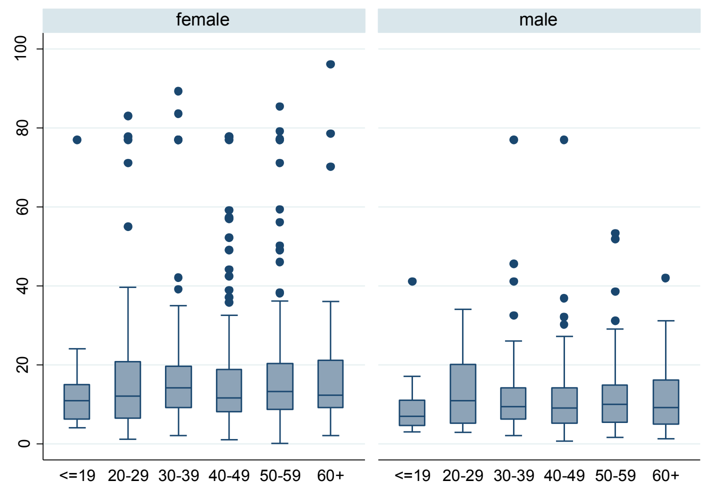


Figure 14f: *Box plot representation showing a significant trend for the triglycerides (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females with elevated triglyceride levels were in the 30-39 age category; whilst in males it was in the 20-29 age group

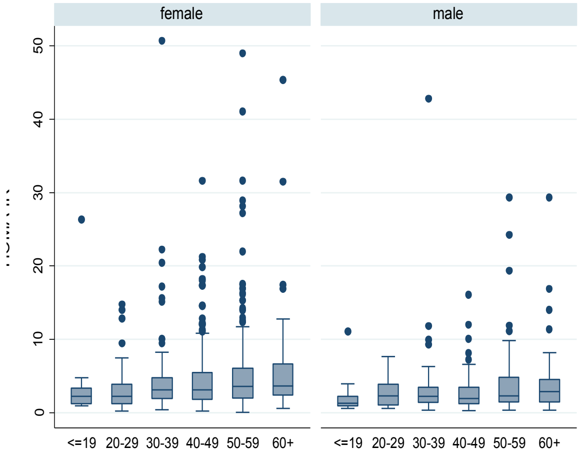


Figure 14g: *Box plot representation showing a significant trend for the HDL-C (vertical axis) with increasing age (horizontal axis) by gender*
The highest proportion of females with elevated HDL-C levels was in the >50 year group; whilst in males it was in the 60+ age category

Figure 14 A-G: Each box represents values between the 25th and 75th percentiles with the bold horizontal line being the median value. The whiskers stretch from the 10th to 90th percentiles. Circles illustrate the outliers value

4.4 The metabolic syndrome in the sample

4.4.1 The prevalence of the metabolic syndrome as defined by the latest descriptors

The prevalence of the MS was 44.3% (95%CI: 41.2-47.5) using the NCEP ATPIII definition, and increased to 51.6% (95%CI: 48.4-54.7) when the IDF criteria were used. The more recent harmonized criteria revealed a prevalence of 49.0% (95%CI: 45.8-52.1). In addition, a slightly higher prevalence of the MS was present in females than males using the NCEP ATPIII (48.1% vs 33.2%), IDF (53.7% vs 45.2%) and harmonized (51.0% vs 42.8%) descriptors (Table XIII).

TABLE XIII: *PREVALENCE OF THE METABOLIC SYNDROME (AS DETERMINED BY THE NCEP ATPIII, IDF, HARMONIZED CRITERIA) BY GENDER*

VARIABLES	NCEP ATP111 (2001)	IDF (2005)	HARMONIZED (2011)
Females n (%)	360/749 (48.1%)	402/749 (53.7%)	382/749 (51.0%)
Males n (%)	83/250 (33.2%)	113/250 (45.2%)	107/250 (42.8%)
Prevalence n (%)	**443/999 (44.3%) (95% CI: 41.2-47.5)**	**515/999 (51.6%) (95% CI: 48.4-54.7)**	**489/999 (49.0%) (95% CI: 45.8- 52.1)**

(Data are presented as prevalence rates with percentages in parenthesis. The overall prevalence highlights the confidence intervals at 95%)

(A higher prevalence of the MS was observed when the IDF (2005) and harmonized (2011) definitions were applied)

The prevalence of the MS further corresponded with advancing age (Figure 15) and females showed a linear increase when the NCEP ATPIII, IDF and harmonized definition was used. However, males showed a linear increase up to the 50-59 year category, followed by a decrease in the prevalence in the 60+ age group when the IDF and harmonized criteria was used. Only the NCEP definition in males showed variations in the prevalence with advancing age.

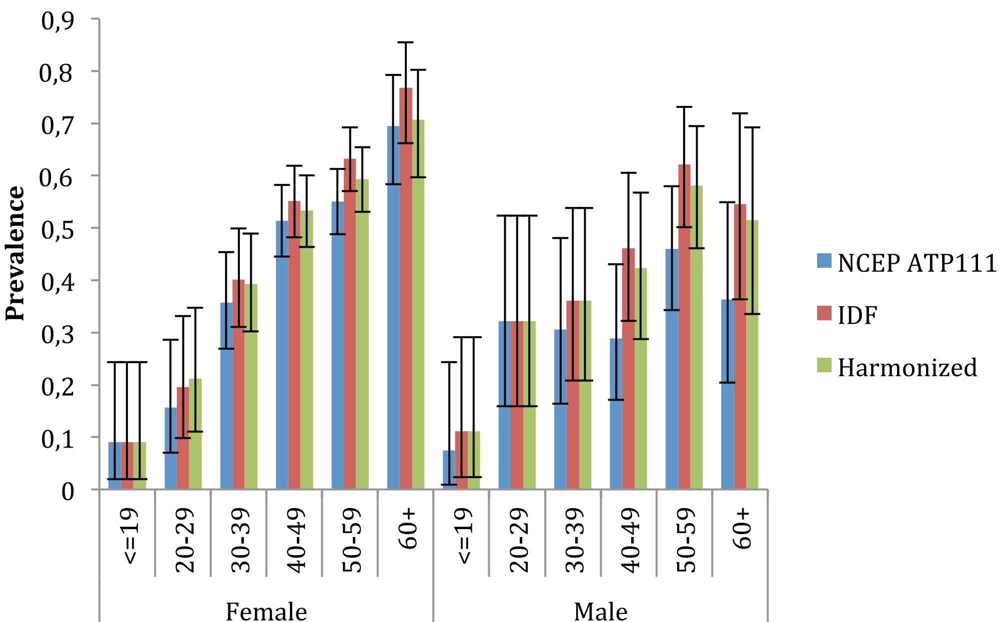


Figure 15*: Prevalence of the MS, as per NCEP ATPIII/IDF/HARMONIZED CRITERIA according to age and gender*

(Age influenced the prevalence of the MS among females and males)

4.4.2 Clustering of the MS componenets sing the NCEP/IDF and harmonized cutoffs in relation to gender and age

Clustering of the components of the MS associated with age and with the latest ethnic specific cutoffs (NCEP ATPIII, IDF and harmonized) is illustrated in Figure 16. We observed that clustering of the components of the

MS (as per NCEP ATPIII, IDF and harmonized cutoffs) in the ≤19 age category for both females and males was relatively low, whilst in the in the 50-59 and 60+ age groups higher frequencies was present. Overall, clustering of the components of MS in females and males was influenced by the definition used.

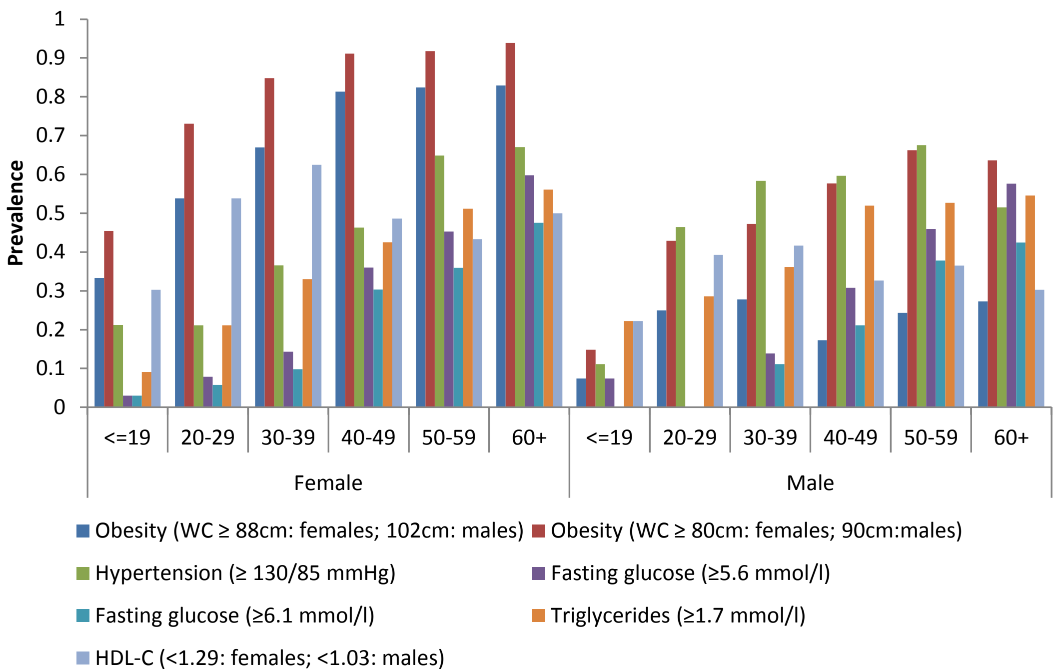


Figure 16*: Clustering of the MS components, as per NCEP ATPIII, IDF and harmonized criteria according to age and gender*

(Age influenced the components and or clustering of the MS among females and males, irrespective of the cutoffs used for diagnosis)

4.4.3 The prevalence of the metabolic components in participants diagnosed with and without metabolic syndrome

The most prevalent variables contributing to the MS using the NCEP ATPIII definition was an increased waist circumference (females: ≥88cm; males: ≥102cm) in 77.1% of subjects, followed by elevated triglycerides (≥1.7mmol/l) in 71.1% of the sample. When the IDF definition was used, mostly all subjects (95.0%) demonstrated an increased waist circumference (females: ≥80cm; males: ≥90cm), followed by elevated triglycerides (≥1.7mmol/l) in 71.1% of the sample. The harmonized definition when applied, showed an increased waist circumference (females: ≥80cm; males: ≥90cm) in 95% of the sample and elevated triglycerides (≥1.7mmol/l) in 71.1%. Less frequent components of the MS, namely reduced HDL-C, elevated blood pressure and elevated blood glucose, were observed when all three definitions (NCEP ATPIII, IDF and harmonized) were used. Table XIV demonstrates the above findings.

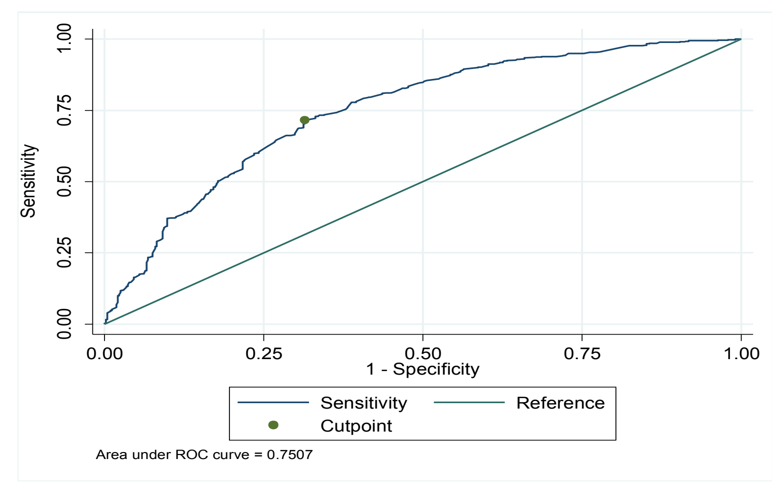


Figure 19a: *ROC curve for the detection of IR using waist circumference*

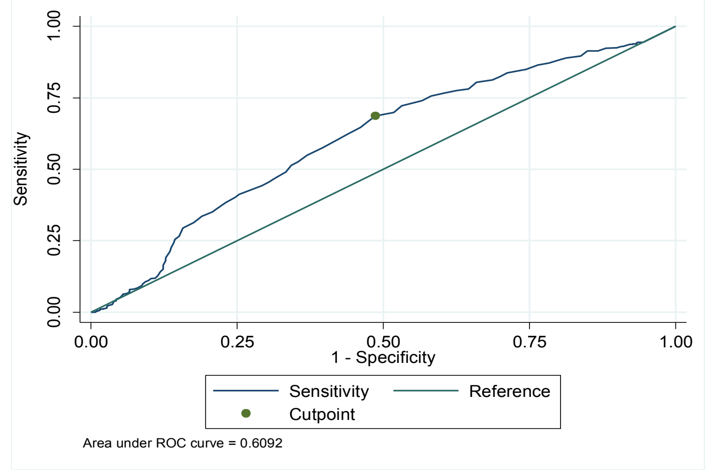


Figure 19b: *ROC curve for the detection of IR using systolic blood pressure*

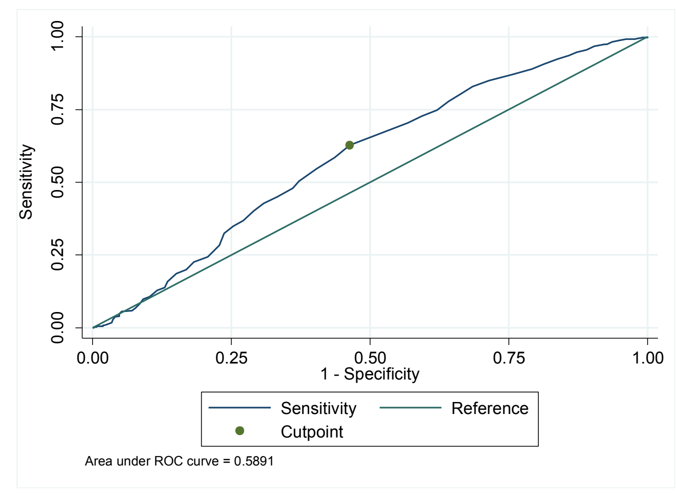


Figure 19c: *ROC curve for the detection of IR using diastolic blood pressure*

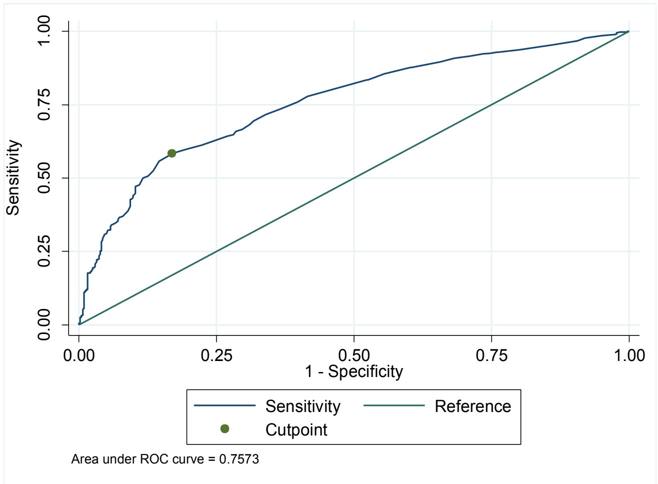


Figure 19d: *ROC curve for the detection of IR using blood glucose*

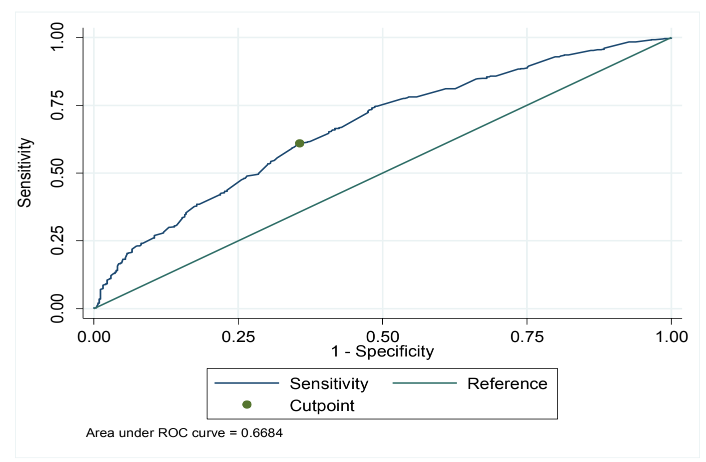


Figure 19e: *ROC curve for the detection of IR using triglycerides*

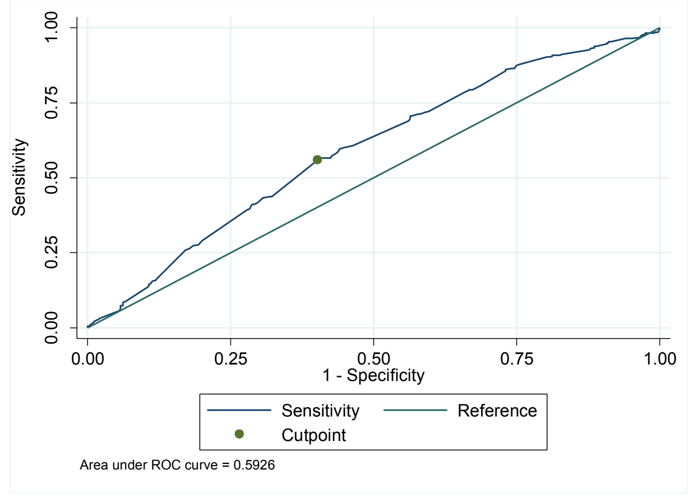


Figure 19f: *ROC curve for the detection of IR using HDL-C*

Application of the latest descriptors i.e. *IDF and harmonized definitions* produced the greatest number of subjects with the MS in this cohort. We therefore present subsequent analysis using the *IDF and harmonized definitions*, due to the greater number of clustering of metabolic risk factors, thereby contributing to a higher risk for CVD.

4.5 Prevalence of insulin resistance as determined by the HOMA model

The prevalence of insulin resistance (IR) was considerably high (IDF: 76.5%; harmonized: 76.3%) in subjects diagnosed with the MS as compared to those subjects without the MS (IDF: 34.5%; harmonized: 36.9%). There was also a greater prevalence of females with IR than males (IDF: 78.4% vs 69.9%; harmonized: 78.3% vs 69.2%) in those diagnosed with MS (Table XV).

TABLE XV: *PREVALENCE OF INSULIN RESISTANCE (AS DETERMINED BY THE HOMA MODEL) IN SUBJECTS DIAGNOSED WITH AND WITHOUT THE METABOLIC SYNDROME*

	IR PREVALENCE **MS DIAGNOSED**		**IR PREVALENCE** **No MS DIAGNOSED**	
	IDF	**HARMONIZED**	**IDF**	**HARMONIZED**
Females	315/402 (78.4%)	299/382 (78.3%)	140/347 (40.3%)	156/367 (42.5%)
Males	79/113 (69.9%)	74/107 (69.2%)	27/137 (19.7%)	32/143 (22.4%)
Prevalence (IR)	**394/515 (76.5%)**	**373/489 (76.3%)**	**167/484 (34.5%)**	**188/510 (36.9%)**

* ***Key***: **MS**- metabolic syndrome; **No MS**- no metabolic syndrome; **IR**- insulin resistance; **IDF**- international diabetes federation definition for the metabolic syndrome

Data are presented as count values of IR subjects with % in parenthesis.

(IR was more prevalent in subjects with the MS, with females being more IR than males)

The prevalence of IR associated with age in subjects with the MS (IDF and harmonized definitions) is illustrated in Figure 17. In the ≤19 year age category the lowest prevalence of IR was present in both females (IDF: 42%; harmonized: 67%) and males (IDF: 22%; harmonized: 33%). As age advanced, the prevalence varied across the sample for both females and males with the MS (as per IDF and harmonized definitions) (Figure 17). This suggests that age and the definition used for diagnosis of the MS influences the prevalence of IR.

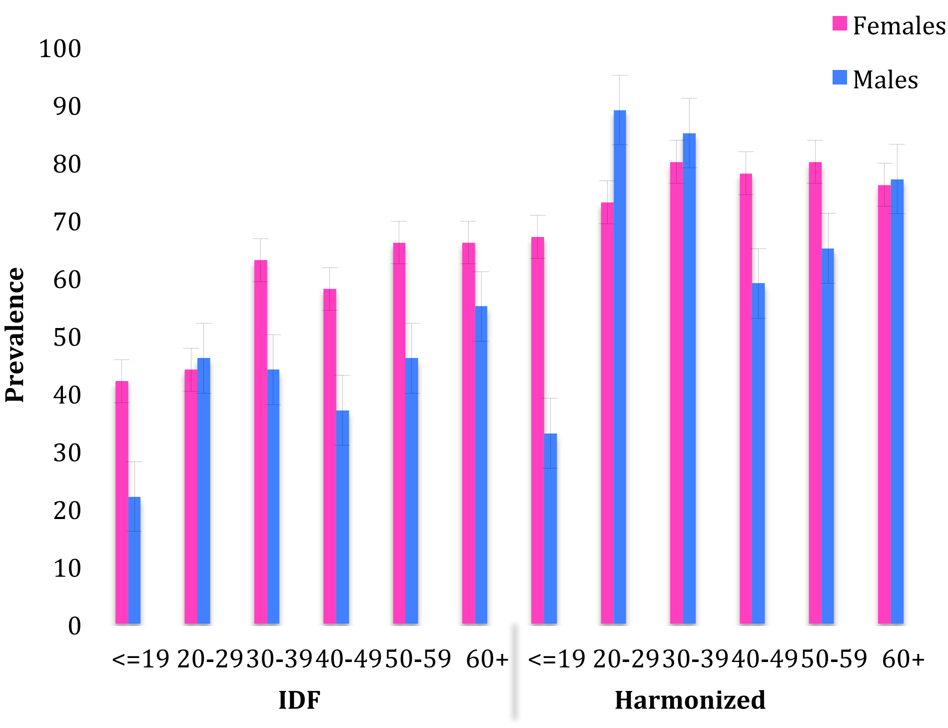


***Figure 17**: Prevalence of insulin resistance (vertical axis) in subjects diagnosed with the metabolic syndrome (IDF and harmonized) by age (horizontal axis)*

(Age influenced the prevalence of IR in females and males with the MS when the IDF and harmonized definitions were applied)

4.6 The pattern of risk factor clustering associated with insulin resistance

The pattern of risk factor clustering in IR subjects is illustrated in Figure 18A and 18B. When the IDF cutoffs was applied the frequencies of risk factor clustering increased with age in females, with the exception of HDL-C which varied as age advanced. Males, on the other hand, displayed varying frequencies for risk factor clustering when the IDF cutoffs was applied. The harmonized definition, on the other hand, showed a low frequency of clustering of the MS components in the 30-39 age category in males, with varying associations being observed in females as age advanced. These findings suggest that clustering of the components of the MS in IR subjects is influenced by age, gender and the definition used for diagnosis of the MS.

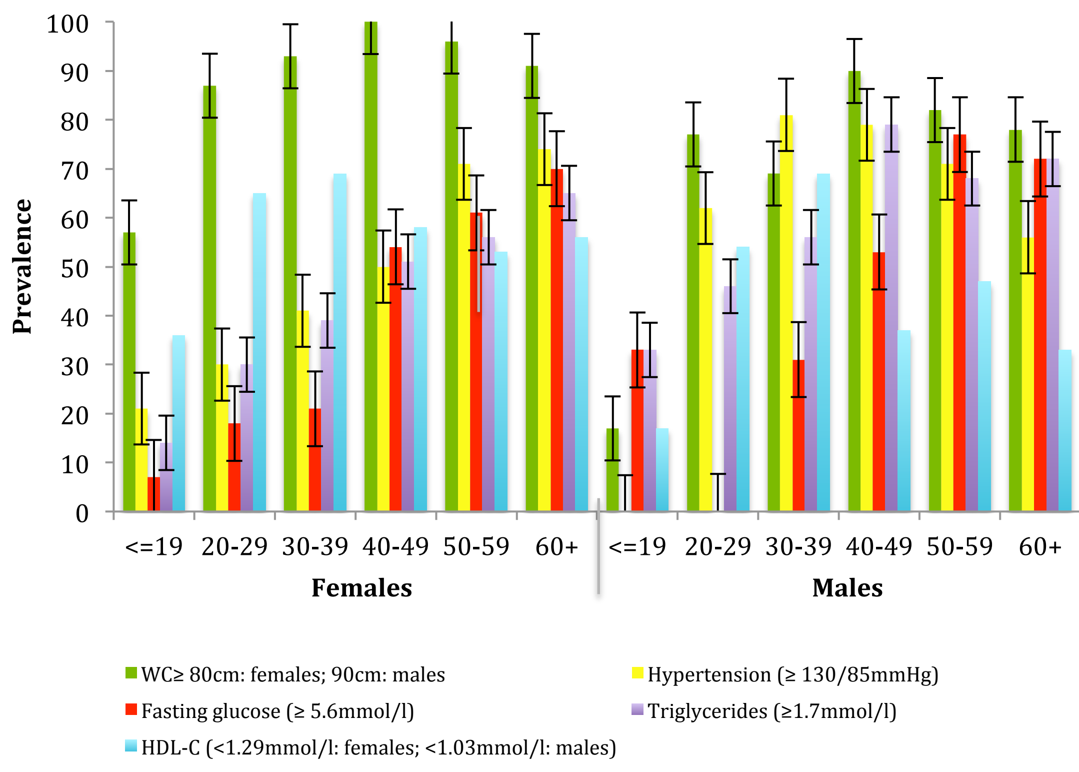


Figure 18A*: Clustering of the MS components, as per IDF criteria in IR subjects by age and gender*

(Age influenced the components of the MS among IR females and males)

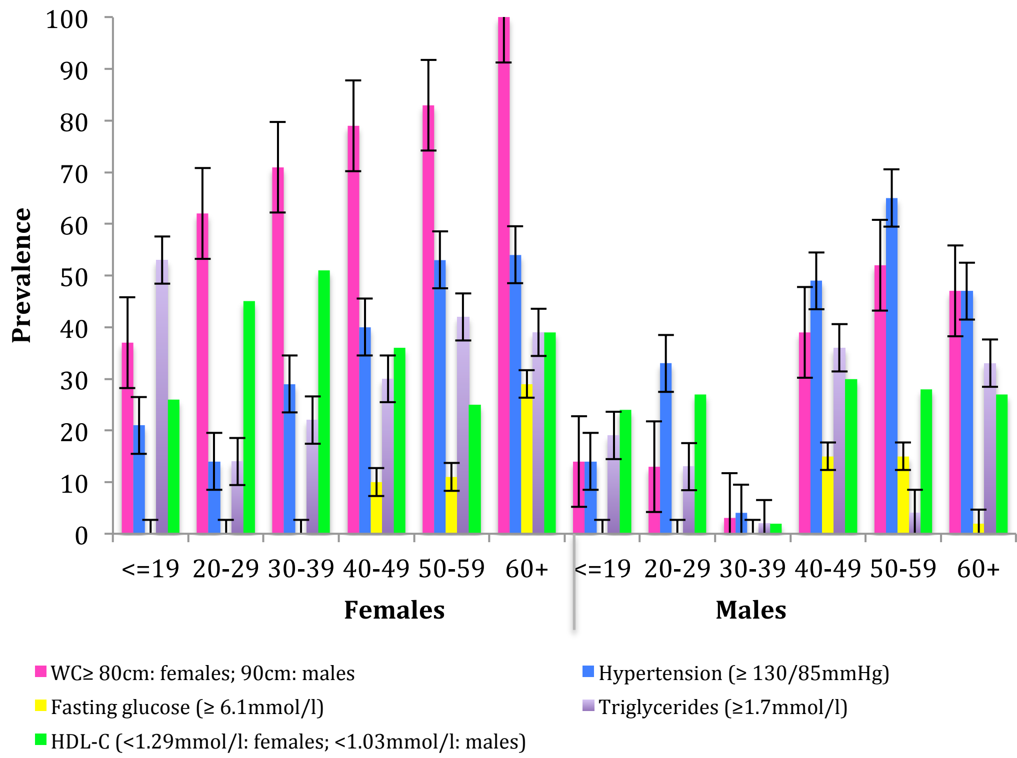


Figure 18B*: Clustering of the MS components, as per harmonized criteria in IR subjects by age and gender*

(Age influenced the components of the MS among IR females and males)

We, additionally produced receiver operator curves (ROC) to demonstrate suitable risk factors contributing to IR in this sample (Figure 19A-F). The highest area under the curve (AUC) was fasting blood glucose levels (AUC: 0.7573) and waist circumference measurements (AUC: 0.7507). This was followed by triglyceride levels (AUC: 0.6684), systolic blood pressure (AUC: 0.6092), HDL-C readings (AUC: 0.5926) and diastolic blood pressure (AUC: 0.5891) (Figure 19A-F). These findings suggest that in this population, fasting blood glucose levels and waist circumference measurements are suitable predictors of IR.

TABLE XIV: DISTRIBUTION OF THE METABOLIC COMPONENTS IN SUBJECTS DIAGNOSED WITH AND WITHOUT THE METABOLIC SYNDROME (ACCORDING TO LATEST DESCRIPTORS)

VARIABLES	MS DIAGNOSED			NO MS DIAGNOSED			P-VALUE	
	TOTAL (n=516)	FEMALES (n=402)	MALES (n=114)	TOTAL (n=483)	FEMALES (n=347)	MALES (n=136)	∞	‡
NCEP ATP111 (2001)								
WC ≥ 88cm (females); 102cm (males)	398 (77.1%)	354 (88.1%)	44 (38.6%)	224 (46.4%)	213 (61.4%)	11 (8.1%)	**<0.001***	**<0.001**
Systolic blood pressure ≥ 130mmHg	335 (64.9%)	252 (62.7%)	83 (72.8%)	115 (23.8%)	78 (22.5%)	37 (27.2%)	**<0.001***	**<0.001**
Diastolic blood pressure ≥ 85mmHg	270 (52.3%)	203 (50.5%)	67 (58.8%)	82 (17.0%)	61 (17.6%)	21 (15.4%)	**<0.001***	**<0.001**
Fasting blood glucose ≥ 6.1mmol/l	238 (46.1%)	190 (47.3%)	48 (42.1%)	30 (6.2%)	21 (6.1%)	9 (6.6%)	**<0.001***	**<0.001**
Triglycerides ≥ 1.7mmol/l	367 (71.1%)	277 (68.9%)	90 (78.9%)	63 (13.0%)	42 (12.1%)	21 (15.4%)	**<0.001***	**<0.001**
HDL-C <1.29 (females); <1.03 (males)	344 (66.7%)	279 (69.4%)	65 (57.0%)	106 (21.9%)	85 (24.5%)	21 (15.4%)	**<0.001***	**<0.001**
IDF (2005)								
WC ≥ 80cm (females); 90cm (males)	490 (95.0%)	391 (97.3%)	99 (86.8%)	298 (61.7%)	264 (76.1%)	34 (25.0%)	**<0.001***	**<0.001**
Systolic blood pressure ≥ 130mmHg	335 (64.9%)	252 (62.7%)	83 (72.8%)	115 (23.8%)	78 (22.5%)	37 (27.2%)	**<0.001***	**<0.001**
Diastolic blood pressure ≥ 85mmHg	270 (52.3%)	203 (50.5%)	67 (58.8%)	82 (17.0%)	61 (17.6%)	21 (15.4%)	**<0.001***	**<0.001**
Fasting blood glucose ≥ 5.6mmol/l	298 (57.8%)	236 (58.7%)	62 (54.4%)	41 (8.5%)	27 (7.8%)	14 (10.3%)	**<0.001***	**<0.001**
Triglycerides ≥ 1.7mmol/l	367 (71.1%)	277 (68.9%)	90 (78.9%)	63 (13.0%)	42 (12.1%)	21 (15.4%)	**<0.001***	**<0.001**
HDL-C <1.29 (females); <1.03 (males)	344 (66.7%)	279 (69.4%)	65 (57.0%)	106 (21.9%)	85 (24.5%)	21 (15.4%)	**<0.001***	**<0.001**
HARMONIZED (2011)								
WC ≥ 80cm (females); 90cm (males)	490 (95.0%)	391 (97.3%)	99 (86.8%)	298 (61.7%)	264 (76.1%)	34 (25.0%)	**<0.001***	**<0.001**
Systolic blood pressure ≥ 130mmHg	335 (64.9%)	252 (62.7%)	83 (72.8%)	115 (23.8%)	78 (22.5%)	37 (27.2%)	**<0.001***	**<0.001**
Diastolic blood pressure ≥ 85mmHg	270 (52.3%)	203 (50.5%)	67 (58.8%)	82 (17.0%)	61 (17.6%)	21 (15.4%)	**<0.001***	**<0.001**
Fasting blood glucose ≥ 6.1mmol/l	238 (46.1%)	190 (47.3%)	48 (42.1%)	30 (6.2%)	21 (6.1%)	9 (6.6%)	**<0.001***	**<0.001**
Triglycerides ≥ 1.7mmol/l	367 (71.1%)	277 (68.9%)	90 (78.9%)	63 (13.0%)	42 (12.1%)	21 (15.4%)	**<0.001***	**<0.001**
HDL-C <1.29 (females); <1.03 (males)	344 (66.7%)	279 (69.4%)	65 (57.0%)	106 (21.9%)	85 (24.5%)	21 (15.4%)	**<0.001***	**<0.001**

* ***Key***: **MS**- metabolic syndrome; **No MS**- no metabolic syndrome; ∞- unadjusted p value; ‡- adjusted p value; ***** best p value chosen from different analysis

(Data are presented as count values of those at risk for each metabolic syndrome criteria with %. ∞- Comparison between metabolic groups was done using Chi-squared (χ^2) test. ‡- Comparison between metabolic groups was done using the Bonferroni test. Variables were considered statistically significant if p<0.05- in bold)

(The distribution of the metabolic components was significantly higher in subjects with the MS, as per the latest descriptors)

We further analyzed the association of environmental factors (current smoking, alcohol consumption and no physical activity) in IR subjects with the MS (IDF and harmonized definitions) (Table XVI). We observed IR to be considerably high in subjects with the MS who performed no physical activity (IDF: 85.8%; harmonized: 85.0%), as compared to subjects with no MS (IDF: 75.9%; harmonized: 78.2%). Current smoking (IDF: 19.0%; harmonized: 18.8%) and alcohol consumption (IDF: 13.7%; harmonized: 13.9%) was shown to contribute to IR in subjects with the MS (Table XVI). These findings indicate that physical activity is a key contributor in the development of IR in this sample.

TABLE XVI: *PREVALENCE OF SMOKING, ALCOHOL CONSUMPTION AND PHYSICAL ACTIVITY IN IR SUBJECTS DIAGNOSED WITH AND WITHOUT THE METABOLIC SYNDROME (IDF AND HARMONIZED CRITERIA)*

VARIABLES	IR PREVALENCE MS DIAGNOSED		IR PREVALENCE NO MS DIAGNOSED	
	IDF (2005)	HARMONIZED (2011)	IDF (2005)	HARMONIZED (2011)
Current smoking	75/394 (19.0%) (95%CI: 15.3-21.8)	70/373 (18.8%) (95%CI: 14.9-23.1)	29/166 (17.5%) § (95%CI: 25.6-34.2)	34/188 (18.1%) (95%CI: 12.9-24.3)
Alcohol consumption	54/394 (13.7%) (95%CI: 10.7-16.4)	52/373 (13.9%) (95%CI: 10.6-17.9)	22/165 (13.3%) § (95%CI: 15.5-22.9)	24/187 (12.8%) § (95%CI: 8.4-18.5)
No physical activity	338/394 (85.8%) (95%CI: 79.6-85.8)	317/373 (85.0%) (95%CI: 81.0-88.5)	126/166 (75.9%) § (95%CI: 74.7-82.4)	147/188 (78.2%) (95%CI: 71.6-83.9)

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **IR**- insulin resistance; **CI**- confidence interval; **IDF**- international diabetes federation definition for the metabolic syndrome
(Data are presented as count values of IR subjects with % in parenthesis and 95% confidence intervals)

§ Missing counts as subject (s) did not disclose smoking status, alcohol consumption and physical activities performed

(IR was more prevalent in subjects with the MS who performed no physical activity)

4.7 Summary

This sample showed highly significant differences between females and males in terms of their age profiles and also found high levels of cardiovascular risk factors (with the exception of HDL-C levels). The anthropometric parameters, physiological measurements and biochemical parameters (blood glucose, insulin and lipid parameters) in the sample was clearly influenced by age. The study further showed a high prevalence of the MS when the IDF and harmonized definitions were applied, (all subsequent analysis was conducted using the *IDF and harmonized definitions*). Females were more susceptible to the MS than males with age and clustering of the metabolic components influencing its progression. Increased waist circumference and elevated triglycerides clustered more frequently in participants with the MS. In addition, IR was prevalent in the sample with the MS and females were more IR than males. Age and risk factor clustering further appeared to influence the progression of IR in participants with the MS. Fasting blood glucose levels and waist circumference measurements were identified as suitable predictors of IR in this sample. IR was further shown to occur more commonly in participants (with the MS) who performed no physical activity.

CHAPTER 5

RESULTS

(GENETICS)

Chapter 5: Results of Genetics Studies

In this chapter the genotype/allele frequencies for the selected candidate gene polymorphisms (apolipoprotein A5 Q139X, lipoprotein lipase HinfI, human paraoxonase1 192Arg/Gln, cholesteryl ester transfer protein Taq1B adiponectin 45T>G and leptin 25CAG) and their association with the metabolic components in the sample have been reported, including a description for Hardy Weinberg equilibrium. Genetic patterns for the selected polymorphisms in participants with and without the MS as per IDF and harmonized definitions are further demonstrated, followed by their associations with the individual metabolic risk factors. Additionally, the genetic patterns in IR participants with and without the MS (IDF and harmonized definitions) have also been demonstrated followed by their association with the individual metabolic risk factors.

5.1 Hardy-Weinberg equilibrium

The frequencies for the genotypes and alleles for the studied polymorphisms were obtained by gene counting, see Table XVII. The lipoprotein lipase HinfI polymorphism deviated from Hardy-Weinberg equilibrium (HWE) ($p<0.05$) but we reported on all genotype/phenotype relations (explanations for this deviation is provided in Chapter 6, page 192). The human paraoxonase1 192Arg/Gln, cholesteryl ester transfer protein Taq1B, adiponectin 45T>G and leptin 25CAG demonstrated no deviation from HWE ($P>0.05$) as per chi-square test in the sample (Table XVII). The six polymorphisms investigated demonstrated similarities in genotype distribution. However, no significant differences were detected (Table XVII).

TABLE XVII: GENOTYPE AND ALLELE FREQUENCIES IN SAMPLE

SNP	GENOTYPE	n (%)	ALLELE FREQUENCIES	P VALUE	
				∞	‡
APOA5 Q139X	CC TT CT	999 (100) 0 (0) 0 (0)	C: 1.0 T: 0.0	-	-
Total		999 (100)	1		
LPL HinfI	CC GG CG	772 (77.3) 53 (5.3) 174 (17.4)	C: 0.86 G: 0.14	*0.00[i]	0.02
Total		999 (100)	1		
PON1 **192 Arg/Gln**	AA GG AG	541 (54.2) 58 (5.8) 400 (40.0)	A: 0.74 G: 0.26	*0.15[ii]	0.97
Total		999 (100)	1		
CETP **Taq1B**	GG AA GA	311 (31.1) 223 (22.3) 465 (46.6)	G: 0.54 A: 0.46	*0.05[ii]	0.90
Total		999 (100)	1		
ADP **45T>G**	TT GG TG	745 (74.5) 18 (1.8) 236 (23.6)	T: 0.86 G: 0.14	*0.89[ii]	0.99
Total		999 (100)	1		
LEP **25CAG**	AA GG AG	991 (99.2) 0 (0) 8 (0.8)	A: 0.996 G: 0.004	*0.90[ii]	0.99
Total		999 (100)	1		

* ***Key***: **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; ∞- unadjusted p value; ‡- adjusted p value; * best p values chosen from different analysis

(Data are presented as prevalence rates with percentages in parenthesis. To test Hardy Weinberg Equilibrium (HWE): ∞- the chi-squared test (x^2) was used and ‡- the Bonferroni test was used)

[i] deviated from HWE ($p<0.05$); [ii] no deviation from HWE ($p>0.05$)

(The genotypes and alleles of the studied polymorphisms showed a small percentage of participants with mutations; and in some instances (APOA5 Q139X and LEP 25CAG) no mutations were present in participants)

5.2 Apolipoprotein A5 polymorphism

5.2.1 Q139X

The homozygous wild type (CC) genotype of the Q139X polymorphism was found in all participants (100%) (Figure 20). No heterozygous (CT) and mutant homozygous (TT) genotypes were detected in the sample (Table XVII) (Figure 20).

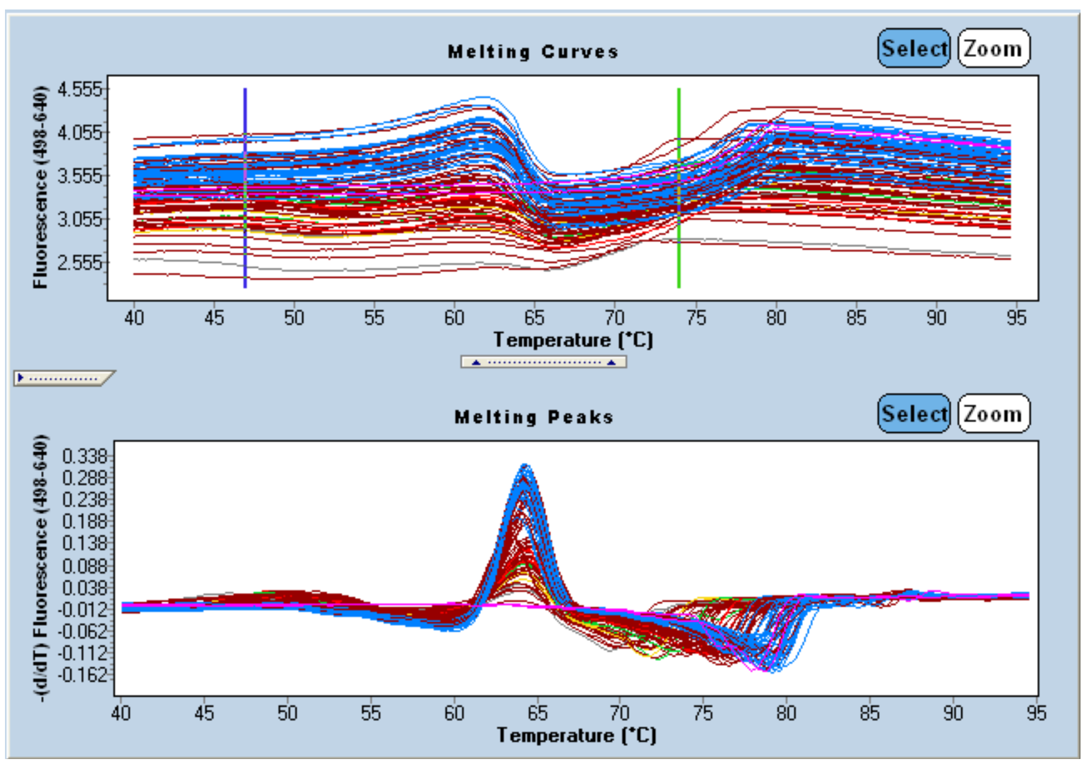
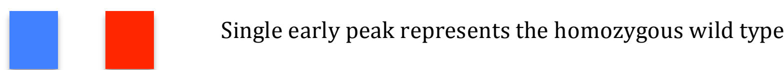


Figure 20*: Illustration showing the Q139X melting peaks*

(The melting peaks differed in color as per Light Cycler 480 software)



5.3 Lipoprotein lipase polymorphism

5.3.1 HinfI

The homozygous wild type (CC) genotype of the HinfI polymorphism was found in 77% of participants. A small number of participants, 5% and 17%, presented with the homozygous mutant (GG) genotype and heterozygous (CG) genotype, respectively. There was a dominance of the C allele, observed in 86% of the cohort, in comparison with the G allele, detected in 14% of the sample (Table XVII) (Figure 21).

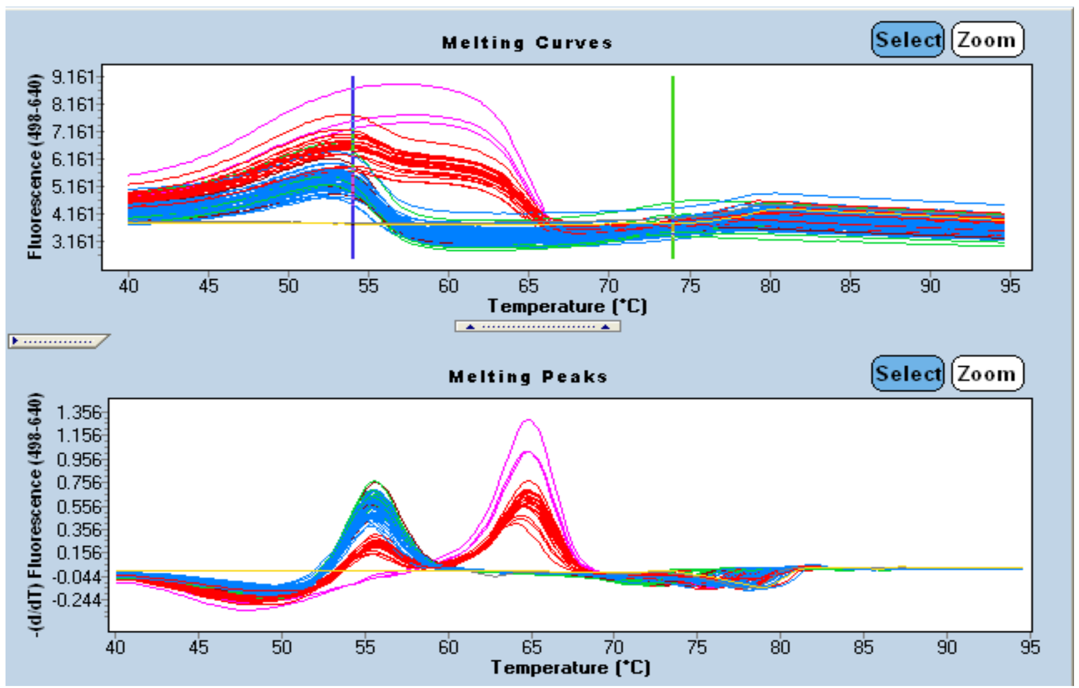
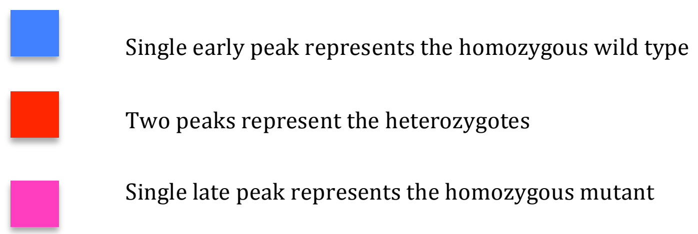


Figure 21: *Illustration showing the HinfI melting peaks*

(The melting curve temperatures differed as per design in order to allow for optimum amplification)
(The melting peaks differed in color as per Light Cycler 480 software)



5.4 Human paraoxonase 1 polymorphism

5.4.1 192Arg/Gln

The homozygous wild type (AA) genotype of the 192Arg/Gln polymorphism was 54% in the sample, whilst the heterozygous (AG) genotype was detected in 40%. There were only 6% of participants with the homozygous mutant genotype. The A allele dominated in the sample (74%), as compared to the G allele, which was observed in 26% of participants (Table XVII) (Figure 22).

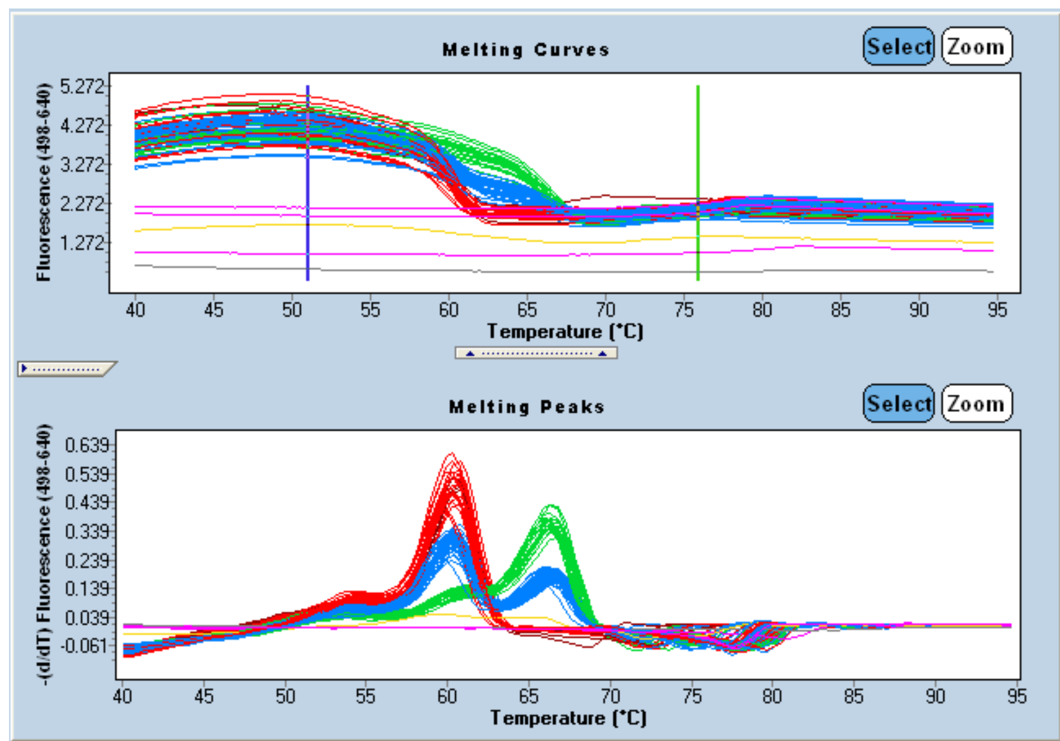


Figure 22*: Illustration showing the 192Arg/Gln melting peaks*

(The melting curve temperatures differed as per design in order to allow for optimum amplification)
(The melting peaks differed in color as per Light Cycler 480 software)

Single early peak represents the homozygous wild type

Two peaks represent the heterozygotes

Single late peak represents the homozygous mutant

5.5 Cholesteryl ester transfer polymorphism

5.5.1 Taq1B

The homozygous wild type (GG) genotype of the Taq1B polymorphism was 31% in the sample, with a higher frequency of the heterozygous (GA) genotype (47%). Interestingly, 22% of participants presented with the homozygous mutant (AA) genotype. The G allele dominated in the sample (54%), as compared to the A allele, which was observed in 46% of participants (Table XVII) (Figure 23).

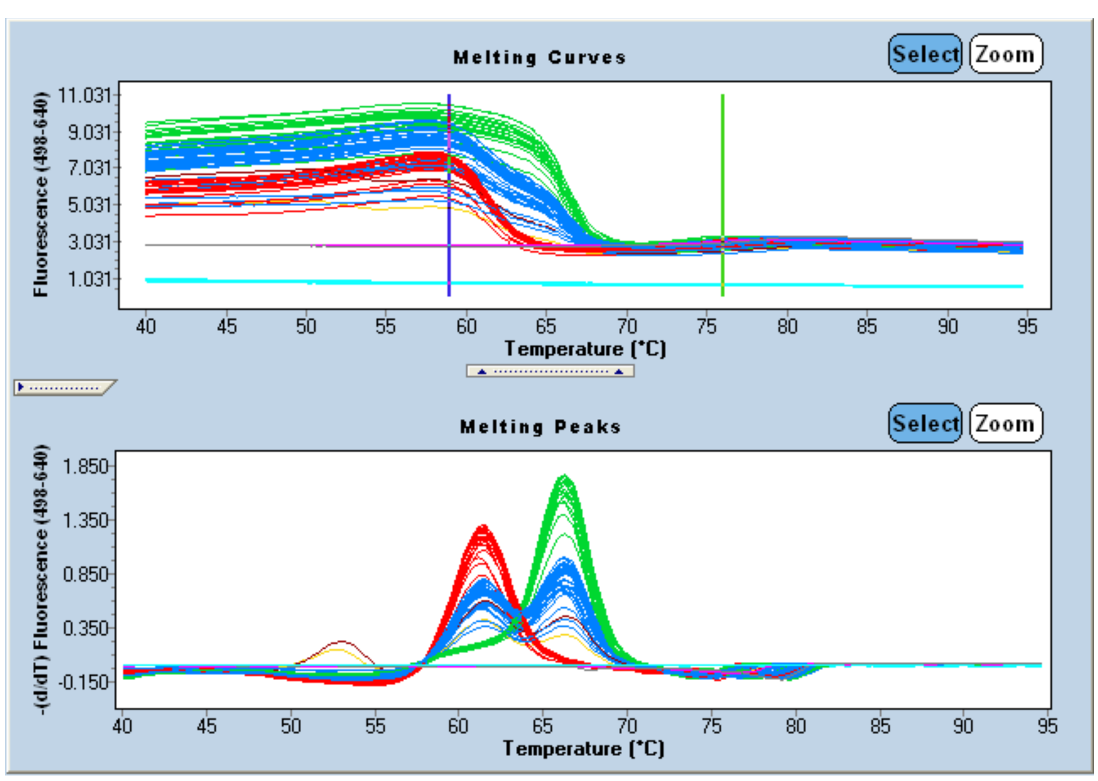


Figure 23: *Illustration showing the Taq1B melting peaks*

(The melting curve temperatures differed as per design in order to allow for optimum amplification)
(The melting peaks differed in color as per Light Cycler 480 software)

Single early peak represents the homozygous wild type

Two peaks represent the heterozygotes

Single late peak represents the homozygous mutant

5.6 Adiponectin polymorphism

5.6.1 45T>G

The homozygous wild type (TT) genotype of the 45T>G polymorphism was present in 75% of the sample, whilst the heterozygous (TG) genotype was detected in 24% of participants. There were only 2% of participants with the homozygous mutant (GG) genotype. The T allele dominated in the sample (86%), as compared to the G allele, which was observed in 14% of participants (Table XVII) (Figure 24).

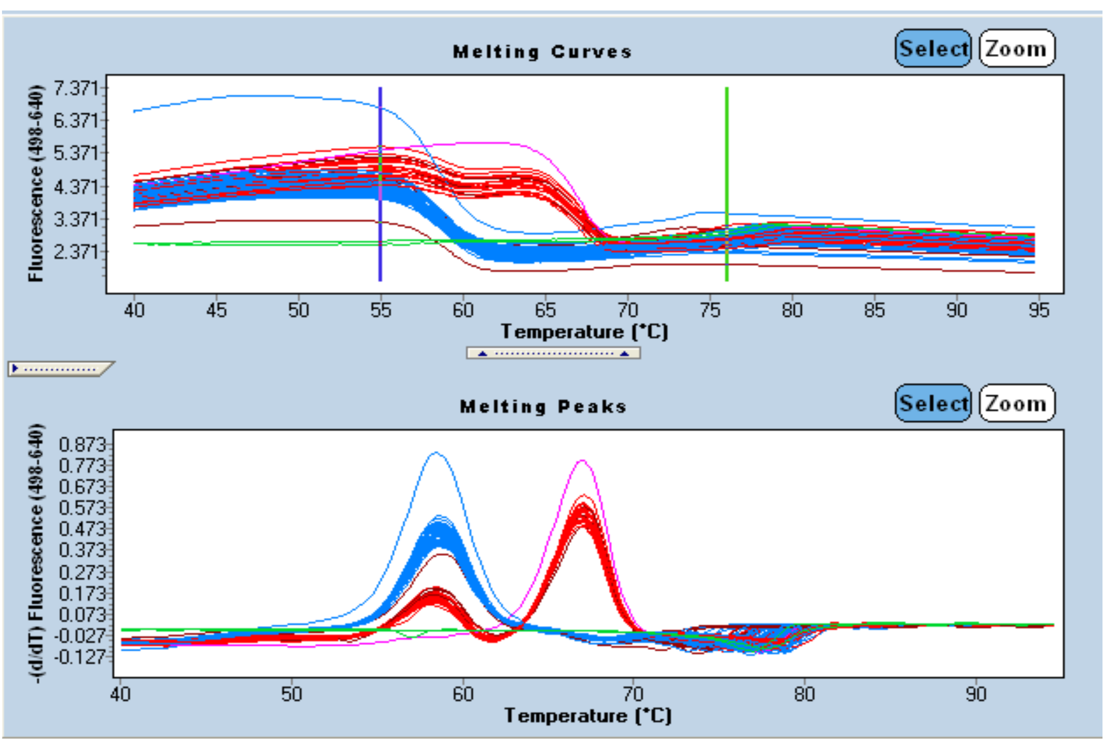


Figure 24*: Illustration showing the 45T>G melting peaks*

(The melting curve temperatures differed as per design in order to allow for optimum amplification)
(The melting peaks differed in color as per Light Cycler 480 software)

Single early peak represents the homozygous wild type

Two peaks represent the heterozygotes

Single late peak represents the homozygous mutant

5.7 Leptin polymorphism

5.7.1 25CAG

A high frequency of the homozygous wild type (AA) genotype of the 25CAG polymorphism was present in the sample (99%), whilst the heterozygous (AG) genotype was detected in 1% of participants. There were no participants with the homozygous mutant (GG) genotype. The A allele dominated in the sample (99.6%), as compared to the G allele, which was observed in 0.4% of participants (Table XVII) (Figure 25).

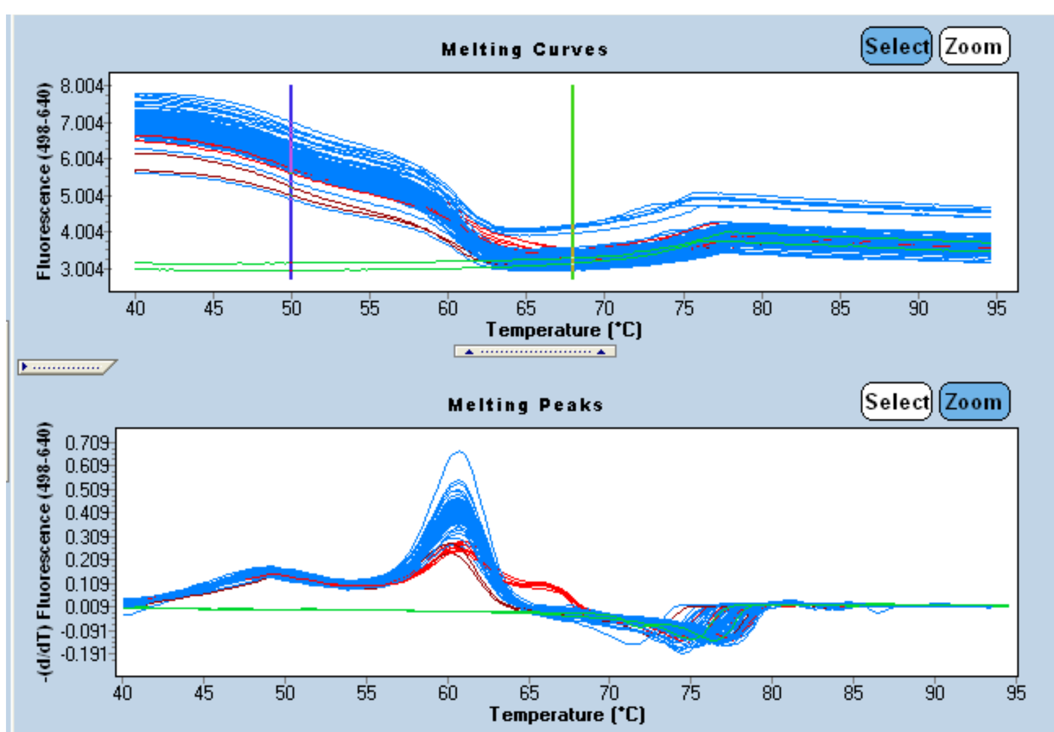
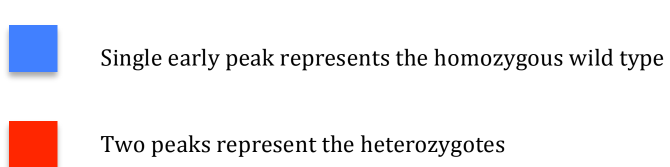


Figure 25*: Illustration showing the 25CAG melting peaks*

(The melting curve temperatures differed as per design in order to allow for optimum amplification)
(The melting peaks differed in color as per Light Cycler 480 software)



5.8 Association between metabolic components and gene polymorphisms in the sample

ANOVA testing associated the metabolic components with the apolipoprotein A5 Q139X, lipoprotein lipase HinfI, human paraoxonase 1 192Arg/Gln, cholesteryl ester transfer protein Taq1B, adiponectin 45T>G and leptin 25CAG polymorphisms, with similar means being observed and non-significant associations detected (Table XVIII). However, worth noting is that participants with the lipoprotein lipase HinfI [homozygous mutant (GG) genotypes] (p=0.06) and cholesteryl ester transfer protein Taq1B [homozygous mutant (AA) genotypes] (p=0.06) polymorphisms were marginally inclined to have higher diastolic blood pressure readings and higher blood glucose levels, respectively, suggesting a possible role in cardiovascular risk (Table XVIII).

TABLE XVIII: *ASSOCIATION BETWEEN GENE POLYMORPHISMS AND METABOLIC COMPONENTS IN SAMPLE*

GENE POLYMORPHISM	METABOLIC COMPONENTS	WTH GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	HET GENOTYPE MEAN±SD	P VALUE	
					∞	‡
APOA5	Waist circumference (cm)	95.5±15.3	-	-	-	-
	Systolic pressure (mmHg)	128.6±23.8	-	-	-	-
Q139X	Diastolic pressure (mmHg)	80.6±12.5	-	-	-	-
	Blood glucose (mmol/l)	6.2±3.6	-	-	-	-
	Triglycerides (mmol/l)	1.8±1.2	-	-	-	-
	HDL-C (mmol/l)	1.4±2.5	-	-	-	-
LPL	Waist circumference (cm)	95.5±15.2	97.0±14.2	95.3±16.0	0.77	0.99
	Systolic pressure (mmHg)	128.5±24.4	133.1±28.8	128.0±18.6	0.36	0.99
HinfI	Diastolic pressure (mmHg)	80.5±12.6	84.5±13.1	80.0±11.6	*0.06**	0.92
	Blood glucose (mmol/l)	6.2±3.7	6.2±2.7	6.3±3.0	0.97	1.00
	Triglycerides (mmol/l)	1.8±1.2	1.8±1.2	1.7±1.1	0.71	0.99
	HDL-C (mmol/l)	1.5±2.8	1.4±0.9	1.3±0.3	0.74	0.99
PON1	Waist circumference (cm)	95.8±15.0	96.2±14.3	95.1±15.9	0.77	0.99
	Systolic pressure (mmHg)	128.7±24.3	126.7±21.0	128.8±23.4	0.81	0.99
192Arg/Gln	Diastolic pressure (mmHg)	81.3±12.9	79.6±10.8	79.8±12.2	0.16	0.97
	Blood glucose (mmol/l)	6.2±2.9	6.8±3.2	6.2±4.3	0.43	0.99
	Triglycerides (mmol/l)	1.8±1.2	1.7±0.8	1.8±1.2	0.59	0.99
	HDL-C (mmol/l)	1.5±3.4	1.3±0.3	1.3±0.6	0.40	0.99
CETP	Waist circumference (cm)	95.5±15.2	95.2±14.2	95.7±15.9	0.91	1.00
	Systolic pressure (mmHg)	129.0±24.2	129.4±23.2	128.0±23.7	0.72	0.99
Taq1B	Diastolic pressure (mmHg)	81.1±13.1	81.9±12.2	79.7±12.2	0.08	0.97
	Blood glucose (mmol/l)	6.1±2.8	6.7±5.6	6.0±2.6	*0.06**	0.92
	Triglycerides (mmol/l)	1.7±1.0	1.9±1.4	1.8±1.2	0.21	0.97
	HDL-C (mmol/l)	1.3±0.5	1.8±5.1	1.3±0.7	0.07	0.95
ADP	Waist circumference (cm)	95.3±15.8	92.1±11.7	96.4±13.8	0.41	0.99
	Systolic pressure (mmHg)	129.3±23.6	121.7±19.4	126.9±24.4	0.18	0.97
45T>G	Diastolic pressure (mmHg)	80.9±12.4	76.3±10.1	80.2±13.0	0.25	0.99
	Blood glucose (mmol/l)	6.2±3.8	5.4±1.3	6.3±2.9	0.60	0.99
	Triglycerides (mmol/l)	1.8±1.2	1.4±0.9	1.9±1.2	0.32	0.99
	HDL-C (mmol/l)	1.4±0.7	1.2±0.3	1.6±5.2	0.32	0.99
LEP	Waist circumference (cm)	95.5±15.3	-	102.8±10.8	0.18	0.97
	Systolic pressure (mmHg)	128.6±23.7	-	126.6±27.3	0.81	0.99
25CAG	Diastolic pressure (mmHg)	80.6±12.6	-	80.4±6.8	0.96	1.00
	Blood glucose (mmol/l)	6.2±3.6	-	5.9±1.4	0.78	0.99
	Triglycerides (mmol/l)	1.8±1.2	-	1.4±0.4	0.31	0.99
	HDL-C (mmol/l)	1.4±2.5	-	1.3±0.3	0.87	0.99

* ***Key***: **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; ∞- unadjusted p value; ‡- adjusted p value;

* best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if p<0.05. P values in italics demonstrate marginal associations)

The p value could not be determined in the APOA5 Q139X as all participants were homozygous wild types

(Participants carrying the LPL HinfI [homozygous mutant (GG) genotypes] and CETP Taq1B [homozygous mutant (AA) genotypes] was marginally associated with elevated diastolic blood pressure readings and higher blood glucose levels, respectively)

5.9 Genetic patterns of gene polymorphisms in participants diagnosed with and without the metabolic syndrome

The distribution of the genotypes of the studied polymorphism in participants diagnosed with and without MS (IDF and harmonized definitions) varied, with no significant differences being detected (Tables XIX-XXI). When we analyzed females and males separately, there were also no significant differences (IDF and harmonized definitions) (Table XXII).

5.9.1 Gene polymorphisms related to lipid and carbohydrate metabolism

Apolipoprotein A5 polymorphism

The distribution of the apolipoprotein A5 Q139X polymorphism was confined to the wild type homozygous (CC) genotype in participants with and with no MS (IDF and harmonized). No homozygous mutant (TT) or heterozygous (CT) genotypes were detected. Overall, only C alleles were observed in the sample and therefore the level of significance could not be analyzed (Table XIX).

TABLE XIX: *GENOTYPE/ALLELE FREQUENCIES OF THE APOLIPOPROTEIN A5 Q139X POLYMORPHISM IN PARTICIPANTS DIAGNOSED WITH AND WITH NO MS (IDF AND HARMONIZED)*

SNP	**GENOTYPE/ ALLELE**		**MS (n%)**		**No MS (n%)**		**P VALUE**	
			IDF	**HARM**	**IDF**	**HARM**	**IDF** ∞/‡	**HARM** ∞/‡
		n (%)						
APOA5								
	CC	999 (100)	516 (100)	489 (100)	483 (100)	510 (100)	-	-
Q139X	TT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
	CT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Total (n%) (genotype)		999 (100)	516 (51.7)	489 (48.9)	483 (48.3)	510 (51.1)		
	C	1998 (100)	1032 (100)	978 (100)	966 (100)	1020 (100)		
	T	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
Total (n%) (allele)		1998 (100)	1032 (51.7)	978 (48.9)	966 (48.3)	1020 (51.1)		

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **IDF-** international diabetes federation definition for the metabolic syndrome; **HARM-** harmonized definition for the metabolic syndrome; **APOA5**- apolipoprotein A5

(Data are presented as prevalence rates with percentages in parenthesis. Comparison between total metabolic groups was done using Chi-squared (χ^2) for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$))

(The distribution of the APOA5 Q139X polymorphism shows no significant differences in genotype/allele frequencies between participants diagnosed with and without the metabolic syndrome, when the IDF and harmonized definitions were applied)

5.9.2 Gene polymorphisms related to insulin resistance

Lipoprotein lipase, human paraoxonsae 1 and cholesteryl ester transfer protein polymorphisms

The distribution of the lipoprotein lipase HinfI and cholesteryl ester transfer protein Taq1B genotypes and alleles was higher in participants diagnosed with the MS than in participants with no MS, when the IDF definition was applied. The harmonized definition, on the other hand, showed varying frequencies for the lipoprotein lipase HinfI and cholesteryl ester transfer protein Taq1B genotypes and alleles in participants with and without the MS (Table XX). However, when the PON1 192 Arg/Gln was analyzed, a reduced number of

homozygous mutant (GG) genotypes and G alleles were distributed to participants with the MS (IDF and harmonized definitions), with no statistical significance being observed (Table XX).

TABLE XX: *GENOTYPE/ALLELE FREQUENCIES OF THE LPL HINFI, PON1 192ARG/GLN AND CETP TAQ1B POLYMORPHISMS IN PARTICIPANTS DIAGNOSED WITH AND WITH NO MS (IDF AND HARMONIZED)*

SNP	GENOTYPE/ ALLELE		MS (n%)		No MS (n%)		P VALUE	
			IDF	HARM	IDF	HARM	IDF ∞/‡	HARM ∞/‡
LPL		**n (%)** *IDF&HARM*						
	CC	772 (77.3)	393 (50.9)	372 (48.2)	379 (49.1)	400 (51.8)	0.63/ 0.99	0.59/ 0.99
HinfI	GG	53 (5.3)	30 (56.6)	29 (54.7)	23 (43.4)	24 (45.3)		
	CG	174 (17.4)	93 (53.4)	88 (50.6)	81 (46.6)	86 (49.4)		
Total (n%) (genotype)		999 (100)	516 (51.7)	489 (48.9)	483 (48.3)	510 (51.1)		
	C	1718 (86.0)	879 (51.2)	832 (48.4)	839 (48.8)	886 (51.6)		
	G	280 (14.0)	153 (54.6)	146 (52.1)	127 (45.4)	134 (47.9)		
Total (n%) (allele)		1998 (100)	1032 (51.7)	978 (48.9)	966 (48.3)	1020 (51.1)		
PON1		**n (%)** *IDF&HARM*						
	AA	541 (54.2)	287 (53.1)	273 (50.5)	254 (47.0)	268 (49.5)	0.60/ 0.99	0.54/ 0.99
192Arg/Gln	GG	58 (5.8)	28 (48.3)	26 (44.8)	30 (51.7)	32 (55.2)		
	AG	400 (40.0)	201 (50.3)	190 (47.5)	199 (49.8)	210 (52.5)		
Total (n%) (genotype)		999 (100)	516 (51.7)	489 (48.9)	483 (48.3)	510 (51.1)		
	A	1482 (74.2)	775 (52.3)	736 (49.7)	707 (47.7)	746 (50.3)		
	G	516 (25.8)	257 (49.8)	242 (46.9)	259 (50.2)	274 (53.1)		
Total (n%) (allele)		1998 (100)	1032 (51.7)	978 (48.9)	966 (48.3)	1020 (51.1)		
CETP		**n (%)**						
	GG	311 (31.1)	158 (50.8)	151 (48.6)	153 (49.2)	160 (51.4)	0.89/ 0.99	0.76/ 0.99
Taq1B	AA	223 (22.3)	118 (52.9)	114 (51.1)	105 (47.1)	109 (48.9)		
	GA	465 (46.5)	240 (51.6)	224 (48.2)	225 (48.4)	241 (51.8)		
Total (n%) (genotype)		999 (100)	516 (51.65)	489 (48.9)	483 (48.3)	510 (51.1)		
	G	1087 (54.4)	556 (51.1)	526 (48.4)	531 (48.9)	561 (51.6)		
	A	911 (45.6)	476 (52.3)	452 (49.6)	435 (47.7)	459 (50.4)		
Total (n%) (allele)		1998 (100)	1032 (51.7)	978 (48.9)	966 (48.3)	1020 (51.1)		

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **IDF-** international diabetes federation definition for the metabolic syndrome; **HARM-** harmonized definition for the metabolic syndrome; **LPL-** lipoprotein lipase; **PON1-** human paraoxonase 1; **CETP-** cholesteryl ester transfer protein; ∞- unadjusted p value; ‡- adjusted p value

Data are presented as prevalence rates with percentages in parenthesis. Comparison between total metabolic groups was done using Chi-squared (χ^2) for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$)

(The distribution of the LPL HinfI, PON1 192Arg/Gln and CETP Taq1B polymorphisms shows no significant differences in genotype/allele frequencies between participants diagnosed with and without the metabolic syndrome)

5.9.3 Gene polymorphisms related to obesity

Adiponectin and leptin polymorphisms

The adiponectin 45T>G when analyzed using the IDF definition, showed a reduced number of homozygous mutant (GG) genotypes being distributed to participants with the MS, with no statistical significance detected. Similarly, when the harmonized definition was applied, a lower frequency of wild type homozygous (TT) genotypes, homozygous mutant (GG) genotypes, and T and G alleles was observed in participants with the MS, with no statistical significance. Additionally, the leptin 25CAG revealed a reduced distribution of heterozygous (AG) genotypes and G alleles when the IDF and harmonized definitions was applied. The harmonized definition also showed a lower frequency of wild type homozygous (AA) genotypes and A alleles of the leptin 25CAG. No homozygous mutant (GG) genotypes was detected, when both the IDF and harmonized definitions was applied, and consequently non-significant associations being present (Table XXI).

TABLE XXI: *DISTRIBUTION OF THE ADIPONECTIN 45T>G AND LEPTIN 25CAG POLYMORPHISMS IN PARTICIPANTS WITH AND WITHOUT METABOLIC SYNDROME (IDF AND HARMONIZED)*

SNP	**GENOTYPE/ ALLELE**		**MS (n%)**		**No MS (n%)**		**P VALUE**	
			IDF	**HARM**	**IDF**	**HARM**	**IDF** ∞/‡	**HARM** ∞/‡
ADP		**n (%)** ***IDF&HARM***						
45T>G	TT	745 (74.6)	385 (51.7)	364 (48.9)	360 (48.3)	381 (51.1)	0.82/ 0.99	0.17/ 0.97
	GG	18 (1.8)	8 (44.4)	5 (27.8)	10 (55.6)	13 (72.2)		
	TG	236 (23.6)	123 (52.1)	120 (50.8)	113 (47.9)	116 (49.2)		
Total (n%) (genotype)		999 (100)	516 (51.7)	489 (48.9)	483 (48.3)	510 (51.1)		
	T	1726 (86.4)	893 (51.7)	848 (49.1)	833 (48.3)	878 (50.9)		
	G	272 (13.6)	139 (51.1)	130 (47.8)	133 (48.9)	142 (52.2)		
Total (n%) (allele)		1998 (100)	1032 (51.7)	978 (48.9)	966 (48.3)	1020 (51.1)		
LEP		**n (%)** ***IDF&HARM***						
25CAG	AA	991 (99.2)	513 (51.8)	486 (49.0)	478 (48.2)	505 (51.0)	0.60/ 0.99	0.52/ 0.99
	GG	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		
	AG	8 (0.8)	3 (37.5)	3 (37.5)	5 (62.5)	5 (62.5)		
Total (n%) (genotype)		999 (100)	516 (51.7)	489 (48.9)	483 (48.3)	510 (51.1)		
	A	1990 (99.6)	1029 (51.7)	975 (49.0)	961 (48.3)	1015 (51.0)		
	G	8 (0.4)	3 (37.5)	3 (37.5)	5 (62.5)	5 (62.5)		
Total (n%) (allele)		1998 (100)	1032 (51.7)	978 (48.9)	966 (48.3)	1020 (51.1)		

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **IDF-** international diabetes federation definition for the metabolic syndrome; **HARM-** harmonized definition for the metabolic syndrome; **ADP-** adiponectin; **LEP-** leptin; ∞- unadjusted p value; ‡- adjusted p value

Data are presented as prevalence rates with percentages in parenthesis. Comparison between total metabolic groups was done using Chi-squared (χ^2) for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$)

(The distribution of the ADP 45T>G and LEP 25CAG polymorphisms shows no significant differences in genotype/allele frequencies between participants diagnosed with and without the metabolic syndrome)

TABLE XXII: DISTRIBUTION OF THE STUDIED POLYMORPHISMS WITH AND WITHOUT THE METABOLIC SYNDROME BY GENDER (IDF&HARM)

SNP	GENOTYPE/ ALLELE	MS n (%)				NO MS n (%)				P VALUE			
		FEMALES		MALES		FEMALES		MALES		FEMALES		MALES	
		IDF	HARM	IDF	HARM	IDF	HARM	IDF	HARM	IDF ∞ ‡	HARM ∞ ‡	IDF ∞ ‡	HARM ∞ ‡
APOA5	CC	402 (100)	382 (100)	114 (100)	107 (100)	347 (100)	367 (100)	136 (100)	143 (100)	-	-	-	-
	TT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)				
Q139X	CT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)				
	C	804 (100)	764 (100)	228 (100)	214 (100)	694 (100)	734 (100)	272 (100)	286 (100)				
	T	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)				
LPL	CC	305 (52.9)	290 (50.3)	88 (44.6)	82 (42.1)	272 (47.1)	287 (49.7)	107 (54.9)	113 (58.0)	0.67	0.70	0.93	0.84
	GG	23 (59.0)	22 (56.4)	7 (50.0)	7 (50.0)	16 (41.0)	17 (43.6)	7 (50.0)	7 (50.0)	0.99	0.99	1.00	0.99
HinfI	CG	74 (55.6)	70 (52.6)	19 (46.3)	18 (43.9)	59 (44.4)	63 (47.4)	22 (53.7)	23 (56.1)				
	C	684 (85.1)	650 (85.1)	195 (85.5)	182 (85.0)	603 (86.9)	637 (86.8)	236 (86.8)	249 (87.1)				
	G	120 (14.9)	114 (14.9)	33 (14.5)	32 (15.0)	91 (13.1)	97 (13.2)	36 (13.2)	37 (12.9)				
PON1	AA	223 (54.0)	214 (51.8)	64 (50.0)	59 (46.1)	190 (46.0)	199 (48.2)	64 (50.0)	69 (53.9)	0.80	0.70	0.10	0.21
	GG	23 (60.5)	21 (55.3)	5 (25.0)	5 (25.0)	15 (39.5)	17 (44.7)	15 (75.0)	15 (75.0)	0.99	0.99	0.97	0.97
192Arg/Gln	AG	156 (52.4)	147 (49.3)	45 (44.1)	43 (42.2)	142 (47.7)	151 (50.7)	57 (55.9)	59 (57.8)				
	A	599 (74.5)	575 (75.3)	173 (75.9)	161 (75.2)	522 (75.2)	549 (74.8)	185 (68.0)	197 (68.9)				
	G	202 (25.1)	189 (24.7)	55 (24.1)	53 (24.8)	172 (24.8)	185 (25.2)	87 (32.0)	89 (31.1)				
CETP	GG	124 (53.2)	120 (51.1)	34 (43.6)	31 (39.7)	109 (46.8)	113 (48.5)	44 (56.4)	47 (60.3)	0.66	0.54	0.68	0.77
	AA	92 (56.8)	88 (54.3)	26 (42.6)	26 (42.6)	70 (43.2)	74 (45.7)	35 (57.4)	35 (57.4)	0.99	0.99	0.99	0.99
Taq1B	GA	186 (52.5)	174 (49.2)	54 (48.7)	50 (45.1)	168 (47.5)	180 (50.9)	57 (51.4)	61 (55.0)				
	G	434 (54.0)	414 (54.2)	122 (53.5)	112 (52.3)	386 (55.6)	406 (55.3)	145 (53.3)	155 (54.2)				
	A	370 (46.0)	350 (45.8)	106 (46.5)	102 (47.7)	308 (44.4)	328 (44.7)	127 (46.7)	131 (45.8)				
ADP	TT	295 (53.5)	279 (50.6)	90 (46.4)	85 (43.8)	256 (46.5)	272 (49.4)	104 (53.6)	109 (56.2)	0.69	0.21	0.88	0.43
	GG	7 (43.8)	5 (31.3)	1 (50.0)	0 (0)	9 (56.3)	11 (68.8)	1 (50.0)	2 (100)	0.99	0.97	0.99	0.99
45T>G	TG	100 (55.0)	98 (53.9)	23 (42.6)	22 (40.7)	82 (45.1)	84 (46.2)	31 (57.4)	32 (59.3)				
	T	690 (85.8)	656 (85.9)	203 (89.0)	192 (89.7)	594 (85.6)	628 (85.6)	239 (87.9)	250 (87.4)				
	G	114 (14.2)	108 (14.1)	25 (11.0)	22 (10.3)	100 (14.4)	106 (14.4)	33 (12.1)	36 (12.6)				
LEP	AA	399 (53.7)	379 (51.0)	114 (45.6)	107 (43.2)	344 (46.4)	364 (49.0)	134 (54.4)	141 (56.9)	0.86	0.96	0.20	0.22
	GG	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.99	1.00	0.97	0.97
25CAG	AG	3 (50.0)	3 (50.0)	0 (0)	0 (0)	3 (50.0)	3 (50.0)	2 (100)	2 (100)				
	A	801 (99.6)	761 (99.6)	226 (100)	214 (100)	691 (99.6)	731 (99.6)	270 (99.3)	284 (99.3)				
	G	3 (0.4)	3 (0.4)	0 (0)	0 (0)	3 (0.4)	3 (0.4)	2 (0.7)	2 (0.7)				

- ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome ; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **IDF-** international diabetes federation definition for the metabolic syndrome; **HARM-** harmonized definition for the metabolic syndrome; **SNP-** single nucleotide polymorphism; ∞- unadjusted p value; ‡- adjusted p value

(Data are presented as prevalence rates with percentages in parenthesis. Comparison between groups was done using Chi-squared (χ^2) for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$))

(The distribution of the studied polymorphisms by gender shows no significant differences in genotype/allele frequencies between participants diagnosed with and with no metabolic syndrome)

5.9.4 *Genetic patterns of studied polymorphisms associated with metabolic risk factors in participants diagnosed with and without the metabolic syndrome*

Gene polymorphisms associated with metabolic risk factors in participants with and with no MS (as defined by the IDF and harmonized)

Associations were demonstrated between the studied gene polymorphisms and the metabolic risk factors in participants diagnosed with and with no MS as per IDF and harmonized definitions (Table XXIII, XXIV).

ANOVA testing detected non-significant associations between the gene polymorphisms and the metabolic risk factors in participants with the MS, when the IDF and harmonized definitiions were applied. Only marginal associations was observed between cholesteryl ester transfer protein Taq1B [homozygous mutant (AA) genotypes] and higher blood glucose levels (IDF: P=0.05; harmonized: P=0.05) in participants with the MS (IDF and harmonized) (Table XXIII, XXIV), suggesting a possible role in the susceptibility to the MS. Participants with no MS (according to IDF and harmonized definition), on the other hand, showed the cholesteryl ester transfer protein Taq1B [heterozygous (GA) genotypes) to be associated with lower systolic blood pressure readings (IDF: P=0.04; harmonized: P=0.02) (Table XXIII, XXIV), indicating a protective influence against systolic pressure and the MS.

When we analyzed gender groups, using either the Kruskal Wallis or ANOVA testing (Table XXV-XXVIII), we observed considerably higher means for waist circumference, systolic and diastolic blood pressure, blood glucose and

triglycerides, with reduced HDL-C, in participants with the MS as compared to those with no MS (according to IDF and harmonized definition), when associated with the gene polymorphisms. Males with the adiponectin 45T>G [homozygous (TT) genotypes], diagnosed with the MS were more inclined to have reduced HDL-C levels [IDF (Kruskal Wallis test: P=0.004); harmonized (ANOVA test:: P=0.001)] (Table XXV, XXVI). Males with the MS carrying the human paraoxonase1 192Arg/Gln [homozygous wild type (AA) genotypes] were more inclined to have elevated systolic blood pressure readings, but only when the harmonized definition was applied (harmonized: ANOVA test: P=0.02). Marginal associations were observed between the cholesteryl ester transfer protein Taq1B [heterozygous (GA) genotypes] and the human paraoxonase1 192Arg/Gln [homozygous wild type (AA) genotypes] with increased waist circumference measurements (P=0.05) and elevated systolic blood pressure (P=0.05) readings respectively in males when the IDF definition was applied (Table XXV). This suggests a possible influence against the susceptibility to the latter metabolic risk factors and the MS.

Female participants, on the other hand, did not show any association between the gene polymorphisms and the MS, when the IDF and harmonized definitions was applied (Table XXVII, XXVIII). We did, however, observe female participants with no MS carrying the adiponectin 45T>G [homozygous mutant (GG) genotypes] to be more inclined to reduced waist circumference measurements, but only when the IDF definition was applied (Kruskal Wallis test: P=0.02) (Table XXVII), suggesting a protective influence against larger

waist measurements and the MS. Similarly, the harmonized definition when applied in females with no MS showed cholesteryl ester transfer protein Taq1B [heterozygous (GA) genotypes] carriers to be more inclined to lower systolic blood pressure readings (ANOVA test: P=0.04) (Table XXVIII), further indicating a protective influence against the risk for the MS.

Overall, these findings suggest that the studied gene polymorphisms associated with the metabolic risk factors in participants with and with no MS is largely influenced by gender and the definition used for diagnosis of the MS.

TABLE XXIII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN PARTICIPANTS WITH AND WITH NO MS (IDF)*

SNP/ METABOLIC RISK FACTORS	MS					NO MS				
	HET GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	WTH GENOTYPE MEAN±SD	P VALUE		HET GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	WTH GENOTYPE MEAN±SD	P VALUE	
				∞	‡				∞	‡
LPL HinfI										
Waist circumference (cm)	100,8±15,6	100,1±9,5	101,3±11,6	0,86	0.99	89,0±14,1	92,9±18,1	89,5±16,2	0,57	0.99
Systolic pressure (mmHg)	132,9±17,4	141,2±27,8	137,9±24,0	0,11	0.96	122,3±18,5	122,5±27,2	118,7±20,7	0,28	0.99
Diastolic pressure (mmHg)	84,5±10,7	89,3±10,9	85,3±12,5	0,16	0.96	74,9±10,5	78,3±13,2	75,5±10,7	0,41	0.99
Blood glucose (mmol/l)	7,4±3,6	7,1±2,9	7,3±4,7	0,97	1.00	5,0±1,3	5,0±1,7	5,0±1,5	1,00	1.00
Triglycerides (mmol/l)	2,2±1,2	2,4±1,3	2,3±1,3	0,53	0.99	1,2±0,6	1,1±0,4	1,3±0,8	0,32	0.99
HDL-C (mmol/l)	1,2±0,3	1,4±1,2	1,4±3,9	0,84	0.99	1,4±0,3	1,3±0,3	1,5±0,7	0,41	0.99
ADP 45T>G										
Waist circumference (cm)	100,9±11,3	99,1±8,0	101,3±12,7	0,88	0.99	91,5±14,6	86,6±12,0	89,0±16,4	0,36	0.99
Systolic pressure (mmHg)	135,5±26,9	128,8±30,0	137,9±21,9	0,41	0.99	117,4±17,0	118,1±11,5	120,2±21,9	0,44	0.99
Diastolic pressure (mmHg)	85,0±13,0	82,7±7,8	85,6±11,9	0,91	1.00	74,8±10,7	71,6±7,5	75,9±10,9	0,29	0.99
Blood glucose (mmol/l)	7,3±3,1	6,5±1,8	7,4±4,8	0,87	0.99	5,1±2,2	4,9±0,6	5,0±1,1	0,71	0.99
Triglycerides (mmol/l)	2,3±1,2	2,1±1,1	2,3±1,3	0,90	0.99	1,4±1,0	1,1±0,5	1,2±0,7	0,23	0.97
HDL-C (mmol/l)	1,8±6,8	1,0±0,3	1,3±0,9	0,32	0.99	1,5±0,9	1,3±0,3	1,5±0,6	0,69	0.99
APOA5 Q139X										
Waist circumference (cm)	-	-	101,1±12,3	-	-	-	-	89,6±15,9	-	-
Systolic pressure (mmHg)	-	-	137,2±23,3	-	-	-	-	119,5±20,7	-	-
Diastolic pressure (mmHg)	-	-	85,4±12,1	-	-	-	-	75,5±10,8	-	-
Blood glucose (mmol/l)	-	-	7,3±4,5	-	-	-	-	5,0±1,4	-	-
Triglycerides (mmol/l)	-	-	2,3±1,3	-	-	-	-	1,3±0,8	-	-
HDL-C (mmol/l)	-	-	1,4±3,4	-	-	-	-	1,5±0,7	-	-
CETP Taq1B										
Waist circumference (cm)	101,5±13,5	99.9±10,4	101,5±11,8	0,47	0.99	89,7±16,0	89,6±15,9	89,5±15,9	0,99	1.00
Systolic pressure (mmHg)	138,4±23,6	135,1±22,1	137,9±23,6	0,45	0.99	117,0±18,4	122,8±22,9	120,9±22,0	**0,04***	0.85
Diastolic pressure (mmHg)	84,7±12,1	85,9±11,3	86,1±12,8	0,49	0.99	74,5±10,1	77,1±11,6	75,9±11,2	0,09	0.97
Blood glucose (mmol/l)	7,0±3,0	8,2±7,2	7,2±3,4	*0,05**	0.95	5,0±1,6	5,0±1,4	5,0±1,2	0,98	1.00
Triglycerides (mmol/l)	2,4±1,3	2,4±1,5	2,1±1,1	0,22	0.96	1,2±0,7	1,3±1,0	1,3±0,8	0,62	0.99
HDL-C (mmol/l)	1,2±0,8	1,9±7,0	1,2±0,6	0,14	0.96	1,5±0,7	1,6±0,9	1,4±0,3	0,16	0.97
PON1 192Arg/Gln										
Waist circumference (cm)	100,8±13,6	103,3±11,3	101,1±11,5	0,62	0.99	89,4±16,0	90,0±14,0	89,7±16,1	0,96	1.00
Systolic pressure (mmHg)	137,0±22,8	129,6±28,1	138,1±23,1	0,19	0.96	120,6±21,1	124,1±11,8	118,0±21,1	0,18	0.97
Diastolic pressure (mmHg)	84,8±11,3	82,4±11,2	86,2±12,7	0,19	0.96	74,8±11,1	77,1±10,0	75,8±10,7	0,43	0.99
Blood glucose (mmol/l)	7,4±5,7	8,3±3,6	7,2±3,4	0,45	0.99	4,9±1,2	5,5±2,0	5,1±1,5	0,14	0.97
Triglycerides (mmol/l)	2,4±1,3	2,0±0,8	2,3±1,3	0,50	0.99	1,3±0,7	1,3±0,6	1,3±0,8	0,88	0.99
HDL-C (mmol/l)	1,2±0,7	1,2±0,2	1,5±4,5	0,61	0.99	1,4±0,3	1,4±0,3	1,5±0,8	0,11	0.97
LEP 25CAG										
Waist circumference (cm)	109,6±14,8	-	101,1±12,3	0,23	0.96	98,8±6,4	-	89,5±16,0	0,20	0.97
Systolic pressure (mmHg)	153,7±3,8	-	137,1±23,3	0,22	0.96	110,4±20,5	-	119,6±20,7	0,32	0.99
Diastolic pressure (mmHg)	86,0±8,0	-	85,4±12,1	0,93	1.00	77,0±3,3	-	75,5±10,8	0,75	0.99
Blood glucose (mmol/l)	7,0±1,9	-	7,3±4,5	0,89	0.99	5,2±0,5	-	5,0±1,5	0,75	0.99
Triglycerides (mmol/l)	1,7±0,2	-	2,3±1,3	0,43	0.99	1,2±0,4	-	1,3±0,8	0,79	0.99
HDL-C (mmol/l)	1,3±0,2	-	1,4±3,4	0,96	1.00	1,3±0,3	-	1,5±0,7	0.51	0.99

Key: **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(All data are represented as mean±SD values. Comparison of the groups was done using one way analysis of variance test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold). P values in italics demonstrate marginal associations)

(The CETP Taq1B [homozygous mutant (AA) genotypes] in participants with the MS was marginally associated with increased blood glucose levels; and in healthy controls with no MS the CETP Taq1B [heterozygous (GA) genotypes was protective against high systolic blood pressure readings and the MS)

TABLE XXIV: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN PARTICIPANTS WITH AND WITH NO MS (HARMONIZED)*

SNP/ METABOLIC RISK FACTORS	MS					NO MS				
	WTH GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	HET GENOTYPE MEAN±SD	P VALUE		WTH GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	HET GENOTYPE MEAN±SD	P VALUE	
				∞	‡				∞	‡
APOA5 Q139X										
Waist circumference (cm)	101.2±12.4	-	-	-	-	90.1±15.9	-	-	-	-
Systolic pressure (mmHg)	137.5±23.2	-	-	-	-	120.1±21.0	-	-	-	-
Diastolic pressure (mmHg)	85.5±12.2	-	-	-	-	75.9±10.9	-	-	-	-
Blood glucose (mmol/l)	7.4±4.6	-	-	-	-	5.1±1.4	-	-	-	-
Triglycerides (mmol/l)	2.4±1.3	-	-	-	-	1.3±0.8	-	-	-	-
HDL-C (mmol/l)	1.4±3.5	-	-	-	-	1.5±0.8	-	-	-	-
LPL HinfI										
Waist circumference (cm)	101.3±11.7	100.1±9.6	101.1±15.5	0.89	0.99	90.1±16.1	93.3±17.8	89.4±14.3	0.56	0.99
Systolic pressure (mmHg)	138.2±23.8	141.8±28.0	133.1±17.7	0.10	0.96	119.4±21.3	122.5±26.6	122.8±18.2	0.35	0.99
Diastolic pressure (mmHg)	85.4±12.6	89.6±11.0	84.6±10.9	0.15	0.96	75.9±10.9	78.4±12.9	75.4±10.6	0.50	0.99
Blood glucose (mmol/l)	7.4±4.9	7.2±3.0	7.4±3.7	0.96	1.00	5.1±1.4	5.1±1.7	5.1±1.2	1.00	1.00
Triglycerides (mmol/l)	2.4±1.3	2.4±1.4	2.2±1.2	0.53	0.99	1.3±0.9	1.1±0.4	1.3±0.6	0.42	0.99
HDL-C (mmol/l)	1.4±4.0	1.4±1.3	1.2±0.3	0.87	0.99	1.5±0.8	1.3±0.2	1.4±0.3	0.33	0.99
PON1 192Arg/Gln										
Waist circumference (cm)	101.0±11.5	103.6±11.4	101.1±13.7	0.59	0.99	90.4±16.2	90.2±13.8	89.8±15.8	0.89	0.99
Systolic pressure (mmHg)	138.4±23.1	129.1±28.5	137.3±22.4	0.15	0.96	118.8±21.4	124.7±12.2	121.1±21.5	0.21	0.97
Diastolic pressure (mmHg)	86.3±12.9	82.6±11.3	84.8±11.2	0.20	0.96	76.2±10.7	77.1±9.9	75.3±11.4	0.55	0.99
Blood glucose (mmol/l)	7.3±3.5	8.4±3.7	7.5±5.9	0.46	0.99	5.1±1.5	5.5±2.0	5.0±1.2	0.16	0.97
Triglycerides (mmol/l)	2.4±1.4	2.1±0.8	2.4±1.3	0.52	0.99	1.3±0.8	1.3±0.6	1.3±0.9	0.94	1.00
HDL-C (mmol/l)	1.5±4.6	1.2±0.2	1.2±0.5	0.56	0.99	1.5±0.9	1.4±0.3	1.4±0.6	0.30	0.99
CETP Taq1B										
Waist circumference (cm)	101.7±11.6	100.1±10.5	101.4±13.7	0.56	0.99	89.7±15.9	90.5±16.0	90.0±15.7	0.89	0.99
Systolic pressure (mmHg)	136.9±24.0	134.6±21.6	139.3±23.4	0.20	0.96	121.5±22.0	117.5±18.7	124.0±23.7	**0.02***	0.78
Diastolic pressure (mmHg)	86.2±13.0	85.8±11.1	84.9±12.2	0.57	0.99	76.2±11.1	74.9±10.2	77.8±12.0	0.07	0.95
Blood glucose (mmol/l)	7.3±3.5	8.3±7.4	7.0±3.1	*0.05**	0.95	5.1±1.2	5.1±1.6	5.1±1.4	0.96	1.00
Triglycerides (mmol/l)	2.2±1.1	2.5±1.5	2.4±1.3	0.20	0.96	1.3±0.8	1.3±0.8	1.3±1.0	0.81	0.99
HDL-C (mmol/l)	1.2±0.6	1.9±7.1	1.2±0.5	0.12	0.96	1.4±0.3	1.5±0.9	1.6±0.9	0.18	0.97
ADP 45T>G										
Waist circumference (cm)	101.2±12.8	101.1±7.1	101.0±11.4	0.98	1.00	89.7±16.4	88.6±11.5	91.7±14.4	0.48	0.99
Systolic pressure (mmHg)	138.0±21.8	132.8±31.6	136.1±26.8	0.66	0.99	121.0±22.2	117.4±11.3	117.4±17.3	0.24	0.99
Diastolic pressure (mmHg)	85.6±11.9	87.6±6.9	85.1±13.2	0.86	0.99	76.3±11.1	71.9±7.3	75.0±10.7	0.22	0.97
Blood glucose (mmol/l)	7.5±5.0	6.6±1.9	7.3±3.2	0.89	0.99	5.1±1.1	4.9±0.6	5.1±2.2	0.78	0.99
Triglycerides (mmol/l)	2.4±1.3	2.2±1.2	2.3±1.2	0.95	1.00	1.3±0.8	1.1±0.5	1.4±1.0	0.32	0.99
HDL-C (mmol/l)	1.2±0.7	1.0±0.3	1.8±6.9	0.28	0.99	1.5±0.7	1.3±0.3	1.5±0.9	0.59	0.99
LEP 25CAG										
Waist circumference (cm)	101.1±12.4	-	109.6±14.8	0.24	0.99	90.1±15.9	-	98.8±6.4	0.22	0.97
Systolic pressure (mmHg)	137.4±23.2	-	153.7±3.8	0.23	0.96	120.2±21.1	-	110.4±20.5	0.30	0.99
Diastolic pressure (mmHg)	85.5±12.2	-	86.0±8.0	0.94	1.00	75.9±11.0	-	77.0±3.3	0.83	0.99
Blood glucose (mmol/l)	7.4±4.6	-	7.0±1.9	0.87	0.99	5.1±1.4	-	5.2±0.5	0.80	0.99
Triglycerides (mmol/l)	2.4±1.3	-	1.7±0.2	0.40	0.99	1.3±0.8	-	1.2±0.4	0.76	0.99
HDL-C (mmol/l)	1.4±3.5	-	1.3±0.2	0.98	1.00	1.5±0.8	-	1.3±0.3	0.52	

Key: **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; ∞- unadjusted p value; ‡- adjusted p value;
* best p value chosen from different analysis
(All data are represented as mean±SD values. Comparison of the groups was done using one way analysis of variance test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold). P values in italics demonstrate marginal associations)

(The CETP Taq1B [homozygous mutant (AA) genotypes] in participants with the MS was marginally associated with increased blood glucose levels; and in healthy controls with no MS the CETP Taq1B [heterozygous (GA) genotypes was protective against high systolic blood pressure readings and the MS)

TABLE XXV: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN MALE PARTICIPANTS WITH AND WITH NO MS (IDF)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE	
					∞	‡				∞	‡
APOA5	Waist circumference (cm)	99.1±10.1	-	-	-	-	82.7±13.1	-	-	-	-
	Systolic pressure (mmHg)	138.0±22.4	-	-	-	-	121.6±19.8	-	-	-	-
Q139X	Diastolic pressure (mmHg)	87.0±12.1	-	-	-	-	74.3±10.8	-	-	-	-
	Blood glucose (mmol/l)	6.8±2.7	-	-	-	-	5.2±1.8	-	-	-	-
	Triglycerides (mmol/l)	2.6±1.3	-	-	-	-	1.3±0.8	-	-	-	-
	HDL-C (mmol/l)	1.1±0.7	-	-	-	-	1.3±0.7	-	-	-	-
LPL	Waist circumference (cm)	99.1±9.2	102.1±10.1	98.2±13.7	0.76	0.99	82.3±13.1	89.8±16.8	82.0±11.4	0.46	0.99
	Systolic pressure (mmHg)	139.2±22.3	143.4±22.8	130.7±22.7	0.52	0.99	121.6±20.1	120.7±25.4	121.7±17.2	0.85	0.99
HinfI	Diastolic pressure (mmHg)	87.1±12.3	90.7±14.6	85.5±10.5	0.68	0.99	74.6±10.9	74.6±13.8	73.2±9.4	0.89	0.99
	Blood glucose (mmol/l)	6.9±2.7	6.4±3.1	6.5±2.3	0.81	0.99	5.2±1.9	5.6±2.9	4.9±0.5	0.75	0.99
	Triglycerides (mmol/l)	2.5±1.2	2.2±0.9	2.9±1.8	0.35	0.99	1.3±0.8	1.2±0.4	1.3±0.7	0.88	0.99
	HDL-C (mmol/l)	1.1±0.8	1.0±0.2	1.1±0.2	0.70	0.99	1.4±0.7	1.2±0.1	1.2±0.2	0.52	0.99
PON1	Waist circumference (cm)	97.6±9.8	102.4±13.6	100.9±9.9	0.17	0.96	81.9±14.9	84.7±9.1	83.0±11.8	0.56	0.99
	Systolic pressure (mmHg)	142.3±20.9	124.0±37.3	133.5±21.7	*0.05*	0.95	118.6±20.1	122.7±14.3	124.6±20.5	0.35	0.99
192Arg/Gln	Diastolic pressure (mmHg)	88.1±12.1	78.8±17.3	86.4±11.3	0.19	0.96	74.3±10.9	74.8±10.9	74.2±10.8	0.96	1.00
	Blood glucose (mmol/l)	6.6±2.6	8.2±3.4	6.9±2.8	0.36	0.99	5.3±2.5	5.4±1.2	4.9±0.6	0.14	0.97
	Triglycerides (mmol/l)	2.6±1.4	2.3±0.6	2.6±1.2	0.83	0.99	1.3±1.0	1.4±0.7	1.3±0.5	0.53	0.99
	HDL-C (mmol/l)	1.2±0.9	1.1±0.2	1.0±0.2	0.42	0.99	1.4±0.9	1.3±0.4	1.2±0.3	0.21	0.97
CETP	Waist circumference (cm)	100.2±9.2	95.1±9.6	100.4±10.5	*0.05*	0.95	83.6±15.3	82.6±14.3	82.0±10.3	0.93	1.00
	Systolic pressure (mmHg)	139.7±27.2	135.0±19.1	138.4±20.9	0.82	0.99	123.4±22.6	123.9±22.0	118.8±15.7	0.54	0.99
Taq1B	Diastolic pressure (mmHg)	87.2±12.2	87.4±10.5	86.8±12.9	0.99	1.00	75.8±9.9	75.7±13.1	72.4±9.7	0.20	0.97
	Blood glucose (mmol/l)	6.6±3.0	7.7±2.9	6.5±2.3	0.25	0.99	5.2±1.5	5.0±1.2	5.2±2.3	0.83	0.99
	Triglycerides (mmol/l)	2.4±1.0	2.9±1.3	2.6±1.4	0.41	0.99	1.2±0.5	1.4±1.2	1.3±0.6	0.82	0.99
	HDL-C (mmol/l)	1.0±0.2	1.1±0.3	1.1±1.0	0.44	0.99	1.3±0.3	1.6±1.2	1.2±0.3	0.08	0.97
ADP	Waist circumference (cm)	99.6±9.9	96.0±0	97.3±11.1	0.61	0.99	82.6±13.0	91.0±0	82.5±13.5	0.60	0.99
	Systolic pressure (mmHg)	140.1±21.3	134.0±0	130.0±25.9	0.29	0.99	122.0±21.0	131.0±0	120.1±15.4	0.57	0.99
45T>G	Diastolic pressure (mmHg)	87.1±11.9	79.0±0	87.2±13.1	0.71	0.99	74.4±10.7	73.0±0	74.1±11.3	0.96	1.00
	Blood glucose (mmol/l)	6.9±2.8	5.6±0	6.6±2.1	0.86	0.99	5.0±1.0	5.1±0	5.6±3.3	0.54	0.99
	Triglycerides (mmol/l)	2.5±1.3	1.2±0	2.8±1.0	0.11	0.96	1.3±0.8	1.0±0	1.2±0.4	0.78	0.99
	HDL-C (mmol/l)	1.1±0.8	1.6±0	1.1±0.2	**0.004***	0.50	1.4±0.7	1.1±0	1.2±0.3	0.40	0.99
LEP	Waist circumference (cm)	99.1±10.1	-	-	-	-	82.4±13.0	-	99.3±4.6	0.06	0.92
	Systolic pressure (mmHg)	138.0±22.4	-	-	-	-	121.9±19.5	-	101.0±33.9	0.37	0.99
25CAG	Diastolic pressure (mmHg)	87.0±12.1	-	-	-	-	74.3±10.8	-	79.5±3.5	0.33	0.99
	Blood glucose (mmol/l)	6.8±2.7	-	-	-	-	5.2±1.8	-	5.5±0.7	0.29	0.99
	Triglycerides (mmol/l)	2.6±1.3	-	-	-	-	1.3±0.8	-	1.2±0.7	0.93	1.00
	HDL-C (mmol/l)	1.1±0.7	-	-	-	-	1.3±0.7	-	1.3±0.3	0.83	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using Kruskal Wallis test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold). P values in italics demonstrate marginal associations)

(The ADP45T>G [heterozygous (TG) genotypes] showed significant associations with reduced HDL-C levels in males with the MS)

***TABLE XXVI:** GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN MALE PARTICIPANTS WITH AND NO MS (HARMONIZED)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	98.9±9.9	-	-	-	-	83.7±13.7	-	-	-	-
	Systolic pressure (mmHg)	138.6±22.3	-	-	-	-	122.0±20.0	-	-	-	-
Q139X	Diastolic pressure (mmHg)	87.1±12.2	-	-	-	-	74.9±11.0	-	-	-	-
	Blood glucose (mmol/l)	6.9±2.7	-	-	-	-	5.2±1.8	-	-	-	-
	Triglycerides (mmol/l)	2.7±1.3	-	-	-	-	1.3±0.8	-	-	-	-
	HDL-C (mmol/l)	1.0±0.2	-	-	-	-	1.4±0.8	-	-	-	-
LPL	Waist circumference (cm)	99.0±9.3	102.1±10.0	96.9±12.7	0.49	0.99	83.3±13.5	89.8±16.8	83.7±13.9	0.47	0.99
	Systolic pressure (mmHg)	140.0±21.9	143.4±22.8	130.1±23.2	0.20	0.96	122.0±20.3	120.7±25.4	122.6±17.3	0.98	1.00
HinfI	Diastolic pressure (mmHg)	87.2±12.4	90.7±14.6	85.3±10.7	0.61	0.99	75.2±11.1	74.6±13.8	73.9±9.7	0.88	0.99
	Blood glucose (mmol/l)	7.0±2.8	6.4±3.1	6.5±2.4	0.75	0.99	5.2±1.9	5.6±2.9	5.0±0.6	0.65	0.99
	Triglycerides (mmol/l)	2.6±1.1	2.2±0.9	3.0±1.8	0.28	0.99	1.3±0.8	1.2±0.4	1.3±0.7	0.94	1.00
	HDL-C (mmol/l)	1.0±0.2	1.0±0.2	1.1±0.2	0.71	0.99	1.4±0.9	1.2±0.1	1.2±0.2	0.53	0.99
PON1	Waist circumference (cm)	97.0±9.3	102.4±13.6	101.0±10.1	0.10	0.96	83.5±15.9	84.7±9.1	83.5±12.0	0.95	1.00
	Systolic pressure (mmHg)	143.8±19.8	124.0±37.3	133.1±22.1	**0.02***	0.83	119.1±20.6	122.7±14.3	125.2±20.4	0.23	0.97
192Arg/Gln	Diastolic pressure (mmHg)	88.5±12.5	78.8±17.3	86.2±11.1	0.20	0.96	75.0±10.9	74.8±10.9	74.8±11.3	0.99	1.00
	Blood glucose (mmol/l)	6.7±2.6	8.2±3.4	7.0±2.8	0.44	0.99	5.4±2.4	5.4±1.2	4.9±0.6	0.30	0.99
	Triglycerides (mmol/l)	2.6±1.4	2.3±0.6	2.7±1.1	0.77	0.99	1.4±0.9	1.4±0.7	1.3±0.5	0.67	0.99
	HDL-C (mmol/l)	1.0±0.2	1.1±0.2	1.0±0.2	0.27	0.99	1.5±1.1	1.3±0.4	1.2±0.3	0.21	0.97
CETP	Waist circumference (cm)	101.1±9.2	95.1±9.6	99.5±10.2	0.07	0.96	84.2±14.9	82.6±14.3	83.9±12.5	0.86	0.99
	Systolic pressure (mmHg)	140.4±28.4	135.0±19.0	139.3±19.8	0.64	0.99	124.0±22.0	123.9±22.0	119.4±17.0	0.41	0.99
Taq1B	Diastolic pressure (mmHg)	87.8±12.6	87.4±10.5	86.5±13.0	0.90	0.99	76.1±9.6	75.7±13.1	73.5±10.7	0.42	0.99
	Blood glucose (mmol/l)	6.6±3.1	7.7±2.9	6.6±2.4	0.24	0.99	5.3±1.4	5.0±1.2	5.2±2.2	0.81	0.99
	Triglycerides (mmol/l)	2.5±1.0	2.9±1.3	2.6±1.4	0.48	0.99	1.2±0.4	1.4±1.2	1.4±0.6	0.42	0.99
	HDL-C (mmol/l)	1.0±0.2	1.1±0.3	1.0±0.2	0.10	0.96	1.3±0.3	1.6±1.2	1.3±0.9	0.16	0.97
ADP	Waist circumference (cm)	99.4±9.6	-	96.9±11.2	0.30	0.99	83.6±13.8	93.5±3.5	83.3±13.8	0.59	0.99
	Systolic pressure (mmHg)	140.2±21.8	-	132.4±23.8	0.14	0.96	122.8±20.9	132.5±2.1	118.7±17.0	0.46	0.99
45T>G	Diastolic pressure (mmHg)	87.0±12.1	-	87.6±13.2	0.82	0.99	75.1±11.1	76.0±4.2	74.2±11.1	0.92	1.00
	Blood glucose (mmol/l)	6.9±2.9	-	6.7±2.2	0.69	0.99	5.1±1.0	5.3±0.4	5.6±3.2	0.37	0.99
	Triglycerides (mmol/l)	2.6±1.3	-	2.8±1.0	0.65	0.99	1.3±0.8	1.1±0.1	1.3±0.6	0.87	0.99
	HDL-C (mmol/l)	1.0±0.2	-	1.1±0.2	**0.001***	0.15	1.4±0.9	1.4±0.4	1.2±0.3	0.44	0.99
LEP	Waist circumference (cm)	98.9±9.9	-	-	-	-	83.4±13.7	-	99.3±4.6	0.11	0.97
	Systolic pressure (mmHg)	138.6±22.3	-	-	-	-	122.3±19.8	-	101.0±33.9	0.14	0.97
25CAG	Diastolic pressure (mmHg)	87.1±12.2	-	-	-	-	74.9±11.0	-	79.5±3.5	0.55	0.99
	Blood glucose (mmol/l)	6.9±2.7	-	-	-	-	5.2±1.8	-	5.5±0.7	0.79	0.99
	Triglycerides (mmol/l)	2.7±1.3	-	-	-	-	1.3±0.8	-	1.2±0.7	0.82	0.99
	HDL-C (mmol/l)	1.0±0.2	-	-	-	-	1.4±0.8	-	1.3±0.3	0.89	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold)).

(The PON1 192Arg/Gln [homozygous wild type (AA) genotypes] and the ADP 45T>G [homozygous wild type (TT) genotypes] showed significant associations with elevated systolic blood pressure and reduced HDL-C levels, respectively, in males with the MS)

TABLE XXVII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN FEMALE PARTICIPANTS WITH AND WITH NO MS (IDF)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	101.7±12.8	-	-	-	-	92.3±16.2	-	-	-	-
	Systolic pressure (mmHg)	137.1±23.3	-	-	-	-	118.4±21.1	-	-	-	-
Q139X	Diastolic pressure (mmHg)	84.9±12.2	-	-	-	-	76.0±10.8	-	-	-	-
	Blood glucose (mmol/l)	7.3±3.4	-	-	-	-	5.2±4.1	-	-	-	-
	Triglycerides (mmol/l)	2.2±1.3	-	-	-	-	1.3±0.9	-	-	-	-
	HDL-C (mmol/l)	1.3±0.7	-	-	-	-	1.7±4.1	-	-	-	-
LPL	Waist circumference (cm)	101.9±12.2	99.5±9.4	101.5±16.1	0.55	0.99	92.3±16.4	94.3±19.1	91.6±14.2	0.83	0.99
	Systolic pressure (mmHg)	137.7±24.2	140.5±29.5	133.5±15.9	0.14	0.96	117.3±21.0	123.3±28.8	122.5±19.1	0.27	0.99
HinfI	Diastolic pressure (mmHg)	84.7±12.6	88.9±9.9	84.3±10.8	0.17	0.96	75.8±10.6	79.9±13.0	75.5±10.9	0.75	0.99
	Blood glucose (mmol/l)	7.2±3.3	7.4±2.9	7.6±3.9	0.79	0.99	5.2±4.6	4.8±0.9	5.1±1.5	0.55	0.99
	Triglycerides (mmol/l)	2.3±1.3	2.5±1.4	2.0±0.9	0.09	0.96	1.3±0.9	1.0±0.3	1.2±0.5	0.09	0.97
	HDL-C (mmol/l)	1.3±0.7	1.5±1.4	1.2±0.3	0.47	0.99	1.8±4.7	1.4±0.3	1.4±0.3	0.85	0.99
PON1	Waist circumference (cm)	102.1±11.7	103.7±10.8	100.9±14.5	0.39	0.99	92.4±15.8	94.1±16.3	91.9±16.8	0.85	0.99
	Systolic pressure (mmHg)	137.1±23.2	131.0±25.9	137.9±23.0	0.78	0.99	117.6±21.8	124.8±9.2	118.9±21.1	0.16	0.97
192Arg/Gln	Diastolic pressure (mmHg)	85.6±12.9	82.5±10.0	84.2±11.3	0.39	0.99	76.3±10.6	80.1±8.7	75.1±11.2	0.19	0.97
	Blood glucose (mmol/l)	7.4±3.6	8.2±3.7	7.0±3.0	0.58	0.99	5.0±1.0	5.5±2.7	5.4±6.2	0.06	0.92
	Triglycerides (mmol/l)	2.3±1.3	2.0±0.8	2.2±1.3	0.64	0.99	1.3±0.8	1.2±0.4	1.3±0.9	0.96	1.00
	HDL-C (mmol/l)	1.3±0.8	1.2±0.3	1.2±0.6	0.83	0.99	1.9±5.5	1.4±0.2	1.5±0.6	0.93	1.00
CETP	Waist circumference (cm)	101.8±12.4	101.3±10.2	101.8±14.2	1.00	1.00	91.8±15.7	93.5±15.5	92.1±16.8	0.78	0.99
	Systolic pressure (mmHg)	136.1±22.6	135.8±22.2	138.3±24.3	0.85	0.99	119.7±21.8	121.4±23.8	116.3±19.3	0.39	0.99
Taq1B	Diastolic pressure (mmHg)	85.7±13.0	85.4±11.7	84.0±11.8	0.48	0.99	76.0±11.8	77.8±10.7	75.2±10.1	0.15	0.97
	Blood glucose (mmol/l)	7.4±3.5	7.6±3.6	7.1±3.2	0.71	0.99	4.9±1.1	6.1±8.7	5.0±1.3	0.81	0.99
	Triglycerides (mmol/l)	2.1±1.1	2.2±1.4	2.3±1.3	0.30	0.99	1.3±0.9	1.4±1.2	1.2±0.7	0.45	0.99
	HDL-C (mmol/l)	1.2±0.6	1.3±0.8	1.3±0.7	0.94	1.00	1.4±0.3	2.7±9.1	1.5±0.7	0.77	0.99
ADP	Waist circumference (cm)	101.8±13.4	100.3±8.1	101.6±11.2	0.90	0.99	91.6±16.9	85.4±11.5	95.1±13.6	**0.02***	0.78
	Systolic pressure (mmHg)	137.1±22.0	129.6±27.3	137.4±26.5	0.87	0.99	119.4±22.3	113.1±8.4	115.9±18.0	0.23	0.97
45T>G	Diastolic pressure (mmHg)	85.0±11.9	83.0±10.0	84.6±13.1	0.80	0.99	76.4±10.9	71.1±8.3	75.1±10.5	0.27	0.99
	Blood glucose (mmol/l)	7.3±3.4	6.3±1.6	7.4±3.3	0.77	0.99	5.3±4.7	4.7±0.5	5.0±1.7	0.23	0.97
	Triglycerides (mmol/l)	2.2±1.3	2.0±1.1	2.2±1.2	0.71	0.99	1.2±0.8	1.1±0.6	1.4±1.1	0.07	0.95
	HDL-C (mmol/l)	1.3±0.8	1.1±0.3	1.2±0.2	0.64	0.99	1.5±0.6	1.3±0.3	2.5±8.4	0.16	0.97
LEP	Waist circumference (cm)	101.6±12.8	-	109.6±14.8	0.30	0.99	92.2±16.2	-	98.5±8.3	0.35	0.99
	Systolic pressure (mmHg)	136.9±23.3	-	153.7±3.8	0.08	0.96	118.4±21.2	-	116.7±10.7	0.86	0.99
25CAG	Diastolic pressure (mmHg)	84.9±12.2	-	86.0±8.0	0.76	0.99	76.0±10.8	-	75.3±2.3	0.94	1.00
	Blood glucose (mmol/l)	7.3±3.4	-	7.0±1.9	0.66	0.99	5.2±4.1	-	5.0±0.03	0.40	0.99
	Triglycerides (mmol/l)	2.2±1.3	-	1.7±0.2	0.48	0.99	1.3±0.9	-	1.2±0.3	0.99	1.00
	HDL-C (mmol/l)	1.3±0.7	-	1.3±0.2	0.35	0.99	1.7±4.1	-	1.2±0.4	0.28	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using Kruskal Wallis test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold))

(None of the studied polymorphisms were associated with the metabolic risk factors in female with MS; however the ADP 45T>G [homozygous mutant (GG) genotypes] was associated with reduced waist circumference measurements and was protective against the MS).

TABLE XXVIII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN FEMALE PARTICIPANTS WITH AND WITH NO MS (HARMONIZED)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	101.8±12.9	-	-	-	-	92.7±16.0	-	-	-	-
	Systolic pressure (mmHg)	137.2±23.4	-	-	-	-	119.4±21.4	-	-	-	-
Q139X	Diastolic pressure (mmHg)	85.1±12.2	-	-	-	-	76.3±10.9	-	-	-	-
	Blood glucose (mmol/l)	7.6±5.0	-	-	-	-	5.0±1.3	-	-	-	-
	Triglycerides (mmol/l)	2.3±1.3	-	-	-	-	1.3±0.8	-	-	-	-
	HDL-C (mmol/l)	1.5±3.9	-	-	-	-	1.5±0.7	-	-	-	-
LPL	Waist circumference (cm)	101.9±12.3	99.4±10.0	102.2±16.1	0.66	0.99	92.8±16.3	94.7±18.5	91.5±14.0	0.72	0.99
	Systolic pressure (mmHg)	137.7±24.4	141.3±30.0	133.8±16.2	0.33	0.99	118.4±21.6	123.3±27.8	122.8±18.6	0.25	0.99
HinfI	Diastolic pressure (mmHg)	84.9±12.6	89.3±9.9	84.4±11.0	0.24	0.99	76.2±10.8	79.9±12.6	76.0±10.9	0.37	0.99
	Blood glucose (mmol/l)	7.6±5.3	7.4±2.9	7.7±4.0	0.97	1.00	5.0±1.2	4.8±1.0	5.1±1.4	0.69	0.99
	Triglycerides (mmol/l)	2.3±1.3	2.5±1.5	2.0±0.9	0.15	0.96	1.3±0.9	1.0±0.4	1.2±0.5	0.43	0.99
	HDL-C (mmol/l)	1.5±4.5	1.5±1.4	1.2±0.3	0.86	0.99	1.5±0.8	1.4±0.3	1.5±0.3	0.59	0.99
PON1	Waist circumference (cm)	102.1±11.8	103.9±11.2	101.1±14.7	0.58	0.99	92.8±15.7	95.0±15.6	92.2±16.5	0.77	0.99
	Systolic pressure (mmHg)	136.9±23.7	130.3±27.0	138.6±22.4	0.31	0.99	118.7±21.8	126.5±10.0	119.6±21.8	0.35	0.99
192Arg/Gln	Diastolic pressure (mmHg)	85.7±13.0	83.5±9.8	84.4±11.2	0.51	0.99	76.7±10.6	79.1±8.7	75.5±11.4	0.35	0.99
	Blood glucose (mmol/l)	7.4±3.6	8.5±3.8	7.6±6.5	0.66	0.99	5.0±1.0	5.5±2.5	5.0±1.4	0.23	0.97
	Triglycerides (mmol/l)	2.3±1.3	2.0±0.8	2.3±1.3	0.66	0.99	1.3±0.8	1.2±0.4	1.3±1.0	0.78	0.99
	HDL-C (mmol/l)	1.6±5.2	1.2±0.2	1.2±0.6	0.58	0.99	1.5±0.8	1.4±0.2	1.5±0.6	0.84	0.99
CETP	Waist circumference (cm)	101.9±12.2	101.6±10.3	101.9±14.5	0.98	1.00	92.0±15.8	93.6±15.1	92.7±16.5	0.82	0.99
	Systolic pressure (mmHg)	136.0±22.7	134.5±22.5	139.3±24.3	0.23	0.96	120.5±22.0	124.1±24.7	116.8±19.3	**0.04***	0.85
Taq1B	Diastolic pressure (mmHg)	85.8±13.1	85.3±11.3	84.4±12.0	0.61	0.99	76.2±11.7	78.7±11.5	73.4±10.0	0.09	0.97
	Blood glucose (mmol/l)	7.5±3.6	8.5±8.2	7.2±3.3	0.12	0.96	5.0±1.1	5.1±1.5	5.0±1.3	0.85	0.99
	Triglycerides (mmol/l)	2.1±1.1	2.3±1.6	2.4±1.3	0.34	0.99	1.3±0.9	1.3±0.8	1.3±0.8	0.94	1.00
	HDL-C (mmol/l)	1.2±0.6	2.2±8.1	1.2±0.6	0.13	0.96	1.4±0.3	1.5±0.8	1.6±0.9	0.43	0.99
ADP	Waist circumference (cm)	101.8±13.5	101.1±7.1	101.9±11.3	0.99	1.00	92.2±16.8	87.7±12.3	94.9±13.4	0.23	0.97
	Systolic pressure (mmHg)	137.3±21.8	132.8±31.6	136.9±27.4	0.90	0.99	120.4±22.8	114.6±10.0	116.9±17.6	0.33	0.99
45T>G	Diastolic pressure (mmHg)	85.2±11.9	87.6±6.9	84.5±13.2	0.80	0.99	76.8±11.1	71.2±7.7	75.4±10.5	0.16	0.97
	Blood glucose (mmol/l)	7.6±5.4	6.6±1.9	7.5±3.4	0.88	0.99	5.0±1.2	4.9±0.6	5.0±1.6	0.83	0.99
	Triglycerides (mmol/l)	2.3±1.3	2.2±1.2	2.2±1.2	0.96	1.00	1.2±0.8	1.2±0.5	1.4±1.1	0.19	0.97
	HDL-C (mmol/l)	1.3±0.8	1.0±0.3	1.9±7.7	0.35	0.99	1.5±0.6	1.3±0.3	1.6±1.0	0.48	0.99
LEP	Waist circumference (cm)	101.7±12.9	-	109.6±14.8	0.30	0.99	92.6±16.0	-	98.5±8.4	0.53	0.99
	Systolic pressure (mmHg)	137.0±23.5	-	153.7±3.8	0.22	0.96	119.4±21.5	-	116.7±10.7	0.82	0.99
25CAG	Diastolic pressure (mmHg)	85.1±12.2	-	86.0±8.0	0.89	0.99	76.3±10.9	-	75.3±2.3	0.87	0.99
	Blood glucose (mmol/l)	7.6±5.0	-	7.0±1.9	0.83	0.99	5.0±1.3	-	5.0±0.0	0.99	1.00
	Triglycerides (mmol/l)	2.3±1.3	-	1.7±0.2	0.47	0.99	1.3±0.8	-	1.2±0.3	0.82	0.99
	HDL-C (mmol/l)	1.5±4.0	-	1.3±0.2	0.95	1.00	1.5±0.7	-	1.2±0.4	0.49	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome ; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold))

(None of the studied polymorphisms were associated with the metabolic risk factors in female with MS; however, the CETP Taq1B [heterozygous (GA) genotypes] was associated with lower systolic blood pressure readings in females and was protective against the MS)

Gene polymorphisms associated with environmental risk factors (smoking, alcohol consumption, physical activity) in participants with and with no MS (as defined by the IDF and harmonized)

To assess if there was any interaction between smoking, alcohol intake and physical activities with the studied polymorphisms and the risk for the MS as per IDF and harmonized definitions, we classified genotypes/ alleles according to smoking/ nonsmoking status, alcohol consumption/ no alcohol intake, physical activities performed/ no physical activities and accordingly assessed their frequencies. See Tables XXIX, XXX. It is clear that the studied polymorphisms at genotype/ allele levels among smokers, alcohol consumers and participants who performed no physical activities were not significant and hence did not increase the risk for the MS.

Interestingly, at the lipoprotein lipase locus, there was no interaction with smoking; but among nonsmokers, the lipoprotein lipase HinfI heterozygous (CG) genotypes and G alleles appear to be protective against the risk for MS (IDF: P=0.04). Similar findings were observed at the human paraoxonase 1 (192Arg/Gln) locus i.e. [heterozygous (AG) genotypes and A allele] among nonsmokers (IDF: P=0.03; harmonized: P=0.04) (Table XXIX, XXX).

We further identified a protective effect between the risk for the MS between non-alcohol consumers with the human paraoxonase 1 192Arg/Gln [heterozygous (AG) genotypes and A alleles] (IDF: P=0.04; harmonized: P=0.04) and leptin 25CAG [homozygous (AA) genotypes and A alleles] polymorphisms (IDF: P=0.01; harmonized: P=0.01) (Table XXIX, XXX).

Overall, these findings suggest that the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms are protective against the risk for MS in non-smokers, irrespective of the definition used for diagnosis. Similarly, the human paraoxonase 1 192Arg/Gln and leptin 25CAG protect against the risk for MS in non-alcohol consumers.

TABLE XXIX: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH OTHER RISK FACTORS (CURRENT SMOKING, ALCOHOL CONSUMPTION AND NO PHYSICAL ACTIVITY) IN PARTICIPANTS WITH AND WITH NO MS (IDF)*

SNP/ OTHER RISK FACTORS		**MS**							**NO MS**						
		WTH GENOTYPE	**MH GENOTYPE**	**HET GENOTYPE**	**MAJOR ALLELE**	**MINOR ALLELE**	**P VALUE**		**WTH GENOTYPE**	**MH GENOTYPE**	**HET GENOTYPE**	**MAJOR ALLELE**	**MINOR ALLELE**	**P VALUE**	
							∞	‡						∞	‡
APOA5 Q139X		CC (n=516)	TT (n=0)	CT (n=0)	C (n=1032)	T (n=0)			CC (n=483)	TT (n=0)	CT (n=0)	C (n=966)	T (n=0)		
Current smoking	Yes	110 (21.3)	0 (0)	0 (0)	220 (21.3)	0 (0)	-	-	125 (25.9)	0 (0)	0 (0)	250 (25.9)	0 (0)	-	-
	No	406 (78.7)	0 (0)	0 (0)	812 (78.7)	0 (0)			358 (74.1)	0 (0)	0 (0)	716 (74.1)	0 (0)		
Alcohol consumption	Yes	83 (16.1)	0 (0)	0 (0)	166 (16.1)	0 (0)	-	-	77 (15.9) §	0 (0)	0 (0)	154 (15.9) §	0 (0)	-	-
	No	433 (83.9)	0 (0)	0 (0)	866 (83.9)	0 (0)			405 (83.9)	0 (0)	0 (0)	810 (83.9)	0 (0)		
No physical activity	Yes	20 (3.9)	0 (0)	0 (0)	40 (3.9)	0 (0)	-	-	62 (12.8)	0 (0)	0 (0)	124 (12.8)	0 (0)	-	-
	No	496 (96.1)	0 (0)	0 (0)	992 (96.1)	0 (0)			421 (87.2)	0 (0)	0 (0)	842 (87.2)	0 (0)		
LPL HinfI		CC (n=392)	GG (n=30)	CG (n=93)	C (n=877)	G (n=153)			CC (n=380)	GG (n=23)	CG (n=81)	C (n=841)	G (n=127)		
Current smoking	Yes	91 (23.2)	4 (13.3)	15 (16.1)	197 (22.5)	23 (15.0)	0.18	0.98	108 (28.4)	4 (17.4)	13 (16.1)	229 (27.2)	21 (16.5)	**0.04***	0.85
	No	301 (76.8)	26 (86.7)	78 (83.9)	680 (77.5)	130 (85.0)			272 (71.6)	19 (82.6)	68 (84.0)	612 (72.8)	106 (83.5)		
Alcohol consumption	Yes	66 (16.8)	5 (16.7)	11 (11.8)	143 (16.3)	21 (13.7)	0.49	0.99	60 (15.8) §	3 (16.1)	15 (18.5)	135 (16.1) §	21 (16.5)	0.94	1.00
	No	326 (83.2)	25 (83.3)	82 (88.2)	734 (83.7)	132 (86.3)			319 (84.0)	20 (87.0)	66 (81.5)	704 (83.7)	106 (83.5)		
Physical activity	Yes	326 (83.2)	27 (90.0)	82 (88.2)	734 (83.7)	136 (88.9)	0.34	0.99	297 (78.2)	19 (82.6)	57 (70.4)	651 (77.4)	95 (74.8)	0.26	0.99
	No	66 (16.8)	3 (10.0)	11 (11.8)	143 (16.3)	17 (11.1)			83 (21.8)	4 (17.4)	24 (29.6)	190 (22.6)	32 (25.2)		
PON1 192Arg/Gln		AA (n=287)	GG (n=27)	AG (n=201)	A (n=775)	G (n=255)			AA (n=254)	GG (n=31)	AG (n=199)	A (n=707)	G (n=261)		
Current smoking	Yes	59 (20.6)	4 (14.8)	47 (23.4)	165 (21.3)	55 (21.6)	0.53	0.99	66 (26.0)	14 (45.2)	45 (22.6)	177 (25.0)	73 (28.0)	**0.03***	0.85
	No	228 (79.4)	23 (85.2)	154 (76.6)	610 (78.7)	200 (78.4)			188 (74.0)	17 (54.8)	154 (77.4)	530 (75.0)	188 (72.0)		
Alcohol consumption	Yes	47 (16.4)	3 (11.1)	32 (15.9)	126 (16.3)	38 (14.9)	0.78	0.99	38 (15.0) §	11 (35.5)	29 (14.6)	105 (14.9) §	51 (19.5)	**0.04***	0.85
	No	240 (83.6)	24 (88.9)	169 (84.1)	649 (83.7)	217 (85.1)			215 (84.7)	20 (64.5)	170 (85.4)	600 (84.9)	210 (80.5)		
Physical activity	Yes	243 (84.7)	22 (81.5)	170 (84.6)	656 (84.6)	214 (83.9)	0.91	1.00	197 (77.6)	20 (64.5)	156 (78.4)	550 (77.8)	196 (75.1)	0.22	0.97
	No	44 (15.3)	5 (18.5)	31 (15.4)	119 (15.4)	41 (16.1)			57 (22.4)	11 (35.5)	43 (21.6)	157 (22.2)	65 (24.9)		
CETP Taq1B		GG (n=157)	AA (n=120)	GA (n=238)	G (n=552)	A (n=478)			GG (n=154)	AA (n=103)	GA (n=227)	G (n=535)	A (n=433)		
Current smoking	Yes	31 (19.8)	26 (21.7)	53 (22.3)	115 (20.8)	105 (22.0)	0.83	0.99	37 (24.0)	30 (29.1)	58 (25.6)	132 (24.7)	118 (27.3)	0.65	0.99
	No	126 (80.3)	94 (78.3)	185 (77.7)	437 (79.2)	373 (78.0)			117 (76.0)	73 (70.9)	169 (74.5)	403 (75.3)	315 (72.7)		
Alcohol consumption	Yes	20 (12.7)	20 (16.7)	42 (17.7)	82 (14.9)	82 (17.2)	0.41	0.99	21 (13.6)	20 (19.4)	37 (16.3) §	79 (14.8) §	77 (17.8) §	0.61	0.99
	No	137 (87.3)	100 (83.3)	196 (82.4)	470 (85.1)	396 (82.8)			133 (86.4)	83 (80.6)	189 (83.3)	455 (85.0)	355 (82.0)		
Physical activity	Yes	135 (86.0)	98 (81.7)	202 (84.9)	472 (85.5)	398 (83.3)	0.60	0.99	118 (76.6)	83 (80.6)	172 (75.8)	408 (76.3)	338 (78.1)	0.62	0.99
	No	22 (14.0)	22 (18.3)	36 (15.1)	80 (14.5)	80 (16.7)			36 (23.4)	20 (19.4)	55 (24.2)	127 (23.7)	95 (21.9)		
ADP 45T>G		TT (n=385)	GG (n=6)	TG (n=124)	T (n=894)	G (n=136)			TT (n=360)	GG (n=12)	TG (n=112)	T (n=832)	G (n=136)		
Current smoking	Yes	86 (22.3)	0 (0)	24 (19.4)	196 (21.9)	24 (17.6)	0.34	0.99	89 (24.7)	6 (50.0)	30 (26.8)	208 (25.0)	42 (30.9)	0.14	0.97
	No	299 (77.7)	6 (100)	100 (80.7)	698 (78.1)	112 (82.4)			271 (75.3)	6 (50.0)	82 (73.2)	624 (75.0)	94 (69.1)		
Alcohol consumption	Yes	68 (17.7)	0 (0)	14 (11.3)	150 (16.8)	14 (10.3)	0.14	0.98	54 (15.0) §	3 (25.0)	21 (18.8)	129 (15.5) §	27 (19.9)	0.75	0.99
	No	317 (82.3)	6 (100)	110 (88.7)	744 (83.2)	122 (89.7)			305 (84.7)	9 (75.0)	91 (81.3)	701 (84.3)	109 (80.1)		
Physical activity	Yes	326 (84.7)	6 (100)	103 (83.1)	755 (84.5)	115 (84.6)	0.52	0.99	281 (78.1)	7 (58.3)	85 (75.9)	647 (77.8)	99 (72.8)	0.26	0.99
	No	59 (15.3)	0 (0)	21 (16.9)	139 (15.5)	21 (15.4)			79 (21.9)	5 (41.7)	27 (24.1)	185 (22.2)	37 (27.2)		
LEP 25CAG		AA (n=512)	GG (n=0)	AG (n=3)	A (n=1027)	G (n=3)			AA (n=479)	GG (n=0)	AG (n=5)	A (n=963)	G (n=5)		
Current smoking	Yes	110 (21.5)	0 (0)	0 (0)	220 (21.4)	0 (0)	0.37	0.99	124 (25.9)	0 (0)	1 (20.0)	249 (25.9)	1 (20.0)	0.71	0.99
	No	402 (78.5)	0 (0)	3 (100)	807 (78.6)	3 (100)			355 (74.1)	0 (0)	4 (80.0)	714 (74.1)	4 (80.0)		
Alcohol consumption	Yes	82 (16.0)	0 (0)	0 (0)	164 (16.0)	0 (0)	0.45	0.99	78 (16.3)	0 (0)	0 (0) §	156 (16.2) §	0 (0) §	**0.01***	0.78
	No	430 (84.0)	0 (0)	3 (100)	863 (84.0)	3 (100)			401 (83.7)	0 (0)	4 (80.0)	806 (83.7)	4 (80.0)		
Physical activity	Yes	432 (84.4)	0 (0)	3 (100)	867 (84.4)	3 (100)	0.46	0.99	368 (76.8)	0 (0)	5 (100)	741 (76.9)	5 (100)	0.22	0.97
	No	80 (15.6)	0 (0)	0 (100)	163 (15.9)	0 (0)			111 (23.2)	0 (0)	0 (0)	222 (23.1)	0 (0)		

Key: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(All data are represented as prevalence rates with percentages in parenthesis. Comparison of the groups was done using Chi-square (x^2) test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold))

§ One participant carrying the genotype did not disclose smoking status, alcohol consumption and physical activities performed, subsequently influencing allele counts

(None of the studied polymorphisms increased the risk for the MS when associated with smoking, alcohol consumption and physical activity. The LPL HinfI [heterozygous (CG) genotypes and G alleles] and PON1 192 Arg/Gln [heterozygous (AG) genotypes and A alleles] in nonsmokers was protective against the MS. The PON1 192 Arg/Gln [heterozygous (AG) genotypes and A alleles] and LEP 25CAG [homozygous (AA) genotypes and A alleles] in non alcohol consumers was protective against the MS)

***TABLE XXX**: GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH OTHER RISK FACTORS (CURRENT SMOKING, ALCOHOL CONSUMPTION AND NO PHYSICAL ACTIVITY) IN PARTICIPANTS WITH AND WITH NO MS (HARMONIZED)*

SNP/ OTHER RISK FACTORS		MS							NO MS						
		WTH GENOTYPE	MH GENOTYPE	HET GENOTYPE	MAJOR ALLELE	MINOR ALLELE	P VALUE ∞	P VALUE ‡	WTH GENOTYPE	MH GENOTYPE	HET GENOTYPE	MAJOR ALLELE	MINOR ALLELE	P VALUE ∞	P VALUE ‡
APOA5 Q139X		CC (n=489)	TT (n=0)	CT (n=0)	C (n=978)	T (n=0)			CC (n=510)	TT (n=0)	CT (n=0)	C (n=1020)	T (n=0)		
Current smoking	Yes	105 (21.5)	0 (0)	0 (0)	210 (21.5)	0 (0)	-	-	130 (25.5)	0 (0)	0 (0)	260 (25.5)	0 (0)	-	-
	No	384 (78.5)	0 (0)	0 (0)	768 (78.5)	0 (0)			380 (74.5)	0 (0)	0 (0)	760 (74.5)	0 (0)		
Alcohol consumption	Yes	80 (16.4)	0 (0)	0 (0)	160 (16.4)	0 (0)	-	-	80 (15.7) §	0 (0)	0 (0)	160 (15.7) §	0 (0)	-	-
	No	409 (83.6)	0 (0)	0 (0)	818 (83.6)	0 (0)			429 (84.1)	0 (0)	0 (0)	858 (84.1)	0 (0)		
No physical activity	Yes	410 (83.8)	0 (0)	0 (0)	820 (83.8)	0 (0)	-	-	398 (78.0)	0 (0)	0 (0)	796 (78.0)	0 (0)	-	-
	No	79 (16.2)	0 (0)	0 (0)	158 (16.2)	0 (0)			112 (22.0)	0 (0)	0 (0)	224 (2.0)	0 (0)		
LPL HinfI		CC (n=372)	GG (n=29)	CG (n=88)	C (n=832)	G (n=146)			CC (n=400)	GG (n=24)	CG (n=86)	C (n=886)	G (n=134)		
Current smoking	Yes	87 (23.4)	4 (13.8)	14 (15.9)	188 (22.6)	22 (15.1)	0.18	0.98	112 (28.0)	4 (16.7)	14 (16.3)	238 (26.9)	22 (16.4)	*0.05*	0.90
	No	285 (76.6)	25 (86.2)	74 (84.1)	644 (77.4)	124 (84.9)			288 (72.0)	20 (83.3)	72 (83.7)	648 (73.1)	112 (83.6)		
Alcohol consumption	Yes	65 (17.5)	5 (17.2)	10 (11.4)	140 (16.8)	10 (6.8)	0.38	0.99	61 (15.3) §	3 (12.5)	16 (18.6)	138 (15.6) §	22 (16.4)	0.90	0.99
	No	307 (82.5)	24 (82.8)	78 (88.6)	692 (83.2)	126 (86.3)			338 (84.5)	21 (87.5)	70 (81.4)	746 (84.2)	112 (83.6)		
Physical activity	Yes	307 (82.5)	26 (89.7)	77 (87.5)	691 (83.1)	129 (88.4)	0.36	0.99	316 (79.0)	20 (83.3)	62 (72.1)	694 (78.3)	102 (76.1)	0.30	0.99
	No	65 (17.5)	3 (10.3)	11 (12.5)	141 (16.9)	17 (11.6)			84 (21.0)	4 (16.7)	24 (27.9)	192 (21.7)	32 (23.9)		
PON1 192Arg/Gln		AA (n=273)	GG (n=26)	AG (n=190)	A (n=736)	G (n=242)			AA (n=268)	GG (n=32)	AG (n=210)	A (n=746)	G (n=274)		
Current smoking	Yes	57 (20.9)	4 (15.4)	44 (23.2)	158 (21.5)	52 (21.5)	0.62	0.99	68 (25.4)	14 (43.8)	48 (22.9)	184 (24.7)	76 (27.7)	**0.04***	0.85
	No	216 (79.1)	22 (84.6)	146 (76.8)	578 (78.5)	190 (78.5)			200 (74.6)	18 (56.3)	162 (77.1)	562 (75.3)	198 (72.3)		
Alcohol consumption	Yes	46 (16.9)	3 (11.5)	31 (16.3)	123 (16.7)	37 (15.3)	0.78	0.99	39 (14.6) §	11 (34.4)	30 (14.3)	108 (14.5) §	52 (19.0)	**0.04***	0.85
	No	227 (83.2)	23 (88.5)	159 (83.7)	613 (83.3)	205 (84.7)			228 (85.1)	21 (65.6)	180 (85.7)	636 (85.3)	222 (81.0)		
Physical activity	Yes	230 (84.3)	21 (80.8)	159 (83.7)	619 (84.1)	201 (83.1)	0.90	0.99	210 (78.4)	21 (65.6)	167 (79.5)	587 (78.7)	209 (76.3)	0.21	0.97
	No	43 (15.8)	5 (19.2)	31 (16.3)	117 (15.9)	41 (16.9)			58 (21.6)	11 (34.4)	43 (20.5)	159 (21.3)	65 (23.7)		
CETP Taq1B		GG (n=151)	AA (n=114)	GA (n=224)	G (n=526)	G (n=452)			GG (n=160)	AA (n=109)	GA (n=241)	G (n=561)	A (n=459)		
Current smoking	Yes	29 (19.2)	25 (21.9)	51 (22.8)	109 (20.7)	101 (22.3)	0.71	0.99	39 (24.4)	31 (28.4)	60 (24.9)	138 (24.6)	122 (26.6)	0.72	0.99
	No	122 (80.8)	89 (78.1)	173 (77.2)	417 (79.3)	351 (77.7)			121 (75.6)	78 (71.6)	181 (75.1)	423 (75.4)	337 (73.4)		
Alcohol consumption	Yes	19 (12.6)	20 (17.5)	41 (18.3)	79 (15.0)	81 (17.9)	0.32	0.99	22 (13.8)	20 (18.4)	38 (15.8) §	82 (14.6) §	78 (17.0) §	0.71	0.99
	No	132 (87.4)	94 (82.5)	183 (81.7)	447 (85.0)	371 (82.1)			138 (86.3)	89 (81.7)	202 (83.8)	478 (85.2)	380 (82.8)		
Physical activity	Yes	130 (86.1)	92 (80.7)	188 (83.9)	448 (85.2)	372 (82.3)	0.50	0.99	123 (76.9)	89 (81.7)	186 (77.2)	432 (77.0)	364 (79.3)	0.59	0.99
	No	21 (13.9)	22 (19.3)	36 (16.1)	78 (14.8)	80 (17.7)			37 (23.1)	20 (18.4)	55 (23.1)	129 (23.0)	95 (20.7)		
ADP 45T>G		TT (n=364)	GG (n=5)	TG (n=120)	T (n=848)	G (n=130)			TT (n=381)	GG (n=13)	TG (n=116)	T (n=878)	G (n=142)		
Current smoking	Yes	81 (22.3)	0 (0)	24 (20.0)	186 (21.9)	24 (18.5)	0.44	0.99	94 (24.7)	6 (46.2)	30 (25.9)	218 (24.8)	42 (29.6)	0.22	0.97
	No	283 (77.8)	5 (100)	96 (80.0)	662 (78.1)	106 (81.5)			287 (75.3)	7 (53.9)	86 (74.1)	660 (75.2)	100 (70.4)		
Alcohol consumption	Yes	66 (18.1)	0 (0)	14 (11.7)	146 (17.2)	14 (10.8)	0.15	0.98	56 (14.7) §	3 (23.1)	21 (18.1)	133 (15.1) §	27 (19.0)	0.80	0.99
	No	298 (81.9)	5 (100)	106 (88.3)	702 (82.8)	116 (89.2)			324 (85.0)	10 (76.9)	95 (81.9)	743 (84.6)	115 (81.0)		
Physical activity	Yes	306 (84.1)	5 (100)	99 (82.5)	711 (83.8)	109 (83.8)	0.57	0.99	301 (79.0)	8 (61.5)	89 (76.7)	691 (78.7)	105 (73.9)	0.30	0.99
	No	58 (15.9)	0 (0)	21 (17.5)	137 (16.2)	21 (16.2)			80 (21.0)	5 (38.5)	27 (23.3)	187 (21.3)	37 (26.1)		
LEP 25CAG		AA (n=486)	GG (n=0)	AG (n=3)	A (n=975)	G (n=3)			AA (n=505)	GG (n=0)	AG (n=5)	A (n=1015)	G (n=5)		
Current smoking	Yes	105 (21.6)	0 (0)	0 (0)	210 (21.5)	0 (0)	0.36	0.99	129 (25.5)	0 (0)	1 (20.0)	259 (25.5)	1 (20.0)	0.78	0.99
	No	381 (78.4)	0 (0)	3 (100)	765 (78.5)	3 (100)			376 (74.5)	0 (0)	4 (80.0)	756 (74.5)	4 (80.0)		
Alcohol consumption	Yes	80 (16.5)	0 (0)	0 (0)	160 (16.4)	0 (0)	0.44	0.99	80 (15.8)	0 (0)	0 (0) §	160 (15.8) §	0 (0) §	**0.01***	0.78
	No	406 (83.5)	0 (0)	3 (100)	815 (83.6)	3 (100)			425 (84.2)	0 (0)	4 (80.0)	854 (84.1)	4 (80.0)		
Physical activity	Yes	407 (83.7)	0 (0)	3 (100)	817 (83.8)	3 (100)	0.47	0.99	393 (77.8)	0 (0)	5 (100)	791 (77.9)	5 (100)	0.23	0.97
	No	79 (16.3)	0 (0)	0 (0)	158 (16.2)	0 (0)			112 (22.2)	0 (0)	0 (0)	224 (22.1)	0 (0)		

Key: **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis (All data are represented as prevalence rates with percentages in parenthesis. Comparison of the groups was done using Chi-square (x^2) test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if p<0.05 (bold). Marginal associations showed in italics)

§ One participant carrying the genotype did not disclose smoking status, alcohol consumption and physical activities performed, subsequently influencing allele counts

(None of the studied polymorphisms increased the risk for the MS when associated with smoking, alcohol consumption and physical activity. PON1 192 Arg/Gln [heterozygous (AG) genotypes and A alleles] in nonsmokers was protective against the MS. The PON1 192 Arg/Gln [heterozygous (AG) genotypes and A alleles] and LEP 25CAG [homozygous (AA) genotypes and A alleles] in non alcohol consumers was protective against the MS)

5.9.5 Gene polymorphisms associated with insulin resistance

Gene polymorphisms associated with IR in participants with and with no MS (as defined by the IDF and harmonized)

Considering IR was relatively high in females (as compared to males) with the MS (IDF: 78.4% vs 69.9%; harmonized: 78.3% vs 69.2%), we analyzed their genotypic/allelic frequencies for the studied polymorphisms using HOMA IR values >2.6 across gender groups (with and with no MS). We observed the distribution of the genotypes/ alleles of the studied polymorphisms in females and males diagnosed with and with no MS to vary, however with no significant statistical differences detected (IDF and harmonized) (Table XXXI).

These findings indicate that there is no interaction between the studied polymorphisms, IR and the MS in females and males in this cohort, irrespective of the definition used for diagnosis.

TABLE XXXI: FREQUENCIES OF POLYMORPHISMS IN IR FEMALES AND MALES WITH AND WITH NO MS (IDF AND HARMONIZED)

SNP	GENO/ ALLELE	MS n (%)						NO MS n (%)					
		FEMALES		MALES		P		FEMALES		MALES		P	
		IDF	HARM	IDF	HARM	IDF ∞ ‡	HARM ∞ ‡	IDF	HARM	IDF	HARM	IDF ∞ ‡	HARM ∞ ‡
APOA5	CC	316 (100)	299 (100)	79 (100)	74 (100)	-	-	139 (100)	156 (100)	27 (100)	32 (100)	-	-
	TT	0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)		
Q139X	CT	0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)		
	C	632 (100)	598 (100)	158 (100)	148 (100)			278 (100)	312 (100)	54 (100)	64 (100)		
	T	0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)		
LPL	CC	237 (75.0)	224 (74.9)	61 (77.2)	57 (77.0)	0.83	0.88	109 (78.4)	122 (78.2)	17 (63.0)	21 (65.6)	0.22	0.32
	GG	17 (5.4)	16 (5.4)	3 (3.8)	3 (4.1)	0.99	0.99	5 (3.6)	6 (3.9)	2 (7.4)	2 (6.3)	0.97	0.99
HinfI	CG	62 (19.6)	59 (19.7)	15 (19.0)	14 (18.9)			25 (18.0)	28 (18.0)	8 (29.6)	9 (28.1)		
	C	536 (84.8)	507 (84.8)	137 (86.7)	128 (86.5)			243 (87.4)	272 (43.6)	42 (77.8)	51 (79.7)		
	G	96 (15.2)	91 (15.2)	21 (13.3)	20 (13.5)			35 (12.6)	40 (6.4)	12 (22.2)	13 (20.3)		
PON1	AA	172 (54.4)	165 (55.2)	42 (53.2)	38 (51.4)	0.87	0.74	80 (57.6)	87 (55.8)	15 (55.6)	19 (59.4)	0.59	0.62
	GG	20 (6.3)	19 (6.4)	4 (5.1)	4 (5.4)	0.99	0.99	8 (5.8)	9 (5.8)	3 (11.1)	3 (9.4)	0.99	0.99
192Arg/Gln	AG	124 (39.2)	115 (38.5)	33 (41.8)	32 (43.2)			51 (36.7)	60 (38.5)	9 (33.3)	10 (31.3)		
	A	468 (74.1)	445 (74.4)	117 (74.1)	108 (73.0)			211 (75.9)	234 (75.0)	39 (72.2)	48 (75.0)		
	G	164 (25.9)	153 (25.6)	41 (25.9)	40 (27.0)			67 (24.1)	78 (25.0)	15 (27.8)	16 (25.0)		
CETP	GG	94 (29.8)	90 (30.1)	21 (26.6)	20 (27.0)	0.77	0.87	48 (34.5)	52 (33.3)	8 (29.6)	9 (28.1)	0.87	0.62
	AA	80 (25.3)	75 (25.1)	19 (24.1)	19 (25.7)	0.99	0.99	26 (18.7)	31 (19.9)	5 (18.5)	5 (15.6)	0.99	0.99
Taq1B	GA	142 (44.9)	134 (44.8)	39 (49.4)	35 (47.3)			65 (46.8)	73 (46.8)	14 (51.9)	18 (56.3)		
	G	330 (52.2)	314 (52.5)	81 (51.3)	75 (50.7)			161 (57.9)	177 (56.7)	30 (55.6)	36 (56.3)		
	A	302 (47.8)	284 (47.5)	77 (48.7)	73 (49.3)			117 (42.1)	135 (43.3)	24 (44.4)	28 (43.8)		
ADP	TT	237 (75.0)	222 (74.3)	63 (79.8)	59 (79.7)	0.52	0.48	98 (70.5)	113 (72.4)	20 (74.1)	24 (75.0)	0.66	0.66
	GG	3 (1.0)	3 (1.0)	0 (0)	0 (0)	0.99	0.99	4 (2.9)	4 (2.6)	0 (0)	0 (0)	0.99	0.99
45T>G	TG	76 (24.1)	74 (24.8)	16 (20.3)	15 (20.3)			37 (26.6)	39 (25.0)	7 (25.9)	8 (25.0)		
	T	550 (87.0)	518 (86.6)	142 (89.9)	133 (89.9)			233 (83.8)	265 (84.9)	47 (87.0)	56 (87.5)		
	G	82 (13.0)	80 (13.4)	16 (10.1)	15 (10.1)			45 (16.2)	47 (15.1)	7 (13.0)	8 (12.5)		
LEP	AA	313 (99.1)	296 (99.0)	79 (100)	74 (100)	0.39	0.39	136 (97.8)	153 (98.1)	26 (96.3)	31 (96.9)	0.63	0.67
	GG	0 (0)	0 (0)	0 (0)	0 (0)	0.99	0.99	0 (0)	0 (0)	0 (0)	0 (0)	0.99	0.99
25CAG	AG	3 (1.0)	3 (1.0)	0 (0)	0 (0)			3 (2.2)	3 (1.9)	1 (3.7)	1 (3.1)		
	A	629 (99.5)	595 (99.5)	158 (100)	148 (100)			275 (98.9)	309 (99.0)	53 (98.1)	63 (98.4)		
	G	3 (0.5)	3 (0.5)	0 (0)	0 (0)			3 (1.1)	3 (1.0)	1 (1.9)	1 (1.6)		

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome ; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **IDF**- international diabetes federation definition for the metabolic syndrome; **HARM**- harmonized definition for the metabolic syndrome; **SNP**- single nucleotide polymorphism; **GENO**- genotype; ∞- unadjusted p value; ‡- adjusted p value
(Data are presented as prevalence rates with percentages in parenthesis. Comparison between groups was done using Chi-squared (χ^2) for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$))

(The distribution of the studied polymorphisms by gender shows no significant differences in genotype/allele frequencies between IR participants diagnosed with and with no MS)

Gene polymorphisms associated with metabolic risk factors in IR participants with and with no MS (as defined by the IDF and harmonized)

Considering we did not observe any association between IR and the MS across gender groups (Table XXXI), we attempted to assess if there was any interaction with the individual metabolic risk factors of the studied gene polymorphisms in IR participants diagnosed with and with no MS (as per IDF and harmonized definitions) (Table XXXII, XXXIII).

ANOVA detected associations with high systolic blood pressure in IR participants carrying the human paraoxonase 1 192Arg/Gln [homozygous wild type (AA) genotypes] polymorphism, when the harmonized definition was applied (P=0.04) (Table XXXIII). Interestingly, among IR participants with no MS, the lipoprotein lipase HinfI [mutant homozygous (GG) genotypes] appeared to be protective against high systolic blood pressure (IDF: P=0.01; harmonized: P=0.02) (Table XXXII, XXXIII). Similarly, the cholesteryl ester transfer protein Taq1B [heterozygous (GA) genotypes] was protective against high diastolic blood pressure when the IDF definition was applied (IDF: P=0.03) (Table XXXII) and high systolic blood pressure when the harmonized definition was used (harmonized: P=0.03) (Table XXXIII).

When we analyzed gender groups (Table XXXIV-XXXVII), we observed considerably higher means for waist circumference, systolic and diastolic blood pressure, blood glucose and triglycerides, with reduced HDL-C, in IR participants with the MS (as compared to those with no MS) (IDF and harmonized), when associated with the gene polymorphisms. IR males diagnosed with the MS carrying the lipoprotein lipase HinfI [mutant

homozygous (GG) genotypes] were more inclined to have elevated systolic (IDF: P=0.02; harmonized: P=0.02) and diastolic blood pressure (IDF: P=0.02; harmonized: P=0.02) (Table XXXIV, XXXV). Similarly, we observed an association between the human paraoxonase 1 192Arg/Gln polymorphism with high systolic [IDF: homozygous wild type (AA) genotypes, P=0.01; harmonized: homozygous wild type (AA) genotypes, P=0.00] and diastolic [IDF: heterozygous (AG) genotypes, P=0.02; harmonized: homozygous wild type (AA) genotypes, P=0.02] blood pressure in IR males with the MS (Table XXXIV, XXXV). In addition, IR males with the MS carrying the adiponectin 45T>G [homozygous wild type (TT) genotypes] were more inclined to have reduced HDL-C levels (P=0.00) when the harmonized definition was applied (Table XXXV). However, when the IDF definition was applied, a marginal association was observed between the adiponectin 45T>G [homozygous wild type (TT) genotypes] with high systolic blood pressure (P=0.05) in IR males with the MS (Table XXXIV). Interestingly, when the harmonized definition was applied, the adiponectin 45T>G [heterozygous (TG) genotypes] polymorphism appeared to be protective against high systolic blood pressure (P=0.02) in IR males with no MS (Table XXXV).

Female participants, on the other hand, did not show any association between the gene polymorphisms with the individual metabolic risk factors, when the IDF and harmonized definitions was applied (Table XXXVI, XXXVII). We did, however, observe female participants with no MS carrying the LPL HinfI [homozygous mutant (GG) genotypes] to be more inclined to lower systolic pressure readings, but only when the IDF definition was applied (P=0.04) (Table XXXVI), suggesting a protective influence against high systolic blood

pressure and the MS. Similarly, the harmonized definition when applied in IR females with no MS showed cholesteryl ester transfer protein Taq1B [heterozygous (GA) genotypes] carriers to be more inclined to lower systolic blood pressure readings (P=0.04) (Table XXXVII), further indicating a protective influence against the risk for the MS.

Overall, these findings suggest that interaction of the studied gene polymorphisms with the individual metabolic risk factors in IR participants is largely influenced by gender and the definition used for diagnosis of the MS.

TABLE XXXII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN IR PARTICIPANTS WITH AND WITH NO MS (IDF)*

SNP/ METABOLIC RISK FACTORS	MS					NO MS				
	WTH GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	HET GENOTYPE MEAN±SD	P VALUE		WTH GENOTYPE MEAN±SD	MH GENOTYPE MEAN±SD	HET GENOTYPE MEAN±SD	P VALUE	
				∞	‡				∞	‡
APOA5 Q139X										
Waist circumference (cm)	102.5±12.5	-	-	-	-	97.5±15.4	-	-	-	-
Systolic pressure (mmHg)	136.6±22.0	-	-	-	-	119.6±21.2	-	-	-	-
Diastolic pressure (mmHg)	84.6±11.2	-	-	-	-	76.2±10.0	-	-	-	-
Blood glucose (mmol/l)	7.7±3.3	-	-	-	-	5.4±2.0	-	-	-	-
Triglycerides (mmol/l)	2.4±1.4	-	-	-	-	1.3±0.8	-	-	-	-
HDL-C (mmol/l)	1.3±0.9	-	-	-	-	1.5±0.8	-	-	-	-
LPL HinfI										
Waist circumference (cm)	102.5±11.8	101.9±8.3	102.5±15.8	0.97	1.00	97.7±15.7	106.6±15.8	94.7±13.7	0.12	0.97
Systolic pressure (mmHg)	137.5±22.5	141.2±28.5	131.8±17.0	0.08	0.97	117.9±20.0	106.9±30.8	128.9±21.1	**0.01***	0.78
Diastolic pressure (mmHg)	84.4±11.4	89.9±11.2	83.6±10.2	0.08	0.97	75.7±10.0	77.0±10.4	77.5±10.3	0.64	0.99
Blood glucose (mmol/l)	7.9±5.2	7.7±2.9	7.8±3.8	0.98	1.00	5.4±2.1	6.1±2.8	5.3±1.7	0.68	0.99
Triglycerides (mmol/l)	2.4±1.4	2.6±1.6	2.2±1.0	0.22	0.97	1.4±0.9	1.1±0.2	1.2±0.5	0.55	0.99
HDL-C (mmol/l)	1.5±4.5	1.5±1.5	1.2±0.3	0.81	0.99	1.5±0.9	1.3±0.1	1.4±0.3	0.68	0.99
PON1 192Arg/Gln										
Waist circumference (cm)	102.8±11.3	104.4±11.4	101.7±14.0	0.51	0.99	98.5±15.7	93.7±18.9	96.4±14.2	0.50	0.99
Systolic pressure (mmHg)	137.3±21.5	126.6±27.8	137.1±21.4	0.07	0.95	118.0±22.8	127.2±12.8	120.8±19.8	0.34	0.99
Diastolic pressure (mmHg)	84.9±11.6	81.2±10.1	84.6±10.8	0.31	0.99	76.2±9.7	74.6±9.2	76.3±10.8	0.87	0.99
Blood glucose (mmol/l)	7.7±3.5	8.8±3.6	7.9±6.3	0.56	0.99	5.5±2.2	6.0±3.0	5.2±1.6	0.41	0.99
Triglycerides (mmol/l)	2.4±1.4	2.1±0.8	2.4±1.4	0.52	0.99	1.4±1.0	1.3±0.3	1.3±0.5	0.68	0.99
HDL-C (mmol/l)	1.6±5.3	1.2±0.2	1.3±0.8	0.59	0.99	1.5±1.0	1.4±0.3	1.4±0.3	0.42	0.99
CETP Taq1B										
Waist circumference (cm)	102.8±11.3	101.0±10.6	103.1±14.0	0.40	0.99	100.4±13.9	95.3±15.4	96.2±16.2	0.21	0.97
Systolic pressure (mmHg)	137.6±21.3	134.6±21.8	137.0±22.6	0.58	0.99	120.7±24.5	126.1±22.1	116.3±17.7	0.08	0.97
Diastolic pressure (mmHg)	85.5±11.3	84.9±10.8	83.8±11.4	0.41	0.99	78.0±11.7	78.3±8.4	74.1±9.0	**0.03***	0.85
Blood glucose (mmol/l)	7.9±3.6	8.6±7.8	7.4±3.0	0.15	0.97	5.4±1.7	5.4±2.2	5.4±2.2	0.99	1.00
Triglycerides (mmol/l)	2.2±1.2	2.5±1.6	2.4±1.3	0.39	0.99	1.3±0.4	1.4±1.2	1.3±0.9	0.85	0.99
HDL-C (mmol/l)	1.2±0.7	2.1±7.7	1.2±0.9	0.17	0.97	1.4±0.3	1.6±1.2	1.5±0.8	0.50	0.99
ADP 45T>G										
Waist circumference (cm)	102.5±13.0	104.4±3.1	102.5±10.9	0.96	1.00	98.1±15.4	96.8±15.6	95.9±15.6	0.73	0.99
Systolic pressure (mmHg)	137.3±20.4	148.0±6.1	133.9±26.8	0.29	0.99	121.3±22.7	117.0±16.0	115.4±17.0	0.29	0.99
Diastolic pressure (mmHg)	84.8±10.5	88.3±2.1	83.7±13.4	0.62	0.99	77.1±10.3	71.0±7.8	74.2±9.1	0.16	0.97
Blood glucose (mmol/l)	7.9±5.2	6.3±1.7	7.8±3.2	0.86	0.99	5.4±1.6	4.9±0.7	5.4±3.0	0.89	0.99
Triglycerides (mmol/l)	2.4±1.4	2.2±1.7	2.3±1.2	0.94	1.00	1.3±0.7	1.0±0.7	1..5±1.1	0.38	0.99
HDL-C (mmol/l)	1.3±1.0	1.0±0.3	2.0±7.9	0.32	0.99	1.5±0.6	1.3±0.3	1.5±1.0	0.85	0.99
LEP 25CAG										
Waist circumference (cm)	102.4±12.5	-	109.6±14.8	0.32	0.99	97.4±15.5	-	97.9±6.9	0.96	1.00
Systolic pressure (mmHg)	136.5±22.0	-	153.7±3.8	0.18	0.97	119.7±21.5	-	118.8±9.7	0.93	1.00
Diastolic pressure (mmHg)	84.5±11.3	-	86.0±8.0	0.82	0.99	76.1±10.1	-	77.0±3.8	0.87	0.99
Blood glucose (mmol/l)	7.9±4.8	-	7.0±1.9	0.75	0.99	5.4±2.1	-	5.0±0.0	0.72	0.99
Triglycerides (mmol/l)	2.4±1.4	-	1.7±0.2	0.42	0.99	1.3±0.8	-	1.3±0.4	0.95	1.00
HDL-C (mmol/l)	1.5±3.9	-	1.3±0.2	0.94	1.00	1.5±0.8	-	1.2±0.3	0.50	0.99

Key: **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis (All data are represented as mean±SD values. Comparison of the groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if p<0.05 (bold).

(None of the studied polymorphisms were associated with the individual metabolic risk factors in IR participants with MS; however, in participants with no MS, the LPL HinfI [mutant homozygous (GG) genotypes] and CETP Taq1B [heterozygous (GA) genotypes] was protective against high systolic and diastolic blood pressure readings, respectively)

Gene polymorphisms associated with other risk factors (smoking, alcohol consumption, physical activity) in IR participants with and with no MS (as defined by the IDF and harmonized)

We further analyzed the interaction of smoking, alcohol intake and physical activities performed with the studied polymorphisms and the risk for the MS (as per IDF and harmonized definitions) in IR participants. We again, classified genotypes/ alleles according to smoking/ nonsmoking status, alcohol consumption/ no alcohol intake, physical activities performed/ no physical activities and accordingly assessed their frequencies, as illustrated in Tables XXXVIII, XXXIX. The results demonstrate that the studied polymorphisms among IR smokers, alcohol consumers and IR participants who did not perform physical activities did not increase the risk for the MS in this cohort.

Interestingly, at the adiponectin locus there was association between the 45T>G polymorphism [homozygous wild type (TT) genotypes and T alleles] with physical activities performed in IR participants; and hence appear to be protective against the risk for MS (IDF: P=0.04; harmonized: P=0.03) (Table XXXVIII, XXXIX). We further identified a protective effect between the risk for the MS in IR participants who did not consume alcohol and carried the Leptin 25CAG [homozygous wild type (AA) genotypes and A alleles] (IDF: P=0.00; harmonized: P=0.00) (Table XXXVIII, XXXIX).

Overall, these findings suggest that an association between physical activities and the adiponectin 45T>G polymorphism is protective against the risk for MS, irrespective of the definition used for diagnosis. IR participants who did not

consume alcohol and carried the leptin 25CAG polymorphism are protected against the MS, using the IDF and harmonized definitions.

TABLE XXXIII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN IR PARTICIPANTS WITH AND WITH NO MS (HARMONIZED)*

SNP/ METABOLIC RISK FACTORS	**MS**					**NO MS**				
	WTH GENOTYPE MEAN±SD	**MH GENOTYPE MEAN±SD**	**HET GENOTYPE MEAN±SD**	**P VALUE**		**WTH GENOTYPE MEAN±SD**	**MH GENOTYPE MEAN±SD**	**HET GENOTYPE MEAN±SD**	**P VALUE**	
				∞	‡				∞	‡
APOA5 Q139X										
Waist circumference (cm)	102.5±12.5	-	-	-	-	98.0±15.0	-	-	-	-
Systolic pressure (mmHg)	136.9±21.8	-	-	-	-	121.1±22.1	-	-	-	-
Diastolic pressure (mmHg)	84.6±11.3	-	-	-	-	77.1±10.4	-	-	-	-
Blood glucose (mmol/l)	7.8±3.4	-	-	-	-	5.4±1.9	-	-	-	-
Triglycerides (mmol/l)	2.4±1.4	-	-	-	-	1.4±0.9	-	-	-	-
HDL-C (mmol/l)	1.2±0.7	-	-	-	-	1.5±1.0	-	-	-	-
LPL HinfI										
Waist circumference (cm)	102.5±11.9	101.9±8.5	102.8±15.6	0.96	1.00	98.3±15.2	106.0±14.8	94.9±14.2	0.14	0.97
Systolic pressure (mmHg)	137.8±22.1	142.2±29.0	131.8±17.4	*0.06**	0.92	119.7±21.7	108.9±29.1	129.2±20.2	**0.02***	0.78
Diastolic pressure (mmHg)	84.4±11.5	90.3±11.3	83.5±10.3	*0.06**	0.92	76.7±10.4	77.5±9.7	78.4±10.4	0.67	0.99
Blood glucose (mmol/l)	8.0±5.3	7.8±2.9	7.9±3.9	0.98	1.00	5.4±2.0	6.0±2.6	5.4±1.6	0.66	0.99
Triglycerides (mmol/l)	2.5±1.4	2.7±1.6	2.2±1.0	0.22	0.97	1.4±1.0	1.2±0.4	1.3±0.5	0.68	0.99
HDL-C (mmol/l)	1.5±4.6	1.5±1.5	1.2±0.3	0.85	0.99	1.6±1.1	1.3±0.1	1.4±0.3	0.53	0.99
PON1 192Arg/Gln										
Waist circumference (cm)	102.6±11.3	104.8±11.5	102.0±14.2	0.60	0.99	99.4±15.5	93.9±18.0	96.6±13.8	0.30	0.99
Systolic pressure (mmHg)	137.7±21.4	125.9±28.2	137.4±20.9	**0.04***	0.85	119.3±23.2	128.6±13.1	122.5±21.5	0.31	0.99
Diastolic pressure (mmHg)	85.0±11.8	81.4±10.3	84.5±10.7	0.34	0.99	76.9±9.7	74.8±8.8	77.6±11.6	0.68	0.99
Blood glucose (mmol/l)	7.8±3.6	8.9±3.6	8.1±6.5	0.55	0.99	5.5±2.1	6.0±2.9	5.3±1.5	0.45	0.99
Triglycerides (mmol/l)	2.4±1.4	2.1±0.8	2.4±1.3	0.55	0.99	1.4±1.0	1.3±0.3	1.4±0.9	0.92	1.00
HDL-C (mmol/l)	1.6±5.4	1.1±0.2	1.2±0.6	0.55	0.99	1.6±1.1	1.4±0.3	1.5±0.8	0.85	0.99
CETP Taq1B										
Waist circumference (cm)	103.0±11.0	101.2±10.6	102.9±14.3	0.52	0.99	100.1±14.1	95.6±14.6	97.6±15.8	0.33	0.99
Systolic pressure (mmHg)	137.6±21.5	134.2±21.3	137.9±22.2	0.38	0.99	122.1±24.3	128.6±24.1	117.5±18.9	**0.03***	0.85
Diastolic pressure (mmHg)	85.0±11.5	84.8±10.6	83.8±11.5	0.41	0.99	78.4±11.4	79.3±9.9	75.3±9.5	0.07	0.95
Blood glucose (mmol/l)	7.9±3.7	8.8±7.9	7.5±3.1	0.16	0.97	5.4±1.6	5.5±2.0	5.4±2.1	0.98	1.00
Triglycerides (mmol/l)	2.3±1.2	2.5±1.6	2.5±1.3	0.35	0.99	1.3±0.4	1.4±1.1	1.4±1.1	0.85	0.99
HDL-C (mmol/l)	1.2±0.7	2.1±7.9	1.2±0.6	0.14	0.97	1.4±0.3	1.6±1.1	1.6±1.2	0.41	0.99
ADP 45T>G										
Waist circumference (cm)	102.5±13.0	104.4±3.1	102.6±11.1	0.96	1.00	98.7±15.1	96.8±15.6	96.2±15.1	0.61	0.99
Systolic pressure (mmHg)	137.5±20.1	148.0±6.1	134.5±26.6	0.36	0.99	123.1±23.3	117.0±16.0	115.4±17.9	0.12	0.97
Diastolic pressure (mmHg)	84.8±10.5	88.3±2.1	83.7±13.6	0.61	0.99	78.0±10.7	71.0±7.8	74.8±9.2	0.09	0.97
Blood glucose (mmol/l)	8.0±5.4	6.3±1.7	7.9±3.3	0.83	0.99	5.5±1.5	4.9±0.7	5.5±2.9	0.87	0.99
Triglycerides (mmol/l)	2.4±1.4	2.2±1.7	2.4±1.2	0.89	0.99	1.3±0.9	1.0±0.7	1.5±1.1	0.55	0.99
HDL-C (mmol/l)	1.2±0.8	1.0±0.3	2.0±8.0	0.28	0.99	1.5±1.0	1.3±0.3	1.5±1.0	0.77	0.99
LEP 25CAG										
Waist circumference (cm)	102.4±12.5	-	109.6±14.8	0.33	0.99	98.0±15.2	-	97.9±6.9	0.98	1.00
Systolic pressure (mmHg)	136.7±21.8	-	153.7±3.8	0.18	0.97	121.1±22.3	-	118.8±9.7	0.83	0.99
Diastolic pressure (mmHg)	84.6±11.3	-	86.0±8.0	0.83	0.99	77.1±10.5	-	77.0±3.8	0.99	1.00
Blood glucose (mmol/l)	8.0±5.0	-	7.0±1.9	0.73	0.99	5.5±1.9	-	5.0±0.0	0.66	0.99
Triglycerides (mmol/l)	2.4±1.4	-	1.7±0.2	0.38	0.99	1.4±0.9	-	1.3±0.4	0.88	0.99
HDL-C (mmol/l)	1.4±4.0	-	1.3±0.2	0.96	1.00	1.5±1.0	-	1.2±0.3	0.50	0.99

Key: **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(All data are represented as mean±SD values. Comparison of the groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if p<0.05 (bold). P values in italics demonstrate marginal associations)

(The PON1 192Arg/Gln [homozygous wild type (AA) genotypes] was associated with high systolic blood pressure readings in IR participants with the MS. In IR participants with no MS, LPL HinfI [mutant homozygous (GG) genotypes] and CETP Taq1B [heterozygous (GA) genotypes] was protective against high systolic and diastolic blood pressure, respectively)

TABLE XXXIV: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN IR MALES WITH AND WITH NO MS (IDF)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	100.9±10.2	-	-	-	-	91.2±15.7	-	-	-	-
	Systolic pressure (mmHg)	136.8±22.8	-	-	-	-	124.5±20.5	-	-	-	-
Q139X	Diastolic pressure (mmHg)	87.3±11.4	-	-	-	-	76.9±10.7	-	-	-	-
	Blood glucose (mmol/l)	7.3±2.7	-	-	-	-	6.0±3.5	-	-	-	-
	Triglycerides (mmol/l)	2.6±1.2	-	-	-	-	1.5±1.3	-	-	-	-
	HDL-C (mmol/l)	1.1±0.8	-	-	-	-	1.6±1.3	-	-	-	-
LPL	Waist circumference (cm)	100.1±9.5	110.5±9.9	102.2±12.2	0.19	0.97	92.5±16.7	97.8±29.1	86.9±11.1	0.60	0.99
	Systolic pressure (mmHg)	137.6±21.8	165.3±11.2	127.6±24.0	**0.02***	0.78	123.8±19.9	101.5±34.6	131.8±16.3	0.17	0.97
HinfI	Diastolic pressure (mmHg)	87.2±11.3	104.3±1.5	84.6±9.8	**0.02***	0.78	77.2±12.2	73.5±4.9	77.1±9.0	0.91	1.00
	Blood glucose (mmol/l)	7.5±2.8	5.8±1.1	6.8±2.4	0.45	0.99	6.3±4.0	8.5±5.0	4.8±0.4	0.34	0.99
	Triglycerides (mmol/l)	2.6±1.2	2.3±1.5	2.6±1.0	0.91	1.00	1.7±1.6	1.0±0.1	1.3±0.6	0.62	0.99
	HDL-C (mmol/l)	1.1±0.9	1.1±0.2	1.0±0.2	0.93	1.00	1.7±1.6	1.2±0.1	1.4±0.2	0.76	0.99
PON1	Waist circumference (cm)	99.5±10.3	104.7±14.6	102.2±9.5	0.39	0.99	92.2±16.0	88.7±16.1	90.3±17.0	0.92	1.00
	Systolic pressure (mmHg)	142.6±21.0	110.8±26.2	132.5±22.1	**0.01***	0.78	119.5±22.9	127.3±20.6	132.0±14.8	0.35	0.99
192Arg/Gln	Diastolic pressure (mmHg)	88.1±11.6	71.8±8.3	88.2±10.2	**0.02***	0.78	76.8±10.9	68.7±5.5	79.8±11.2	0.31	0.99
	Blood glucose (mmol/l)	6.8±2.5	9.0±3.4	7.7±2.8	0.16	0.97	6.9±4.5	5.0±0.8	4.9±0.4	0.37	0.99
	Triglycerides (mmol/l)	2.5±1.2	2.5±0.5	2.8±1.3	0.59	0.99	1.8±1.7	1.1±0.4	1.2±0.4	0.44	0.99
	HDL-C (mmol/l)	1.2±1.1	1.1±0.2	1.0±0.2	0.49	0.99	1.7±1.7	1.5±0.7	1.3±0.3	0.71	0.99
CETP	Waist circumference (cm)	102.3±8.6	96.8±10.0	102.1±10.7	0.14	0.97	98.8±18.2	96.0±7.1	85.1±14.7	0.12	0.97
	Systolic pressure (mmHg)	113.8±26.1	134.5±19.9	139.5±22.5	0.59	0.99	121.8±26.6	134.8±13.6	122.4±18.7	0.48	0.99
Taq1B	Diastolic pressure (mmHg)	86.1±11.2	86.3±10.4	88.5±12.0	0.69	0.99	78.6±11.4	80.6±7.7	74.6±11.4	0.50	0.99
	Blood glucose (mmol/l)	7.6±3.4	7.6±2.8	7.0±2.2	0.59	0.99	6.5±2.7	4.8±0.4	6.2±4.4	0.69	0.99
	Triglycerides (mmol/l)	2.4±1.1	3.0±1.5	2.5±1.0	0.19	0.97	1.4±0.6	2.6±2.9	1.3±0.5	0.15	0.97
	HDL-C (mmol/l)	1.0±0.2	1.1±0.3	1.2±1.1	0.83	0.99	1.2±0.2	2.7±2.8	1.3±0.2	*0.06**	0.92
ADP	Waist circumference (cm)	100.7±10.3	-	101.5±9.8	0.78	0.99	92.5±14.3	-	87.4±20.0	0.46	0.99
	Systolic pressure (mmHg)	139.3±20.6	-	126.9±28.5	*0.05**	0.90	127.7±21.5	-	115.6±15.1	0.18	0.97
45T>G	Diastolic pressure (mmHg)	87.9±10.9	-	85.0±13.2	0.36	0.99	78.9±10.6	-	71.3±9.8	0.11	0.97
	Blood glucose (mmol/l)	7.4±2.9	-	6.8±1.9	0.37	0.99	5.3±1.6	-	7.9±6.1	0.09	0.97
	Triglycerides (mmol/l)	2.5±1.2	-	2.9±1.1	0.36	0.99	1.6±1.5	-	1.4±0.7	0.82	0.99
	HDL-C (mmol/l)	1.1±0.9	-	1.1±0.2	0.79	0.99	1.7±1.4	-	1.2±0.3	0.45	0.99
LEP	Waist circumference (cm)	100.9±10.2	-	-	-	-	91.0±16.0	-	96.0±0.0	0.76	0.99
	Systolic pressure (mmHg)	136.8±22.8	-	-	-	-	124.5±20.9	-	125.0±0.0	0.98	1.00
25CAG	Diastolic pressure (mmHg)	87.3±11.4	-	-	-	-	76.7±10.9	-	82.0±0.0	0.64	0.99
	Blood glucose (mmol/l)	7.3±2.7	-	-	-	-	6.1±3.5	-	5.0±0.0	0.77	0.99
	Triglycerides (mmol/l)	2.6±1.2	-	-	-	-	1.5±1.3	-	1.7±0.0	0.90	0.99
	HDL-C (mmol/l)	1.1±0.8	-	-	-	-	1.6±1.3	-	1.1±0.0	0.72	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$) (bold). P values in italics demonstrate marginal associations)

(The LPL HinfI [mutant homozygous (GG) genotypes] was associated with high systolic and diastolic blood pressure; and the PON1 192 Arg/Gln polymorphism with high systolic [homozygous wild type (AA) genotypes] and diastolic [heterozygous (AG) genotypes] blood pressure in IR males with the MS)

TABLE XXXV: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN IR MALE PARTICIPANTS WITH AND NO MS (HARMONIZED)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	100.5±10.0	-	-	-	-	93.6±16.1	-	-	-	-
	Systolic pressure (mmHg)	137.4±22.4	-	-	-	-	124.9±21.5	-	-	-	-
Q139X	Diastolic pressure (mmHg)	87.3±11.5	-	-	-	-	78.7±11.4	-	-	-	-
	Blood glucose (mmol/l)	7.4±2.8	-	-	-	-	6.0±3.2	-	-	-	-
	Triglycerides (mmol/l)	2.7±1.2	-	-	-	-	1.6±1.3	-	-	-	-
	HDL-C (mmol/l)	1.0±0.2	-	-	-	-	1.7±1.6	-	-	-	-
LPL	Waist circumference (cm)	99.9±9.5	110.5±9.9	100.7±11.3	0.20	0.97	94.5±16.1	97.8±29.1	90.8±15.6	0.80	0.99
	Systolic pressure (mmHg)	138.6±20.9	165.3±11.2	126.6±24.6	**0.02***	0.78	123.8±21.8	101.5±34.6	132.8±15.6	0.16	0.97
HinfI	Diastolic pressure (mmHg)	87.1±11.4	104.3±1.5	84.3±10.1	**0.02***	0.78	79.3±12.7	73.5±4.9	78.4±9.3	0.80	0.99
	Blood glucose (mmol/l)	7.6±2.9	5.8±1.1	6.9±2.5	0.44	0.99	6.3±3.6	8.5±5.0	4.9±0.6	0.29	0.99
	Triglycerides (mmol/l)	2.7±1.2	2.3±1.5	2.6±1.0	0.87	0.99	1.7±1.5	1.0±1.1	1.3±0.5	0.59	0.99
	HDL-C (mmol/l)	1.0±0.2	1.1±0.2	1.0±0.2	0.75	0.99	1.9±2.0	1.2±0.1	1.3±1.2	0.63	0.99
PON1	Waist circumference (cm)	98.6±9.6	104.7±14.6	102.2±9.7	0.22	0.97	95.6±16.6	88.7±16.1	91.4±16.4	0.70	0.99
	Systolic pressure (mmHg)	144.8±18.9	110.8±26.2	132.0±22.3	**0.00***	**0.00**	119.9±23.9	127.3±20.6	133.6±14.9	0.27	0.99
192Arg/Gln	Diastolic pressure (mmHg)	88.5±12.0	71.8±8.3	87.7±9.9	**0.02***	0.78	78.4±10.4	68.7±5.5	82.3±13.2	0.19	0.97
	Blood glucose (mmol/l)	6.9±2.6	9.0±3.4	7.7±2.8	0.22	0.97	6.7±4.0	5.0±0.8	5.0±0.5	0.36	0.99
	Triglycerides (mmol/l)	2.6±1.2	2.5±0.5	2.8±1.3	0.63	0.99	1.8±1.6	1.1±0.4	1.2±0.4	0.36	0.99
	HDL-C (mmol/l)	1.0±0.2	1.1±0.2	1.0±0.2	0.22	0.97	2.0±2.1	1.5±0.7	1.3±0.3	0.56	0.99
CETP	Waist circumference (cm)	102.9±8.3	96.8±10.0	101.1±10.5	0.15	0.97	97.9±17.3	96.0±7.1	90.9±17.4	0.55	0.99
	Systolic pressure (mmHg)	134.3±26.7	134.5±19.9	140.9±21.1	0.47	0.99	122.1±24.9	134.8±13.6	123.6±21.7	0.54	0.99
Taq1B	Diastolic pressure (mmHg)	86.4±11.5	86.3±10.4	88.3±12.3	0.77	0.99	79.0±10.7	80.6±7.7	78.0±12.9	0.90	0.99
	Blood glucose (mmol/l)	7.7±3.4	7.6±2.8	7.1±2.3	0.69	0.99	6.4±2.5	4.8±0.4	6.1±3.8	0.65	0.99
	Triglycerides (mmol/l)	2.5±1.1	3.0±1.5	2.6±1.0	0.29	0.99	1.4±0.6	2.6±2.9	1.4±0.6	0.14	0.97
	HDL-C (mmol/l)	1.0±0.2	1.1±0.3	1.0±0.2	0.13	0.97	1.2±0.2	2.7±2.8	1.7±1.6	0.23	0.97
ADP	Waist circumference (cm)	100.3±10.0	-	101.2±10.0	0.74	0.99	95.0±15.1	-	89.6±19.6	0.43	0.99
	Systolic pressure (mmHg)	139.3±21.2	-	130.2±26.1	0.16	0.97	129.6±20.4	-	110.8±19.5	**0.03***	0.85
45T>G	Diastolic pressure (mmHg)	87.7±11.0	-	85.5±13.5	0.52	0.99	80.9±11.3	-	72.0±9.3	*0.05**	0.90
	Blood glucose (mmol/l)	7.5±2.9	-	6.8±1.9	0.36	0.99	5.5±1.5	-	7.7±5.7	0.08	0.97
	Triglycerides (mmol/l)	2.6±1.2	-	2.8±1.2	0.57	0.99	1.5±1.4	-	1.7±0.9	0.79	0.99
	HDL-C (mmol/l)	1.0±0.2	-	1.1±0.2	**0.00***	**0.00**	1.9±1.9	-	1.2±0.3	0.36	0.99
LEP	Waist circumference (cm)	100.5±10.0	-	-	-	-	93.6±16.4	-	96.0±0.0	0.89	0.99
	Systolic pressure (mmHg)	137.4±22.4	-	-	-	-	124.9±21.9	-	125.0±0.0	1.00	1.00
25CAG	Diastolic pressure (mmHg)	87.3±11.5	-	-	-	-	78.6±11.5	-	82.0±0.0	0.77	0.99
	Blood glucose (mmol/l)	7.4±2.8	-	-	-	-	6.0±3.2	-	5.0±0.0	0.75	0.99
	Triglycerides (mmol/l)	2.7±1.2	-	-	-	-	1.5±1.3	-	1.7±0.0	0.91	1.00
	HDL-C (mmol/l)	1.0±0.2	-	-	-	-	1.7±1.6	-	1.1±0.0	0.71	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold). P values in italics demonstrate marginal associations)

(In IR males with the MS, the LPL HinfI [mutant homozygous (GG) genotypes] was associated with high systolic and diastolic blood pressure, the PON1 192 Arg/Gln [homozygous wild type (AA) genotypes] with high systolic and diastolic blood pressure; and the ADP 45T>G [homozygous wild type (TT) genotypes] with reduced HDL-C. In IR males with MS, the ADP 45T>G [heterozygous (TG) genotypes] was protective against high systolic blood pressure)

TABLE XXXVI: GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN IR FEMALES WITH AND WITH NO MS (IDF)

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	102.9±13.0	-	-	-	-	98.7±15.1	-	-	-	-
	Systolic pressure (mmHg)	136.5±21.8	-	-	-	-	118.7±21.3	-	-	-	-
Q139X	Diastolic pressure (mmHg)	83.9±11.1	-	-	-	-	76.0±9.9	-	-	-	-
	Blood glucose (mmol/l)	7.8±3.5	-	-	-	-	5.3±1.6	-	-	-	-
	Triglycerides (mmol/l)	2.3±1.4	-	-	-	-	1.3±0.7	-	-	-	-
	HDL-C (mmol/l)	1.3±0.9	-	-	-	-	1.4±0.6	-	-	-	-
LPL	Waist circumference (cm)	103.1±12.2	100.3±7.2	102.6±16.6	0.67	0.99	98.5±15.4	110.2±10.4	97.2±13.7	0.21	0.97
	Systolic pressure (mmHg)	137.5±22.8	136.9±28.6	132.8±15.0	0.32	0.99	117.0±20.0	109.0±33.2	128.0±22.7	**0.04***	0.85
HinfI	Diastolic pressure (mmHg)	83.7±11.4	87.3±10.1	83.4±10.3	0.42	0.99	75.5±9.6	78.4±12.1	77.7±10.8	0.54	0.99
	Blood glucose (mmol/l)	8.0±5.6	8.0±3.0	8.1±4.1	0.99	1.00	5.2±1.6	5.1±1.2	5.5±1.9	0.68	0.99
	Triglycerides (mmol/l)	2.4±1.5	2.7±1.6	2.1±1.0	0.16	0.97	1.3±0.7	1.1±0.2	1.2±0.5	0.75	0.99
	HDL-C (mmol/l)	1.6±5.0	1.6±1.6	1.2±0.3	0.83	0.99	1.4±0.7	1.3±0.1	1.4±0.3	0.86	0.99
PON1	Waist circumference (cm)	103.6±11.5	104.4±11.1	101.6±15.1	0.34	0.99	99.7±15.5	95.5±20.5	97.5±13.5	0.60	0.99
	Systolic pressure (mmHg)	136.0±21.5	129.8±27.6	138.3±21.2	0.25	0.99	117.7±22.9	127.1±10.6	118.9±20.0	0.50	0.99
192Arg/Gln	Diastolic pressure (mmHg)	84.1±11.5	83.1±9.5	83.6±10.8	0.88	0.99	76.1±9.5	76.9±9.5	75.7±10.7	0.95	1.00
	Blood glucose (mmol/l)	7.9±3.7	8.7±3.7	8.0±6.9	0.79	0.99	5.2±1.2	6.3±3.5	5.2±1.7	0.15	0.97
	Triglycerides (mmol/l)	2.4±1.5	2.0±0.9	2.3±1.4	0.55	0.99	1.3±0.8	1.4±0.2	1.3±0.5	0.94	1.00
	HDL-C (mmol/l)	1.7±5.8	1.2±0.2	1.3±0.9	0.65	0.99	1.5±0.8	1.4±0.2	1.4±0.3	0.59	0.99
CETP	Waist circumference (cm)	102.9±11.9	102.0±10.5	103.4±14.9	0.76	0.99	100.6±13.3	95.2±16.6	98.6±15.6	0.34	0.99
	Systolic pressure (mmHg)	138.4±20.1	134.7±22.4	136.4±22.7	0.53	0.99	120.5±24.5	124.5±23.2	115.0±17.4	0.12	0.97
Taq1B	Diastolic pressure (mmHg)	85.4±11.4	84.5±11.0	82.5±10.9	0.12	0.97	77.9±11.8	77.8±8.6	73.9±8.5	0.07	0.95
	Blood glucose (mmol/l)	7.9±3.7	8.9±8.5	7.6±3.2	0.20	0.97	5.2±1.4	5.6±2.3	5.2±1.4	0.58	0.99
	Triglycerides (mmol/l)	2.2±1.2	2.3±1.6	2.4±1.4	0.54	0.99	1.3±0.4	1.2±0.3	1.3±0.9	0.65	0.99
	HDL-C (mmol/l)	1.3±0.7	2.3±8.5	1.3±0.8	0.17	0.97	1.4±0.3	1.4±0.3	1.5±0.8	0.65	0.99
ADP	Waist circumference (cm)	102.9±13.6	104.4±3.1	102.7±11.2	0.97	1.00	99.2±15.4	96.8±15.6	97.5±14.3	0.82	0.99
	Systolic pressure (mmHg)	136.8±20.3	148.0±6.1	135.4±26.4	0.59	0.99	120.0±22.8	117.0±16.0	115.4±17.5	0.53	0.99
45T>G	Diastolic pressure (mmHg)	83.9±10.3	88.3±2.1	83.4±13.5	0.74	0.99	76.7±10.3	71.0±7.8	74.8±9.0	0.36	0.99
	Blood glucose (mmol/l)	8.0±5.7	6.3±1.7	8.0±3.4	0.86	0.99	5.4±1.6	4.9±0.7	5.0±1.7	0.38	0.99
	Triglycerides (mmol/l)	2.3±1.5	2.2±1.7	2.2±1.2	0.83	0.99	1.2±0.4	1.0±0.7	1.5±1.1	0.15	0.97
	HDL-C (mmol/l)	1.4±1.0	1.0±0.3	2.2±8.7	0.35	0.99	1.4±0.3	1.3±0.3	1.5±1.1	0.52	0.99
LEP	Waist circumference (cm)	102.8±13.0	-	109.6±14.8	0.37	0.99	98.7±15.2	-	98.5±8.4	0.98	1.00
	Systolic pressure (mmHg)	136.4±21.9	-	153.7±3.8	0.17	0.97	118.7±21.5	-	116.7±10.7	0.87	0.99
25CAG	Diastolic pressure (mmHg)	83.8±11.1	-	86.0±8.0	0.74	0.99	76.0±10.0	-	75.3±2.3	0.90	0.99
	Blood glucose (mmol/l)	8.0±5.2	-	7.0±1.9	0.73	0.99	5.3±1.6	-	5.0±0.0	0.81	0.99
	Triglycerides (mmol/l)	2.3±1.4	-	1.7±0.2	0.47	0.99	1.3±0.7	-	1.2±0.3	0.76	0.99
	HDL-C (mmol/l)	1.5±4.4	-	1.3±0.2	0.92	1.00	1.4±0.6	-	1.2±0.4	0.57	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value;
* best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold))

(None of the studied polymorphisms were associated with the individual metabolic risk factors in IR females with MS; however the LPL HinfI [homozygous mutant (GG) genotypes] was protective against high systolic blood pressure)

TABLE XXXVII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH METABOLIC COMPONENTS IN IR FEMALES WITH AND WITH NO MS (HARMONIZED)*

SNP	METABOLIC RISK FACTORS	MS					NO MS				
		WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡	WTH GENO MEAN±SD	MH GENO MEAN±SD	HET GENO MEAN±SD	P VALUE ∞	P VALUE ‡
APOA5	Waist circumference (cm)	103.0±13.1	-	-	-	-	98.9±14.7	-	-	-	-
	Systolic pressure (mmHg)	136.7±21.7	-	-	-	-	120.3±22.2	-	-	-	-
Q139X	Diastolic pressure (mmHg)	83.9±11.1	-	-	-	-	76.7±10.2	-	-	-	-
	Blood glucose (mmol/l)	7.9±3.5	-	-	-	-	5.3±1.5	-	-	-	-
	Triglycerides (mmol/l)	2.4±1.4	-	-	-	-	1.3±0.8	-	-	-	-
	HDL-C (mmol/l)	1.3±0.8	-	-	-	-	1.5±0.8	-	-	-	-
LPL	Waist circumference (cm)	103.1±12.3	100.2±7.5	103.3±16.5	0.69	0.99	99.0±15.0	108.7±10.0	96.3±13.8	0.17	0.97
	Systolic pressure (mmHg)	137.6±22.5	137.8±29.4	133.1±15.2	0.35	0.99	119.0±21.7	111.3±30.3	128.0±21.6	0.09	0.97
HinfI	Diastolic pressure (mmHg)	83.8±11.4	87.7±10.3	83.4±10.4	0.37	0.99	76.2±90.0	78.8±10.9	78.4±10.8	0.53	0.99
	Blood glucose (mmol/l)	8.1±5.7	8.1±3.0	8.2±4.2	1.00	1.00	5.3±1.5	5.2±1.2	5.5±1.8	0.71	0.99
	Triglycerides (mmol/l)	2.4±1.4	2.7±1.7	2.1±1.0	1.15	1.00	1.3±0.9	1.3±0.5	1.3±0.5	0.90	0.99
	HDL-C (mmol/l)	1.6±5.1	1.6±1.7	1.2±0.3	0.84	0.99	1.5±0.9	1.3±0.1	1.4±0.3	0.78	0.99
PON1	Waist circumference (cm)	103.6±11.5	104.8±11.2	101.9±15.3	0.48	0.99	100.2±15.2	95.6±19.2	97.5±13.2	0.43	0.99
	Systolic pressure (mmHg)	136.1±21.6	129.1±28.2	138.9±20.4	0.16	0.97	119.2±23.1	129.0±11.4	120.6±22.0	0.45	0.99
192Arg/Gln	Diastolic pressure (mmHg)	84.2±11.6	83.4±9.6	83.6±10.8	0.89	0.99	76.6±9.6	76.9±8.9	76.9±11.2	0.99	1.00
	Blood glucose (mmol/l)	8.0±3.8	8.9±3.8	8.2±7.2	0.78	0.99	5.3±1.2	6.3±3.3	5.3±1.6	0.15	0.97
	Triglycerides (mmol/l)	2.4±1.5	2.0±0.9	2.3±1.3	0.56	0.99	1.3±0.8	1.3±0.2	1.4±1.0	0.72	0.99
	HDL-C (mmol/l)	1.8±6.0	1.1±0.2	1.2±0.6	0.58	0.99	1.5±0.7	1.4±0.2	1.5±0.9	0.86	0.99
CETP	Waist circumference (cm)	103.1±11.5	102.3±10.6	103.3±15.2	0.87	0.99	100.5±13.7	95.5±15.5	99.2±15.0	0.32	0.99
	Systolic pressure (mmHg)	138.3±20.3	134.1±21.7	137.1±22.5	0.44	0.99	122.1±24.5	127.5±25.4	116.0±18.0	**0.04***	0.85
Taq1B	Diastolic pressure (mmHg)	85.5±11.5	84.4±10.7	82.6±11.1	0.16	0.97	78.3±11.7	79.1±10.3	74.6±8.5	*0.05**	0.90
	Blood glucose (mmol/l)	8.0±3.8	9.1±8.8	7.6±3.3	0.18	0.97	5.2±1.4	5.6±2.1	5.3±1.4	0.53	0.99
	Triglycerides (mmol/l)	2.2±1.2	2.4±1.7	2.4±1.4	0.56	0.99	1.3±0.4	1.2±0.4	1.4±1.2	0.50	0.99
	HDL-C (mmol/l)	1.3±0.7	2.4±8.8	1.2±0.6	0.15	0.97	1.4±0.3	1.4±0.4	1.6±1.1	0.49	0.99
ADP	Waist circumference (cm)	103.0±13.7	104.4±3.1	102.8±11.3	0.98	1.00	99.4±15.0	96.8±15.6	97.5±14.0	0.75	0.99
	Systolic pressure (mmHg)	137.0±19.8	148.0±6.1	135.4±26.8	0.57	0.99	121.8±23.7	117.0±16.0	116.4±17.6	0.41	0.99
45T>G	Diastolic pressure (mmHg)	84.1±10.3	88.3±2.1	83.4±13.7	0.71	0.99	77.4±10.5	71.0±7.8	75.4±9.1	0.30	0.99
	Blood glucose (mmol/l)	8.1±5.9	6.3±1.7	8.1±3.4	0.84	0.99	5.5±1.5	4.9±0.7	5.0±1.6	0.29	0.99
	Triglycerides (mmol/l)	2.4±1.4	2.2±1.7	2.3±1.2	0.83	0.99	1.3±0.7	1.0±0.7	1.4±1.1	0.56	0.99
	HDL-C (mmol/l)	1.3±0.9	1.0±0.3	2.2±8.8	0.33	0.99	1.5±0.7	1.3±0.3	1.5±1.1	0.80	0.99
LEP	Waist circumference (cm)	102.9±13.0	-	109.6±14.8	0.38	0.99	98.9±14.8	-	98.5±8.4	0.96	1.00
	Systolic pressure (mmHg)	136.5±21.7	-	153.7±3.8	0.17	0.97	120.4±22.4	-	116.7±10.7	0.78	0.99
25CAG	Diastolic pressure (mmHg)	83.9±11.2	-	86.0±8.0	0.75	0.99	76.8±10.3	-	75.3±2.3	0.81	0.99
	Blood glucose (mmol/l)	8.1±5.4	-	7.0±1.9	0.71	0.99	5.3±1.6	-	5.0±0.0	0.74	0.99
	Triglycerides (mmol/l)	2.4±1.4	-	1.7±0.2	0.44	0.99	1.3±0.9	-	1.2±0.3	0.73	0.99
	HDL-C (mmol/l)	1.5±4.5	-	1.3±0.2	0.93	1.00	1.5±0.8	-	1.2±0.4	0.57	0.99

* ***Key***: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome ; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **GENO-** genotype; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; ∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis

(Data are presented as MEANS±SD. Comparison between genotype groups was done using ANOVA test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if $p<0.05$ (bold). P values in italics demonstrate marginal associations)

(None of the studied polymorphisms were associated with the individual metabolic risk factors in IR females with MS; however, the CETP Taq1B [heterozygous (GA) genotypes] was protective against high systolic blood pressure)

TABLE XXXVIII: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH OTHER RISK FACTORS (CURRENT SMOKING, ALCOHOL CONSUMPTION AND NO PHYSICAL ACTIVITY) IN IR PARTICIPANTS WITH AND WITH NO MS (IDF)*

SNP/ OTHER RISK FACTORS		MS							NO MS						
		WTH GENOTYPE	MH GENOTYPE	HET GENOTYPE	MAJOR ALLELE	MINOR ALLELE	P VALUE ∞	P VALUE ‡	WTH GENOTYPE	MH GENOTYPE	HET GENOTYPE	MAJOR ALLELE	MINOR ALLELE	P VALUE ∞	P VALUE ‡
APOA5 Q139X		CC (n=394)	TT (n=0)	CT (n=0)	C (n=788)	T (n=0)			CC (n=166)	TT (n=0)	CT (n=0)	C (n=332)	T (n=0)		
Current smoking	Yes	75 (19.0)	0 (0)	0 (0)	150 (19.0)	0 (0)	-	-	29 (17.5)	0 (0)	0 (0)	58 (17.5)	0 (0)	-	-
	No	319 (81.0)	0 (0)	0 (0)	638 (81.0)	0 (0)			137 (82.5)	0 (0)	0 (0)	274 82.5)	0 (0)		
Alcohol consumption	Yes	54 (13.7)	0 (0)	0 (0)	108 (13.7)	0 (0)	-	-	22 (13.3) §	0 (0)	0 (0)	44 (13.3) §	0 (0)	-	-
	No	340 (86.3)	0 (0)	0 (0)	680 (86.3)	0 (0)			143 (86.7)	0 (0)	0 (0)	286 (86.7)	0 (0)		
No physical activity	Yes	338 (85.8)	0 (0)	0 (0)	676 (85.8)	0 (0)	-	-	126 (75.9)	0 (0)	0 (0)	252 (75.9)	0 (0)	-	-
	No	56 (14.2)	0 (0)	0 (0)	112 (14.2)	0 (0)			40 (24.1)	0 (0)	0 (0)	80 (24.1)	0 (0)		
LPL HinfI		CC (n=298)	GG (n=20)	CG (n=77)	C (n=673)	G (n=117)			CC (n=126)	GG (n=7)	CG (n=33)	C (n=285)	G (n=47)		
Current smoking	Yes	61 (20.5)	3 (15.0)	11 (14.3)	133 (19.8)	17 (14.5)	0.42	0.99	23 (18.3)	1 (14.3)	5 (15.2)	51 (17.9)	7 (14.9)	0.89	0.99
	No	237 (79.5)	17 (85.0)	66 (85.7)	540 (80.2)	100 (85.5)			103 (81.8)	6 (85.7)	28 (84.9)	234 (82.1)	40 (85.1)		
Alcohol consumption	Yes	45 (15.1)	1 (5.0)	8 (10.4)	98 (14.6)	10 (8.5)	0.29	0.99	15 (11.9) §	2 (28.6)	5 (15.2)	35 (12.3)§	9 (19.1)	0.73	0.99
	No	253 (84.9)	19 (95.0)	69 (89.6)	575 (85.4)	107 (91.5)			110 (87.3)	5 (71.4)	28 (84.9)	248 (87.0)	38 (80.9)		
Physical activity	Yes	253 (84.9)	17 (85.0)	68 (88.3)	574 (85.3)	102 (87.2)	0.75	0.99	97 (77.0)	6 (85.7)	23 (69.7)	217 (76.1)	35 (74.5)	0.56	0.99
	No	45 (15.1)	3 (15.0)	9 (11.7)	99 (14.7)	15 (12.8)			29 (23.0)	1 (14.3)	10 (30.3)	68 (23.9)	12 (25.5)		
PON1 192Arg/Gln		AA (n=214)	GG (n=24)	AG (n=157)	A (n=585)	G (n=205)			AA (n=95)	GG (n=11)	AG (n=60)	A (n=250)	G (n=82)		
Current smoking	Yes	35 (16.4)	4 (16.7)	36 (22.9)	106 (18.1)	44 (21.5)	0.27	0.99	19 (20.0)	2 (18.2)	8 (13.3)	46 (18.4)	12 (14.6)	0.57	0.99
	No	179 (83.6)	20 (83.3)	121 (77.1)	479 (81.9)	161 (78.5)			76 (80.0)	9 (81.8)	52 (86.7)	204 (81.6)	70 (85.4)		
Alcohol consumption	Yes	29 (13.6)	2 (8.3)	23 (14.7)	81 (13.8)	27 (13.2)	0.70	0.99	14 (14.7)§	2 (18.2)	6 (10.0)	34 (13.6)§	10 (12.2)	0.78	0.99
	No	185 (86.5)	22 (91.7)	134 (85.4)	504 (86.2)	178 (86.8)			80 (84.2)	9 (81.8)	54 (90.0)	214 (85.6)	72 (87.8)		
Physical activity	Yes	184 (86.0)	19 (79.2)	135 (86.0)	503 (86.0)	173 (84.4)	0.65	0.99	72 (75.8)	7 (63.6)	47 (78.3)	191 (76.4)	61 (74.4)	0.58	0.99
	No	30 (14.0)	5 (20.8)	22 (14.0)	82 (14.0)	32 (15.6)			23 (24.2)	4 (36.4)	13 (21.7)	59 (23.6)	21 (25.6)		
CETP Taq1B		GG (n=115)	AA (n=99)	GA (n=181)	G (n=411)	A (n=379)			GG (n=56)	AA (n=31)	GA (n=79)	G (n=191)	A (n=141)		
Current smoking	Yes	17 (14.8)	19 (19.2)	39 (21.6)	73 (17.8)	77 (20.3)	0.35	0.99	9 (16.1)	5 (16.1)	15 (19.0)	33 (17.3)	25 (17.7)	0.89	0.99
	No	98 (85.2)	80 (80.8)	142 (78.5)	338 (82.2)	302 (79.7)			47 (83.9)	26 (83.9)	64 (81.0)	158 (82.7)	116 (82.3)		
Alcohol consumption	Yes	10 (8.7)	14 (14.1)	30 (16.6)	50 (12.2)	58 (15.3)	0.16	0.97	8 (14.3)	3 (9.7)	11 (13.9) §	27 (14.1) §	17 (12.1) §	0.82	0.99
	No	105 (91.3)	85 (85.9)	151 (83.4)	361 (87.8)	321 (84.7)			48 (85.7)	28 (90.3)	67 (84.8)	163 (85.3)	123 (87.2)		
Physical activity	Yes	101 (87.8)	83 (83.8)	154 (85.1)	356 (86.6)	320 (84.4)	0.69	0.99	43 (76.8)	26 (83.9)	57 (72.2)	143 (74.9)	109 (77.3)	0.43	0.99
	No	14 (12.2)	16 (16.2)	27 (14.9)	55 (13.4)	59 (15.6)			13 (23.2)	5 (16.1)	22 (27.9)	48 (25.1)	32 (22.7)		
ADP 45T>G		TT (n=300)	GG (n=3)	TG (n=92)	T (n=692)	G (n=98)			TT (n=118)	GG (n=4)	TG (n=44)	T (n=280)	G (n=52)		
Current smoking	Yes	61 (20.3)	0 (0)	14 (15.2)	136 (19.7)	14 (14.3)	0.39	0.99	16 (13.6)	2 (50.0)	11 (25.0)	43 (15.4)	15 (28.8)	*0.05**	0.90
	No	239 (79.7)	3 (100)	78 (84.8)	556 (80.3)	84 (85.7)			102 (86.4)	2 (50.0)	33 (75.0)	237 (84.6)	37 (71.2)		
Alcohol consumption	Yes	46 (15.3)	0 (0)	8 (8.7)	100 (14.5)	8 (8.2)	0.21	0.97	16 (13.6) §	0 (0)	6 (13.6)	38 (13.6) §	6 (11.5)	0.90	0.99
	No	254 (84.7)	3 (100)	84 (91.3)	592 (85.5)	90 (91.8)			101 (85.6)	4 (100)	38 (86.4)	240 (85.7)	46 (88.5)		
Physical activity	Yes	257 (85.7)	3 (100)	78 (84.8)	592 (85.5)	84 (85.7)	0.76	0.99	89 (75.4)	1 (25.0)	36 (81.8)	214 (76.4)	38 (73.1)	**0.04***	0.85
	No	43 (14.3)	0 (0)	14 (15.2)	100 (14.5)	14 (14.3)			29 (24.6)	3 (75.0)	8 (18.2)	66 (23.6)	14 (26.9)		
LEP 25CAG		AA (n=392)	GG (n=0)	AG (n=3)	A (n=787)	G (n=3)			AA (n=162)	GG (n=0)	AG (n=4)	A (n=328)	G (n=4)		
Current smoking	Yes	75 (19.1)	-	0 (0)	150 (19.1)	0 (0)	0.40	0.99	28 (17.3)	-	1 (25.0)	57 (17.4)	1 (25.0)	0.69	0.99
	No	317 (80.9)	-	3 (100)	637 (80.9)	3 (100)			134 (82.7)	-	3 (75.0)	271 (82.6)	3 (75.0)		
Alcohol consumption	Yes	54 (13.8)	-	0 (0)	108 (13.7)	0 (0)	0.49	0.99	22 (13.6)	-	0 (0) §	44 (13.4) §	0 (0) §	**0.00***	0.14
	No	338 (86.2)	-	3 (100)	679 (86.3)	3 (100)			140 (86.4)	-	3 (75.0)	283 (86.3)	3 (75.0)		
Physical activity	Yes	335 (85.5)	-	3 (100)	673 (85.5)	3 (100)	0.48	0.99	122 (75.3)	-	4 (100)	248 (75.6)	4 (100)	0.25	0.99
	No	57 (14.5)	-	0 (0)	114 (14.5)	0 (0)			40 (24.7)	-	0 (0)	80 (24.4)	0 (0)		

Key: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP**- single nucleotide polymorphism;
∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis
(All data are represented as prevalence rates with percentages in parenthesis. Comparison of the groups was done using Chi-square (x^2) test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if p<0.05 (bold))

§ One subject carrying the genotype did not disclose smoking status, alcohol consumption and physical activities performed, subsequently influencing allele counts

(None of the studied polymorphisms increased the risk for the MS when associated with smoking, alcohol consumption and physical activity in IR participants. The ADP 45T>G [homozygous wild type (TT) genotypes and T alleles] and LEP 25CAG [homozygous wild type (AA) genotypes and A alleles] in IR participants who performed physical activities and who did not consume alcohol, respectively, was protective against the MS. The ADP 45T>G [homozygous wild type (TT) genotypes and T alleles] in IR nonsmokers were marginally protective against the MS)

TABLE XXXIX: *GENETIC PATTERNS OF POLYMORPHISMS ASSOCIATED WITH OTHER RISK FACTORS (CURRENT SMOKING, ALCOHOL CONSUMPTION AND NO PHYSICAL ACTIVITY) IN IR PARTICIPANTS WITH AND WITH NO MS (HARMONIZED)*

SNP/ OTHER RISK FACTORS		MS							NO MS						
		WTH GENOTYPE	MH GENOTYPE	HET GENOTYPE	MAJOR ALLELE	MINOR ALLELE	P VALUE		WTH GENOTYPE	MH GENOTYPE	HET GENOTYPE	MAJOR ALLELE	MINOR ALLELE	P VALUE	
							∞	‡						∞	‡
APOA5 Q139X		CC (n=373)	TT (n=0)	CT (n=0)	C (n=746)	T (n=0)			CC (n=188)	TT (n=0)	CT (n=0)	(n=376)	T (n=0)		
Current smoking	Yes	70 (18.8)	0 (0)	0 (0)	140 (18.8)	0 (0)	-	-	34 (18.1)	0 (0)	0 (0)	68 (18.1)	0 (0)	-	-
	No	303 (81.2)	0 (0)	0 (0)	606 (81.2)	0 (0)			154 (81.9)	0 (0)	0 (0)	308 (81.9)	0 (0)		
Alcohol consumption	Yes	52 (13.9)	0 (0)	0 (0)	104 (13.9)	0 (0)	-	-	24 (12.8) §	0 (0)	0 (0)	48 (12.8) §	0 (0)	-	-
	No	321 (86.1)	0 (0)	0 (0)	642 (86.1)	0 (0)			163 (87.2)	0 (0)	0 (0)	326 (87.2)	0 (0)		
No physical activity	Yes	317 (85.0)	0 (0)	0 (0)	634 (85.0)	0 (0)	-	-	147 (78.2)	0 (0)	0 (0)	294 (78.2)	0 (0)	-	-
	No	56 (15.0)	0 (0)	0 (0)	112 (15.0)	0 (0)			41 (21.8)	0 (0)	0 (0)	82 (21.8)	0 (0)		
LPL HinfI		CC (n=281)	GG (n=19)	CG (n=73)	C (n=635)	G (n=111)			CC (n=143)	GG (n=8)	CG (n=37)	C (n=323)	G (n=53)		
Current smoking	Yes	57 (20.3)	3 (15.8)	10 (13.7)	124 (19.5)	16 (14.4)	0.41	0.99	27 (18.9)	1 (12.5)	6 (16.2)	60 (18.6)	8 (15.1)	0.85	0.99
	No	224 (79.7)	16 (84.2)	63 (86.3)	511 (80.5)	95 (85.6)			116 (81.1)	7 (87.5)	31 (83.8)	263 (81.4)	45 (84.9)		
Alcohol consumption	Yes	44 (15.7)	1 (5.3)	7 (9.6)	95 (15.0)	9 (8.1)	0.22	0.97	16 (11.2) §	2 (25.0)	6 (16.2)	38 (11.8) §	10 (18.9)	0.72	0.99
	No	237 (84.3)	18 (94.7)	66 (90.4)	540 (85.0)	102 (91.9)			126 (88.1)	6 (75.0)	31 (83.8)	283 (87.6)	43 (81.1)		
Physical activity	Yes	237 (84.3)	16 (84.2)	64 (87.7)	538 (84.7)	96 (86.5)	0.77	0.99	113 (79.0)	7 (87.5)	27 (73.0)	253 (78.3)	41 (77.4)	0.59	0.99
	No	44 (15.7)	3 (15.8)	9 (12.3)	97 (15.3)	15 (13.5)			30 (21.0)	1 (12.5)	10 (27.0)	70 (21.7)	12 (22.6)		
PON1 192Arg/Gln		AA (n=203)	GG (n=23)	AG (n=147)	A (n=553)	G (n=193)			AA (n=106)	GG (n=12)	AG (n=70)	A (n=282)	G (n=94)		
Current smoking	Yes	33 (16.3)	4 (17.4)	33 (22.5)	99 (17.9)	41 (21.2)	0.34	0.99	21 (19.8)	2 (16.7)	11 (15.7)	53 (18.8)	15 (16.0)	0.78	0.99
	No	170 (83.7)	19 (82.6)	114 (77.6)	454 (82.1)	152 (78.8)			85 (80.2)	10 (83.3)	59 (84.3)	229 (81.2)	79 (84.0)		
Alcohol consumption	Yes	28 (13.8)	2 (8.7)	22 (15.0)	78 (14.1)	26 (13.5)	0.72	0.99	15 (14.2) §	2 (16.7)	7 (10.0)	37 (13.1) §	11 (11.7)	0.80	0.99
	No	175 (86.2)	21 (91.3)	125 (85.0)	475 (85.9)	167 (86.5)			90 (84.9)	10 (83.3)	63 (90.0)	243 (86.2)	83 (88.3)		
Physical activity	Yes	174 (85.7)	18 (78.3)	125 (85.0)	473 (85.5)	161 (83.4)	0.64	0.99	82 (77.4)	8 (66.7)	57 (81.4)	221 (78.4)	73 (77.7)	0.50	0.99
	No	29 (14.3)	5 (21.7)	22 (15.0)	80 (14.5)	32 (16.6)			24 (22.6)	4 (33.3)	13 (18.6)	61 (21.6)	21 (22.3)		
CETP Taq1B		GG (n=110)	AA (n=94)	GA (n=169)	G (n=389)	A (n=357)			GG (n=61)	AA (n=36)	GA (n=91)	G (n=213)	A (n=163)		
Current smoking	Yes	15 (13.6)	18 (19.2)	37 (21.9)	67 (17.2)	73 (20.4)	0.22	0.97	11 (18.0)	6 (16.7)	17 (18.7)	39 (18.3)	29 (17.8)	0.97	1.00
	No	95 (86.4)	76 (80.9)	132 (78.1)	322 (82.8)	284 (79.6)			50 (82.0)	30 (83.3)	74 (81.3)	174 (81.7)	134 (82.2)		
Alcohol consumption	Yes	9 (8.2)	14 (14.9)	29 (17.2)	47 (12.1)	57 (16.0)	0.10	0.97	9 (14.8)	3 (8.3)	12 (13.2) §	30 (14.1) §	18 (11.0) §	0.75	0.99
	No	101 (91.8)	80 (85.1)	140 (82.8)	342 (87.9)	300 (84.0)			52 (85.3)	33 (91.7)	78 (85.7)	182 (85.4)	144 (88.3)		
Physical activity	Yes	97 (88.2)	78 (83.0)	142 (84.0)	336 (86.4)	298 (83.5)	0.52	0.99	47 (77.1)	31 (86.1)	69 (75.8)	163 (76.5)	131 (80.4)	0.43	0.99
	No	13 (11.8)	16 (17.0)	27 (16.0)	53 (13.6)	59 (16.5)			14 (23.0)	5 (13.9)	22 (24.2)	50 (23.5)	32 (19.6)		
ADP 45T>G		TT (n=281)	GG (n=3)	TG (n=89)	T (n=651)	G (n=95)			TT (n=137)	GG (n=4)	TG (n=47)	T (n=321)	G (n=55)		
Current smoking	Yes	56 (19.9)	0 (0)	14 (15.7)	126 (19.4)	14 (14.7)	0.48	0.99	21 (15.3)	2 (50.0)	11 (23.4)	53 (16.5)	15 (27.3)	0.11	0.97
	No	225 (80.1)	3 (100)	75 (84.3)	525 (80.6)	81 (85.3)			116 (84.7)	2 (50.0)	36 (76.6)	268 (83.5)	40 (72.7)		
Alcohol consumption	Yes	44 (15.7)	0 (0)	8 (9.0)	96 (14.7)	8 (8.4)	0.22	0.97	18 (13.1) §	0 (0)	6 (12.8)	42 (13.1) §	6 (10.9)	0.91	1.00
	No	237 (84.3)	3 (100)	81 (91.0)	555 (85.3)	87 (91.6)			118 (86.1)	4 (100)	41 (87.2)	277 (86.3)	49 (89.1)		
Physical activity	Yes	239 (85.1)	3 (100)	75 (84.3)	553 (84.9)	81 (85.3)	0.75	0.99	107 (78.1)	1 (25.0)	39 (83.0)	253 (78.8)	41 (74.5)	**0.03***	0.85
	No	42 (14.9)	0 (0)	14 (15.7)	98 (15.1)	14 (14.7)			30 (21.9)	3 (75.0)	8 (17.0)	68 (21.2)	14 (25.5)		
LEP 25CAG		AA (n=370)	GG (n=0)	AG (n=3)	A (n=743)	G (n=3)			AA (n=184)	GG (n=0)	AG (n=4)	A (n=372)	G (n=4)		
Current smoking	Yes	70 (18.9)	-	0 (0)	140 (18.8)	0 (0)	0.40	0.99	33 (17.9)	-	1 (25.0)	67 (18.0)	1 (25.0)	0.72	0.99
	No	300 (81.1)	-	3 (100)	603 (81.2)	3 (100)			151 (82.1)	-	3 (75.0)	305 (82.0)	3 (75.0)		
Alcohol consumption	Yes	52 (14.1)	-	0 (0)	104 (14.0)	0 (0)	0.48	0.99	24 (13.0)	-	0 (0) §	48 (12.9) §	0 (0) §	**0.00***	1.14
	No	318 (86.0)	-	3 (100)	639 (86.0)	3 (100)			160 (87.0)	-	3 (75.0)	323 (86.8)	3 (75.0)		
Physical activity	Yes	314 (84.9)	-	3 (100)	631 (84.9)	3 (100)	0.47	0.99	143 (77.7)	-	4 (100)	290 (78.0)	4 (100)	0.29	0.99
	No	56 (15.1)	-	0 (0)	112 (15.1)	0 (0)			41 (22.3)	-	0 (0)	82 (22.0)	0 (0)		

Key: **MS**- metabolic syndrome; **NO MS**- no metabolic syndrome; **WTH**- wild type homozygotes; **MH**- mutant homozygotes; **HET**- heterozygotes; **APOA5**- apolipoprotein A5; **LPL**- lipoprotein lipase; **PON1**- human paraoxonase 1; **CETP**- cholesteryl ester transfer protein; **ADP**- adiponectin; **LEP**- leptin; **SNP-** single nucleotide polymorphism;
∞- unadjusted p value; ‡- adjusted p value; * best p value chosen from different analysis
(All data are represented as prevalence rates with percentages in parenthesis. Comparison of the groups was done using Chi-square (x^2) test for ∞ and Bonferroni test for ‡. Variables were considered statistically significant if p<0.05 (bold))

§ One subject carrying the genotype did not disclose smoking status, alcohol consumption and physical activities performed, subsequently influencing allele counts

(None of the studied polymorphisms increased the risk for the MS when associated with smoking, alcohol consumption and physical activity in IR participants. The ADP 45T>G [homozygous wild type (TT) genotypes and T alleles] and LEP 25CAG [homozygous wild type (AA) genotypes and A alleles] in IR participants who performed physical activities and who did not consume alcohol, respectively, was protective against the MS.

5.10 Summary

The six polymorphisms investigated demonstrated similarities in genotype distribution with no significant differences being detected. There was no deviation from HWE for the human paraoxonase1 192Arg/Gln, cholesteryl ester transfer protein Taq1B, adiponectin 45T>G and leptin 25CAG polymorphisms. However, the lipoprotein lipase HinfI polymorphism was shown to deviate from HWE.

This sample showed no association between the polymorphisms studied and the risk for MS (IDF and harmonized definitions) but showed significant associations with the individual metabolic risk factors. This was observed between the adiponectin 45T>G with low HDL in males (IDF and harmonized definitions) and the human paraoxonase 1 192Arg/Gln with elevated systolic blood pressure in males (harmonized definition). Interestingly, this study showed a protective influence against larger waist circumference measurements and high systolic blood pressure in females with no MS carrying the adiponectin 45T>G (IDF definition) and cholesteryl ester transfer protein Taq1B polymorphisms (harmonized definition), respectively.

Smoking, alcohol intake and physical activity did not increase the risk for the MS in this study. However, the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms appeared to be protective against the risk for MS in non-smokers; and the human paraoxonase 1 192Arg/Gln and leptin 25CAG in non-alcohol consumers (IDF and harmonized definition).

This study further showed no association between the studied polymorphisms in IR participants with the MS (IDF and harmonized definitions). However, IR males with the

MS carrying the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln were more inclined to have hypertension (IDF and harmonized definitions). Similarly, IR males with the MS carrying the adiponectin 45T>G polymorphism were more inclined to have reduced HDL-C (harmonized definition). When IR females were analyzed, a protective influence against high systolic blood pressure in LPL HinfI (IDF definition) and cholesteryl ester transfer protein Taq1B carriers (harmonized definition) were observed. Additionally, IR participants who performed physical activity and who did not consume alcohol and carried the adiponectin 45T>G and leptin 25CAG, respectively, appeared to be protected against the MS (IDF and harmonized definition).

CHAPTER 6

DISCUSSION, CONCLUSION & LIMITATIONS

Chapter 6: Discussion, Conclusion and Limitations

This study reports on gene polymorphisms in 1000 DNA samples from the Phoenix Lifestyle Project (PLP), a community survey of 1500 participants. It describes the prevalence of the MS as determined by the most recent descriptors and is the first study that examines the genetic risk of the MS in this community.

In keeping with the original study, this sample demonstrates a high prevalence of the MS in relation to clustering of the components of the MS, which was notably associated with age, gender and the definition used for diagnosis, ie NCEP ATPIII, IDF or harmonized. A high prevalence of IR as per the HOMA model was shown in this sample, with gender and age again being contributory factors. Moreover, risk factor clustering associated with IR showed blood glucose and waist circumference measurements to be the most suitable predictors for IR in this population. Genotyping of the selected candidate gene polymorphisms showed no significant associations.

6.1 The metabolic syndrome

Many definitions for the MS have been proposed by health organizations (Alberti *et al*., 2009), but have been inconsistent and therefore impractical for use in clinical practice. Until recently, there has been no universally approved definition for the MS, and much debate as to whether the MS represents a specific syndrome or is a surrogate of combined metabolic risk factors that increases the risk of CVD exists (Alberti, 2005). The present investigation indicates that the risk for the MS may lie in its components rather than the MS as an entity *(discussed below)*.

6.1.1 The prevalence of the metabolic syndrome

This investigation diagnosed the MS according to the NCEP ATPIII, IDF and harmonized definition. Regardless of whichever of the three definitions was applied, a very high prevalence of the MS was present in the South African Indian population, albeit the findings indicate that more participants were diagnosed with the MS when the IDF (51.6%) and harmonized (49.0%) criteria was applied than when the NCEP ATPIII (44.3%) definition was used (Table XIII). These observations are not surprising as the IDF and harmonized definitions use many of the same variables such as dyslipidemia and hypertension, but highlight differences in fasting blood glucose cutoffs and include population or country specific cutoffs for central obesity in the form of waist circumference. By including these cutoffs explicitly, the IDF and harmonized definitions identify participants with the MS more directly. Asian Indians for example have a predominance of visceral adipose tissue and larger waist circumference measurements (Bajaj *et al*., 2004) (IDF and harmonized: 95% prevalence (Table XIV)), extrapolating the need for population or country specific measures of central obesity for diagnosing MS. The NCEP ATPIII definition (Table IV) in contrast, does not include population or country specific measures to identify central obesity in Asian Indians, and thus it does not reflect the true prevalence of the MS of this cohort.

These findings show a high prevalence of MS compared to studies conducted in the United States, which demonstrate a 24% (Ford *et al*., 2002) and 34.2% (from the NHANES III survey) (Mozumdar *et al*., 2011) prevalence. Other populations, such as Italians (22.2%) (Bo *et al*., 2005), the Portuguese

(23.9%) (Santos *et al*., 2003) and the Greeks (24.5%) (Athyros *et al*., 2005) show similar results to that documented in the United States. Indians from Mumbai (19.5%) (Sawant *et al*., 2011) and Chennai (18.3%) (Deepa *et al*., 2011) (NCEP-ATP III definition was applied), South Asians (20%–25%) (Corti *et al*., 2004; Fuster *et al*., 2005), Black South Africans (18.5%) (Motala *et al*., 2011) and corporate South Africans (31%) (Ker *et al*., 2007) further demonstrated a lower prevalence of the MS than that observed in our cohort. In contrast to these studies, one study in South African Indians by Ranjith *et al* (2011) showed a high prevalence of the MS (NCEP ATPIII (61%) and IDF (60%)), similar to our results. Therefore the high prevalence of the MS in this study may explain the high frequency of CVD in South African Indians as compared to other ethnic groups, corroborating Baguleyan's (1996) study, which showed myocardial infarctions to occur more frequently in Asian Indians with CVD.

Gender further influenced the susceptibility to the MS in this study i.e. females were more likely to have the MS than males (Table XIII), corroborating Motala *et al's* (2011) study in Black South Africans and Ranjith *et al's* (2011) study in South African Indians. In contrast, Chow *et al's* (2008) study in Southern India showed males (26.9%) to be at an increased risk for the MS than females (18.4%). These gender differences are expected as ethnic cutoffs when applied differs between gender and ethnicity. Lifestyle factors may also be a contributory factor for the observed differences in prevalence.

The prevalence of the MS according to all three definitions in this study (Figure 15) increases with advancing age, which was more apparent in females, irrespective of the definition applied (Figure 15). Males, on the other hand, showed the highest prevalence of the MS in the 50-59 year category, with a slight decrease in the 60+ age group (IDF and harmonized criteria). When the NCEP ATPIII definition was applied in males no such pattern was observed. Similar findings were observed in a study by Sundstrom *et al* (2006) in 2207 Swedish males who showed a higher risk for the MS in the 50-69 age group (NCEP ATP III: 1.36; 1.17-1.58; WHO: 1.26, 1.05-1.52) and a lower risk in the +70 year age categories (NCEP ATPIII: 1.20, 0.90-1.59; WHO: 1.37, 1.03-1.83). In contrast, Wilson's (2004) study demonstrated a two-fold risk only in younger adults with the MS, whilst Eberly *et al* (2006) stated that the risk for the MS is not dependent on age (<50 years: 1.54, 1.25-1.89; ≥50 years: 147, 1.31-1.64). These findings indicate that advancing age increases the risk of cardiometabolic factors, which is dependent on ethnicity.

6.1.2 Risk factor clustering associated with the metabolic syndrome

The results of the present study demonstrate that in the 60+ age category females are the worst affected with regards to clustering of the MS components, whilst in males the prevalence of the five components of the MS that clustered together varied as seen in the 50-59 and 60+ age groups (Figure 16). This is probably due to the differing cutoffs that were used for diagnosing the MS and furthermore, the etiology of the MS which involves more than one underlying pathway (Song *et al*., 2012), could have contributed to a higher risk of cardiometabolic risk factors during the later stages of life. For example,

central obesity in the form of waist circumference and hypertriglyceridemia in this study clustered more frequently across the gender groups irrespective of the definition and or cutoffs applied (Figure 16), which is consistent with studies by Bard *et al* (2001) and Pimenta *et al* (2008) who demonstrated positive associations between hypertriglyceridemia and central obesity. Interestingly, in the different age groups, clustering of central obesity and hypertriglyceridemia increased with age in females but not in males (Figure 16). This may be attributed to higher blood glucose levels observed in females as compared to males in this study (Table XIV). High blood glucose mediates lipid metabolism leading to hypertriglyceridemia (Woodley, 2014) and obesity (Forouhi *et al*., 1999) hence confirming our findings. What emerged is that central obesity and hypertriglyceridemia in this study interplays with insulin sensitivity ((Forouhi *et al*., 1999) and interestingly IR in this study occurred in more than half of our participants with the MS (Table XV).

6.2 The prevalence of insulin resistance as determined by the HOMA model

The prevalence of IR (HOMA-IR>2.6) in this study was higher (IDF: 76.5%; harmonized: 76.3%) in participants diagnosed with the MS (Table XV) compared to studies conducted in France (35.8%: HOMA-IR>4) (Tresaco *et al*., 2003), Italy (40.8% in children: HOMA-IR>2.5, 41.2% in adolescents: HOMA-IR>4.0) (Valerio *et al*., 2006), Spain (45.4%: HOMA-IR≥3.8) (Tapia-Ceballos *et al*., 2007) and in the United States (52.1%: HOMA-IR>4.39) (Lee *et al*., 2006). It is postulated that these differences in prevalence are due to the differing cutoff levels used for HOMA-IR in the assessment of IR (Gungor *et al*., 2004) with gender contributing to these differences (Table XV). This study showed a greater number of females (IDF: 78.4%;

harmonized: 78.3%) with IR compared to males (IDF: 69.9%; harmonized: 69.2%) (Table XV), corroborating Ramachandran *et al's* (2003) study in Asian Indians who showed a higher prevalence of IR in females than males. However, one study in 61 obese children and adolescents aged 5-19 years demonstrated a higher prevalence of IR in males (50%) than females (29%) (Caceres *et al*., 2008). A possible explanation for gender differences may be associated with age groups that were selected for study. When we assessed the age-gender relationship with IR we observed the least number of IR participants with the MS in the ≤19 year age category [IDF (females: 42%, males: 22%); harmonized (females: 67%, males: 33%)] but as age advanced varying frequencies were demonstrated (Figure 17). A possible explanation for these differences is that IR mediates the clustering of several metabolic risk factors with advancing age (Ferrannini *et al*., 1996; Reaven, 1998).

6.3 Pattern of risk factor clustering associated with insulin resistance

IR females in the 40-49, 50-59 and 60+ age categories in this study are the worst affected with regards to clustering of the MS components (IDF), whilst IR males showed varying patterns of clustering of the components (IDF) (Figure 18A). In contrast, the harmonized definition showed varying patterns of clustering of the metabolic components in both females and males (Figure 18B).

This study shows central obesity (in the form of waist circumference measurements) to cluster more frequently with the other metabolic risk factors including elevated blood pressure, elevated triglycerides, reduced HDL-C and elevated fasting blood glucose with frequency increased with advancing age, which was most discernable in IR females when the harmonized definition was applied (Figure 18B). Our findings

overall suggests that central obesity (in the form of waist circumference measurements) is a suitable predictor for IR in participants diagnosed with the MS (Figure 19a), corroborating Hotamisligil's (2006) study. Hence, we indicate that central obesity may provide greater overall diagnostic accuracy when predicting IR in this sample, than counting the other categorical components of the MS such as TG, HDL-C and blood pressure, which demonstrates low sensitivity or specificity in the identification of insulin-resistant individuals (Figures 19b, c, e, f).

Additionally, IR and obesity collectively contribute to T2D (Lillioja *et al*., 1993; Pyorala *et al*., 2000; Shulman, 2000; Sharma *et al*., 2006) due to impairment of insulin metabolism at the different signaling pathways (Saltiel *et al*., 2001; Hribal *et al*., 2002; Song *et al*., 2012). In this regard, we found the frequency of fasting blood glucose to increase with age only in females (IDF) (Figure 18A), and overall highlighted fasting blood glucose to be a predictor for IR in this sample (Figure 19 d). Our findings are in agreement with Bajaj *et al* (2004) who showed IR (in participants with central obesity) to underlie the development of T2D.

We therefore have sufficient evidence to support the notion that IR associated with central obesity, leading to T2D, may be the core metabolic risk factor that characterizes the MS in this sample, which is further exaggerated by the definition used for diagnosis of the MS, lifestyle factors and genetics (discussion to follow). Therefore, knowledge of the mechanisms underlying the pathogenesis of the MS, along with lifestyle patterns may help elucidate the origins of IR that contribute to metabolic outcomes.

6.4 Genetics patterns in the metabolic syndrome

There is evidence linking genetics with the MS (Pollex *et al*., 2006; Povel *et al*., 2011). Studies indicate that 90% of heritability is associated with the components of the MS and ~30% with the MS entity (Teran-Garcia *et al*., 2007; Joy *et al*., 2008; Monda *et al*., 2010) mediated by underlying pathophysiological mechanisms (Aizawa *et al*., 2006; Benyamin *et al*., 2007; Sjogren *et al*., 2008). Genetic studies show an ethnic and lifestyle influence on susceptibility to the MS (Schwandt *et al*., 2010). The South African Indian population in particular, presents with high CV risk profile in relation to the MS, and a resultant increase in CV mortality rate (Seedat, 1995). Therefore, we determined the prevalence of gene polymorphisms that were related to the MS and associated their genotypes/alleles with the metabolic risk factors. Furthermore, as central obesity is core for the development of IR, leading to the MS and T2D, we studied gene polymorphisms that were specifically related to lipid and carbohydrate metabolism, IR and obesity.

6.4.1 Genes related to lipid and carbohydrate metabolism

Apolipoprotein A5 polymorphisms

Apolipoprotein A5 plays a key role in the pathophysiology of dyslipidemia due to its association with triglyceride expression (Laurila *et al*., 2010), which is modulated by oxygen free radicals (Gianturco *et al*., 1994), LDL (Hokanson *et al*., 1997) and cholesteryl ester transfer protein (Brewer *et al*., 2004). However, gene-gene interactions have shown significant associations with dyslipidemia (Laurila *et al*., 2010). Considering, hypertriglyceridemia is a contributory factor for CHD (Hokanson *et al*., 1997) familial hyperlipidemia, the MS and atherogenic lipoprotein profiles (Durrington *et al*., 2003), gene

polymorphisms related to lipid metabolism were studied to determine their propensity to CV risk factors in relation to the MS.

Frequency of the Q139X polymorphism

There is a lack of information on the allele frequencies of the rare Q139X polymorphism in the literature. One study by Marcais *et al* (2005), however, revealed that 200 participants had allele frequencies <0.25%. Our study reports the genotype/allele frequencies of the Q139X polymorphism being distributed only to homozygous wild type (CC) (100%) and or C allele participants (100%) (Table XVII), with no heterozygous (CT) and or mutant homozygous (TT) genotypes and or T alleles being detected. We cannot make any other allelic associations due to limited studies on the Q139X polymorphism being observed in the literature.

Association of the apolipoprotein A5 gene polymorphism with the metabolic syndrome

The apolipoprotein A5 gene is regarded as a candidate gene for triglycerides (Talmud *et al*., 2002; Kao *et al*., 2003), HDL-C modulation (Nabika *et al*., 2002; Baum *et al*., 2003; Zhao *et al*., 2010) and CHD risk (Lai *et al*., 2004). The Q139X polymorphism in particular, has been reported to contribute to severe hyperchylomicronemia and dyslipidemia (Marcais *et al*., 2005). Five of nine heterozygous carriers and one homozygous carrier were associated with hyperchylomicronemia as shown in Marcais *et al*'s (2005) study. Our findings suggest that the Q139X polymorphism is not a common polymorphism within the apolipoprotein A5 gene that influences dyslipidemia in South African

Indians as we found no association with the MS (Table XIX, XXII), the individual metabolic risk factors (Table XXIII-XXVIII) and or environmental risk factors such as smoking, alcohol consumption and physical activity (XXIX, XXX).

Additionally, Zhai *et al* (2007) and Yin *et al* (2014) reported that apolipoprotein A5 gene is associated with an increased susceptibility to T2D, due to its association with insulin levels by phosphorylation de-phosphorylation mechanisms at the insulin-signaling cascade (Nowak *et al*., 2005). Because IR is associated with T2D (Meigs *et al*., 2007) we attempted to associate the apolipoprotein A5 gene polymorphism with IR in the sample. Our findings demonstrate no association with the Q139X and IR. Hence, we propose that these factors are not likely to be responsible for the marked differences in IR susceptibility in Q139X carriers in this sample.

6.4.2 Genes related to insulin resistance

Lipoprotein Lipase polymorphisms

Lipoprotein lipase plays a key role in central obesity, insulin sensitivity, foam cell formation (Mead, 2002), cardiovascular risk factors (Mead, 2002) and the MS (Aguilera *et al*., 2008). Its expression is modulated by gene-gene (Brunzell *et al*., 1995) and or gene-environmental variations (Jemaa *et al*., 1995; Gagne *et al*., 1999; Sing *et al*., 1999; Arca *et al*., 2000; Clee *et al*., 2001; Wittrup *et al*., 2002; Yang *et al*., 2004). Considering IR is the driving factor for the development of the MS (Meigs *et al*., 2007), polymorphisms

related to IR were investigated to determine their propensity to CV risk factors in relation to the MS.

Frequency of the HinfI polymorphism

The distribution of the HinfI varied in the sample, with a higher frequency of the homozygous wild type genotype being detected (77.3%) (Table XVII). There have been similar observations with regards to the allele frequencies of the HinfI polymorphism. Huang *et al* (2011) reported a frequency of 88.0% in the Chinese, whilst Stocks *et al* (1992) showed an allelic frequency of 89.1% in Caucasians and Mattu *et al* (1994) a 91.0% in Caucasians for the C allele. The present findings report an allelic frequency of 86%, which is similar to that reported by Ranjith *et al* (1994) (89.0%) in Asian South Africans. The varying frequencies observed are attributed to racial factors, inclusion criteria that were used to define the control group and/or environmental factors that influenced the association of the HinfI in the respective studies.

Association of the lipoprotein lipase gene polymorphism with the metabolic syndrome

The lipoprotein lipase gene is a candidate gene for lipid metabolism (Marcais *et al*., 2000; Ma *et al*., 2003; Sorquard *et al*., 2006; Cho *et al*., 2008). The HinfI polymorphism, in particular, located at exon 9 (Salah *et al*., 2009) encodes the lipoprotein lipase enzyme, which contributes to increased lipolytic activity (Ross *et al*., 2005). Earlier studies have shown significant associations between the G allele and reduced triglycerides, elevated HDL-C levels (Jemaa *et al*., 1995; Groenemeijer *et al*., 1997; Gagne *et al*., 1999;

Wittrup *et al*., 1999; Garenc *et al*., 2000; Huang *et al*., 2006) and overall a protective effect against the MS (Goodarzi *et al*., 2004; Komurcu-Bayrak *et al*., 2007; Jensen *et al*., 2009), thus conferring a cardio-protective effect (Franceschini *et al*., 1985; Lacoviello *et al*., 1998; Margaglione *et al*., 1998). The present findings however, did not suggest any protective effects against the MS (Table XX) and therefore may not influence cardiovascular events in this sample.

Recently, Kusunoki *et al* (2012) reported that an imbalance of lipoprotein lipase activity alters triglycerides contributing to IR and thus increased adipocytes. In this regard, the G allele has been associated with adiposity in the form of increased waist circumference, elevated triglycerides and elevated blood glucose levels for the MS (Wood *et al*., 2011). This is in contrast with our findings, as we found no relationship between HinfI with the metabolic parameters (Table XXIII-XXVIII). Further evidence suggests that the C allele is associated with hypertension (Clee *et al*., 2001; Talmud *et al*., 2007) and the G allele is protective against hypertension. In spite of the elevated diastolic and systolic readings found in C allele carriers diagnosed with the MS in our study (Table XXIII, XXVIII), we found no significant associations between the polymorphism and hypertension, suggesting that the C allele in this sample is not directly involved in the pathophysiology of the MS. We propose that inconsistencies in the effect brought about by HinfI are likely to be associated with linkage disequilibrium between functional lipoprotein lipase polymorphisms and HinfI at the lipoprotein lipase locus (Liu *et al*., 2005) and, more importantly, as a result of environmental factors (Jemaa *et*

al., 1995; Gagne *et al*., 1999; Sing *et al*., 1999; Arca *et al*., 2000; Clee *et al*., 2001; Wittrup *et al*., 2002; Yang *et al*., 2004).

Smoking is associated with lower plasma LPL activity (Freeman *et al*., 1998) and delayed metabolism of triglyceride rich lipoproteins that reduces HDL-C levels (Wilson *et al*., 1983) and in this way predisposes to the MS. When we associated smoking, alcohol consumption and physical activity with HinfI we did not observe an increased risk for the MS in this sample (Table XXIX, XXX), which is in contrast with studies by Lee *et al* (2004) who showed an increased risk of the MS in participants with the HinfI polymorphism who smoked and consumed alcohol. Interestingly, in the present study, non-smokers with the CG genotypes and G alleles appeared to be protected against the risk for MS (Table XXIX). This is probably due to G allele (X447) carriers having higher HDL-C levels and a greater degree of protection against the risk for the MS (Komurcu-Bayrak *et al.,* 2007). Our findings demonstrate a larger proportion of participants in the control group (with no MS) who were non-smokers and carried the G allele (Table XXIX, XXX) corroborating the latter studies. Because the G allele in this study was not associated directly with the MS, we attempted to assess the relationship between the HinfI polymorphism and IR in participants with the MS due to IR being the driving factor for the pathogenesis of the MS (Reaven, 1995). This study demonstrated no association between HinfI, IR and the risk for the MS (Table XXXI), contradicting Wood *et al's* (2011) study where a positive relationship was demonstrated between the G allele and IR. However, we identified positive relationships between IR and the metabolic parameters. IR males with

the GG genotype were significantly associated with hypertension, increasing the risk for the MS (IDF and harmonized) (Table XXXIV, XXXV), whilst females with this genotype were protected against high systolic blood pressure and the MS (IDF) (Table XXXVI). These findings probably occurred due to lifestyle and or environmental influences in the sample. We showed IR non-smokers with the CG genotypes and G alleles to be protected against the risk for MS (Table XXIX, XXX), corroborating Komurcu-Bayrak *et al's* (2007) study where the genotypic/allelic impact of HinfI was modulated by cigarette smoking. This links smoking to beta cell function and IR (Daniel *et al*., 2005) and may explain why our large IR sample who were non-smokers did not develop the MS.

<u>*Human paraoxonase 1 polymorphisms*</u>

Human Paraoxonase 1 plays a key role in the pathophysiology of atherosclerosis by its involvement in oxidative stress (Eckersen *et al*., 1989). Its regulation has been well established in the literature (Holvoet *et al*., 2001; Holvoet *et al*., 2003, Holvoet *et al*., 2004; Hoogeveen *et al*., 2007; Njajou *et al*., 2009) and is reported to be modulated by gene-gene (Mackness *et al*., 1997; Leviev *et al*., 2000) and or gene-environment interactions, which contribute significantly to the susceptibility of CAD (Osei-Hyiaman *et al*., 2001). Since oxidative stress is a significant factor contributing to metabolic risk factors (Holvoet *et al*., 2001; Holvoet *et al*., 2003, Holvoet *et al*., 2004; Hoogeveen *et al*., 2007; Njajou *et al*., 2009), we examined the association of polymorphisms with the MS and its components.

Frequency of the 192Arg/Gln polymorphism

The distribution of the 192Arg/Gln varied in the sample, with a higher frequency of the homozygous wild type genotype being detected (Table XVII). The literature highlights variability in allele frequencies of 192Arg/Gln among different geographic populations. For example, Scacchi *et al* (2003) reported 192Arg allelic frequencies of 31.3% in the Italians, 24.8% in Sardinia participants, 40.8% in Ethopians and 61.2% in participants from Benin. The present findings however, report a comparatively high allelic frequency of 74% for the 192Arg allele (Table XVII), which is similar to the 78.9% frequency observed in Ecuador participants (Scacchi *et al*., 2003).

Association of the human paraoxonase 1 gene polymorphism with the metabolic syndrome

There is substantial evidence linking CAD with HDL-C levels occurring as a result of inhibition of LDL oxidation (Mertens *et al*., 2001) by attachment of the 192Arg allele to HDL. This modulates cholesterol efflux and influences the risk for CV related disorders (Bhattacharyya *et al*., 2008), namely IR and adiposity. These factors are considered key components for the MS, but there are few studies associating this polymorphism with the MS, as studies to date focus primarily on human paraoxonase 1 activity. For example, Senti *et al* (2003) and Yilmaz *et al* (2010) reported low levels of human paraoxonase 1 in participants with the MS due to increased oxidative stress. In terms of the polymorphism, the 192R allele has been associated with reduced human paraoxonase 1 activity (Tomas *et al*., 2002). The present study did not record human paraoxonase 1 activity and we therefore cannot make any comparisons

in this regard, but instead we report no significant associations between the 192Arg/Gln polymorphism and the MS (Table XX). We cannot associate our findings with other published studies due to limited reports being available. One study by Martinelli *et al* (2005) showed an increased risk for the MS in 192Arg allele carriers due to reduced protection from lipid peroxidation, which is in contrast with our findings as similar genotype/allele distributions were observed in participants with and with no MS (Table XX).

When the metabolic components were analyzed, we showed significant associations between the polymorphism and systolic blood pressure in males, when the harmonized definition was applied (Table XXVI). Since AA homozygotes had higher systolic blood pressure readings (Table XXVI), male participants with hypertension and with this genotype, may possibly have increased oxidative stress that alters the functioning of paraoxonase (Irace *et al*., 2008) by inactivating nitric oxide by reactive oxygen species during endothelial dysfunction (Consentino *et al*., 2001). Environmental factors may further influence the effect of paraoxonase expression (Hashemi *et al*., 2011). Our findings showed no gene-environmental associations with the MS (Table XXIX, XXX). We did, however, observe a reduced risk of the MS in non-smokers with the human paraoxonase 1 192Arg/Gln polymorphism (AG genotypes and A alleles: IDF and harmonized) (Table XXIX, XXX). This is to be expected as smoking is known to reduce human paraoxonase 1 activity resulting in reduced HDL-C levels (Nishio *et al*., 1997). The human paraoxonase 1 Arg allele in this regard elevates paraoxonase 1 levels and

increases HDL-C (Mackness *et al*., 1996), so protecting low density lipoproteins from lipid peroxidation (Mackness *et al*., 1997).

Additionally, we found that participants who did not consume alcohol who had the human paraoxonase 1 192Arg/Gln polymorphism (AG genotypes and A alleles) were protected against the MS (Table XXIX, XXX). However we were not able to demonstrate a relationship between the genotype/alleles and HDL-C levels. This is in contrast to studies which show that non-alcohol consumers have lower paraoxonase 1 and reduced HDL-C levels (Gouedard *et al*., 2004), resulting in an increase in the risk for the MS. It is known that the human paraoxonase 1 192 Arg allele increases PON1 activity and elevates HDL-C levels so protecting against the MS. Our findings may be explained by the possibility that the protective effect of alcohol may be related to the amount of alcohol consumed. Further studies need to be done in this field.

To our knowledge there are no studies in Asian Indians associating IR with the 192Arg/Gln polymorphism. Our study found a positive relationship between AA homozygote carriers with the MS and high systolic blood pressure according to the harmonized definition (Table XXXIII). Evaluation of gender groups confirmed an increased risk of high systolic and diastolic blood pressure in insulin resistant males with the MS carrying the homozygous AA and heterozygous AG genotypes respectively (IDF) (Table XXXIV). When the harmonized definition was applied, insulin resistant males with the MS carrying the homozygous AA genotypes were more susceptible to

hypertension (Table XXXV). One possibility for our results is attributed to elevated fasting blood glucose levels (considered a predictor of IR) in this community (Figure 19D), which interrelates with hypertension (due to increased oxidative stress) and genetics (Epstein, 1992). In terms of environmental associations, we did not identify any relationship between the 192Arg/Gln polymorphism with smoking status, alcohol consumption and physical activities performed in insulin resistant participants with the MS (Table XXXVIII, XXXIX). These findings are in contrast with Bortolasci *et al* (2014) study in which smoking was shown to increase the risk for the MS in 192Arg/Arg or 192 QQ carriers. To our knowledge, there are no studies associating alcohol consumption and physical activity with the MS, IR and the 192Arg/Gln polymorphism. This underscores the importance of considering IR and T2D when investigating the association between human paraoxonase 1 192Arg/Gln polymorphism and the risk for MS and CAD.

Cholesteryl ester transfer polymorphisms

Cholesteryl ester transfer proteins plays a role in the pathway of "reverse cholesterol transport" (Mohrschladt *et al*., 2005), and has been implicated in atherosclerosis (Austin *et al*., 1988; Sharrett *et al*., 2001; Harder *et al*., 2007). As a result, there has been interest in the cholesteryl ester transfer protein polymorphisms that confer to the risk or protection caused by the reverse cholesterol transport pathway. Previous studies have reported that cholesteryl ester transfer protein polymorphisms are associated with cholesteryl ester transfer protein measures (Parthasarathy *et al*., 1990) that contribute

significantly to atherosclerosis (Austin *et al*., 1988; Sharrett *et al*., 2001; Harder *et al*., 2007).

Cholesteryl ester transfer protein has been reported to possess anti-atherogenic properties (Miller *et al*., 2003; Plengpanich *et al*., 2009) reducing the risk for CAD. The effects of the cholesteryl ester transfer protein polymorphism have been shown in Asian Indians to be associated with increased HDL-C levels (Ranjith *et al*., 2009). It is well recognized that cholesteryl ester transfer protein plays a key role in reducing HDL-C levels during "reverse cholesterol transport" (Mann *et al*., 1991; Lagrost *et al*., 1994) and since reduced HDL-C is a component of the MS, we examined the relationship of a common cholesteryl ester transfer protein polymorphism with the MS and its components.

Frequency of the Taq1B polymorphism

The frequency of Taq1B varied in the sample, with no statistical significance being detected. There was a higher frequency for the GA (B1B2) genotype in this sample as compared to the GG (B1B1) (46.6% vs 31.1%) (Table XVII), which is similar to Bernard *et al's* (1998) (43.8% vs 38.1%) and Carlquist *et al's* (2003) (50.3% vs 32.9%) findings.

The G (B1) allele has been reported in 58.5% of Japanese (Meguro *et al*., 2000), 57.7% of Chinese (Hsu *et al*., 2002), 74.5% of African Americans

(Cuchel *et al*., 2002) and in 50% of Indians (Mukherjee *et al*., 2004). This allele appeared to be the dominant allele in most populations, concurring with our findings, where we report a 54% frequency (Table XVII). The frequency of the A (B2) allele in our sample was similar to those reported by Freeman *et al* (1994) (46%), and was observed in 46% of our participants.

Association of the cholesteryl ester transfer protein gene polymorphism with the metabolic syndrome

Cholesteryl ester transfer protein polymorphisms, particulalry Taq1B, have been inconsistently associated with CAD (as a result of the MS) as observed in population studies. For example, Rahimi *et al* (2013) reported no association with CAD in Iranian participants with the Taq1B polymorphism, whereas in contrast Padmaja *et al* (2009) reported an increased risk of CAD in participants with the G (B1) allele. These differences are attributed to cholesteryl ester transfer protein activity, which influences HDL-C levels (Kondo *et al*., 1989; Freeman *et al*., 1990; Freeman *et al*., 1994; Hannuksela *et al*., 1994; Mitchell *et al*., 1994; Fumeron *et al*., 1995). For example, studies show GG (B1B1) carriers with reduced HDL-C levels (Noone *et al*., 2000; Boekholdt *et al*., 2004) and AA (B2B2) carriers with higher HDL-C levels (Dixit *et al*., 2005; Ranjith *et al*., 2009; Kashari *et al*., 2010). Several other studies showed no association of TaqIB polymorphism with HDL-C levels (Goto *et al*., 2001; Kakko *et al*., 2001; Meguro *et al*., 2001), which is similar to our findings (Table XXIII- XXVIII). Additionally, in the present study we found no association of Taq1B with the MS (Table XXIII- XXVIII) and we

believe that similarities in the distribution of Taq1B in participants with and with no MS may have influenced our findings (Table XX).

However, we found that GA (B1B2) genotypes are protective for hypertension, specifically systolic blood pressure, in participants diagnosed with no MS (IDF and harmonized) (Table XXIII, XXIV), which appears contradictory to reports by Dixit *et al* (2005) who found the GA (B1B2) genotype to be a risk factor for hypertension and the GG (B1B1) genotype was found to be protective. When gender groups were assessed, we observed GA (B1B2) genotypes of IR females to significantly reduce the risk of hypertension, again specifically high systolic blood pressure, in participants with no MS (harmonized) (Table XXXVII). In fact, this is the first study to record such an association in this population and more studies are required in order to confirm these findings.

Interestingly, this study demonstrated GA (B1B2) carriers to be marginally associated with central obesity in males diagnosed with the MS (IDF) (Table XXV, XXVI), in direct contrast with Pachocka *et al's* (2012) study, which showed positive associations with central obesity (in the form of waist circumference) in GA (B1B2) male carriers. We cannot link our findings to environmental influences, as this study demonstrates no significant association with smoking, alcohol consumption and or physical activity performed with the Taq1B polymorphism (Table XXIX-XXX), contradicting Thu *et al's* (2005) study, which showed environmental factors to increase the

risk for the MS in obese participants. However, our findings may be attributed to the 1:2 ratio of males: females that were randomly selected for this study.

6.4.3 Genes related to obesity

<u>*Adiponectin polymorphisms*</u>

Adioponectin plays a key role in the pathophysiology of atherosclerosis (Yung *et al*., 2006; Knudson *et al*., 2007) as its levels are believed to promote FFA oxidation in peripheral tissue (Fruebis *et al*., 2005) thereby modulating energy homeostasis and contributing to metabolic diseases (Yoon *et al*., 2006). As a result, much focus has been given to establishing whether the adiponectin polymorphisms to confer increased risk or provide protection from the effects of FFA oxidation.

Many population studies (Table III) have identified significant associations between adiponectin polymorphisms and obesity-associated metabolic risk factors due to the mechanisms of FFA oxidation which, in turn contribute to the development of CVD as a result of the MS. Adiponectin possesses anti-inflammatory and anti-atherogenic properties (Yung *et al*., 2006; Knudson *et al*., 2007), and hence contributes to a reduced risk for CVD (Arita *et al*., 1999; Weyer *et al*., 2001).

Evidence further indicates that adiponectin ensures insulin sensitivity (Kodowaki *et al*., 2005) by inhibiting gluconeogenesis by FFA oxidation, thereby stimulating glucose uptake by skeletal muscle which in turn contributes to the risk for T2D (Hotta *et al*., 2000; Lindsay *et al*., 2002;

Spranger *et al*., 2003; Li *et al*., 2009). Also, adiponectin has been further linked to dyslipidemia in the MS (Schneider *et al*., 2005) which is probably mediated by PPARα (Lewis *et al*., 2004). Since obesity is a determinant in the pathogenesis of these features of the MS (Reaven *et al*., 1995), occurring by FFA oxidation, we examined the association of common polymorphism with the MS and its components.

Frequency of the 45T>G polymorphism

The frequency of the 45T>G polymorphism varied in the sample with no significance being detected (Table XVII). We assume that the lack of statistical differences between the groups is due to the low frequency of the studied polymorphism and it is suggested that a larger group of participants should be analyzed in future studies. There was a six times higher frequency for the T allele in this sample as compared to G allele (86% vs 14%) (Table XVII), which is similar to Namvaran *et al's* (1998) (87% vs 13%) and Sobouri *et al's* (2011) (88.3% vs 11.7%) findings in Iranians.

The common TT genotype has been reported in 49.3% of Koreans (Chung *et al*., 2009), 72.6% of Greeks (Melistas *et al*., 2009) and in 77.2% of Iranians (Sabouri *et al*., 2011). This genotype appeared to be the dominant genotype in these populations, concurring with our findings where we report a 74.5% frequency (Table XVII). The frequency of the less common GG genotype in our sample (1.8%) (Table XVII) was similar to that reported by Melistas *et al* (2009) (2.0%).

Association of the adiponectin gene polymorphism with the metabolic syndrome

Adiponectin polymorphisms have been inconsistently associated with the risk for CAD (Sobouri *et al.*, 2011). For example, in a cross sectional study of Whites with T2D from Switzerland and France, a positive association with CAD and the 45T>G has been reported (Qi *et al.*, 2006), whereas in contrast, Chang *et al* (2004) reported a protective effect and a reduced risk for CAD in diabetic participants with the +45G allele whilst Bacci *et al* (2004) reported no association. These studies probably occurred due to endothelial inflammation by TNF-α that increased monocyte production, hence adhering to the arterial endothelium (Maeda *et al.*, 1996; Matsuzawa *et al.*, 1999; Ouchi *et al.*, 1999; Okamoto *et al.*, 2000; Ouchi *et al.*, 2000; Yokata *et al.*, 2000).

Evidence indicates that development of CAD is ascribed to adiponectin levels, which play a key role in inhibiting the production and action of TNF-α (Okamoto *et al.*, 2000). Since CAD develops due to the MS (Goldberg *et al.*, 2012), we provide subsequent analysis on the association of the polymorphism with the MS. However, we report no significant association between the 45T>G and the MS compared to those with no MS, irrespective of the definition used (Table XXI). These finding are similar to Ranjith *et al's* (2011) and Suriyaprom *et al's* (2014) studies in their Asian Indians and Thai participants who observed no link between the 45T>G and the MS. In contrast, a study in the Chinese population showed a positive relationship between the 45T>G and the MS (Li *et al.*, 2012). The reason for the

discrepant findings in the present study and in previous studies may be due to the differing genetic backgrounds of the study groups.

In terms of the association with the components of the MS, we observed the TG (IDF) and TT (harmonized) genotypes to influence reduced HDL-C levels in males with the MS (Table XXV, XXVI). This may be plausible since adiponectin levels have been positively associated with HDL-C (Hotta *et al.*, 2000), mainly due to hepatic lipase activity in the liver (Laakso *et al.*, 1987; Deeb *et al.*, 2013; Sibley *et al.*, 2003), mediated by PPARα (Lewis *et al.*, 2004). To our knowledge, no other study has observed this association, and we propose that larger studies are required to confirm whether the 45T>G confers a greater risk for dyslipidemia in male participants with the MS. In females with no MS (IDF) a reduced risk for central obesity was shown in GG carriers (Table XXVII) which is in contrast with studies by Yang *et al* (2003), Menzaghi *et al* (2002), Ronconi *et al* (2010) and Ukkola *et al* (2003) who all showed the T allele (TT+TG) to correlate with a reduced risk (in the form of BMI). These findings are plausible as central obesity is known to contribute to the MS (Alberti *et al.*, 2005) and is ascribed to adiponectin levels by systemic low-grade inflammation (Guzman-Ornelas *et al.*, 2012).

Additionally, central obesity has been linked with IR and collectively these variables contribute to the MS (Reaven *et al.*, 1995). Studies suggest that the pathogenesis of IR correlates with altered FFA oxidation as a result of adiponectin levels. Therefore, much interest has been invested on the association between adiponectin levels and IR (Blaslov *et al.*, 2013). In terms

of the polymorphisms associated with IR, Li *et al* (2007), Wang *et al* (2008) and Melistas *et al* (2009) found that participants with the +45G allele were at an increased risk for IR (ascribed to reduced adiponectin levels) whereas in contrast, Yang *et al* (2003) and Zacharova *et al* (2005) reported the +45T allele to correlate with increased IR. Lee *et al* (2005) found no association with IR and the polymorphism. We report no link between IR and the 45T>G in females and males with and with no MS (Table XXXI), concurring with Lee *et al's* (2005) study who demonstrated no association with the 45T>G and IR. These discrepant findings in previous reports and in the present study may be due to the differing ethnic backgrounds or environmental factors of the study groups (Curti *et al*., 2012). This study found no link with environmental factors, namely smoking status, alcohol consumption and physical activities performed, and the risk for the MS. This is in contrast with Curti *et al* (2012) who showed lifestyle factors to delay the metabolic effect of the 45T>G in high risk cardiometabolic Brazilians.

When the metabolic components of the MS were evaluated in IR participants in this study, we found IR males with the TT genotypes to be at an increased risk for reduced HDL-C levels and the MS (harmonized) (Table XXXV). Additionally, IR males with TG genotypes with no MS (harmonized) were protected against high systolic blood pressure (Table XXXV). A possible explanation for these findings may be due to the interaction of the 45T>G with environmental factors such as physical activity (Melistas *et al*., 2009), which is similar to the present findings as we found the impact of the 45T>G on IR to be dependent on physical activity i.e. IR participants who perform

physical activity with the TT genotype and T alleles had a reduced risk of developing the MS according to the IDF and harmonized definitions (Table XXXIX). These findings probably occurred due to changes in mRNA stability and adiponectin levels (Li *et al*., 2007). However, limited data is available in the literature and we therefore cannot provide any comparisons in this regard. Further studies are therefore needed to assess the influence of the 45T>G with the metabolic components in participants with IR.

Leptin polymorphisms

Leptin plays a key role in energy homeostasis (Elmquist *et al*., 1998; Friedman *et al*., 1998; Bates *et al*., 2003), occurring by FFA oxidation (Wauters *et al*., 2000; Minokoshi *et al*., 2002) and inhibition of insulin secretion (Seufert *et al*., 1999). Leptin levels are modulated by gene-gene (Considine *et al*., 1995; Hager *et al*., 1998; Li *et al*., 1999) and gene-environmental (Ragin *et al*., 2009; Martins *et al*., 2012) factors and are indeed subjected to variations. Polymorphisms associated with adiposity were analyzed to establish their role with the MS and its components.

Frequency of the 25CAG polymorphism

The distribution of the 25CAG was more prevalent in homozygous wild types than in heterozygotes, with no mutant homozygotes being detected in the study (Table XVII). There is a lack of information with regard to the allele frequencies of the rare 25CAG polymorphism. Our study reports a low frequency for the G allele (0.004%) (Table XVII). In contrast, Kongmacheep *et al* (2009) reported an allelic frequency of 3% for the G mutation. We

cannot make any other allelic associations due to limited association studies being present in the literature, as only one study by Kongmacheep *et al* (2009) reports on the 25CAG frequencies.

Association of the leptin gene polymorphism with the metabolic syndrome

The leptin gene is considered a candidate gene for obesity as a result of leptin modulation (Reaven *et al*., 1988; Bandin *et al*., 2000; Sousa *et al*., 2009) occurring due to the synthesis and secretion of neuropeptide Y and hormones that expresses α-melanocyte, which are key elements for appetite (Yildiz, 2010). The 25CAG, in particular has been reported as a genetic marker for obesity susceptibility in the Japanese (Ohshiro *et al*., 2000), but is a rare polymorphism, which requires further investigation in other populations. Kongmacheep *et al* (2009) reported that the 25CAG alters the signaling pathways as a result of environmental influences, namely, hunger sensations and dietary behavior but showed no significant associations in this regard. Kongmacheep *et al* (2009) added that studies on a larger scale need to be conducted in order to provide substantial conclusions between environmental influences on the 25CAG polymorphism. The present study did not record the eating behavior of participants and therefore cannot make any comparisons in terms of the association with the polymorphism. However, this study found participants with the AA genotypes and A alleles who do not consume alcohol to be protected against developing the MS (IDF and harmonized) (Table XXIX, XXX). Considering a larger proportion of participants in the control group (with no MS) were non-alcohol consumers (Table XXIX, XXX), we suggest that non-alcohol consumption reduces leptin levels and protects

against IR, concurring with Roth *et al* (2003) who showed an inverse relationship with leptin levels and alcohol consumption. There is only one study (Ohshiro *et al.,* 2000) on the 25 CAG polymorphism which showed no association with environmental factors".

Since, central obesity is considered a key component of the MS which contributes to the other metabolic risk factors (Wabitschet *et al*., 1997), we evaluated the association of the individual components of the MS with the 25CAG, and found no significant relationship (Table XXIII-XXVIII). This suggests that the 25CAG may not be a major polymorphism within the leptin locus that influences obesity related effects in this population study. Similarly, in terms of the association of the 25CAG with the metabolic components in IR participants, no association was detected in this study (Table XXXII-XXXVII). However, we found IR participants with the AA genotypes and A alleles who did not consume alcohol to be protected against developing the MS (IDF and harmonized) (Table XXXVIII, XXXIX). It is evident from our findings that there is a gene-environment association with the risk for the MS and we propose that studies on a larger scale need to be conducted in this cohort in order to confirm our findings.

6.5 Hardy-Weinberg equilibrium

In our study, the lipoprotein lipase HinfI polymorphism deviated from Hardy-Weinberg equilibrium (HWE) ($p<0.05$). These findings are in contrast with other studies, which demonstrate no deviation from HWE (Lee *et al*., 2004; Bhanushali *et*

al., 2010). We suggest that the deviation from HWE in this sample is possibly due to "null alleles" being present resulting in false observation of homozygotes, inbreeding (Karlsson *et al*., 2005) and/or phenotypic assortative mating as a result of religion, age, socioeconomic status, intelligence and physical traits (Kail *et al*., 2010). These published factors are indicative of this community, in terms of their immigration from the Indian subcontinent in 1860 and their cultural practices, dietary habits and beliefs have evolved due to their adoption of the Western way of living.

The other polymorphisms in this study (apolipoprotein A5 Q139X, human paraoxonase 1 192Arg/Gln, cholesteryl ester transfer protein Taq1B, adiponectin 45T>G and leptin 25CAG) demonstrated no deviation from HWE ($p>0.05$). With regard to the apolipoprotein A5 Q139X, no other study in the literature described HWE, whilst studies of the human paraoxonase 1 192Arg/Gln (Agrawal *et al*., 2009), cholesteryl ester transfer protein Taq1B (Bhanushali *et al*., 2010) and adiponectin 45T>G (Ranjith *et al*., 2011) reported no deviation from HWE, concurring with the present findings. These findings suggest that there were no evolutionary changes in this community, based on five published factors viz. communities are isolated from others (no emigration or immigration occurs), with no mutations, and or random mating occurring, and no natural selection (all participants in this community have an equal survival rate) (Andrews, 2010). The Phoenix community comprises 85% Asian Indians (Census, 2011) indicating and confirming the latter assumptions. This study shows no deviation from HWE for the rare leptin 25CAG. However the one study that reported on the rare leptin 25CAG (Kongacheep *et al*., 2009) failed to document HWE and we therefore cannot comment in this regard.

6.6 Conclusion

Our results demonstrate that South African Indians from the Phoenix community present with an increased risk for the MS (51.6%: IDF; 49.0%: harmonized). Age gender and clustering of the metabolic components influenced the progression of the MS in this sample. IR was observed to be the driving factor for its pathogenesis with age, gender, clustering patterns and physical activity serving as key contributing factors. Since central obesity was the highest contributor to the MS and IR in our study (irrespective of the definition used), we propose that approaches need to be implemented in order to improve diet and nutrition, overcome sedentary lifestyles and increase physical activity in the sample. Health authorities may contribute significantly in preventing the development of the MS by educating and training participants on weight management and physical activities. Since the MS is a reversible condition (Barnard *et al*., 1994), preventative interventions may contribute immensely in averting T2D, CVD and other clinical complications.

Since the prevalence of IR is high in this community and is the underlying factor for the MS (Reaven, 1988) and T2D Lillioja *et al*., 1993; Pyorala *et al*., 2000; Shulman, 2000; Sharma *et al*., 2006), an emphasis on its development (as a result of metabolic disturbances) will contribute to the understanding of its origin. One of the key observations in this study was that fasting blood glucose levels appeared to serve as a suitable predictor for IR in this community and hence, health professionals should be vigilant for glucose intolerance in patients at hospitals and or clinics. This approach will allow for early detection of IR and will improve metabolic outcomes, thus, preventing the progression of CVD in this community.

Overall, the MS represents a complex entity, consisting of several metabolic risk factors (Park *et al*., 2003) and identification of possible candidate gene polymorphisms associated with the MS may be of utmost importance. It will allow researchers and clinicians to elucidate the pathogenesis of the MS, leading to the formulation of treatments and or strategies that will predispose to the MS in this sample.

In contrast to previous studies we did not observe any relationship between the studied polymorphisms (apolipoprotein A5 Q139X, lipoprotein lipase HinfI, human paraoxonase 1 192Arg/Gln, cholesteryl ester transfer protein Taq1B, adiponectin 45T>G and leptin 25CAG) and the MS entity, but we found associations with gender and components of the MS. Previous studies did not employ random sampling in the community as we did and also comprised smaller samples. For example, the apolipoprotein A5 Q139X polymorphism was evaluated only in 9 White participants (Marcais *et al*., 2005), the lipoprotein lipase HinfI in 99 Caucasians (Garenc *et al*., 2000), the human paraoxonase 1 192Arg/Gln in 434 French (Ruiz *et al*., 1995), the cholesteryl ester transfer protein Taq1B in 365 Taiwanese (Hsieh *et al*., 2007), the adiponectin 45T>G in 151 Uygur (Li *et al*., 2007) and the leptin 25CAG in 30 Thai participants (Komgmacheep *et al*., 2009). Population stratification could have confounded the genetic findings in these studies since false-positive findings were observed. Although our study was performed on 1000 Indian subjects the larger sample made population stratification less likely and increased the robustness of our findings which show no significant relationship between the polymorphisms studied and the MS. The larger randomized sample in our study compared to previous studies

(Ruiz *et al*., 1995; Marcais *et al*., 2005; Hsieh *et al*., 2007; Li *et al*., 2007; Komgmacheep *et al*., 2009; Ranjith *et al*., 2009) also strengthens our findings.

Ours is the first study that showed males diagnosed with MS with the adiponectin 45T>G and the human paraoxonase 1 192Arg/Gln are more inclined to have reduced HDL-C levels and elevated systolic blood pressure, respectively. Similarly, the presence of the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms in insulin resistant participants increased the risk for high systolic and diastolic blood pressure in males with the MS; whilst the adiponectin 45T>G was associated with reduced HDL-C in insulin resistant males with MS.

Gene-environmental associations showed no association with the MS or with IR in this study. However, to our knowledge this is the first study in this sample to show the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms to exert a protective effect against the risk for MS in non-smokers and the human paraoxonase 1 192Arg/Gln and leptin 25CAG in non-alcohol consumers. Similarly, IR participants who performed physical activities and who did not consume alcohol and carried the adiponectin 45T>G and leptin 25CAG, respectively, appeared to be protected against the MS.

We therefore conclude that the adiponectin 45T>G and the human paraoxonase 1 192Arg/Gln polymorphisms are genetic markers that may assist in identifying participants who are susceptible to hypocholesterolemia (in males with the MS and with IR) and hypertension (in males with the MS), respectively. The lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms associated with IR may

also serve as genetic markers that may assist in identifying males with MS who are susceptible to hypertension. On the other hand, non-smokers with the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln polymorphisms and non-alcohol consumers with the human paraoxonase 1 192Arg/Gln and leptin 25CAG polymorphisms are protected from developing the MS. Similarly, IR participants with the adiponectin 45T>G and leptin 25CAG polymorphisms who perform physical activities and who are non-alcohol consumers, respectively, are also protected from developing the MS. Hence, the genetic risk for the MS may lie in its components (which includes environmental factors) rather than the MS as an entity. We propose that genetic studies on larger sample sizes with advanced laboratory techniques and bioinformatics may produce more meaningful results for future candidate gene studies, allowing for effective interventions that will reduce the prevalence of the MS and CVD in this community.

Since the MS is a growing public health burden in Phoenix, we recommend that future studies need to be undertaken, and should address novel molecular mechanisms, gene-gene and gene-environmental influences in order for us to appreciate the complexity of the MS, and to prevent its development.

6.7 Limitations of this study

The present study has several limitations, which should be noted:

- Firstly, this investigation used cross sectional data, which provided information on a once-off basis. Previous investigations employed cross sectional designs (Motala *et al*., 2011; Ranjith *et al*., 2011). It is therefore recommended that

longitudinal studies should be considered as this could contribute immensely to the understanding of the MS and its association with genetic factors.

- Secondly, we used stored whole blood for the extraction of DNA. We must bear in mind that degradation may possibly have occurred during transportation from the Lifestyle Centre, Inkosi Albert Luthuli Central Hospital to the Chemical Pathology Laboratory at Inkosi Albert Lutuli Central Hospital and finally to the Pfizer Molecular Biology Unit, Doris Duke Medical Research Institute (DDMRI), University of KwaZulu Natal. Our suggestions are in line with Makowski *et al* (1996) who reported that DNA from whole blood deteriorates with prolonged transportation and storage.
- Thirdly, modulation of blood lipids by gene-gene and gene-environmental influences has been reported to influence enzymatic activity (Ordovas, 2009), and our study did not consider these factors. We believe that a more detailed individual analysis could have been conducted in this community in order to address the MS and to facilitate therapeutic approaches.
- Fourthly, there was a weak publication bias for the apolipoprotein Q139X, human paraoxonase 1 192Arg/Gln and the leptin 25CAG polymorphism in MS participants and more data would have allowed for more conclusive comparisons.
- Fifthly, the participants of the Phoenix Lifestyle Project study are predominantly females (due to random sampling), and this could have limited the generalizability of our findings. This high female to male ratio (2:1) has been employed in a previous community study (Motala *et al*., 2011), which evaluated the metabolic risk factors, and is, hence, consistent with this study.
- Sixthly, there is a lack of haplotype analysis in this study. It will be valuable to replicate these findings and test for haplotypes associated with the risk for the MS.

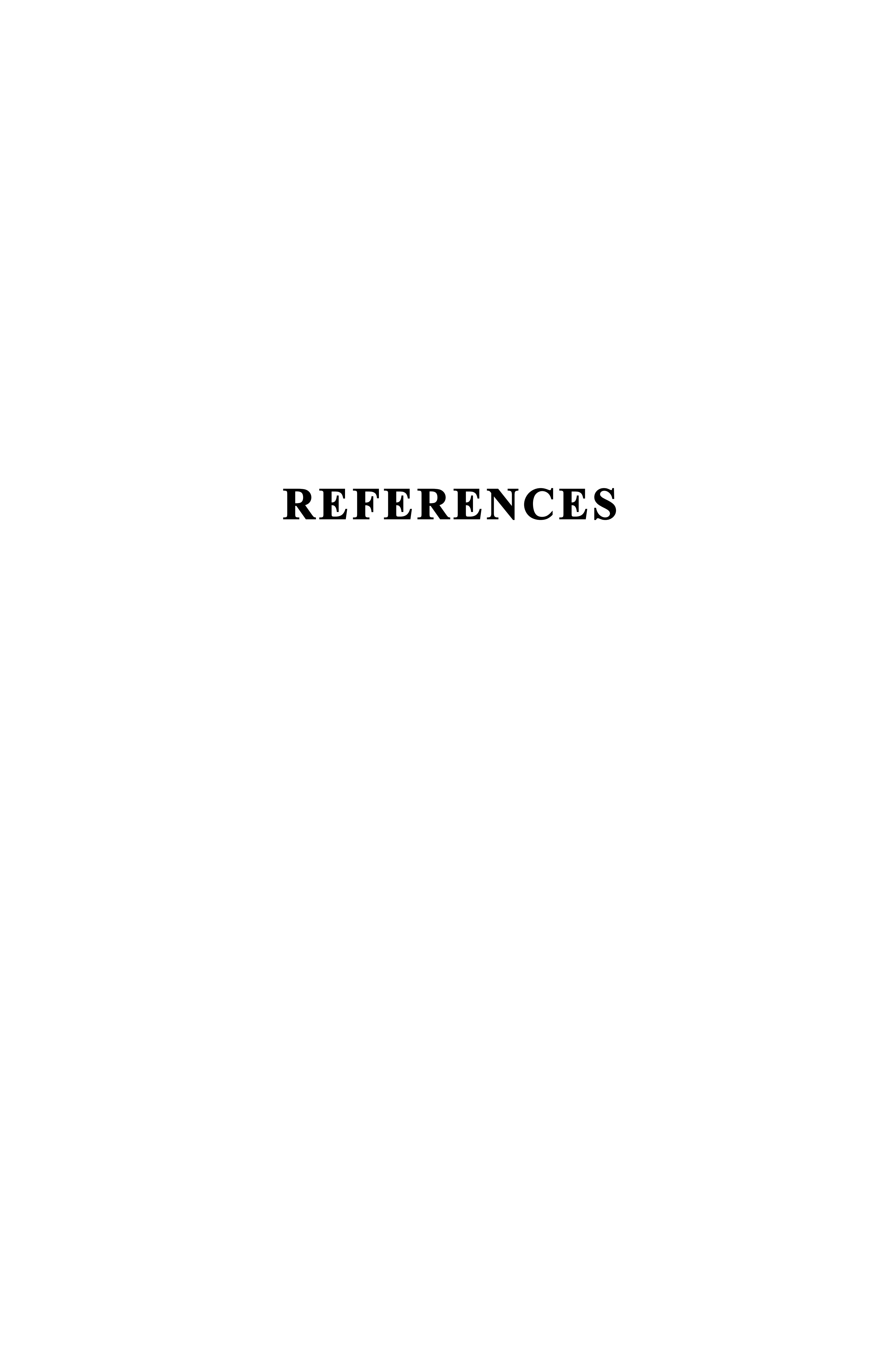
This could improve our understanding of the genetic associations of the MS in South African Indians.

- Seventhly, we did not measure the circulating intermediate phenotypes for the phenotypic expression of the studied polymorphisms. This could account for the lack of relationship between the genotype and the MS, its individual components, or IR due to the fact that the genotype may have no effect on the intermediate molecule itself in the Indian population. We did not observe any studies in the Indian population that recorded the circulating intermediate phenotypes for the phenotypic expression of the studied polymorphisms. In this regard, future studies are warranted.
- Additionally, the genotype-phenotype interactions that we have found were not a priori hypotheses but were discovered through exploratory analysis and these findings should therefore be interpreted with caution. Furthermore, the suggested mechanisms underlying the interactions cannot be confirmed from our findings because we did not measure the intermediate phenotypes for these genes.
- Lastly, our study analyzed six candidate gene polymorphisms. Since the MS is a complex entity, occurring due to lipid and carbohydrate metabolism, it is likely that there are numerous candidate gene polymorphisms that influence the metabolic effects in this sample, and genome wide association scans may provide more accurate results, hence, allowing for the eradication of this syndrome in this community.

6.8 Summary

A high number of Indians living in Phoenix have the MS with risk factor clustering, age and gender influencing its progression. No association between the

polymorphisms studied and the risk for MS was observed, except for an association with the adiponectin 45T>G and low HDL; and the human paraoxonase 1 192Arg/Gln with hypertension (in males). Additionally, no association between the polymorphisms studied and the risk for MS in IR participants was observed, except for an association with the lipoprotein lipase HinfI and human paraoxonase 1 192Arg/Gln with hypertension (in IR males). The genetic risk for MS may therefore lie in its components rather than the MS as an entity. Gene-environmental associations exerted a degree of protection against the risk for the MS and IR. Studies on larger samples will allow for effective interventions thereby reducing the MS and CVD in this community.



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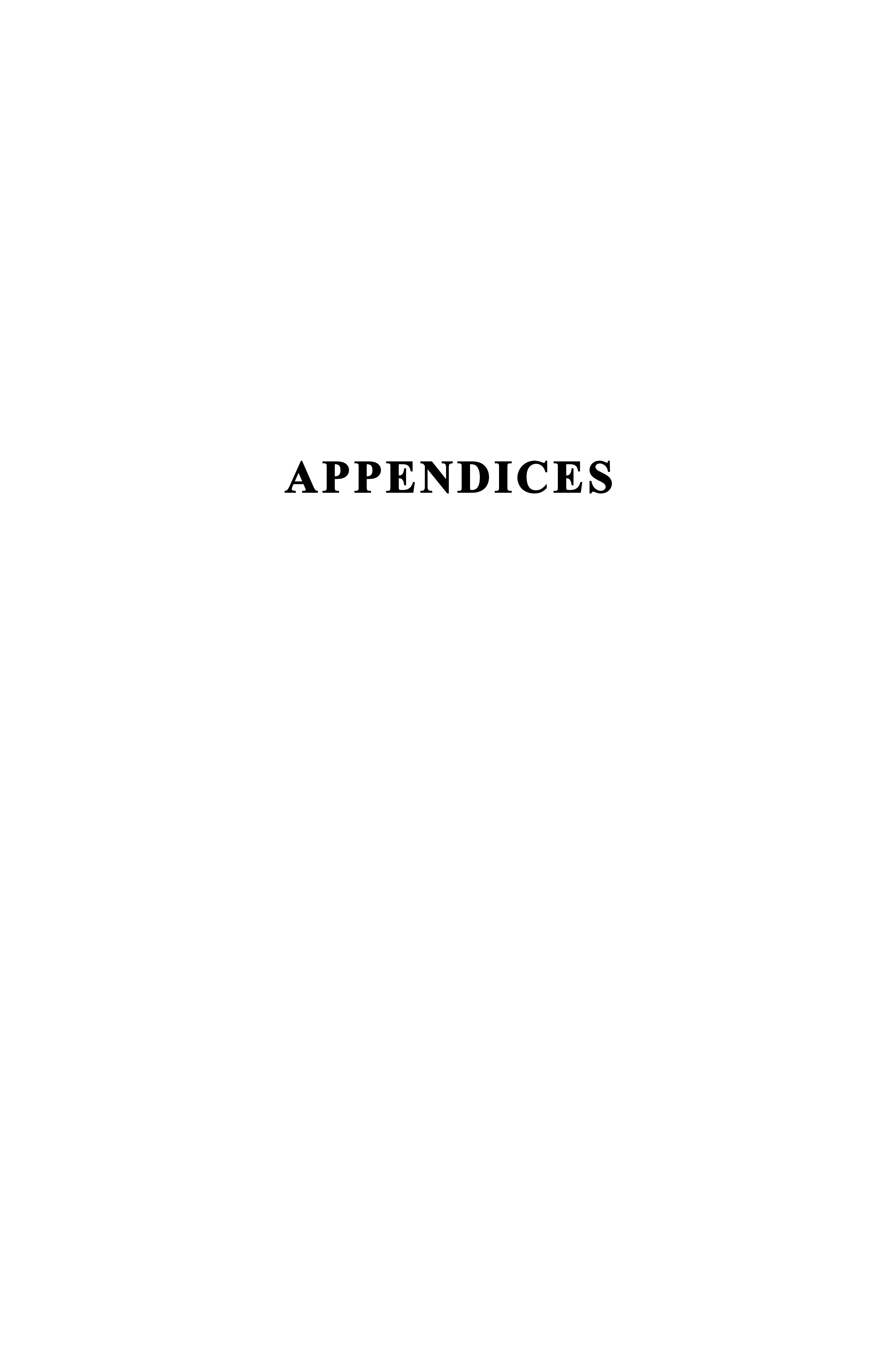

APPENDICES

Participant Identification Number └─┴─┘ └─┴─┘ └─┴─┘

WHO STEPS Instrument
for Chronic Disease
Risk Factor Surveillance

Phoenix, South Africa

Survey Information

	Location and Date	Response	Code
1	Interviewer Identification	└─┴─┴─┘	I4
2	Date of completion of the instrument	└─┴─┘ └─┴─┘ └─┴─┴─┴─┘ dd mm year	I5

Participant Id Number └─┴─┴─┴─┴─┴─┴─┘

	Consent, Interview Language and Name	Response	Code
3	Consent has been read out to participant	Yes 1 No 2 **If NO, read consent**	I6
4	Consent has been obtained (verbal or written)	Yes 1 No 2 **If NO, END**	I7
5	Interview Language [Insert Language]	English 1 [Add others] 2 [Add others] 3 [Add others] 4	I8
6	Time of interview (24 hour clock)	└─┴─┘ : └─┴─┘ hrs mins	I9
7	Family Name		I10
8	First Name		I11
9	Contact phone number where possible		I12
10	Specify whose phone	Work 1 Home 2 Neighbour 3 Other (specify) 4	I13
		Other └─┴─┴─┴─┴─┴─┴─┘	I13 other
11	Household no.		I14
12	Street Name		I15

Record and file identification information (I6 to I13) separately from the completed questionnaire.

Step 1 Demographic Information

CORE: Demographic Information			
Questions		**Response**	**Code**
13	Sex (*Record Male / Female as observed)*	Male 1 Female 2	C1
14	What is your date of birth? *Don't Know 77 777 7777*	└─┴─┘ └─┴─┘ └─┴─┴─┴─┘ dd mm year	C2
15	How old are you?	Years └─┴─┘	C3
16	In total, how many years have you spent at school or in full-time study (excluding pre-school)?	Years └─┴─┘	C4
17	What is the highest level of education you have completed?	No formal schooling 01 Less than primary school 02 Primary school completed 03 Secondary school completed 04 High school completed 05 College/University completed 06 Post graduate degree 07 Refused 08	C6
18	Which of the following best describes your <u>main</u> work status over the last 12 months? *(USE SHOWCARD)*	Government employee 01 Non-government employee 02 Self-employed 03 Non-paid 4 Student 05 Homemaker 06 Retired 07 Unemployed (able to work) 08 Unemployed (unable to work) 09 Refused 88	C7
19	How many people older than 15 years, including yourself, live in your household?	Number of people └─┴─┘	C8
20	Taking **the past year**, can you tell me what the average earnings of the household have been? *(RECORD ONLY ONE, NOT ALL 4)*	Per week └─┴─┴─┴─┴─┴─┴─┘ *Go to T1* OR per month └─┴─┴─┴─┴─┴─┴─┘ *Go to T1* OR per year └─┴─┴─┴─┴─┴─┴─┘ *Go to T1* Refused 8	C9a C9b C9c C9d
21	If you don't know the amount, can you give an **estimate** of the annual household income if I read some options to you? Is it	≤4800 1 1 More than 4800, ≤ 19200 2 2 More than 19200, ≤ 38400 3 3 More than 38400, ≤76800 4 4 More than 76800 4 5 Don't Know 7 Refused 8	C10

Step 1 Behavioural Measurements

CORE: Tobacco Use

Now I am going to ask you some questions about various health behaviours. This includes things like smoking, drinking alcohol, eating fruits and vegetables and physical activity. Let's start with tobacco.

	Questions	Response	Code
22	Do you currently smoke any **tobacco products**, such as cigarettes, cigars or pipes?	Yes 1 No 2 *If No, go to T6*	T1
23	**If Yes,** Do you currently smoke tobacco products **daily**?	Yes 1 No 2 *If No, go to T6*	T2
24	How old were you when you **first started** smoking daily?	Age (years) └─┴─┴─┘ *If Known, go to T5a* Don't remember 777	T3
25	Do you remember how long ago it was? *(RECORD ONLY 1, NOT ALL 3)* Don't remember 777	In Years └─┴─┴─┘ *If Known, go to T5a*	T4a
		OR in Months └─┴─┴─┘ *If Known, go to T5a*	T4b
		OR in Weeks └─┴─┴─┘	T4c
26	On average, **how many** of the following do you smoke each day? *(RECORD FOR EACH TYPE)* Don't remember 777	Manufactured cigarettes └─┴─┴─┘	T5a
		Hand-rolled cigarettes └─┴─┴─┘	T5b
		Pipes full of tobacco └─┴─┴─┘	T5c
		Cigars, cheroots, cigarillos └─┴─┴─┘	T5d
		Other └─┴─┴─┘ *If other, go to T5 other*	T5e
		Other (please specify): └─┴─┴─┴─┴─┴─┴─┴─┘	T5other
27	In the past, did you **ever** smoke **daily**?	Yes 1 No 2 *If No, go to T9*	T6
28	**If Yes,** How old were you when you **stopped** smoking **daily**?	Age (years) └─┴─┴─┘ *If Known, go to T9* Don't remember 777	T7
29	How **long ago** did you stop smoking daily? *(RECORD ONLY 1, NOT ALL 3)* Don't remember 777	Years ago └─┴─┘ *If Known, go to T9*	T8a
		OR Months ago └─┴─┘ *If Known, go to T9*	T8b
		OR Weeks ago └─┴─┘	T8c
30	Do you **currently use** any **smokeless tobacco** such as [*snuff, chewing tobacco, betel*]?	Yes 1 No 2 *If No, go to T12*	T9
31	**If Yes,** Do you **currently use smokeless tobacco** products **daily?**	Yes 1 No 2 *If No, go to T12*	T10
32	On average, how many **times a day** do you use …. *(RECORD FOR EACH TYPE)*	Snuff, by mouth └─┴─┴─┘	T11a
		Snuff, by nose └─┴─┴─┘	T11b
		Chewing tobacco └─┴─┴─┘	T11c
		Betel, quid └─┴─┴─┘	T11d

	Don't Know 777	Other └──┴──┴──┘ *If Other, go to T11 other*	T11e
		Other (specify) └──┴──┴──┴──┴──┴──┴──┘	T11other
33	In the past, did you **ever use** smokeless tobacco such as [*snuff, chewing tobacco, or betel*] **daily**?	Yes 1 No 2	T12
34	During the past 7 days on how many days have people smoked in your presence, where you live?	Number of days └──┴──┘	T13
35	During the past 7 days on how many days have people smoked in your presence, other than where you live?	Number of days └──┴──┘	T14

CORE: Alcohol Consumption

The next questions ask about the consumption of alcohol.

	Questions	Response	Code
36	Have you consumed alcohol (such as beer, wine, spirits, fermented cider or *[add other local examples]* within the **past 12 months**? *(USE SHOWCARD OR SHOW EXAMPLES)*	Yes 1 No 2 *If No, go to D1*	A1
37	In the past 12 months, **how frequently** have you had at least one drink? *(READ RESPONSES USE SHOWCARD)*	Daily 1 5-6 days per week 2 1-4 days per week 3 1-3 days per month 4 Less than once a month 5	A2
38	When you drink alcohol, **on average**, how many drinks do you have during one day?	Number └──┴──┘ Don't know 77	A3
39	Have you consumed alcohol (such as beer, wine, spirits, fermented cider or *[add other local examples]* within the **past 30 days**? *(USE SHOWCARD OR SHOW EXAMPLES)*	Yes 1 No 2 *If No, go to A 6*	A4
40	During each of the **past 7 days**, how many standard drinks of any alcoholic drink did you have each day? *(RECORD FOR EACH DAY USE SHOWCARD)* Don't Know 77	Monday └──┴──┘	A5a
		Tuesday └──┴──┘	A5b
		Wednesday └──┴──┘	A5c
		Thursday └──┴──┘	A5d
		Friday └──┴──┘	A5e
		Saturday └──┴──┘	A5f
		Sunday └──┴──┘	A5g
41	In the past 12 months, what was the **largest number** of drinks you had on a single occasion, counting all types of standard drinks together?	Largest number └──┴──┘	A6
42	**For men only:** In the past 12 months, on how many days did you have **five or more** standard drinks in a single day?	Number of days └──┴──┴──┘	A7
43	**For women only**: In the past 12 months, on how many days did you have **four or more** standard drinks in a single day?	Number of days └──┴──┴──┘	A8

CORE: Diet			
The next questions ask about the fruits and vegetables that you usually eat. I have a nutrition card here that shows you some examples of local fruits and vegetables. Each picture represents the size of a serving. As you answer these questions please think of a typical week in the last year.			
Questions		**Response**	**Code**
44	In a typical week, on how many days do you **eat fruit**? *(USE SHOWCARD)*	Number of days └─┴─┘ *If Zero days, go to D3* Don't Know 77	D1
45	How many **servings** of fruit do you eat on **one** of those days? (*USE SHOWCARD)*	Number of servings └─┴─┘ Don't Know 77	D2
46	In a typical week, on how many days do you **eat vegetables**? *(USE SHOWCARD)*	Number of days └─┴─┘ *If Zero days, go to D5* Don't Know 77	D3
47	How many **servings** of vegetables do you eat on one of those days? *(USE SHOWCARD)*	Number of servings └─┴─┘ Don't Know 77	D4
48	What type of **oil or fat is most often** used for meal preparation in your household? *(USE SHOWCARD SELECT ONLY ONE)*	Vegetable oil 0 1 Lard or suet 0 2 Butter or ghee 0 3 Margarine 0 4 Other 0 5 *If Other, go to D5 other* None in particular 0 6 None used 0 7 Don't know 7 7	D5
		Other └─┴─┴─┴─┴─┴─┴─┘	D5other

CORE: Physical Activity			
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed].* In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.			
Questions		**Response**	**Code**
Activity at work			
49	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work*] for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 4*	P1
50	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days └─┘	P2
51	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P3 (a-b)

52	Does your work involve moderate-intensity activity, that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads*] for at least 10 minutes	Yes 1	P4

	continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	No 2 *If No, go to P 7*	
53	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days └─┘	P5
54	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P6 (a-b)
Travel to and from places			
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. [insert other examples if needed]			
55	Do you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
56	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days └─┘	P8
57	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P9 (a-b)
Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure),[insert relevant terms].			
58	Do you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like [*running or football,*] for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 13*	P10
59	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (*leisure*) activities?	Number of days └─┘	P11
60	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P12 (a-b)

Physical Activity (recreational activities) contd.

Questions		**Response**	**Code**
61	Do you do any moderate-intensity sports, fitness or recreational *(leisure*) activities that causes a small increase in breathing or heart rate such as brisk walking,(*cycling, swimming, volleyball*)for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P16*	P13
62	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (*leisure*) activities?	Number of days └─┘	P14
63	How much time do you spend doing moderate-intensity sports, fitness or recreational (*leisure*) activities on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P15 (a-b)
Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD)*			
64	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs min s	P16 (a-b)

History of High Blood Pressure

Questions		Response	Code
65	When was your blood pressure last measured by a health professional?	Within past 12 months 1 1-5 years ago 2 Not within past 5 years 3	H1
66	During the past 12 months have you been told by a doctor or other health worker that you have elevated blood pressure or hypertension?	Yes 1 No 2	H2
67	Are you currently receiving any of the following treatments/advice for high blood pressure prescribed by a doctor or other health worker?		
	Drugs (medication) that you have taken in the last 2 weeks	Yes 1 No 2	H3a
	Special prescribed diet	Yes 1 No 2	H3b
	Advice or treatment to lose weight	Yes 1 No 2	H3c
	Advice or treatment to stop smoking	Yes 1 No 2	H3d
	Advice to start or do more exercise	Yes 1 No 2	H3e
68	During the past 12 months have you seen a traditional healer for elevated blood pressure or hypertension	Yes 1 No 2	H4
69	Are you currently taking any herbal or traditional remedy for your high blood pressure?	Yes 1 No 2	H5

History of Diabetes

Questions		Response	Code
70	Have you had your blood sugar measured in the last 12 months?	Yes 1 No 2	H6
71	During the past 12 months, have you ever been told by a doctor or other health worker that you have diabetes?	Yes 1 No 2	H7
72	Are you currently receiving any of the following treatments/advice for diabetes prescribed by a doctor or other health worker?		
	Insulin	Yes 1 No 2	H8a
	Oral drug (medication that you have taken in the last 2 weeks	Yes 1 No 2	H8b
	Special prescribed diet	Yes 1 No 2	H8c
	Advice or treatment to lose weight	Yes 1 No 2	H8d
	Advice or treatment to stop smoking	Yes 1 No 2	H8e
	Advice to start or do more exercise	Yes 1 No 2	H8f
	During the past 12 months have you seen a traditional healer for diabetes?	Yes 1 No 2	H9
	Are you currently taking any herbal or traditional remedy for your diabetes?	Yes 1 No 2	H10

Step 2 Physical Measurements

Height and Weight		Response	Code
73	Technician ID	└─┴─┴─┘	M1
74	Device IDs for height and weight	Height └─┴─┘	M2a
		Weight └─┴─┘	M2b
75	Height	in Centimetres (cm) └─┴─┴─┘.└─┘	M3
76	Weight *If too large for scale, code 666.6*	in Kilograms (kg) └─┴─┴─┘.└─┘	M4
77	***(For women)*** Are you pregnant?	Yes *1* No *2 If Yes, go to M 9*	M5

Waist			
78	Device ID for waist	└─┴─┘	M6
79	Waist circumference	in Centimetres (cm) └─┴─┴─┘.└─┘	M7

Blood Pressure			
80	Technician ID	└─┴─┴─┘	M8
81	Device ID for blood pressure	└─┴─┘	M9
82	Cuff size used	Small 1 Normal 2 Large 3	M10
83	Reading 1	Systolic (mmHg) └─┴─┴─┘	M11a
		Diastolic (mmHg) └─┴─┴─┘	M11b
84	Reading 2	Systolic (mmHg) └─┴─┴─┘	M12a
		Diastolic (mmHg) └─┴─┴─┘	M12b
85	Reading 3	Systolic (mmHg) └─┴─┴─┘	M13a
		Diastolic (mmHg) └─┴─┴─┘	M13b
86	During the past two weeks, have you been treated for high blood pressure with drugs (medication) prescribed by a doctor or other health worker?	Yes 1 No 2	M14

Other measures			
87	Hip circumference	in Centimetres (cm) └─┴─┴─┘.└─┘	M15
88	Mid Arm circumference	in Centimetres (cm) └─┴─┴─┘.└─┘	M17
89	Neck circumference	in Centimetres (cm) └─┴─┴─┘.└─┘	M18
90	Triceps Skinfold Thickness		
	Reading 1	in milliimetres (mm) └─┴─┴─┘	M19a
	Reading 2	in millimetres (mm) └─┴─┴─┘	M19b

	Reading 3	in millimetres (mm) └─┴─┴─┘	M19c

91	Heart Rate (Record if automatic blood pressure device is used)		
	Reading 1	Beats per minute └─┴─┴─┘	M16a
	Reading 2	Beats per minute └─┴─┴─┘	M16b
	Reading 3	Beats per minute └─┴─┴─┘	M16c

Step 3 Biochemical Measurements

Blood Glucose		Response	Code
92	During the last 12 hours have you had anything to eat or drink, other than water?	Yes 1 No 2	B1
93	Technician ID	└─┴─┴─┘	B2
94	Device ID	└─┴─┘	B3
95	Time of day blood specimen taken (24 hour clock)	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	B4
96	Blood glucose	mmol/l └─┴─┘ . └─┴─┘	B5a
		Low 1 High 2 Unable to assess 3	B5b

Blood Lipids			
97	Device ID	└─┴─┘	B6
98	Total cholesterol	mmol/l └─┴─┘ . └─┴─┘	B7a
		Low 1 High 2 Unable to assess 3	B7b
99	Triglycerides	mmol/l └─┴─┘ . └─┴─┘	B8a
		Low 1 High 2 Unable to assess 3	B8b
100	HDL Cholesterol	mmol/l └─┘ . └─┴─┘	B9a
		Low 1 High 2 Unable to assess 3	B9b
Urine proteins			
101	Device ID	└─┴─┘	X
102	Proteins (Dipstick) If positive give reading (eg + or ++ etc)	Positive └─┘ Negative └─┘	X

	Microalbumin Device ID	└─┴─┘ mg/mMCRT └─┘ . └─┴─┘	X