ASSOCIATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) α, γ AND δ GENE POLYMORPHISMS WITH OBESITY AND METABOLIC SYNDROME IN THE SUB-URBAN KAMPAR POPULATION, MALAYSIA

By

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ABSTRACT

ASSOCIATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) *α, γ* AND *δ* GENE POLYMORPHISMS WITH OBESITY AND METABOLIC SYNDROME IN THE SUB-URBAN KAMPAR POPULATION, MALAYSIA

Chia Phee Phee

Obesity and metabolic syndrome (Met-S) are worldwide health problems with increasing prevalence. PPARs are ligand-activated transcription factors that belong to the superfamily of nuclear receptors and form heterodimers with retinoid X receptor, bind to DNA, thereby regulating the expression of target genes involved in lipid and carbohydrate metabolism, inflammatory response and energy homeostasis. PPAR genes *PPARα, γ* and *δ* are said to be associated with obesity and Met-S. Therefore, this study aimed to investigate the association of *PPARα* L162V, *γ2* C161T, *δ* T294C single nucleotide polymorphisms (SNPs) with obesity and Met-S in a multi-ethnic population in Kampar. Socio-demographic data, anthropometric and biochemical measurements (plasma lipid profile, adiponectin and interleukin-6 (IL-6) levels) were taken from 307 subjects (124 males, 183 females; 180 obese; 249 Met-S; 97 Malays, 85 Chinese, 55 Indians). The overall minor allele frequencies were 0.08, 0.22 and 0.30 for *PPARα* L162V, *γ2* C161T, *δ* T294C, respectively. All SNPs were not associated with obesity, Met-S and obesity with/without Met-S by Chi-square, ethnicity-stratified and logistic regression analysis. Subjects with LV genotype/V162 allele carrier of *PPARα* L162V

showed significantly higher IL-6 levels as compared to LL genotype/L162 allele, while homeostatic model assessment-estimated insulin resistance (HOMA-IR) was significantly different among *PPARγ2* C161T genotypes and alleles, with CC/CT genotype/C161 allele > TT genotype/T161 allele. Adiponectin level was significantly lower in male subjects and smokers. IL-6 level was significantly different among ethnicities, with Malays showing the highest, followed by Indians and Chinese. Meanwhile, Chinese was found to have significantly lower total cholesterol/HDL ratio compared to Indians (highest total cholesterol/HDL ratio) and Malays. In conclusion, all *PPAR* SNPs were not associated with obesity and Met-S in the sub-urban Kampar population, Malaysia. *PPARα* V162 allele carriers were associated with significant higher plasma IL-6 level, whereas *PPARγ2* T161 allele carriers were associated with significantly lower HOMA-IR.

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FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

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SUBMISSION OF DISSERTATION

It is hereby certified that **CHIA PHEE PHEE** (ID No: **11ADM03675**) has completed this dissertation entitled "ASSOCIATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) α, γ AND δ GENE POLYMORPHISMS WITH OBESITY AND METABOLIC SYNDROME IN THE SUB-URBAN KAMPAR POPULATION, MALAYSIA" under the supervision of Dr Say Yee How (Supervisor) from the Department of Biomedical Science, Faculty of Science, and Dr Chew Choy Hoong (Co-Supervisor) from the Department of Biomedical Science, Faculty of Science.

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DECLARATION

I hereby declare that the dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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LIST OF ABBREVIATIONS

A	Adenine
ApoA1	Apolipoprotein A-1
ANOVA	Analysis of Variance
AVF	Abdominal Visceral Fat
BAT	Brown Adipose Tissue
BCAA	Branched Chain Amino Acids
BLAST	Basic Local Alignment Search Tool
BMI	Body Mass Index
bp	Base Pair
BP	Blood Pressure
C	Cytosine
cDNA	Complement Deoxyribonucleic Acid
CI	Confidence Interval
DBD	DNA Binding Domain
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
ELISA	Enzyme-linked Immuno Sorbent Assay
FAO	Fatty Acid Oxidation
FBG	Fasting Blood Glucose
FI	Fasting Insulin
G	Guanine
glc	Glucose

GSIS	Glucose Stimulated Insulin Secretion
HC	Hip Circumference
HDL-C	High-density Lipoprotein Cholesterol
HPA	Hypothalamic-pituitary-adrenal
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IASO	International Association for the Study of Obesity
IOTF	International Obesity Task Force
IL-6	Interleukin-6
IRS	Insulin Receptor Substrates
L	Leucine
K_2EDTA	Dipotassium Ethylene Diamine Tetraacetic Acid
LBD	Ligand Binding Domain
LDL-C	Low-density lipoprotein cholesterol
MAF	Minor Allele Frequency
Met-S	Metabolic Syndrome
$MgCl_2$	Magnesium Chloride
MHO	Metabolically Healthy Obese
mRNA	Messenger Ribonucleic Acid
NCEP ATP III	National Cholesterol Education Program's Adult Treatment Panel III
NHMS	National Health and Morbidity Survey
NIH	National Institutes of Health
NTD	N-terminal domain
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PPARs	Peroxisome Proliferator Activated Receptors

PPARα	Peroxisome Proliferator Activated Receptor Alpha Gene
PPARβ	Peroxisome Proliferator Activated Receptor Beta Gene
PPARγ	Peroxisome Proliferator Activated Receptor Gamma Gene
PPARδ	Peroxisome Proliferator Activated Receptor Delta Gene
PPRE	Peroxisome Proliferator Response Element
PUFA	Polyunsaturated Fatty Acid
RBC	Red Blood Cell
RFLP	Restriction Fragment Length Polymorphism
RM	Resting Metabolism
RXR	Retinoic Acid Receptor
SBP	Systolic Blood Pressure
SD	Standard Deviation
SE	Standard Errors
SF	Subcutaneous Fat
SM	Skeletal Muscle
SNP	Single Nucleotide Polymorphism
T	Thymine
T2DM	Type 2 Diabetes Mellitus
TAF	Total Abdominal Fat
Taq	*Thermus aquaticus*
TBE	Tris-Borate-EDTA
TBF	Total Body Fat
TC	Total Cholesterol
TC/HDL-C	Total Cholesterol/HDL Cholesterol Ratio
TG	Triglyceride

TNF-α	Tumour Necrosis Factor Alpha
UTR	Untranslated Region
UV	Ultraviolet
V	Valine
VFL	Visceral Fat Level
WAT	White Adipose Tissue
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist-to-hip Ratio
WHtR	Waist-to-Height Ratio
χ^2	Chi-Square

CHAPTER 1

INTRODUCTION

Overweight/obesity is defined as abnormal or excessive fat accumulation which may adversely affect health (WHO, 1998). Overweight/obesity is now a global health challenge which increases at an unprecedented rate, causing morbidity and mortality in both developed and developing countries. Worldwide, the prevalence of overweight/obesity increased from 857 million in 1980, to 2.1 billion in 2013, rising by 27.5 % between 1980 and 2013 in adults (Ng et al., 2014). If recent trends continue unabated, by 2030, it is projected that up to 57.8 % of the world's adult population (3.3 billion people) could be overweight/obese (Kelly et al., 2008). This global pandemic is also threatening Malaysia, with drastic rising prevalence from 20.6 % in 1996 (NHMS II) to 44.5 % in 2011 (NHMS IV), ranking Malaysia as the fattest nation in South-East Asia (WHO, 2011).

Accompanied with overweight/obesity are metabolic abnormalities, including hypertension, dyslipidemia (high levels of triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C)), hyperglycemia, insulin resistance and inflammation (Hotamisligil, 2006). The constellation of these metabolic complications of obesity is often known as metabolic syndrome (Met-S) and it has been related to increased risk for type II diabetes and cardiovascular disease. So far, the National Cholesterol Education Program's Adult

Treatment Panel III (NCEP ATP III) is the most commonly used criteria to define Met-S in most epidemiological studies, which includes clinical findings of abdominal obesity, hypertension, hyperglycemia and dyslipidemia (Grundy et al., 2005). Although the report of worldwide prevalence of Met-S is not as established as obesity, it is undoubtedly that the upsurge of obesity will drive the increase of Met-S. It is noteworthy that the determination of the Met-S prevalence in different regions depends on its defining criteria, particularly the waist circumference (WC). The available evidence indicates that between 20 % and 30 % of the adult population in most countries can be characterized as having Met-S and the prevalence is even higher in some populations or segments of the population (Grundy, 2008). With the projections of a greater prevalence of obesity in the future, the likelihood of a further increase in the Met-S can be anticipated (Hossain et al., 2007). Indeed, in Malaysia, a nationwide survey reported that the prevalence of Met-S as 34.3 % in year 2008 (Mohamud et al., 2011a).

Obesity is a complex multifactorial and chronic low-grade inflammation disease resulting from a long-term energy surplus, in which both genetic and environmental factors are involved (Sorensen, 1995; Maes et al., 1997; Barsh et al.; 2000; Strohacker and McFarlin, 2010). As established risk factors for obesity, environmental factors such as low economic status, high carbohydrates food intake, smoking, alcohol consumption, and physical inactivity can also lead to the development of the Met-S (Mayer et al., 1996; Zhu et al., 2004). Individual with obesity and Met-S has excess adipose tissues which secrete increased amount of pro-inflammatory cytokines like

interleukin-6 (IL-6); while the secretion of anti-inflammatory cytokines, like adiponectin decreases (Bastard et al., 2000; Hotamisligil, 2006). Evidences indicate that genetic component governing metabolic process and inflammation are implicated in the development of obesity and Met-S (Phillips et al., 2006; Pollex and Hegele, 2006; Sale et al., 2006). One of the candidate gene family involved is the peroxisome proliferator-activated receptors (PPARs) (Evans et al., 2004; Fernandez, 2004).

PPARs are transcriptional factors belonging to the ligand-activated nuclear receptor superfamily. Upon activation by endogenous fatty acids or specific pharmacological synthetic ligands such as fibrates (dyslipidemia drugs) and thiazolidinediones (anti-diabetic drugs) (reviewed in Grygiel-Gorniak, 2014), PPARs form heterodimers with retinoid X receptor, bind to DNA consensus sequence known as the peroxisome proliferator response element (PPRE) and regulate the expression of target genes, depending on the PPAR isoforms - PPARα, PPARδ (also known as β), and PPARγ (Issemann and Green, 1990; Wahli et al., 1995; Desvergne and Wahli, 1999; Berger and Moller, 2002). PPARα and PPARδ regulate energy combustion, lipoprotein synthesis, inflammatory responses and regulation of blood cholesterol and glucose levels, whereas PPARγ facilitates energy storage by adipogenesis as it helps to convert unspecialised cells to adipocyte particularly when in high fat diet. PPARγ also plays a role in insulin sensitivity, glucose and protein metabolism, cell proliferation, cycle, and growth (Pyper et al., 2010; Grygiel-Gorniak, 2014). The association of PPAR with obesity and Met-S was reported by a pathway analysis via Genome-Wide Association Study (Shim et

al., 2014). Thus, considering the great functional diversity of PPARs in metabolic and inflammation responses, single nucleotide polymorphisms (SNPs) in *PPAR* genes may therefore modulate the risk and susceptibility to obesity and Met-S.

Polymorphisms within the human *PPARα, γ* and δ genes are implicated in the development of obesity and Met-S (Evans et al., 2004; Fernandez, 2004). Thus, alteration in the genes will potentially affect the pathophysiology of obesity and Met-S. In this study, three SNPs namely *PPARα* L162V *PPARγ2* C161T and *PPARδ* T294C were investigated. *PPARα* L162V SNP (rs1800206) is identified as 484C/G transversion in exon 5 which results in a missense mutation of leucine to valine at codon 162 (Flavell et al., 2000; Vohl et al., 2000). *PPARγ2* C161T SNP (rs3856806; also known as C1431T or CAC478CAT) is a silent His477His polymorphism in exon 6 (Meirhaeghe et al., 1998), while *PPARδ* T294C SNP (rs2016520; also known as +15 C/T, -87 T/C, or c.1-87T>C) is located at the 5'- untranslated region (UTR) of exon 4, 87 nucleotides upstream of the start codon (Skogsberg et al., 2003a). There are conflicting findings with regard to the association of these *PPAR* SNPs with obesity and Met-S, due to ethnic population and social cultural differences. In view of the fast expanding prevalence of obesity and Met-S, more basic, clinical and population studies are urgently needed to confirm and elucidate the complex pathophysiological of obesity and Met-S as well as to help in better therapeutic interventions.

At the moment, there is limited study investigating the association of SNPs with the obesity, Met-S and metabolic risk factors in multi-ethnic Malaysia, while most of the data available are confined to the Western countries. A pilot study was performed previously to investigate the association of *PPARα* L162V and *PPARγ*2 C161T SNPs with overweight among university students who were majority of the Chinese ethnicity (n = 256), with no association found (Yiew et al., 2010). Expanding the study to a relative larger and more multi-ethnic sample population, this study was performed to screen the aforementioned SNPs and additional *PPARδ* T294C SNP, together with more anthropometric measurements, biochemical variables. Therefore, the main and secondary objectives of this study were:

Main objectives

a) To determine the prevalence of the *PPARα* L162V, *PPARγ*2 C161T and *PPARδ* T294C SNPs and their possible association with obesity and Met-S alone, or with comorbidities of obesity and Met-S in a multi-ethnic Malaysian population in Kampar, Perak, regardless of ethnicity.

b) To investigate the influence of socio-demographic characteristics, dietary habits and lifestyle factors on obesity and Met-S.

c) To correlate the anthropometric and biochemical measurements of subjects with obesity, Met-S and SNPs.

Secondary objective

a) To further investigate the possible association of the *PPARα* L162V, *PPARγ*2 C161T and *PPARδ* T294C SNPs with obesity and Met-S alone, or with comorbidities of obesity and Met-S by stratified analysis based on ethnicity due to the heterogeneity of the subjects.

CHAPTER 2

LITERATURE REVIEW

2.1 Overweight/obesity

2.1.1 Defining Overweight/obesity

Overweight/obesity is a condition of abnormal or excessive fat accumulation to the extent that may impair health. This chronic disease is characterized by energy imbalance with a positive energy balance in which energy intake has exceeded energy expenditure over time. The excess energy results in increased body adiposity. Beside the degree of excess fat storage, obese individuals also differ in the regional distribution of fat within the body, particularly the excess abdominal fat, attributing to risk of negative health consequences (WHO, 1998).

2.1.2 Assessment of Overweight /obesity

Overweight/obesity is commonly assessed by body mass index (BMI) and waist circumference (WC). BMI is a simple index of weight-for-height measure of obesity in adult that is widely used in most epidemiological studies. BMI is calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). WC can be measured at the midpoint between the lower border of the rib cage and the iliac crest (WHO, 2008b). WC is an additional measurement to complement BMI, to measure abdominal fat mass

adiposity (referred to as central or visceral obesity) which is related to obesity-related morbidity (WHO, 2000). WC cut-off point for men is higher than women as it is established that generally men have on average twice the amount of abdominal fat when compared to pre-menopausal women (Lemieux et al., 1993).

However, the international standard for BMI and WC cut-off points may underestimate obesity-related risks in Asians populations. Asian populations have lower mean or median BMI but greater tendency towards abdominal obesity as compared to Caucasians, with a higher percentage of total body fat (TBF) at lower BMI. Evidences showed that the BMI cut-off points for observed increased risk in different Asian populations varies from 22 to 25 kg/m^2 and for high risk varies from 26 to 31 kg/m^2 (Deurenberg-Yap et al., 2000; Deurenberg-Yap et al., 2001). Therefore, in 2000, the Regional Office for the Western Pacific Region of WHO with the International Association for the Study of Obesity (IASO) and the International Obesity Task Force (IOTF) has recommended another classification of BMI and WC for Asian adults. Table 2.1 shows the comparison of weight status classification of overweight/obesity to the risk of co-morbidities in adults according to BMI and WC based on the International standard and the redefined standard for Asian adults.

Table 2.1: Comparison of weight status and risk of co-morbidities of BMI and WC according to classification of International and Asian adults

Classification	International (WHO, 1998)	Asian (WHO/IASO/IOTF, 2000)	Risk of co-morbidities
BMI (kg/m^2)			
Underweight	< 18.5	< 18.5	
Normal range	18.5–24.9	18.5-22.9	Low
Overweight	≥ 25.0	≥ 23.0	
Pre-obesity	25.0–29.9	23.0-24.9	Increased
Obesity class I	30.00–34.9	25.0-29.9	Moderate
Obesity class II	35.00–39.9	≥ 30.0	Severe
Obesity class III	≥ 40.0		Very severe
WC (cm)			
Normal	Men < 102; Women < 88	Men < 90; Women < 80	Low
Central obesity	Men ≥ 102; Women ≥ 88	Men ≥ 90; Women ≥ 80	Increased

Other alternative ways to assess obesity include waist to hip ratio (WHR), waist to height ratio (WHtR) and percent total body fat (TBF) which can be measured by skin-fold thickness, underwater weighing and bioelectrical impedance (NIH, 1998; WHO, 1998). The National Institutes of Health Practical guide to obesity (NHLBI Obesity Education Initiative, 2000) suggests that WC is particularly useful in patients with normal or overweight

on BMI scale, but has little added predictive power of disease risk beyond severely obese individuals with BMI ≥35 kg/m^2. The use of WC is favored over WHR and WHtR due to the relative ease of obtaining. Due to high cost and inconveniency, the measurement of percent TBF is rarely used in clinical practice (WHO, 2008).

2.1.3 Worldwide Prevalence of Overweight/obesity

The Global Burden of Disease Study 2013 has portrayed overweight and obesity as global pandemic which has risen substantially in the past three decades in both developed and developing countries, with an absolute increase from 857 million in 1980 to 2.1 billion in 2013. The proportion of men who were overweight increased from 28.8 % in 1980, to 36.9 % in 2013, and the proportion of women who were overweight increased from 29.8 % to 38.0 % (Ng et al., 2014). In developed countries, overweight/obesity is more prevalent in men than in women, whereas in developing countries, more women than men are either overweight or obese. Compared to developed countries, the prevalence of developing overweight/obesity is much lower, however, the age pattern of overweight/obesity is similar. The highest level of obesity (BMI ≥ 30 kg/m^2) age was at about 45 years for men and 55 years for women (Ng et al., 2014). If the rising trends continue, the absolute numbers is projected to rise to a total of 2.16 billion overweight and 1.12 billion obese individuals, or 58 % (38 % overweight and 20 % obese) of the world's adult population by 2030 (Kelly et al., 2008). The worldwide prevalence of men and women in 2013 is shown in maps in Figure 2.1 and Figure 2.2.

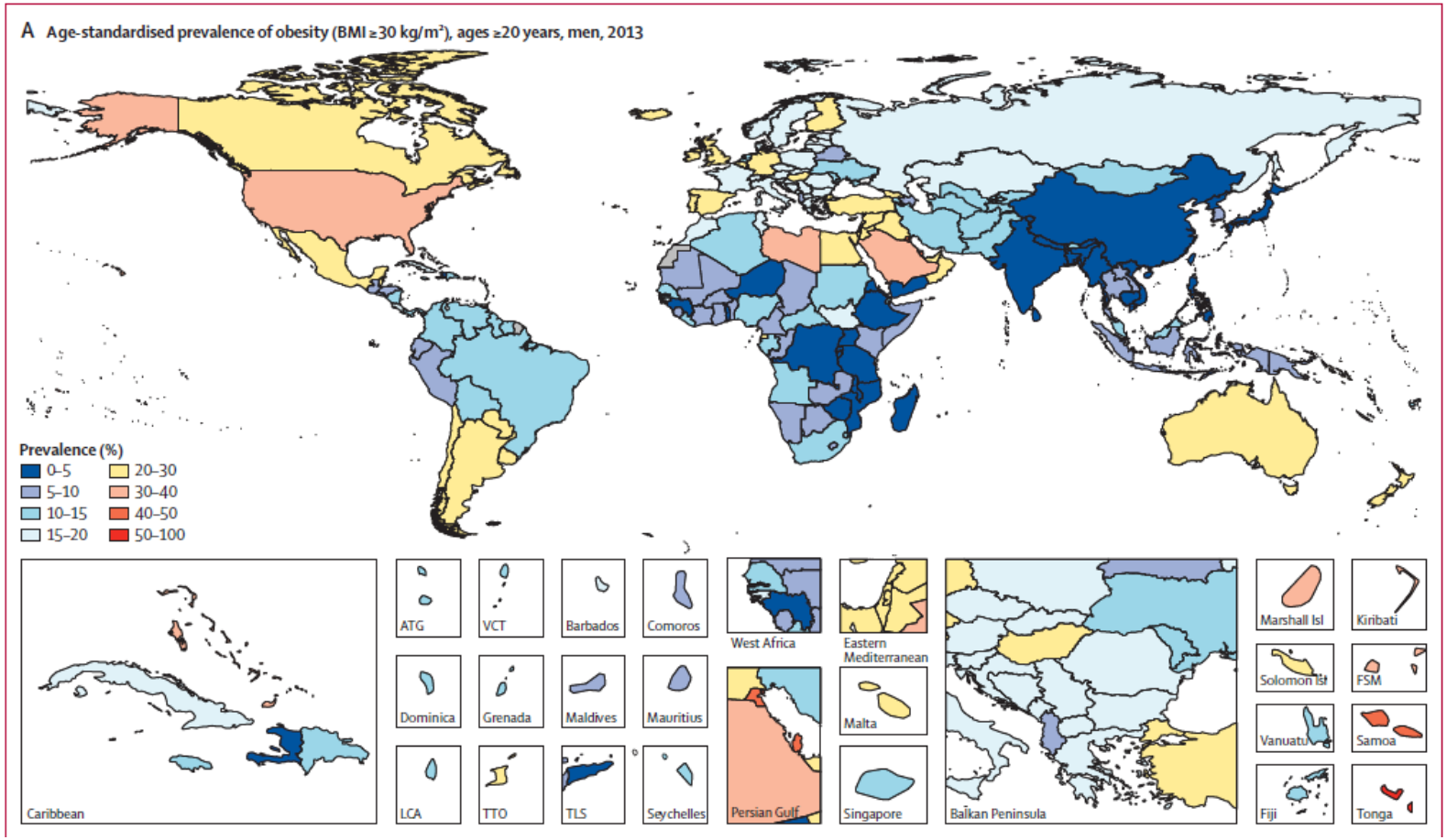


Figure 2.1 Worldwide prevalence of obesity of men in 2013 (Adopted from Ng et al., 2014)

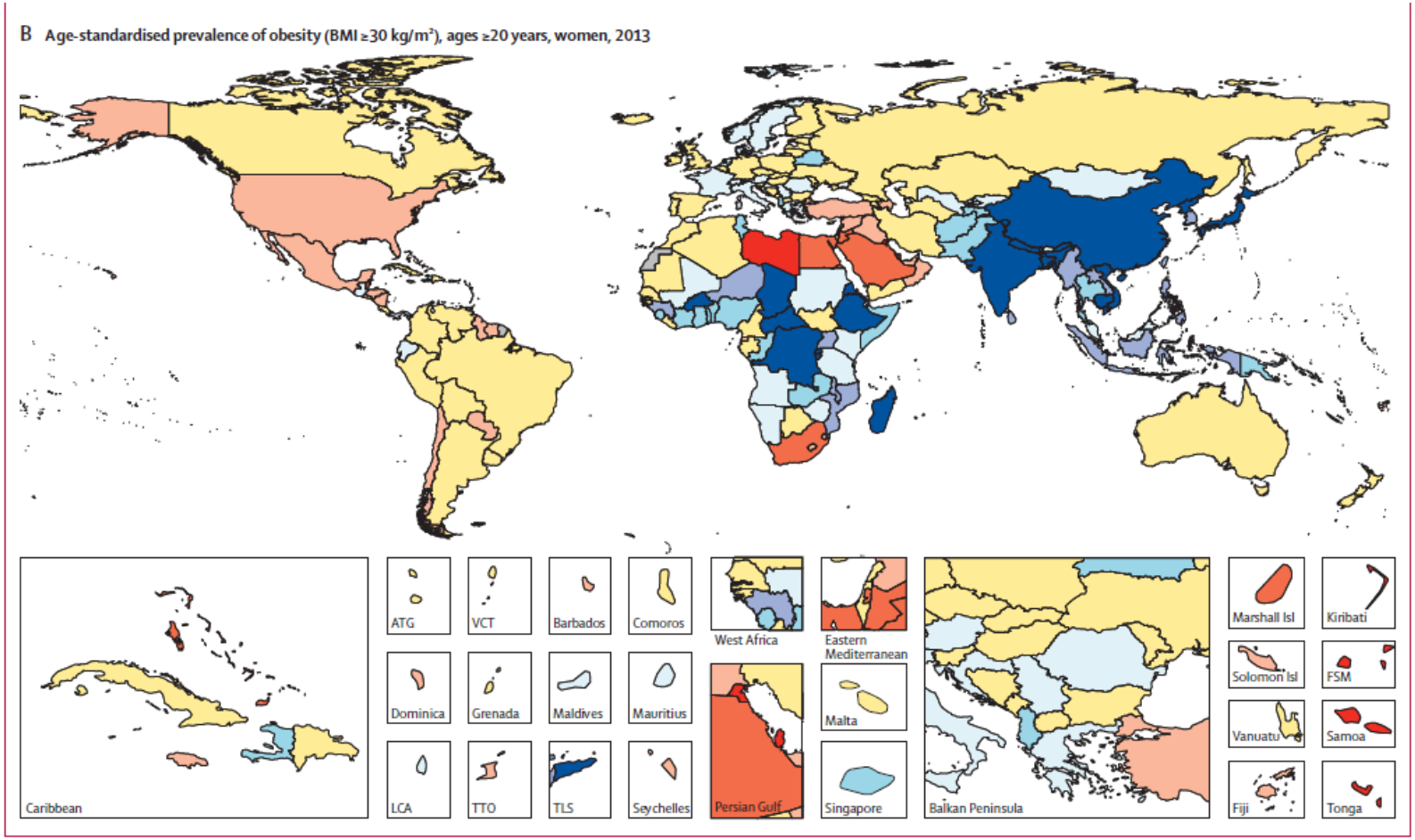


Figure 2.2 Worldwide prevalence of obesity of women in 2013 (Adopted from Ng et al., 2014)

2.2 Met-S

2.2.1 Defining Met-S

Met-S is a cluster of metabolic abnormalities which includes central (abdominal) obesity, raised TG, reduced HDL-C, raised fasting glucose and raised blood pressure (BP). Indeed, all these metabolic abnormalities are the health complications of obesity. Individuals with Met-S are at twice the risk for cardiovascular disease and 5-fold the risk for type II diabetes (Grundy, 2008). Met-S is also known as Syndrome X, the insulin resistance syndrome, cardio-metabolic syndrome, dysmetabolic syndrome and the deadly quartet syndrome (Reaven, 1988; Kaplan, 1989; DeFronzo and Ferrannini, 1991).

Met-S was first described by Reaven (1988) as Syndrome X whereby he noted that several risk factors for cardiovascular diseases (eg, dyslipidemia, hypertension, hyperglycemia) commonly cluster together. Later, a number of expert groups have developed clinical criteria to define Met-S, and over the past two decades, the detection criteria have evolved to allow simplicity for clinical practice. The first proposal came in 1998 by WHO, followed by the European group for the Study of Insulin Resistance (EGIR) in 1999, then by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) in 2001, the American Association of Clinical Endocrinologists (AACE) in 2003, International Diabetes Federation (IDF) in 2005 and the harmonized ATP III and the IDF consensus in 2009 (Table 2.2) (reviewed in Oladejo, 2011).

Table 2.2 Clinical diagnosis of Met-S

Clinical measures	WHO (1998)	EGIR (1999)	ATPIII (2001)	AACE (2003)	IDF (2005)	IDF/ATPIII (2009)
Insulin resistance	IGT, IFG, T2DM or lowered insulin sensitivity plus any two of the following	Plasma insulin > 75^{th} percentile plus any 2 of the following	None but any 3 of the following 5 features	IGT or IFG plus any of the following	None	None Any 3 of the following
Body weight	WHR: Men $\geq$ 0.90; Women $\geq$ 0.85 and/or BMI $\geq$ 30 kg/m^2	WC: Men $\geq$ 94 cm; Women $\geq$ 80 cm	WC: Men $\geq$ 102cm; Women $\geq$ 88cm	BMI $\geq$ 25 kg/m^2	↑ WC (population specific) plus any 2 of the following	↑ WC (population specific)
Lipid	TG $\geq$ 1.7 mmol/L and/or HDL-C < 0.91 mmol/L in men ; 1.01 mmol/L in women	TG $\geq$ 1.7 mmol/L. HDL-C < 1.01 mmol/L in men and women	TG $\geq$ 1.7 mmol/L. HDL-C < 1.04 mmol/L in men; 1.29 mmol/L in women	TG $\geq$ 1.7 mmol/L. HDL-C < 1.04 mmol/L in men; 1.29 mmol/L in women	TG $\geq$ 1.7 mmol/L. or on Rx. HDL-C < 1.04 mmol/L in men; 1.29 mmol/L in women or on Rx	Similar to IDF
Blood pressure	140/90 mmHg	140/90 mmHg	130/85 mmHg	130/85 mmHg	130/85 mmHg Or on Rx	Similar to IDF
Glucose	IGT, IFG, T2DM	IGT, IFG (not diabetes)	FBG $\geq$ 5.6 mmol/L includes diabetes	IGT or IFG but not diabetes	FBG $\geq$ 5.6 mmol/L including diabetes	Similar to IDF
Others	Microalbuminuria			Other features of insulin resistance		

TG- Triglyceride, HDL-C- High density lipoprotein, IGT- Impaired glucose tolerance, IFG- Impaired fasting glucose, FBG- Fasting blood glucose T2DM- Type 2 Diabetes Mellitus, BMI- Body mass index, Rx- Treatment (Adapted from IDF, 2006; Oladejo, 2011)

2.2.2 Prevalence of Met-S

On par with the rise of the prevalence of obesity, there has been a striking increase in the number of people with Met-S worldwide over the past two decades. The likelihood of its prevalence to increase is anticipated in view of the projected increment in the prevalence of obesity in the future. The estimation of the prevalence of Met-S has been a difficult one due to the rapid changes in the diagnosis criteria and definition, region-specific cut-points for the level of obesity (WC) of the studied populations. It is estimated that around 20-25 % of the world's adult population have Met-S. Similar to obesity, Met-S has been described as a pandemic, and 1 out of 4 adults has Met-S with the prevalence rises up to 40 % among old patients (age over > 60 years) (Grundy, 2008). The prevalence of the Met-S increases by age, peaks around the age of 60–75 years, thereafter it decreases. This decrease is likely to be explained by differential survival of those with and without Met-S.

2.3 Etiology of Obesity and Met-S

Figure 2.3 shows the etiology of obesity and Met-S. The etiology of obesity and Met-S is multi-factorial, involving a complex interaction of genetics, behavioral and environment factors (Park et al., 2003). Urbanization and industrialization have resulted in economic and social circumstances, such as excess energy consumption (energy-dense foods that are high in fats and simple sugars and low in dietary fibers), cigarette smoking, excessive alcohol

consumption and sedentary lifestyles, of all may increase the risk of both the obesity and Met-S (Grundy, 1998).

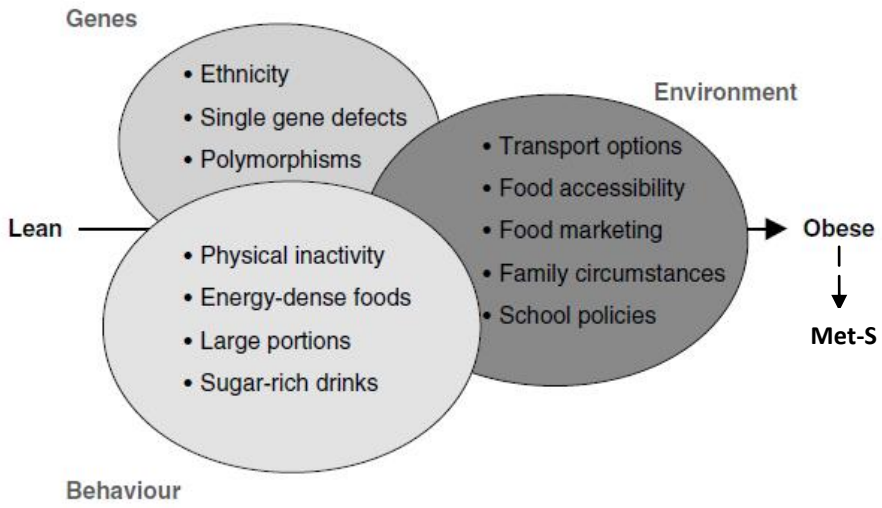


Figure 2.3 Etiology of obesity and Met-S (Adapted from Jebb and Kreb, 2004).

In addition, a second set of factors, metabolic susceptibility, usually is required for the Met-S (Grundy, 2007). Susceptibility factors include adipose tissue disorders that are typically manifested as abdominal obesity, endocrine disorders, pro-inflammatory state, aging, genetic and racial factors. Besides, genetic aberrations that affect the specific metabolic risk factors can further modify expression of both the obesity and Met-S (Grundy, 1998; Bergstrom and Hernell, 2001).

2.4 Ethnicity, obesity and Met-S

Ethnicity may account for some of the disparity in obesity and obesity-related disease such as Met-S. According to the Harvard Encyclopedia of American Ethnic Groups, ethnicity is a socially defined category of people

with commonality on ancestry, social, culture or national experience. The list of features characterizing ethnicity includes common geographical origin, language, religious faith, cultural ties (shared traditions, symbols, values, music, literature and food preferences), and shared political interests regarding the homeland (Thernstrom, 1980). Biological distinct characteristics on the basis of genetics and phenotypic traits that play a part in racial differences may be visible in an ethnic group (Schulman, 1995; Williams 1997). With its complex interplay encompassing environmental, cultural and genetic factors, ethnicity categories had been suggested to be considered as an attempt to extend the taxonomic classification below the level of species rather than to define a clear-cut biological entity in research studies (Watt 1981; Cooper 1984). However, if based on the assumption that ethnicity differences are a manifestation of biology, this can help to imply a genetic explanation for differences in the incidence, severity, or outcome of diseases as in obesity and Met-S (Osborn and Feit 1992).

Of particular concern, evidences had shown that at any given BMI, the risk of comorbidities is markedly higher in some ethnic groups than others (Deurenberg-Yap, 2000). Shai et al. (2006) reported that Asians had more than double the risk of developing type II diabetes than whites at the same BMI; Hispanics and blacks also had higher risks of diabetes than whites, but to a lesser degree. At the same BMI, Asians was found to have higher risks of hypertension and cardiovascular disease than their white European counterparts, and a higher risk of dying early from cardiovascular disease or any cause from several other studies (Deurenberg-Yap et al., 2000; Pan et

al.,2004; Wen et al., 2009). South Asians have especially high levels of body fat and are more prone to developing abdominal obesity, which may account for their very high risk of Met-S (Misra and Vikram, 2004; Misra and Khurana, 2009). Meanwhile, some studies found that blacks have lower TBF and higher lean muscle mass than whites at the same BMI. Thus, at the same BMI, they may be at lower risk of obesity-related diseases including Met-S (Aloia et al., 1999; Rush et al., 2007). While genetic aberrations among different ethnicity may still play its role, ethnic specific criteria for assessing abdominal obesity as a component of Met-S and lower BMI cut-off points for Asians had been implemented by expert groups.

2.5 Metabolically Healthy Obese (MHO)

There is a subset of apparently healthy obese subjects who have a lower burden of adiposity related metabolic abnormalities, and is referred to as metabolically healthy obese (MHO). These individuals display a favorable metabolic profile, characterized by high levels of insulin sensitivity, a favorable lipid and inflammation profile and a low prevalence of hypertension (Sims, 2001; Karelis, 2008; Hinnouho et al., 2013; Hinnouho et al., 2014). It is estimated that about 10-40 % of obese individuals has MHO phenotype, depends on the definition used for metabolic status (Hinnouho et al., 2013; Primeau et al., 2011). However, the exact mechanisms underlying the favorable metabolic profile of MHO individuals remain unclear (Hinnouho et al., 2013).

2.6 The Putative Pathogenesis of Dyslipidemia, Insulin Resistance, High Plasma IL-6 and Low Plasma Adiponectin in Obesity and Met-S

Adipose tissue is well known as the energy storage depot of fat. However, excess adipose tissue mass particularly white adipose tissue (WAT) results in obesity disorder, with the continuous differentiation of new adipocyte which is the main cellular component of WAT throughout life. (Gregoire, 2001). As a complex interplay process known as adipogenesis, obesity involves the alterations in adipose tissue masses with the changes either in mature adipocyte size and/or number (Rosen, 2007). Obesity has long been characterized by a cluster of lipid disturbances includes high total cholesterol (TC), TG, LDL-C levels and low HDL-C level (Miettinen, 1971). However, the etiology of obesity as a risk factor for many diseases including Met-S has not been ascertained. The later discovered role of adipose tissue as an endocrine gland (Kershaw and Flier, 2004) shed light on this matter. In obesity, excessive triglyceride accumulation within adipocytes leads to adipocyte hypertrophy and cause dysregulation of adipokine secretion which results in induction of inflammation, attributing to the development of insulin resistance and Met-S (Dandona et al., 2004).

Insulin resistance is characterized by impairments in insulin-mediated suppression of liver glucose production, skeletal muscle glucose disposal and inhibition of lipolysis. This leads to hyperglycemia and increased plasma free fatty acids (Olefsky and Glass, 2010). Adiponectin is an anti-inflammatory adipokine that increases insulin sensitivity (Yamauchi et al., 2001), while IL-6

is a pro-inflammatory adipokine that can lead to insulin resistance (Kern et al., 2001).

Adiponectin is expressed in adipose tissue, and is also known as ACRP30, AdipoQ, and GBP28. It is the product of the human apM1 gene and is a 247 amino acid protein consisting of a N-terminal collagenous region and a C-terminal globular domain (Maeda et al., 1996). Adiponectin appears abundant in the circulating plasma with levels in the range 5-30 μg/ml in humans (Scherer et al., 1995; Nakano et al., 1996). Adiponectin levels are inversely correlated with BMI (Arita et al., 1999), decreased in obesity, and increased with weight loss (Kadowaki et al., 2006). Adiponectin improves insulin sensitivity and lipid profile by monophosphate-activated protein kinase in skeletal muscle cells and the liver (Yamauchi et al., 2002, Beltowski et al., 2008). On the other hand, insulin resistance which is related to low plasma levels of adiponectin, lowers HDL-C concentration by stimulating the transcriptional activity of apolipoprotein A-1 (ApoA1), and decreases VLDL-C production, as well as increases the expression of lipoprotein lipase (Lara-Castro et al., 2006; Beltowski et al., 2008).

The exact role of IL-6 on insulin resistance and subsequent disturbance on lipid profile in obesity and Met-S has not been fully elucidated. Nonetheless, IL-6 signaling has been recently described in its role in insulin regulation. IL-6 is produced by a number of different cells including mature adipocytes and the stromal-vascular fraction. It induces insulin resistance by inhibiting insulin signal transduction pathway in liver. In brief, IL-6 results in

the formation of suppressor of cytokine signaling-3 (SOC-3) protein that blocks the interaction between insulin receptor and insulin receptor substrates (IRS), leading to the inhibition *Akt* activation and thus attenuates normal insulin signaling (Kalupahana et al., 2012).

2.7 PPARs

2.7.1 Background of PPARs

PPARs are transcriptional factors belonging to the ligand-activated nuclear receptor superfamily. PPARs are implicated in obesity and Met-S by regulating the expression of target genes involved in lipid and carbohydrate metabolism, inflammatory response and energy homeostasis. The PPARs family consists of three isoforms in lower vertebrates and mammals: PPARα (NR1C1), PPARβ (also known as PPARδ, NR1C2), and PPARγ (NR1C3) (Escriva et al., 2000).

In 1983, a peroxisome proliferator biding protein was identified in the rat liver (Lalwani et al., 1983) and its function is to mediate the action of peroxisome proliferator was suggested. Then, Reddy et al. (1986) postulated that the peroxisome proliferators act in a manner similar to steroid hormones. A few years later, Issemann and Green (1990) identified four new orphan members of the nuclear hormone-like receptor family when screening a rodent liver cDNA library with a probe derived from the combined nucleotide sequences of several hormone receptors. As one of these receptors could be activated by a variety of peroxisome proliferators, therefore it is being named

as peroxisome proliferator-activated receptor (PPAR). In 1990, the Wahli laboratory reported cloning the *PPARα* from *Xenopus laevis* frog as well as two closely related orphan receptors which were encoded by distinct genes, named *PPARβ* and *PPARγ*. These three receptors share a high degree of homology (Dreyer et al., 1992). At the same year, *PPARδ* was identified in humans by Schmidt et al. (1992), which turned out to be closely related to *PPARβ* cloned by Wahli's laboratory.

2.7.2 Structure of PPARs

Similar to other members of the nuclear receptor family, PPARs share a similar modular structure with functionally distinct domains called A/B (ligand-independent activation domain /N-terminal domain-NTD), C (DNA binding domain-DBD), D (hinge domain), and E/F (ligand-binding domain-LBD) (Owen and Zelent, 2000) (Figure 2.4). The N-terminal domain A/B contains a ligand-independent activating function called AF-1. The DNA-binding domain (DBD, C domain) is highly conserved. It contains two zinc fingers, binding to specific sequences of DNA known as response elements when activated. The hinge region (D domain) is highly flexible and allows the receptor to change conformation. The E/F domain is the ligand binding domain (LBD) and it has the ligand dependent activation function, AF-2 (Poulsen et al., 2012).

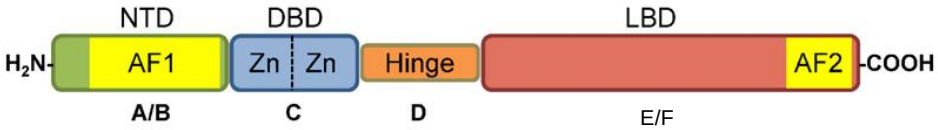


Figure 2.4 The domain structure of PPARs (Adopted from Poulsen et al., 2012)

2.7.3 Gene Map, Messenger RNA (mRNA) and Amino Acid of PPARs

The gene for PPARα is located on chromosome 22q12-13.1 and has one main isoform (Sher et al., 1993). A splice variant lacking exon 6 is found in the liver, but its function is unknown (Palmer et al., 1998). The gene for PPARδ is located on chromosome 6p21.2-p21.1 and encodes one known isoform (Yoshikawa et al., 1996). The gene for PPARγ is located on chromosome 3p25 (Beamer et al., 1997; Greene et al., 1995). Four different subtypes of PPAR-γ mRNA (-γ1, -γ2, -γ3, and -γ4) can be transcribed from four different promoters in human (Fajas et al., 1997; Fajas et al., 1998; Sundvold and Lien, 2001; Zieleniak et al., 2008). *PPARγ1*, *PPARγ3* and *PPARγ4* are regulated by separate promoters, but differ only in their transcription initiation start site so the mature proteins are identical (Sundvold and Lien, 2001). The *PPARγ2* isoform is also regulated by its own promoter and but has a unique first exon, encoding for an additional 28 amino acids in the N-terminal (Beamer et al., 1997; Fajas et al., 1997), results in a product of 505 amino acids instead of 477 amino acids. The domain structure of each PPAR isoforms is shown in Figure 2.5 while the schematic genomic structure of *PPARγ* subtypes and their corresponding protein structure are shown in Figure 2.6.

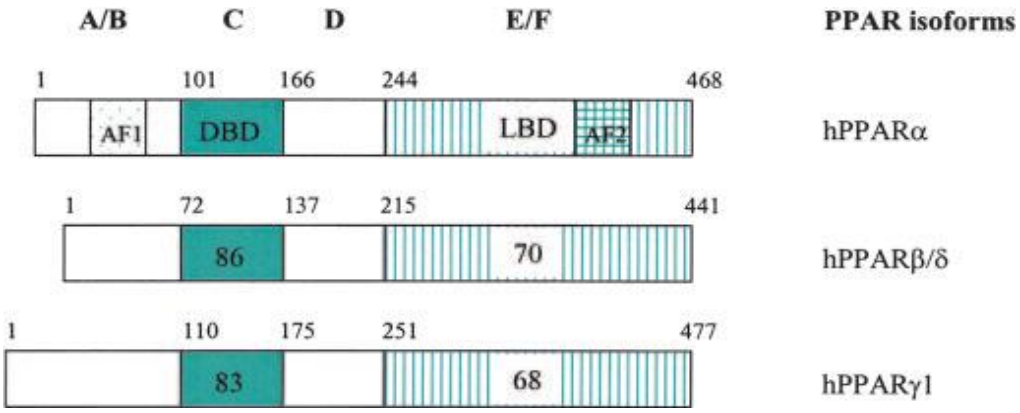


Figure 2.5 Schematic representation of the domain structure of human PPARα, δ/β, and γ. The number inside each domain corresponds to amino acid sequence identity of human PPARδ/β and γ1 relative to PPARα. The numbers above the boxes indicate amino acid positions. (Adopted from Guan, 2004)

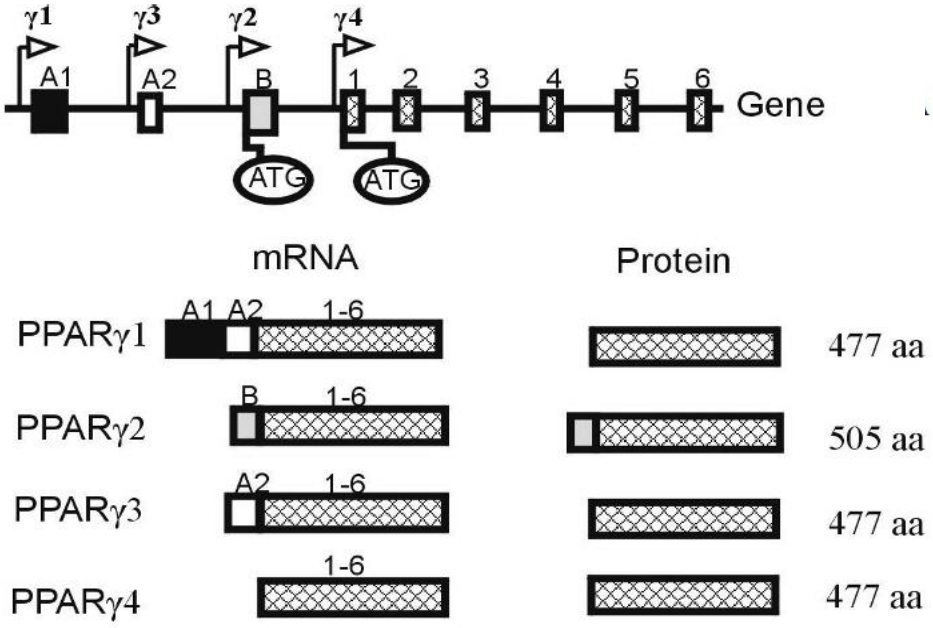


Figure 2.6 Schematic representation schematic genomic structure of *PPARγ* subtypes and their corresponding protein structure. The location of the ATG start-codon is indicated. γ1, γ2, γ3, and γ4 represent the promoters of PPAR-γ1, -γ2 -γ3, and -γ4 mRNA, respectively. The four subtypes of mRNA give rise to two different PPAR-γ proteins. Transcription of promoters γ1, γ3, and γ4 produces the same protein of 477 amino acids. Transcription from promoter γ2 results in 505 amino acids. (Adopted from Zieleniak et al., 2008)

2.7.4 Mode of Action and Ligands of PPARs

Upon activation by natural or synthetic ligands (Figure 2.7), PPARs form heterodimers with the 9-*cis* retinoic acid receptor (RXR) and bind to DNA consensus sequence (AGGTCANAGGTCA, N being any nucleotide) known as the peroxisome proliferator response element (PPRE), with PPARs always oriented to the DNA's 5'-end, while RXR to the 3'-end of PPRE (Issemann and Green, 1990; Wahli et al., 1995; Desvergne and Wahli, 1999; Berger and Moller, 2002; Coll et al., 2009; Tovar-Palacio et al., 2012; Grygiel-Gorniak, 2014).

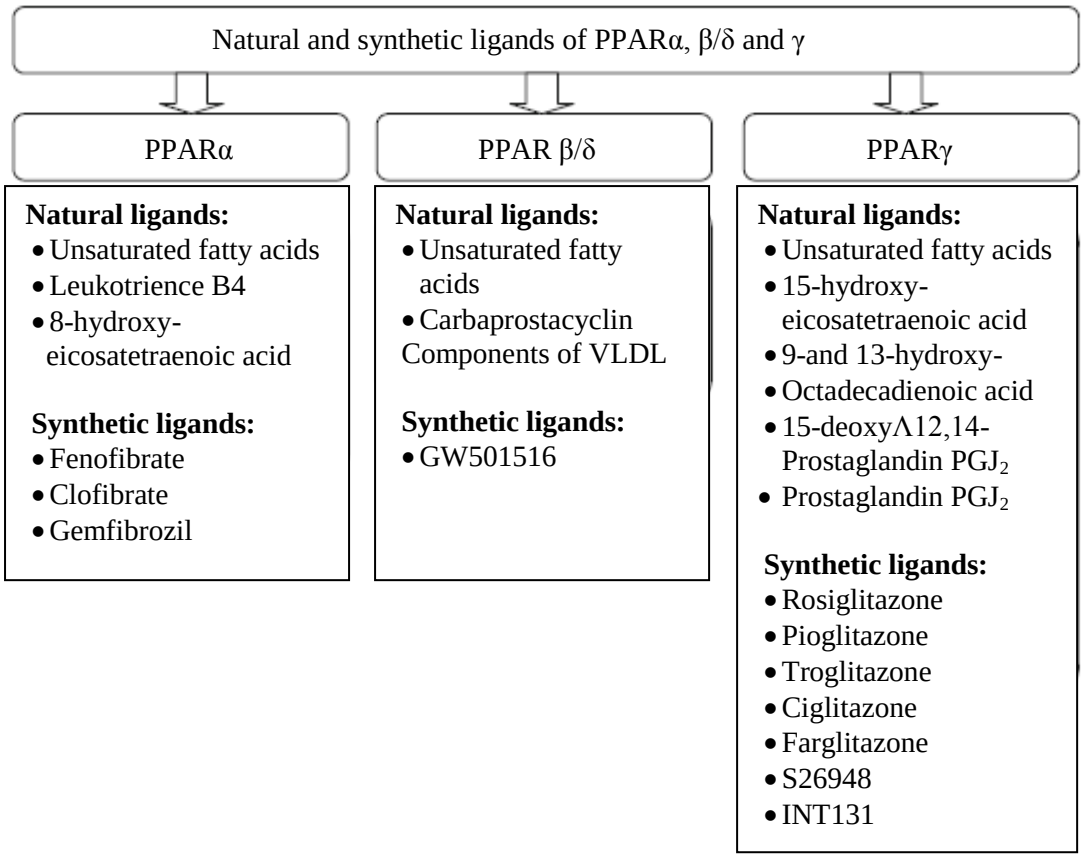


Figure 2.7 Natural and synthetic ligands of PPARs (Adopted from Grygiel-Gorniak, 2014).

The biological activity of each PPAR isoform is regulated by the availability of (natural or synthetic) ligands, the recruitment of co-activators and repressors, and the phosphorylation of PPAR (Coll et al., 2009). PPAR-RXR heterodimers and nuclear receptor co-repressor proteins form high-affinity complexes to block transcriptional activation by sequestering the heterodimer from the promoter in the absence of ligand. Binding of the ligand to PPAR induces a conformational change and results in dissociation of co-repressor proteins and recruitment of coactivator proteins, thus promoting the PPAR-RXR heterodimer to bind to PPRE to initiate transcription. PPAR-RXR heterodimers may bind to PPREs in the absence of a ligand, depending on the context of promoter and cell type (Guan et al., 2005).

Hormones such as insulin can modulate PPAR (α and γ) activity by phosphorylation of two mitogen-activated protein (MAP) kinase sites in the modulator region of the PPAR receptor (Shalev et al., 1996; Juge-Aubry et al., 1999,). Other than these, PPARs may also regulate gene expression of target gene through other mechanisms such as ligand dependent trans-repression, ligand independent transrepression and alteration of lipid environment (Varga et al., 2011).

2.7.5 Tissue Distribution and Primary Metabolic Functions of PPARs

PPARα is mainly expressed in metabolically active tissues, such as liver, heart, skeletal muscle, intestinal mucosa and brown adipose tissue (Issemann and Green, 1990; Braissant et al., 1996). PPARβ/δ is expressed

ubiquitously in virtually all tissues and is particularly abundant in the liver, intestine, kidney, abdominal adipose tissue, and skeletal muscle, all of which are involved in lipid metabolism (Escher et al., 2001). Meanwhile, PPARγ is expressed in white and brown adipose tissue, human skeletal and cardiac muscle, liver, kidney, small intestine, bladder, large intestine and spleen. In particular, PPARγ1 predominates quantitatively (Fajas et al., 1997; Mukherjee et al., 1997; Fajas et al., 1998). In human, mRNA of PPARγ1 is in highest level in adipose tissue and the large intestine but it is barely detectable in skeletal muscle (Fajas et al., 1997). The mRNA PPARγ2 and γ4 are restricted to adipose tissue (Sundvold and Lien, 2001), whereas the PPARγ3 is expressed in white adipose tissue, the large intestine, and macrophages (Braissant et al., 1996; Fajas et al., 1998; Ricote et al., 1998).

Generally, PPARs activity affects body weight due to its major involvement in fat metabolism, glucose metabolism and inflammation. PPARs are effective molecular targets for treating some aspects of Met-S, such as PPARα receptors for treating hypertriglyceridemia (fibrates) and PPARγ receptors for type II diabetes mellitus (thiazolidinedione) (Kersten et al., 2000; Kersten, 2002). PPARα and PPARβ/δ regulates energy combustion, lipoprotein synthesis, inflammatory responses, increases insulin sensitising and regulates blood cholesterol and glucose levels, whereas PPARγ facilitates energy storage by adipogenesis as it helps to convert unspecialised cells to adipocyte particularly when in high fat diet. PPARγ also plays a role in insulin sensitivity, glucose and protein metabolism, cell proliferation, cycle, and growth (reviewed in Khoo et al., 2008; Pyper et al. 2010; Grygiel-Gorniak,

2014). Figure 2.8 illustrates the distribution and functions of the PPARs in metabolic tissues.

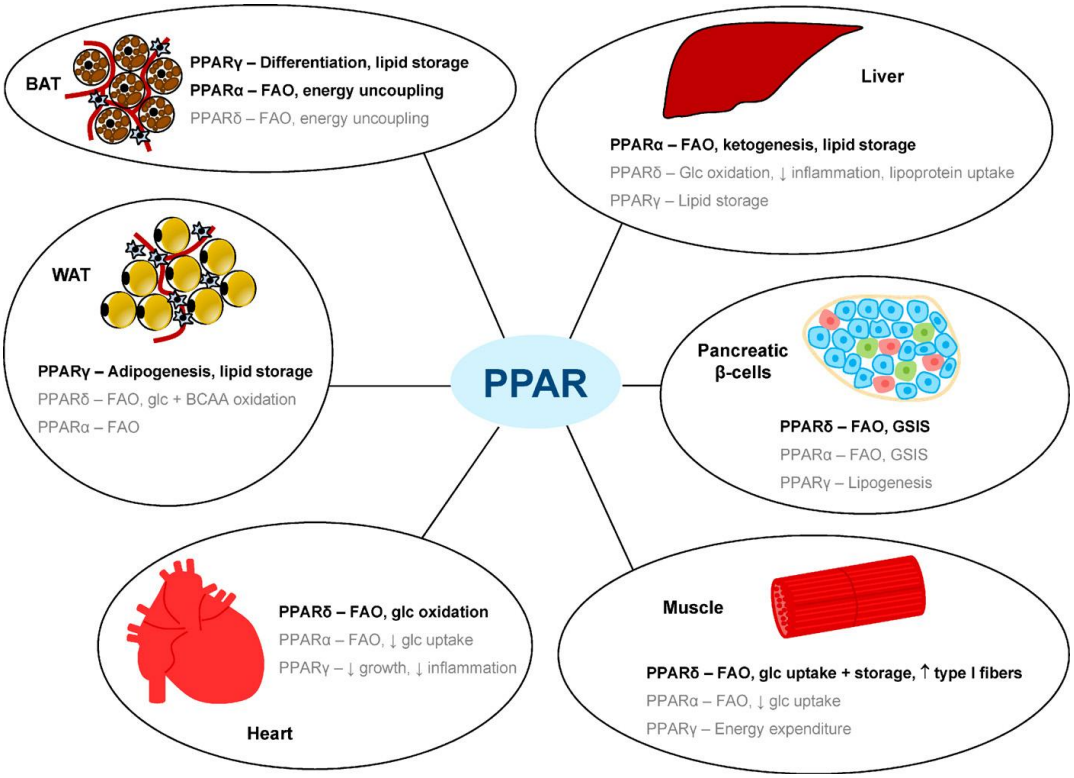


Figure 2.8 Distinct but overlapping distribution and function of the PPARs in metabolic tissues. The most important PPAR subtype(s) expressed at highest levels in each tissue are indicated in bold and black, while the subtypes expressed at lower levels are indicated in gray. BAT, brown adipose tissue; BCAA, branched chain amino acids; FAO, fatty acid oxidation; glc, glucose; GSIS, glucose stimulated insulin secretion; WAT, white adipose tissue. (Adopted from Poulsen et al., 2012)

2.8 *PPARs* SNP

2.8.1 *PPARα* L162V SNP

PPARα L162V SNP (rs1800206) is a missense mutation of leucine to valine at codon 162 due to 484C/G transversion in exon 5 (Flavell et al., 2000; Vohl et al., 2000). The functional activity of *PPARα* L162V SNP is affected by ligand concentration. In *in vitro* transfection assays performed, *PPARα* L162V variant is unresponsive to a low concentration of ligand compared with the wild type. However, the transcriptional activity of the V162 allele is higher compared with that of the L162 allele in the presence of a high concentration of the synthetic ligand Wy14643 (>25 mM) (Flavell et al., 2000; Sapone et al., 2000).

Table 2.3 summarizes minor allele frequency (MAF) *PPARα* L162V SNP and the findings of the association study on the obese and Met-S related phenotypes. The MAF of *PPARα* L162V SNP was found to be ranging from 0.13 to 0.16 in Chinese Han population from China while the Caucasians have reported a MAF of 0.06 to 0.12 from different populations. The MAF was found to be low at 0.02 for African Americans. Overall, this SNP has been previously associated with dyslipidemia.

Table 2.3: Association studies between *PPARα* L162V SNP and metabolic phenotypes

Population	No. of subjects/ Gender	MAF	Metabolic phenotypes	Reference
Caucasians				
Quebec, Canadian	T2DM: 121/Mix Non-diabetic subject:193/Men	0.12 0.07	↑LDL-C No association with type2 diabetes mellitus (T2DM), BMI and WC	Vohl et al. (2000)
Hamburg, German	911 Caucasians /Mix	0.06	In women with T2DM: ↓BMI No association with lipid profile	Evans et al. (2001)
Framingham American	2372 Caucasians/Mix	0.07	In men ↑TC ↑LDL-C	Tai et al. (2002)
Quebec, Canadian	632 Caucasians/Men	0.11	↑TG No association with Met-S	Robitaille et al. (2004)
Polish	Healthy subject: 51 Caucasians/Men Coronary heart disease: 48 Caucasians/Men	Healthy men:0.06 Patients : 0.25	No association with BMI and lipid profile	Skoczynska et al. (2005)
Framingham American	2106 Caucasians/Mix	0.07	*PPARα* L162V X low polyunsaturated fatty acid (PUFA) diet interaction ↑TG *PPARα* L162V X high PUFA diet interaction ↓ TG	Tai et al. (2005)
Hamburg, German	462 Caucasians/Mix	0.11	No association with obesity and lipid profile	Aberle et al. (2006a)

Table 2.3 continued

Population	No. of subjects/ Gender	MAF	Metabolic phenotypes	Reference
Danish	5799 Caucasians /Mix	0.06	↑TG No association with obesity and T2DM	Sparso et al. (2007)
Spanish	59 Caucasians/Men	0.10	↑TC ↓ HDL-C ↑TG	Tanaka et al. (2007)
Washington, American	610 Caucasians /Mix	0.08	In men ↑BMI ↑SF ↑TG ↓ HDL-C	Uthurralt et al. (2007)
German	4779 Caucasians /Mix	N/A	No association with obesity and T2DM	Silbernagel et al. (2009)
Brazilian	570Caucasians/Mix	0.05	↑Dyslipedemia	Mazzotti et al. (2011)
Brazilian	206 Caucasians /Mix	0.06	No association with obesity, T2DM, insulin resistance and dyslipidemia, adiponectin level	Domenici et al. (2013)
Asians				
Chinese (Jiangsu, China)	820 Chinese Han/Mix Obese:307 Non-obese:513	0.13	No association with obesity(BMI> 24kg/m^2)	Luo et al. (2013)
Chinese (Jiangsu, China)	647 Chinese Han/Mix	0.16	No association with obesity	Gu et al. (2014)
Mixed				
European and Asian	Healthy subjects: 2508 Caucasians /Men; T2DM:129/Mix-86 European , 43 Asian	European: 0.07 Asians: 0.03	In subjects with T2DM: ↑TC	Flavell et al. (2000)
Los Angeles & San Francisco, American	Caucasians: 609/Mix, African American: 335/Mix	0.06, 0.02	In African-Americans ↑TG	Shin et al. (2008)

2.8.2 *PPARγ2* C161T SNP

PPARγ2 C161T SNP (rs3856806; also known as C1431T or CAC478CAT) is a silent His477His polymorphism in exon 6 (Meirhaeghe et al., 1998). To date, there is no functional information on this polymorphism available. It is probably implicated in obesity and Met-S by linkage disequilibrium with nearby Pro12Ala polymorphism (rs1801282) (Doney et al., 2002; Doney et al., 2004; Meirhaeghe et al., 2005; Meirhaeghe et al., 1998; Tai et al., 2004; Yang et al., 2009; Youssef et al., 2014). Masud et al. (2003) and Paracchini et al. (2005) who performed meta-analysis study on PPARγ gene polymorphisms found no association of PPARγ2 C161T SNP with obesity/BMI.

Table 2.4 shows the MAF of *PPARγ2* C161T SNP and some findings of the association study on the obese and Met-S related phenotypes. The MAF of this SNP was found to be highest among Chinese populations from various countries (0.20 to 0.30), followed by Malays (0.22), Koreans (0.20, Tunisian (0.18) and Japanese (0.17). The MAF for Indians is similar to Caucasians (0.12 to 0.17). Generally, *PPARγ2* C161T SNP has been previously associated with decreased risk for type II diabetes mellitus (Tai et al., 2004) and hyperglycemia (Jaziri et al., 2006) as well as improved insulin sensitivity (Liu et al., 2008).

Table 2.4: Association studies between *PPARγ2* C161T SNP and metabolic phenotypes

Population	No. of subjects/ Gender	MAF	Metabolic phenotypes	Reference
Caucasians				
Australian	647 Caucasians/Mix	0.16	No association with obesity	Wang et al. (1999)
Scottish	T2DM: 1107 Caucasians /Mix Non-T2DM: 440 Caucasians /Mix	0.12	No association with BMI Allelic linkage disequilibrium with *PPARγ2* Pro12Ala (rs 1801282): BMI of Pro-T161 & Ala-T161 > Pro-C161 & Ala-C161	Doney et al. (2002)
French	Control: 855 Caucasians /Mix; Met-S: 279 Caucasians /Mix	0.14	No association with Met-S Allelic linkage disequilibrium with *PPARγ2* Pro12Ala and *PPARγ2* P-689T	Meirhaeghe et al. (2005)
French	3914 Caucasians/Mix	0.12	↓ risk to develop hyperglycemia	Jaziri et al. (2006)
Asians				
Singaporean	568 Indians/Mix; 740 Malays/Mix; 2729 Chinese/Mix	Indians:0.17 Malays: 0.22 Chinese: 0.25	↓ risk for T2DM In normal subjects ↑BMI No association with lipid profile and glucose parameter in normal, impaired glucose tolerance and T2DM subjects Allelic linkage disequilibrium with *PPARγ2* Pro12Ala (rs 1801282)	Tai et al. (2004)
Korean (Seoul, Korea)	253 Korean/Women	0.20	No association with adiposity indices, blood pressure, lipid profile, glucose parameters and Met-S	Rhee et al. (2006)
Chinese (Beijing, China)	729 Chinese Han /Mix	0.21	No association with obesity and Met-S ↓ HOMA-IR	Liu et al. (2008)
Indians (South India)	699 Indians/Mix	0.14	No association with obesity and Met-S	Haseeb et al. (2009)
Chinese (Beijing, China)	423 Chinese Han/Mix	0.25	↑FBG ↑TG Affect risk for Met-S by allelic linkage disequilibrium with *PPARγ2* Pro12Ala (rs 1801282): Pro/Ala + CT or TT of controls was higher than that of subjects with Met-S.	Yang et al. (2009)

Table 2.4 continued

Population	No. of subjects/ Gender	MAF	Metabolic phenotypes	Reference
Taiwanese	600 Chinese/Mix	0.25	No association with obesity and Met-S	Chen et al. (2011)
Japanese	716 Japanese/Mix	0.17	No association with BMI, blood pressure, lipid profile, glucose parameters and Met-S	Sanada et al. (2011)
Indians (North India)	642 Indians/Mix	0.15	↑ TBF ↑FI No association with obesity	Prakash et al. (2012)
Chinese (Jiangsu, China)	820 Chinese/Mix Obese:307 Non-obese:513	0.29	No association with obesity(BMI> 24kg/m^2)	Luo et al. (2013)
Others				
Tunisian	522 subjects/Mix Met-S subjects:264 Non-Met-S subjects: 258	0.18	All subjects: ↑FI ↑FBG ↑HOMA-IR Separate analysis between subjects with and without Met-S: No significant difference on adiposity indices, blood pressure, lipid profile, glucose parameters , respectively ↑ risk for Met-S by allelic linkage disequilibrium with *PPARγ2* Pro12Ala (rs 1801282) Ala-C161 : ↓ BMI, ↓FBG, ↓FI, ↓HOMA-IR	Youssef et al. (2014)

2.8.3 *PPARδ* T294C SNP

PPARδ T294C SNP (rs2016520; also known as +15 C/T, -87 T/C, or c.1-87T>C) is located at the 5'- untranslated region (UTR) of exon 4, 87 nucleotides upstream of the start codon. This SNP influences the binding of a transcription factor Sp-1 and the rare C allele is associated with higher transcriptional activity (Skogsberg et al., 2003a). A meta-analysis performed by Ye et al. (2013) suggested a lack of association of this SNP with the risk of coronary heart disease.

Table 2.5 presents the MAF of *PPARδ* T294C SNP and some findings of the association study on the obese and Met-S related phenotypes. The MAF of this SNP was found to be relatively common (0.25 to 0.30) among Chinese populations from China and Koreans (0.23). For Caucasians, its MAF varies widely across different populations. As shown in Table 2.5, this SNP is related to lower risk of obesity, decreased BMI and HDL-C in various studies.

Table 2.5: Association studies between *PPARδ* T294C SNP and metabolic phenotypes

Population	No. of subjects/ Gender	MAF	Metabolic phenotypes	Reference
Caucasians				
Swedish	543 Caucasians/Men	0.16	↑LDL-C	Skogsberg et al. (2003a)
Swedish	Caucasians/Men		In control group	Skogsberg et al. (2003b)
	Coronary heart disease : 501	0.21	↓ HDL-C	
	Controls: 1118	0.18	Neither controls nor cases groups associated with BMI, inflammation markers PLA2, CRP, fibrinogen and white cell count. No association between plasma lipid and lipoprotein concentrations in subjects below or above the median value for BMI (25.3 kg/m^2)	
German	838 Caucasians/Mix T2DM: 402 Controls: 436	0.04	No association with lipid profile and BMI	Gouni-Berthold et al. (2005)
German	517 Caucasians/Mix	0.25	↓ body weight ↓BMI	Aberle et al. (2006a)
German	967 Caucasians/Women Coronary heart disease: 514 Healthy subjects: 453	0.24	↓VLDL 561 subjects with BMI below median (26.5kg/m^2): ↓HDL-C	Aberle et al. (2006b)
Danish	7495 Caucasians/Mix	0.16	No association with all Met-S traits	Grarup et al. (2007)
Quebec-Canadian	340 Caucasians/Mix	0.20	↑ HDL-C in women ↓TC/HDL-C ratio when exposed to a low-fat diet (less than 34.4% of energy from fat)	Robitaille et al. (2007)

Table 2.5 continued

Population	No. of subjects/ Gender	MAF	Metabolic phenotypes	Reference
European	11074 Caucasians/Mix T2DM: 5224 Control: 5850	0.24	No association with BMI, lipid profile, TNF-α and adiponectin in whole study population Men: ↓BMI ↓TNF-α ↑HDL-C Female: ↑BMI ↓HDL-C	Burch et al. (2010)
Asians				
Korean (Seoul, Korea)	492 Korean/Mix	0.23	No association with adiposity indices, lipid profile and with T2DM Non-T2DM subjects: ↑FBG	Shin et al. (2004)
Chinese (Guangxi, China)	1729 Bai Ku Yao/Mix	0.26	No association with obesity (>28 kg/m^2) and blood pressure	Yin et al. (2012a)
Chinese (Jiangsu, China)	820 Chinese/Mix Abdominal obese: 404 Control: 416	0.30	↓ risk for abdominal obesity (WC ≥85 cm for males and ≥80 cm for females)	Ding et al. (2012b)
Chinese (Jiangsu, China)	820 Chinese/Mix Obese: 307 Non-obese: 513	0.30	↓ risk for obesity (BMI> 24kg/m^2)	Luo et al. (2013)

CHAPTER 3

MATERIALS AND METHODS

3.1 Subjects and Study Population

This study was registered under the National Medical Research Registry (NMRR-09-826-4266) which was extension from a previous study in the same laboratory (Fan and Say, 2014) and the protocol was approved by the Medical Research and Ethnics Committee, Ministry of Health Malaysia (Appendix A). A total of 286 subjects were recruited from the patrons of clinic who attended the Kampar Health Clinic from June to December 2011 and 27 subjects from previous study. Informed consent (Appendix B) was obtained from each participants. Whereas the samples were taken in accordance with the Declaration of Helsinki (revised in Seoul, 2008). The subjects were recruited on a voluntary basis based on their willingness to attend to a standard questionnaire (Appendix C) and availability of their fasting blood samples.

A standard questionnaire which consisted of the subject's medical history, socio-demographic data, dietary habits and lifestyle factors was given to each participant. The questionnaire with three languages was designed and pilot run previously by undergraduate students of Universiti Tunku Abdul Rahman, Malaysia. The socio-demographic information obtained were age, gender, ethnicity, marital status, occupation, monthly household income, educational status and family history of obesity. Of note, the identification of

ethnic group by self-declaration was obtained by selection from the four choices (Malays, Chinese, Indians and others) in the questionnaire. In this study, four subjects were self-reported as 'Others' in the questionnaire and they were identified as *Orang Asli* of Peninsular Malaysia. As the number of *Orang Asli* subjects recruited in this study was low, they were grouped together in the Malays ethnic group in the statistical analysis. As such, three categories of ethnic groups (Malays, Chinese and Indians) were employed in all statistical analysis throughout this study of multi-ethnic population with 'Malays' group denoting both Malaysian Malays and *Orang Asli* from Peninsular Malaysia. Meanwhile, *Orang Asli* from Sabah and Sarawak, mixed-race as well as non-Malaysian foreigners were excluded from this study.

As for dietary habits and lifestyle factors, a few components which can lead to obesity and/or Met-S were covered in the questionnaire. For instance, high salty food intake was used as a component to check for its possible hypertensive effect which is a defined criterion for Met-S. Strict vegetarian practice as a component to investigate the possible connection of diet particularly low in protein content with the incidence of obesity and Met-S. The frequency of weekly fast food consumption was used to check for its possible association with obesity and/or Met-S as fast food is characterized by a particular high energy density with high fat and salt content. Meanwhile, caffeine intake was investigated to check for its possible implication on energy expenditure or thermogenesis. In this study, salty food preference (intake of at least a serving or dish of high sodium foods such as salted vegetables, salted fish or salted egg once a week), strict vegetarian practice (diet absent of any

meat, poultry, seafood or any animal origins, excluding egg and milk products), caffeine intake as in coffee consumption (at least a cup per day) and weekly fast food intake (at least once a week) were evaluated based on either ‘yes’ or ‘no’ to find out the dietary habits of subjects.

Besides, physical activity, smoking habit and alcohol consumption were evaluated as the lifestyle factors. In particular, simple assessment on physical activity was included to assess the possible influence of adopting active lifestyle on the overall health status of the subject. The presence of smoking habit and alcohol was employed to assess for its possible influence on the metabolic rates as well as their substantial effects on any other biochemical parameters. Subjects were categorized as physically active if they exercised everyday or more than three times a week, physically inactive if they exercised occasionally or two to three days a week. The smoking habits of subjects were evaluated by the options: ‘yes’ (current smokers), ‘quit/smoked before’ (subjects who had changed their smoking habits or stopped smoking less than five years before the start of the study) and ‘no’ (subjects who had never smoked or stopped smoking more than five years before the start of the study). Whereas the alcohol consumption was categorised into ‘yes’ (current drinker), ‘stopped’ (subjects who stopped drinking for more than five years prior to the start of the study), ‘no’ (never drank) and social drinker (occasionally). The details on frequency and duration of drinking and smoking habits were excluded for analysis due to the low number of current smokers and drinkers reported.

After the questionnaire-interview session, anthropometric, blood pressure and fasting blood glucose measurements were also obtained from the participants. The exclusion criteria for the subjects of this study included known medical conditions such as pituitary diseases, chronic liver or renal diseases, hyperthyroidism, acute infection hematologic diseases, patients undergoing dialysis, people with a fever or swelling, patients with osteoporosis who have very low bone density, pregnancy and body builders or highly trained athletes.

Raosoft® Sample size calculator software (Raosoft Inc, USA, available at http://www.raosoft.com/samplesize.html) was used to determine the minimum sample size of subjects needed to recruit based on the Kampar population. Kampar is a suburban area of Perak state with multi-ethnic residents consisted of Malays, Chinese and Indians. Based on the 2010 population and housing census (Department of Statistics, Malaysia 2010), there is 88,638 residents in Kampar with a ratio of 3:5:1 for the Malays, Chinese and Indians. Using the Raosoft® Sample size calculator software (Raosoft Inc, USA), a minimum sample size of 288 had to be recruited, assuming a margin of error of 5 %, with confidence level of 95 % and response distribution of at least 25 %.

To achieve the minimum sample size, this study had recruited a total of 307 subjects, of which 6 subjects were excluded from the study due to incomplete data, other ethnic group background or technical errors (low DNA concentration) from the earlier total of 313 subjects. The subjects recruited in

the study showed a ratio of 2:3:1 for Malays, Chinese and Indians which was considered to be approximately representative on the ethnicities composition of the Kampar local residents. The high ratio of Chinese population in Kampar census could be due to the inclusion of university students (Kolej Universiti Tunku Abdul Rahman/Universiti Tunku Abdul Rahman, Perak campus) who are predominantly Chinese, and therefore were less likely to be the participants from the Kampar Health Clinic who were mainly attended by the local residents of Kampar.

3.2 Defining Outcome Variables

Obesity and Met-S are the two main outcome variables examined in this PPAR polymorphisms study. To better understand the possible association of both obese and Met-S with the PPAR polymorphisms, the obese subjects were further grouped based on the presence or absence of Met-S.

3.2.1 Obesity

The obesity classification of this study was based on the redefined classification for Asian populations (WHO/IASO/IOTF, 2000) as shown in Table 3.1. The recommendation was proposed based on the increased risks of co-morbidities with obesity occurs at a lower BMIs in Asians. In general, BMI < 25 kg/m^2 was classified as non-obese whereas BMI ≥ 25 kg/m^2 as obese. The BMI was then calculated as the weight in kilograms divided by the square of the height in metres (kg/m^2).

Table 3.1: Classification of weight status according to BMI in Asian adults

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal range	18.5 - 22.9
Overweight	≥ 23.0
At Risk	23.0 - 24.9
Obese class I	25.0 - 29.9
Obese class II	≥ 30.0

Source: WHO/IASO/IOTF (2000)

3.2.2 Met-S

Met-S was diagnosed using the criteria proposed by the U.S. National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III). The five criteria under NCEP ATP III were included central obesity, high TG, low HDL-C, high blood pressure (BP) and high fasting blood glucose (FBG). In consideration of Malaysia as an Asian country, a lower cut-off point for central obesity would be more appropriate. Based on a previous study by Tan et al. (2011) to redefine classification criteria on Met-S in Malaysia, a subject was categorized under Met-S in the presence of at least three out of the five criteria listed in Table 3.2.

Table 3.2: Clinical identification guidelines of Met-S according to the definition of NCEP ATP III

Criteria	Defining guidelines
Central obesity	WC for Asians: Male ≥ 90 cm (35 inches) ; Female ≥ 80 cm (31.5 inches)
TG	TG ≥ 1.7 mmol/L (150 mg/dL) or specific treatment for this lipid abnormality
HDL-C	Male < 1.03 mmol/L (40 mg/dL) ; Female < 1.29 mmol/L (50 mg/dL); or specific treatment for this lipid abnormality
BP	Systolic BP ≥ 130 mmHg or Diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension
FBG	FBG ≥ 5.6 mmol/L (100 mg/dL) or previously diagnosed type 2 diabetes

Sources: (Alberti et al., 2006; Grundy et al., 2004; Tan et al., 2011)

3.3 Anthropometric and BP Measurements

The anthropometric measurements investigated from the subjects were waist and hip circumference (WC and HC), weight, height and body compositions including total body fat (TBF), subcutaneous fat (SF), visceral

fat level (VFL), resting metabolism (RM) and skeletal muscle (SM) percentage. BP and pulse rate were also obtained as part of the measurements.

The weight of the subject was measured using a bio-impedance body fat weighing scale (Model HBF-362 Karada Scan Body Composition Monitor with Scale, Omron, Japan). The scale also displayed TBF, SF and VFL, RM and SM percentage during the measurement. The height of the subject was measured using a standard height scale provided by the Kampar Health Clinic with the subject's footwear removed, standing straight and looking forward obtained. WC was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of iliac crest while HC was taken around the widest portion of the buttocks (WHO, 2008b) using a measuring tape to the nearest 0.1 cm. The Waist-Hip Ratio (WHR) was calculated by dividing the WC by the HC. The average reading of Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and pulse rate were obtained from two repeated reading using an automated blood pressure monitor (SEM-1, Omron, Japan) after sitting and resting for ≥ 10 min.

3.4 Blood Sampling

Overnight fasting blood samples of participants were collected under routine phlebotomy techniques by professional nurses into K_2EDTA tubes (BD Vacutainer, USA). The blood samples were then transported back on cool packs and immediately centrifuged to prevent lysis of erythrocytes at 3300 *g* for 10 min (Centrifuge Sigma 2-16PK, Sartorius, Germany). The whole blood

was fractionated into upper plasma, middle buffy coat and bottom erythrocytes layers through centrifugation. The upper plasma layer was collected into three microcentrifuge tubes (1.5 ml) and was stored at -80 ˚C for further biochemical analysis. For all biochemical measurement analysis, assays were performed within 6 months after stored at -80 ˚C. The middle buffy coat layer (leucocytes-enrich fraction) on the concentrated erythrocytes layer was carefully drawn out using a pipette for the use of genomic DNA extraction. The purified DNA was stored at -20 ˚C until further use.

3.5 Biochemical Analysis

3.5.1 Fasting Blood Glucose Determination

Fasting blood glucose concentration of the subjects was tested using One Touch® Ultra-Easy blood glucose monitoring system (LifeScan, Inc. USA). The meter was calibrated as instructed using the code numbers on the test strip vial. The test was performed using whole blood of the subjects at the clinic on the spot. During the measurement, a test strip was inserted to turn on the meter. The test strip was lined up with the edge of a blood drop from the subject to allow the blood drop to be drawn into the narrow channel of the test strip. The blood glucose level displayed on the display was then recorded.

3.5.2 Fasting Insulin Level Determination

Fasting insulin level was measured using commercial insulin Enzyme-linked Immuno Sorbent Assay (ELISA) kit (Diagnostic Automation/ Cortez

Diagnostics, Inc. USA). This assay employed the principle of competitive ELISA technique to measure to insulin levels in human plasma or serum.

Firstly, reagents, standards and samples were prepared as instructed and brought to room temperature (20-27 ºC) before proceeding with the assay. The microplate wells were formatted for standards and samples in duplicated. A volume of 50 µl of the appropriate standards and samples were pipetted into the assigned wells, followed by 100 µl of Insulin Enzyme Reagent. The microplate was covered with a plastic wrap and was swirled gently for 20-30 sec to mix. Subsequently, the microplate was incubated for 120 min at room temperature. Then, the contents were discarded by decantation (tapped and blotted dry with absorbent papers).

After that, 350 µl of wash buffer was added followed by decantation. This washing step was repeated up to a total of 3 washes. Next, 100 µl of working substrate solution was added to all wells followed by 15 min incubation at room temperature. Lastly, 50 µl of stop solution was added to each well and the microplate was gently mixed for 15-20 sec. Using a miroplate reader (Infinite M200, Tecan, Switzerland), the absorbance in each well was read at 450 nm with a reference wavelength of 620 nm to minimize well imperfections.

A dose response curve was used to ascertain the concentration of insulin in unknown specimens. The absorbance of each duplicated serum reference versus the corresponding insulin concentration in µIU/ml was

plotted using Microsoft Office Excel 2007. The best-fit curve was drawn through the plotted points. To determine the concentration of insulin for an unknown, the average absorbance of the duplicated for each unknown was located on the vertical axis of the graph, the intersecting point on the curve was found, then the concentration (in μIU/ml) was read from the horizontal axis of the graph.

3.5.3 Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)

Insulin resistant was calculated via homeostatic model assessment (HOMA-IR), where the product of fasting glucose (mmol/L) and fasting insulin (μIU/ml) was divided by 22.5 (Matthews, 1985).

3.5.4 Lipid Profile Analysis

Lipid profile analysis was performed to determine the plasma concentration of total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG) and total cholesterol/HDL cholesterol ratio (TC/HDL-C). The analysis was performed through an outsourced service by Pathology and Clinical Laboratory (M) Sdn. Bhd. Plasma TC, TG and HDL-C were measured standards enzymatic methods using an automated biochemistry analyzer while LDL-C was calculated with the Friedewald formula.

3.5.5 Human Plasma Adiponectin Level Determination

Human plasma adiponectin level was identified using Quantikine® Human Total Adiponectin/Acrp30 Immunoassay (R&D Systems, USA). This assay employed the quantitative sandwich enzyme immunoassay technique.

Firstly, all the reagents, standards and samples were prepared as directed and were brought to room temperature before use. Plasma samples were prepared by adding 990 µl of Calibrator Diluent RD6-39 into 10 µl of each sample (100-fold dilution). The assay was performed in duplicate. A volume of 100 µl of Assay Diluent RD1W was added to each well. Then, 50 µl of standards and samples were added per well. The covered plate was incubated for 2 hr at room temperature. Next, the contents were discarded and the plate was washed for a total of 4 times. The plate was washed by filling approximately 400 µl of Wash Buffer into each well, inverted and blotted against clean absorbent papers.

Then, 200 µl of Adiponectin Conjugate was added to each well, followed by 2 hr of incubation at room temperature. Afterward, the contents were discarded and washed for 4 times. Next, 200 µl of Substrate Solution was added to each well, followed by another 30 min of incubation at room temperature but this time with protection from light.

Lastly, 50 µl of Stop Solution was added to each well and the plate was gently tapped to ensure thorough mixing. The absorbance was determined

within 30 min, using a microplate reader (Infinite M200, Tecan, Switzerland) set to 450 nm. Wavelength correction was performed at 540 nm.

To determine the plasma adiponectin concentration, the duplicated readings for each standard and sample were averaged and subtracted with average zero standard optical density. The optical density for the standards versus the concentration of the standards was plotted using Microsoft Office Excel 2007 and a best curve was drawn. The concentration read from the standard curve was multiplied by the dilution factor of 100-fold.

3.5.6 Human Plasma IL-6 Level Determination

The measurement of human plasma IL-6 level was done using Quantikine® ELISA Human IL-6 (R&D Systems). The principle of the assays was based on the quantitative sandwich enzyme immunoassay technique.

All reagents, standards and samples were prepared as directed and were brought to room temperature before use. Initially, 100 µl of Assay Diluent RD1W was added to each well. Duplicated standards and samples were then added by a volume of 100 µl per well. The plate was covered by an adhesive strip and incubated for 2 hr at room temperature. The contents were discarded and the plate was washed for a total of 4 times by inverting the plate and blotting dry.

Next, 200 µl of IL-6 Conjugate was added into each well and the plate was covered using a new adhesive strip. After 2 hr of incubation at room temperature, the plate was decanted and washed for 4 times. Then, 200 µl of Substrate Solution was added to each well followed by 20 min of incubation at room temperature with the plate protected from light.

Finally, 50 µl of Stop Solution was added to each well and the plate was gently tapped to ensure thorough mixing. Using a microplate reader (Infinite M200, Tecan, Switzerland) set to 450 nm, the optical density was determined within 30 min. Wavelength correction was performed by setting at 540 nm.

The duplicated readings for each standard and sample were averaged and subtracted with average zero standard optical density. The optical density for the standards versus the concentration of the standards was plotted using Microsoft Office Excel 2007. A best fit curve was drawn through the points. The concentration of human plasma IL-6 was determined from the standard curve.

3.6 Genomic DNA Extraction

Genomic DNAs of the subjects were extracted using Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid, USA). Following the buffy coat protocol, the RBC lysis steps were performed to remove non-nucleated red blood cells and reduce hemoglobin contamination. This was done by adding

and mixing 3 times the sample volume (approximately 600 µl) of RBC Lysis Buffer to a 1.5 ml microcentrifuge tube containing about 200 µl of buffy coat. The tube was incubated for 10 min at room temperature. During incubation, the tube was inverted every 3 min. The tube was centrifuged using Sorvall Legend Micro 17R Centrifuge (Thermo Scientific, USA) at full speed for 1 min and the supernatant was discarded completely. Then, 500 µl of RBC Lysis Buffer was added to resuspend the white pellet before another full speed of centrifugation for 1 min. Again, the supernatant was completely discarded. Another 200 µl of RBC Lysis Buffer was added to resuspend the white pellet completely.

The next few steps were done for white blood cell lysis. A volume of 250 µl of GB Buffer was added into the tube and was mixed by vortex. The sample lysate was incubated up to 30 min or until the content was clear. During the incubation, the tube was inverted every 3 min. At this time, the required Elution Buffer (200 µl per sample) was preheated in a 70 ºC water bath to prepare for the last DNA elution step. A volume of 5 µl of RNase A (10 mg/ml) was added to the sample lysate, followed by vortexing and 5 min of incubation at room temperature.

The subsequent steps were performed for DNA binding. A volume of 250 µl of absolute ethanol was added to the sample lysate followed by 10 seconds of immediate vortex. All the mixture (including precipitate) was transferred to a GD column placed in a 2 ml Collection Tube. The tube was

centrifuged at full speed for 5 min. Then, the flow-through was discarded and the GD Column was placed in a new 2 ml Collection Tube.

For the washing steps, 400 µl of W1 Buffer was added to the GD column followed by 1 min of full speed centrifugation. The flow-through was discarded while the GD column was placed back in the 2 ml Collection Tube. Next, 600 µl of Wash Buffer was added to the GD column followed by 1 min of full speed centrifugation. The flow-through was discarded and the GD column was placed back again in the 2 ml Collection Tube. The tube was centrifuged again for 3 min at full speed to dry the column matrix.

Finally, DNA was eluted through the following steps. The dried GD column was transferred to a clean 1.5 ml microcentrifuge tube. Then, 100 µl of preheated Elution Buffer was added to the center of the column matrix followed by 10 min incubation at 37 ºC. The purified DNA was eluted by 1 minute of full speed centrifugation. The concentration of the extracted DNA was measured by Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA) and DNA purity was confirmed with A260/A280 ratio of 1.8 to 2.0. The genomic DNA extracted for the first batch of extraction was resolved by agarose gel electrophoresis to check for their integrity.

3.7 Genotyping

3.7.1 Polymerase Chain Reaction-restriction Fragment Length Analysis (PCR-RFLP)

The genotyping of *PPARα, γ* and *δ* polymorphisms were performed by PCR-RFLP. The genes of *PPARα, γ* and *δ* were amplified by PCR using MJ Mini^{TM} Personal Thermal Cycler (Biorad, US) prior to the restriction enzyme treatment. Table 3.3 summarizes the conditions of PCR amplification. PCR reaction was performed in a final volume of 25 µl with approximately 100 ng of genomic DNA per reaction. The final concentrations of each PCR reaction composition are listed in Table 3.4.

Table 3.3: Conditions of PCR amplification

PCR Conditions	**SNP**		
	***PPARα* L162V**	***PPARγ2* C161T**	***PPARδ* T294C**
Cycles	30	30	30
Initial denaturation	94 ˚C, 3 min	94 ˚C, 3 min	94 ˚C, 3 min
Denaturation	94 ˚C, 30 sec	94 ˚C, 30 sec	94 ˚C, 30 sec
Annealing	55 ˚C, 40 sec	56 ˚C, 40 sec	60 ˚C, 40 sec
Extension	72 ˚C, 1 min	72 ˚C, 1 min	72 ˚C, 1 min
Final extension	72 ˚C, 10 min	72 ˚C, 10 min	72 ˚C, 10 min

Table 3.4: Final concentration of PCR composition

Reagents (manufacturer)	Reaction Volume (µl)	Final Concentration
Genomic DNA	Appropriate amount	100 ng
Forward and Reverse primers (100 µM, Bioneer, NHK Bioscience, Malaysia)	0.25	1 µM
10× Pol Buffer B with 15 mM $MgCl_2$ (EURx Ltd., Poland)	2.5	1 X
dNTP mixture (10 mM of each, Bioron, Germany)	0.5	200 µM
Taq DNA polymerase (5 U/µl, EURx Ltd., Poland)	0.1	0.5 U
Sterile deionised water	Appropriate amount	-
Total	25	

Table 3.5 shows the sequence of primers and restriction enzymes used in RFLP analysis as adopted from previous studies. After PCR amplification, a total of 10 µl of the PCR products were digested with 3 U of restriction enzymes for overnight. Restriction enzymes *Bgl* I (10 U/µl, New England Biolabs, USA) and *Pml* I (10 U/µl, Fermentas, Lithuania) were used to digest PCR products of *PPARα* L162V and *PPARγ2* C161T respectively at 37 ºC. Whereas restriction enzyme *Bsl* I (10 U/µl, Fermentas, Lithuania) was used to cut PCR products of *PPARδ* T294C at 55 ºC. All the restriction enzymes were diluted with a ratio of 1:10 using the manufacturer diluents buffer prior to the digestion. These restriction enzymes cut the PCR products of *PPARα*, *γ* and *δ* polymorphisms at specific recognition site, producing fragments of different

sizes as shown in Table 3.6. A PCR product with known genotypes from previous genotyping was included in subsequent batch of digestion as a control to ensure proper digestion.

Table 3.5: Primer sequences and temperature restriction enzymes digestion in PCR-RFLP analysis

SNP (SNP ID)	Primer Sequence	RE, temperature	Adopted from
PPARα L162V (rs1800206)	Forward: 5'-AACAATAAGTGAGCAACA AAAAAG-3' Reverse: 5'-CGTTGTGTGACATCCCGCCA GAAA-3'	*Bgl* I, 37 ºC	Lacquemant et al., 2000
PPARγ2 C161T (rs3856806)	Forward: 5'- CAAGACAACCTGCTACA AGC-3' Reverse: 5'-TCCTTGTAGATCTCCTG CAG-3'	*Pml* I, 37 ºC	Wang et al., 1999
PPARδ T294C (rs2016520)	Forward: 5'-CATGGTATAGCACTGCAGG AA-3' Reverse: 5'-CTTCCTCCTGTGGCTGCTC-3'	*Bsl* I, 55 ºC	Wei et al., 2011

Table 3.6: Restriction enzyme recognition site and the size of RFLP products

SNP	RE	Restriction Site	RFLP Products
PPARα L162V	*Bgl* I	5′...GCCNNNNNGGC...3′ 3′...CGGNNNNNCCG...5′	L162: 206, 28 V162: 234
PPARγ2 C161T	*Pml* I	5′...CACGTG...3′ 3′...GTGCAC...5′	C161: 120, 77 T161: 197
PPARδ T294C	*Bsl* I	5′...CCNNNNNNNGG...3′ 3′...GGNNNNNNNCC...5′	T294: 269 C294: 167, 102

3.7.2 Determination of Genotypes via Agarose Gel Electrophoresis

The digested PCR products were resolved by electrophoresis on 2.5 % agarose gel. An appropriate amount of agarose powder (SeaKem® LE Agarose, Lonza, Switzerland) was added and 50 bp /100 bp DNA ladder (Mini Sizer 50 bp Ladder DNA marker, Norgen, Canada/ 100 bp Ladder DNA marker, Axygen, USA) was used alongside each gel. The gel was electrophoresed at 90 V for about 45 min. After that, the gel was stained with ethidium bromide and was visualized under ultraviolet light using a UV transilluminator documentation system (MultiDoc-It Digital Imaging System, UVP, UK).

3.7.3 Confirmation of Genotypes via DNA Sequencing

The PCR products of three samples from each SNP were purified (AxyPrep DNA Gel Extraction Kit) according to the manufacturer's protocol.

The purified products were sent to First BASE Laboratories Sdn. Bhd. (Malaysia) for DNA sequencing for confirmation of the genotyping, using the same PCR primers as the sequencing primers.

3.8 Statistical Analysis

3.8.1 Power Calculation of Sample Size

The minimum sample size of the PPARs genotype variants in association with obesity and Met-S and the power of sample size achieved by each ethnic group in stratified analysis in this study were calculated by using Quanto version 1.2.4 (available at http://hydra.usc.edu/GxE).

According to the National Health and Morbidity Survey 2011 (NHMS IV, 2011), 29.4 % of Malaysia population was overweight (definition by WHO 1998: BMI = 25.0-29.9 kg/m^2) and 15.1 % was obese (definition by WHO 1998: BMI $\geq$ 30.0 kg/m^2). A nationwide survey conducted by Wan-Nazaimoon et al. (2011) reported the prevalence of Met-S based on NCEP ATP III definition as 34.3 %. Thus, the population risk of obesity and Met-S in this study was set as 44.5 % (29.4 % + 15.1 %) and 34.3 % respectively. The statistical power was set to be 80 % (two-sided) at 5 % level of significance by using the disease as outcome parameter. The sample size was calculated with unmatched case-control (1:0.7). Power is estimated with the hypothesis of gene effect only and under the log-additive model of inheritance. As an overall, the calculated minimum sample size required to achieved

desired 80 % of statistical power as shown in Table 3.7 indicates that the total recruited subjects of 307 in this study was adequate.

Table 3.7: Parameters, data/values required and results analysis by Quanto program

Parameter	**Data/values**		
Outcome	Disease		
Design	Unmatched case control (1: 0.7)		
Hypothesis	Gene only		
Desired power	0.80		
Significance	0.05, 2 sided		
Gene			
Mode of inheritance	Log-additive		
Allele frequency	MAF:		
	0.08 (*PPARα* L162V)		
	0.22 (*PPARγ2* C161T)		
	0.30 (*PPARδ* T294C)		
Prevalence	44.5 % (obesity) / 34.3 % (Met-S)		
Population risk	0.445 /0.343		
Genetic effect	2		
Minimum sample size	Genotype	Total obesity/ non-obese	Total Met-S/ non-Met-S
	PPARα L162V	281	265
	PPARγ2 C161T	121	117
	PPARδ T294C	99	97

Based on the minor allele frequency of the three PPARs SNP from respective ethnicity and the same setting for the rest of the parameters, the power of samples size achieved by each ethnic group in the stratified analysis of the association of the three PPARs SNP with obesity and Met-S was also calculated. The power achieved by each ethnic group for the stratified analysis was indicated in the footnote of Table 4.10.

3.8.2 Statistical Analysis of Data

Statistical Package for Social Sciences (SPSS) for Windows® Version 17.0 (SPSS Inc, Chicago) was employed to analyse the collected data in this study. Descriptive statistics were performed on the baseline variables to better understand the social-demographic data. The frequency and percentage of categorical variables were calculated. For the continuous variables, the data was reported as mean ± standard deviations (SD). Normality of data for continuous variables was checked with histogram and Kolmogorov-Smirnov test ($p > 0.05$). Logarithmic transformation was performed on non-normally distributed data to reduce the skewness. Homogeneity of variances was tested using Levene's test ($p > 0.05$). Allele frequencies of all the three SNPs were calculated. Chi-square (χ^2) test was performed to calculate whether the allelic frequencies and genotypic distributions of the PPAR variants conform to Hardy-Weinberg equilibrium.

Pearson's chi-square (χ^2) test or Fisher's exact test (for an expected value of less than 5) was applied to look for the association between pair of

categorical variables. In this study, the χ^2 test was used to determine if there were any gender or ethnic differences in the frequency of the categorical variables (obesity/non-obese, Met-S/non-Met-S and obese with/without Met-S). Besides, χ^2 test was performed to calculate the association of genotype and allele distributions with categorical variables (obesity/non-obese, Met-S/non-Met-S and obese with/without Met-S, gender and ethnicity). To further explore the effect of ethnicity on the SNPS, separate analysis of allele distribution of the three PPAR SNPs with categorical variables (obese/non-obese, Met-S/non-Met-S, obese with/without Met-S and gender) within each ethnic group was also performed either using χ^2 or Fisher's exact test.

Univariate analysis (General Linear Model) was conducted to test for the association of outcome variables (anthropometric measurements, fasting glucose, fasting insulin, lipid profiles, plasma adiponectin and plasma IL-6) with the categorical variables (obesity/non-obese, Met-S/non-Met-S and obese with/without Met-S). Data was reported as mean ± standard deviations (SD). The mean of age and ethnicity adjusted analysis was presented as adjusted mean ± standard errors (SE).

Logistic regression analysis (enter method) was done with adjustment for age, gender and ethnicity to evaluate if the three SNPs could predict the risk of obesity, Met-S and obese with Met-S.

Univariate analysis (General Linear Model) adjusted for age and ethnicity was used to test for the association between the genotypic factors

(homozygous wild-type, heterozygous and homozygous variant) and allelic factor with all the continuous variables. Post-hoc test was performed using ANOVA Bonferroni adjustment for multiple comparisons if significant effect ($p < 0.05$) was found across the three genotypic factors.

The association of plasma IL-6, plasma adiponectin, HOMA-IR and TC/HDL-C with gender and ethnicity was performed using Student's *t*-test and One-way ANOVA respectively and were then graphed as bar charts. If significant effect for One-way ANOVA test was observed among ethnicity, post-hoc test ANOVA Bonferroni adjustment for multiple comparisons was performed.

To assess the association of dietary habits and lifestyle factors with obesity/non-obese, Met-S/non-Met-S and obese with/without Met-S, Pearson's chi-square (χ^2) test or Fisher's exact test was done. Meanwhile, the association of dietary habits and lifestyle factors with plasma IL-6, plasma adiponectin, HOMA-IR and TC/HDL-C was evaluated by univariate analysis.

In addition, Pearson's Correlation test was used to estimate the association of plasma IL-6, plasma adiponectin, HOMA-IR, TC/HDL-C with baseline and biochemical variables. Partial correlation was used to control for ethnicity as the confounding factor of both plasma IL-6 and TC/HDL-C, respectively. Meanwhile, partial correlation was also performed to adjust the significant effect of gender on plasma adiponectin level. Bivariate correlation was calculated for HOMA-IR as there was no the confounding factor found to

significantly affecting this variable. The results were presented as heat map correlation matrix constructed using Microsoft Excel 2007.

Lastly, univariate analysis (General Linear Model) was performed to determine the relationship of genotype and allele combinations with all the continuous predictor variables. All subjects with homozygous wild type for all the three PPAR SNPs were grouped under one category, while the rest were included into the category of subjects without homozygous wild type for all the three PPAR SNPs. The analysis of allele combination with the anthropometric and biochemical variables was according to the sequence of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C. In all statistical analysis, $p < 0.05$ was considered statistically significant.

CHAPTER 4

RESULTS

4.1 Socio-demographic Characteristics and Baseline Characteristics of Subjects

A total of 307 subjects were recruited in this study, of which 6 subjects were excluded from the study due to incomplete data, other ethnic group background or technical errors (low DNA concentration) from the earlier total of 313 subjects. A total of 183 subjects (56.9 %) were females and 124 subjects (43.1 %) were males. The ethnic composition of the sample is representative of Kampar population with 31.6 % of Malays, 50.5 % of Chinese and 17.9 % of Indians. Table 4.1 presents the socio-demographic characteristics of the subjects with the classification of the subjects based on obesity (non-obese/obese), Met-S (non-Met-S/Met-S) and obesity with Met-S (obese without/with Met-S). There were 180 subjects (58.6 %) found to be obese in this study whereas 249 subjects (81.1 %) had Met-S. Besides, 90.6 % (n = 163) of the obese subjects were found to have Met-S.

The age of the subjects ranged from 21 to 84 years old, with a mean ± SD of 53.30 ± 14.17 years. The mean ± SD of non-obese and obese subjects was similar with 52.89 ± 17.58 years and 53.59 ± 11.19 years old, respectively. The mean ± SD age of subjects with Met-S was found to be higher than those without Met-S (56.28 ± 11.14 *vs.* 40.50 ± 18.23). The mean ± SD age of obese subject with Met-S was 54.40 ± 10.60 while for obese

subject without Met-S was 45.76 ± 13.85. The highest number of subjects of all groups was within the range of 51 to 60 years old.

Majority of the recruited subjects were retired or not working (*n* = 175, 57 %). Most of the recruited subjects were found to have low monthly household income (below RM 3000), 43.00 % (*n* = 132) with monthly household income less than RM 1000; 44.30 % (*n* = 136) with monthly household income ranging from RM 1001 to RM 3000. Most of the recruited subject attained education up to primary level. Healthy subjects (non-obese and without Met-S) were generally found to attain higher level of education (data not shown). Meanwhile, obese subjects reported a slightly higher family history of obesity.

Table 4.1: Socio-demographic characteristics of the subjects

Group	Obesity (*n* = 307) *n* (%)		Met-S (*n* = 307) *n* (%)		Obesity with Met-S (*n* = 180) *n* (%)	
Variable	**Non-Obese**	**Obese**	**Absent**	**Present**	**Absent**	**Present**
N	127 (41.4)	180 (58.6)	58 (18.9)	249 (81.1)	17 (9.4)	163 (90.6)
Gender						
Male	53 (41.7)	71 (39.4)	28 (48.3)	96 (38.6)	9 (52.9)	62 (38.0)
Female	74 (58.3)	109 (60.6)	30 (51.7)	153 (61.4)	8 (47.1)	101 (62.0)
Race						
Malays	25 (19.7)	72 (40.0)	10 (17.2)	87 (34.9)	6 (35.3)	66 (40.5)
Chinese	82 (64.6)	73 (40.6)	37 (63.8)	118 (47.4)	8 (47.1)	65 (39.9)
India	20 (15.7)	35 (19.4)	11 (19.0)	44 (17.7)	3 (17.6)	32 (19.6)
Age group						
21-30	22 (17.3)	8 (4.4)	23 (39.7)	7 (2.8)	3 (17.6)	5 (3.1)
31-40	7 (5.5)	12 (6.7)	8 (13.8)	11 (4.4)	4 (23.5)	8 (4.9)
41-50	19 (15.0)	54 (30.0)	6 (10.3)	67 (26.9)	4 (23.5)	50 (30.7)
51-60	31 (24.4)	63 (35.0)	13 (22.4)	81 (32.5)	4 (23.5)	59 (36.2)
61-70	30 (23.6)	35 (19.4)	5 (8.6)	60 (24.1)	2 (11.8)	33 (20.2)
>70	18 (13.8)	8 (4.4)	3 (5.2)	23 (9.2)	0 (0.0)	8 (4.9)

Table 4.1 continued.

Group	Obesity (*n* = 307) *n* (%)		Met-S (*n* = 307) *n* (%)		Obesity with Met-S (*n* = 180) *n* (%)	
Variable	**Non-Obese**	**Obese**	**Absent**	**Present**	**Absent**	**Present**
Occupation						
Professional	3 (2.4)	11 (6.1)	2 (3.4)	12 (4.8)	1 (5.9)	10 (6.1)
White-collar	7 (5.5)	12 (6.7)	6 (10.3)	13 (5.2)	1 (5.9)	11 (6.7)
Blue-collar	19 (15.0)	28 (15.6)	10 (17.2)	37 (14.9)	3 (17.6)	25 (15.3)
Retired/not working	67 (52.8)	108 (60.0)	15 (25.9)	160 (64.3)	7 (41.2)	101 (62.0)
Own/others	31 (24.4)	21 (11.7)	25 (43.1)	27 (10.8)	5 (29.4)	16 (9.8)
Monthly household income						
<RM1000	52 (40.9)	80 (44.4)	17 (29.3)	115 (46.2)	7 (41.2)	73 (44.8)
RM1001-3000	52 (40.9)	84 (46.7)	24 (41.4)	112 (45.0)	10 (58.8)	74 (45.4)
>RM3000	23 (18.1)	16 (8.9)	17 (29.3)	22 (8.8)	0 (0.0)	16 (9.8)
Education						
No formal education	21 (16.5)	26 (14.4)	3 (5.2)	44 (17.7)	1 (5.9)	25 (15.3)
Primary level	51 (40.2)	69 (38.3)	17 (29.3)	103 (41.4)	8 (47.1)	61 (37.4)
Lower secondary level	19 (15.0)	43 (23.9)	8 (13.8)	54 (21.7)	4 (23.5)	39 (23.9)
Upper secondary level	12 (9.4)	30 (16.7)	5 (8.6)	37 (14.9)	1 (5.9)	29 (17.8)
University	24 (18.9)	12 (6.6)	25 (43.1)	11 (4.4)	3 (17.7)	9 (5.6)
Family history of obesity						
Yes	31 (24.4)	94 (52.2)	19 (32.8)	106 (42.6)	10 (58.8)	84 (51.5)
No	96 (75.6)	86 (47.8)	39 (67.2)	143 (57.4)	7 (41.2)	79 (48.5)

( ) % within obesity/Met-S/obese with Met-S group

4.2 Distribution of Obesity, Met-S and Obesity with Met-S Groups according to Gender and Ethnicity

As shown in Table 4.2, the distribution of obesity (non-obese/obese), Met-S (non-Met-S/Met-S) and obesity with Met-S (obese without/with Met-S) groups was found to be not significantly associated with gender. The prevalence of obesity was found to be significantly different among ethnicities with the highest prevalence within ethnic group for Malays, followed by Indians and Chinese. The prevalence of Met-S was also significantly different among ethnicities, with Chinese showing the highest, followed by Indians and Malays.

Table 4.2: Obesity (non-obese/obese), Met-S (non-Met-S/Met-S) and obesity with Met-S (obese without/with Met-S) groups according to gender and ethnicity

	Gender		Ethnicity		
	Male	**Female**	**Malays**	**Chinese**	**Indians**
Obesity					
Non-obese	53 (42.7)	74 (40.4)	25 (25.8)	82 (52.9)	20 (36.4)
Obese	71 (57.3)	109 (59.6)	72 (74.2)	73 (47.1)	35 (63.6)
χ^2; *p*	0.162; 0.687			18.80; **<0.001**	
Met-S					
Absent	28 (22.6)	30 (16.4)	87 (89.7)	118 (76.1)	44 (80.0)
Present	96 (77.4)	153 (83.6)	10 (10.3)	37 (23.9)	11 (20.0)
χ^2; *p*	1.847; 0.174			7.215; **0.027**	
Obese with Met-S					
Absent	9 (12.7)	8 (7.3)	66 (91.7)	65 (89.0)	32 (91.4)
Present	62 (87.3)	101 (92.7)	6 (8.3)	8 (11.0)	3 (8.6)
χ^2; *p*	1.432; 0.231			NP	

( ) - % within gender/ethnicity.
All values by Chi-Square Test, significant at $p < 0.05$ and indicated in bold font.
NP- not performed because 1 cell (16.7%) has expected count less than 5.

4.3 Anthropometric and Biochemical Measurements of Obesity, Met-S and Obesity with Met-S Groups

4.3.1 Anthropometric and Biochemical Measurements between Non-obese and Obese Subjects

Table 4.3 presents the mean and adjusted mean measurements of anthropometric and biochemical measurements between obese and non-obese subjects. All the continuous variables which were non-normally distributed were log-transformed prior to statistical analysis. Weight, BMI, WC, WHR, TBF, SF, VFL, RM, DBP, FI, HOMA-IR, TG and TC/ HDL-C were significantly higher in obese subjects compared to non-obese subjects even after the variables were adjusted for age and ethnicity. Meanwhile, SBP and plasma IL-6 which were significantly higher in obese subjects were found to be not significant after adjustment for age and ethnicity. SM and HDL were found to be significantly lower in obese subjects; the latter was found to be not significant after adjustment. Pulse rate, FBG, TC, LDL-C and plasma adiponectin of both groups showed no significant difference.

Table 4.3: Means of variables between non-obese and obese subjects (Unadjusted and Adjusted)

Variables	Mean ± SD		Unadjusted	Adjusted Mean ± SEM		Adjusted
	Non-obese	Obese	*p*	Non-obese	Obese	p^{ψ}
Weight[#] (kg)	56.60 ± 7.58	75.13 ± 13.36	**<0.001**	56.18 ± 1.19	74.89 ± 0.88	**<0.001**
BMI[#] (kg/m^2)	22.03 ± 2.19	30.28 ± 4.69	**<0.001**	22.17 ± 0.40	30.30 ± 0.30	**<0.001**
WC (cm)	82.51 ± 8.99	98.46 ± 11.11	**<0.001**	84.12 ± 1.06	98.44 ± 0.79	**<0.001**
WHR[#]	0.88 ± 0.09	0.93 ± 0.12	**<0.001**	0.89 ± 0.01	0.93 ± 0.01	**<0.001**
TBF[#] (%)	28.55 ± 7.24	36.19 ± 5.88	**<0.001**	29.68 ± 0.64	36.64 ± 0.48	**<0.001**
SF (%)	22.20 ± 6.51	30.61 ± 8.13	**<0.001**	23.01 ± 0.79	31.00 ± 0.58	**<0.001**
VFL[#] (%)	6.47 ± 3.17	15.98 ± 5.57	**<0.001**	6.49 ± 0.49	15.97 ± 0.36	**<0.001**
RM [#](kcal)	1282.09 ± 181.00	1516.16 ± 235.42	**<0.001**	1265.00 ± 22.58	1509.11 ± 16.74	**<0.001**
SM[#] (%)	26.47 ± 4.46	23.71 ± 3.70	**<0.001**	25.90 ± 0.40	23.44 ± 0.30	**<0.001**
SBP[#] (mmHg)	137.51 ± 24.47	142.97 ± 20.63	**0.016**	139.01 ± 2.19	141.63 ± 1.62	0.209
DBP (mmHg)	78.06 ± 13.08	83.87 ± 11.53	**<0.001**	78.50 ± 1.30	84.00 ± 0.96	**0.001**
Pulse Rate (bpm)	77.08 ± 14.03	78.04 ± 13.11	0.541	77.60 ± 1.42	78.12 ± 1.06	0.770
FBG[#] (mg/dL)	7.48 ± 0.29	7.83 ± 0.25	0.215	8.20 ± 0.35	7.83 ± 0.26	0.536

Table 4.3 continued.

Variables	Mean ± SD		Unadjusted	Adjusted Mean ± SEM		Adjusted
	Non-obese	Obese	*p*	Non-obese	Obese	p^{Ψ}
FI[#] (μIU/ml)	10.40 ± 14.76	14.26 ± 14.05	**<0.001**	9.18 ± 1.72	14.86 ± 1.23	**<0.001**
HOMA-IR[#]	3.50 ± 5.22	5.22 ± 6.53	**<0.001**	3.28 ± 0.73	5.44 ± 0.52	**<0.001**
TC (mmol/L)	4.29 ± 0.99	4.51 ± 0.89	0.058	4.40 ± 0.11	4.50 ± 0.08	0.451
TG[#] (mmol/L)	1.31 ± 0.82	1.61 ± 1.27	**0.001**	1.34 ± 0.13	1.58 ± 0.09	**0.029**
HDL-C[#](mmol/L)	1.26 ± 0.34	1.18 ± 0.25	**0.049**	1.23 ± 0.03	1.17 ± 0.02	0.132
LDL-C (mmol/L)	2.44 ± 0.78	2.60 ± 0.76	0.093	2.57 ± 0.9	2.62 ± 0.06	0.646
TC/ HDL-C[#]	3.54 ± 0.89	3.91 ± 0.82	**<0.001**	3.69 ± 0.10	3.95 ± 0.07	**0.023**
Plasma Adiponectin[#] (ng/ml)	6903.67 ± 5576.44	5806.60 ± 4127.05	0.239	7172.21 ± 571.25	5888.57 ± 407.83	0.237
Plasma IL-6[#] (pg/ml)	7.81 ± 9.16	9.83 ± 20.84	**0.017**	7.73 ± 2.06	9.86 ± 1.47	0.074

All values by univariate analysis (General Linear Model), significant at $p < 0.05$ and indicated in bold font; unadjusted values presented as mean ± SD (mean ± standard deviation); adjusted values presented as adjusted mean ± SEM (estimated marginal means ± standard error of the mean); $^{\Psi}$ Adjusted to age and ethnicity; [#] Values were log transformed before analysis.

n = non-obese/obese for: Anthropometric and BP measurements = 127/180; lipid profile analysis and FBG = 116/164; HOMA-IR, FI, adiponectin and IL-6 = 104/156 ;BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TBF, total body fat; SF, subcutaneous fat; VFL, visceral fat level, RM, resting metabolism, SM, skeletal muscle; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; FI, fasting insulin; HOMA-IR, homeostatic model assessment insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC/HDL-C, total cholesterol to high-density lipoprotein cholesterol ratio; IL-6, interleukin 6.

4.3.2 Anthropometric and Biochemical Measurements between Subjects with or without Met-S

The mean and adjusted means of anthropometric and biochemical measurements between obese and non-obese subjects are shown in Table 4.4. Weight, BMI, WC, TBF, SF, VFL, SBP, DBP, pulse rate, FBG, FI, HOMA-IR, TG and TC/ HDL-C were significantly higher in Met-S subjects compared to non-Met-S subjects even after the variables were adjusted for age and ethnicity. WHR and TC were significantly higher in Met-S subjects; however, both were found to be not significantly different after adjustment for age and ethnicity. SM, HDL and plasma adiponectin were found to be significantly lower in obese subjects. The mean measurements of RM, LDL-C and plasma IL-6 were found to be not significantly different between both groups.

Table 4.4: Means of variables between subjects without Met-S and with Met-S (unadjusted and adjusted)

Variables	Mean ± SD		Unadjusted	Adjusted Mean ± SEM		Adjusted
	Non Met-S	Met-S	*p*	Non Met-S	Met-S	p^{ψ}
Weight[#] (kg)	62.87 ± 14.95	68.54 ± 14.27	**0.002**	63.13 ± 2.24	69.21 ± 0.96	**0.010**
BMI[#] (kg/m^2)	23.76 ± 5.76	27.58 ± 5.32	**<0.001**	24.54 ± 0.83	27.94 ± 0.35	**<0.001**
WC (cm)	82.70 ± 15.25	94.00 ± 11.34	**<0.001**	86.43 ± 1.91	94.31 ± 0.82	**<0.001**
WHR[#]	0.85 ± 0.10	0.92 ± 0.11	**<0.001**	0.89 ± 0.02	0.92 ± 0.01	0.068
TBF[#] (%)	27.05 ± 8.53	34.42 ± 6.49	**<0.001**	29.92 ± 1.06	34.99 ± 0.45	**<0.001**
SF (%)	21.83 ± 8.24	28.37 ± 8.18	**<0.001**	23.21 ± 1.28	29.09 ± 0.55	**<0.001**
VFL[#] (%)	8.07 ± 6.93	12.97 ± 6.25	**<0.001**	9.82 ± 1.01	13.07 ± 0.43	**<0.001**
RM[#] (kcal)	1388 ± 255.02	1426.57 ± 240.56	0.232	1365.14 ± 38.56	1432.12 ± 16.56	0.104
SM[#] (%)	27.88 ± 4.90	24.15 ± 3.75	**<0.001**	26.29 ± 0.62	23.94 ± 0.27	**0.001**
SBP[#] (mmHg)	124.22 ± 18.83	144.55 ± 21.46	**0.016**	131.34 ± 3.27	142.48 ± 1.40	**0.001**
DBP (mmHg)	75.24 ± 13.68	82.92 ± 11.78	**<0.001**	75.82 ± 1.94	83.43 ± 0.83	**<0.001**
Pulse Rate (bpm)	74.17 ± 12.68	78.45 ± 13.56	**0.029**	72.72 ± 2.13	79.25 ± 0.91	**0.006**
FBG[#] (mg/dL)	6.04 ± 1.70	8.04 ± 3.30	**<0.001**	6.15 ± 0.57	8.21 ± 0.22	**<0.001**

Table 4.4 continued.

Variables	Mean ± SD		Unadjusted	Adjusted Mean ± SEM		Adjusted
	Non Met-S	Met-S	*p*	Non Met-S	Met-S	p^{Ψ}
FI[#] (μIU/ml)	8.63 ± 9.77	13.55 ± 15.09	**<0.001**	8.66 ± 2.71	13.98 ± 1.10	**<0.001**
HOMA-IR[#]	2.32 ± 2.93	4.98 ± 6.46	**<0.001**	2.25 ± 1.14	5.21 ± 0.46	**<0.001**
TC (mmol/L)	4.18 ± 1.22	4.47 ± 0.86	**0.047**	4.38 ± 0.17	4.49 ± 0.07	0.574
TG[#] (mmol/L)	0.97 ± 0.73	1.59 ± 1.15	**<0.001**	0.93 ± 0.20	1.58 ± 0.08	**<0.001**
HDL-C[#](mmol/L)	1.33 ± 0.45	1.19 ± 0.24	**0.011**	1.33 ± 0.05	1.17 ± 0.02	**0.013**
LDL-C (mmol/L)	2.41 ± 0.95	2.56 ± 0.73	0.222	2.64 ± 0.14	2.60 ± 0.06	0.832
TC/ HDL-C[#]	3.32 ± 1.07	3.85 ± 0.79	**<0.001**	4.38 ± 0.17	4.49 ± 0.07	**0.002**
Plasma Adiponectin[#] (ng/ml)	7765.05 ± 6009.10	5935.87 ± 4442.45	**0.015**	8502.40 ± 879.79	5888.11 ± 356.62	**0.006**
Plasma IL-6[#] (pg/ml)	8.65 ± 12.87	9.10 ± 17.93	0.093	9.18 ± 3.22	9.24 ± 1.31	0.683

All values by univariate analysis (General Linear Model), significant at $p < 0.05$ and indicated in bold font.
Unadjusted values presented as mean ± SD (mean ± standard deviation).
Adjusted values presented as adjusted mean ± SEM (estimated marginal means ± standard error of the mean).
$^{\Psi}$ Adjusted to age and ethnicity; [#] Values were log transformed before analysis.
n = non-Met-S/Met-S for: Anthropometric and BP measurements= 58/249; lipid profile analysis and FBG= 49/231; HOMA-IR, FI, adiponectin and IL-6 = 44/216
Non-Met-S, non-metabolic syndrome; Met-S, metabolic syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment insulin resistance; T-chol, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC/HDL-C, total cholesterol to high-density lipoprotein cholesterol ratio; FBG, fasting blood glucose; FI, fasting insulin; IL-6, interleukin 6.

4.3.3 Anthropometric and Biochemical Measurements between Obese subjects with Met-S and Obese subjects without Met-S

Table 4.5 shows the mean and adjusted mean of anthropometric and biochemical measurements between Met-S and non-Met-S among the obese subjects. The mean measurement for weight which was significantly different between both groups however showed no significant difference after adjustment for age and ethnicity. SBP, FBG, FI and HOMA-IR were significantly higher in obese-Met-S subjects. Nonetheless, pulse rate and TG were significantly higher in obese-Met-S subjects only after adjustment for age and ethnicity. Meanwhile, BMI, WC, WHR, TBF, SF, VFL, RM, SM, DBP, TC, HDL-C, LDL-C, TC/ HDL-C, plasma adiponectin and IL-6 were not significantly different between Met-S and non-Met-S of the obese subjects.

Table 4.5: Means of variables between obese-Met-S and obese non-Met-S subjects (unadjusted and adjusted)

Variables	Mean ± SD		Unadjusted	Adjusted Mean ± SEM		Adjusted
	Obese with Met-S	Obese without Met-S	*p*	Obese with Met-S	Obese without Met-S	p^{ψ}
Weight[#] (kg)	74.52 ± 13.43	81.04 ± 11.42	**0.038**	74.46 ± 1.05	78.38 ± 3.40	0.198
BMI[#] (kg/m^2)	30.18 ± 4.73	31.25 ± 4.29	0.305	30.19 ± 0.36	31.00 ± 1.17	0.449
WC (cm)	98.40 ± 10.72	98.97 ± 14.73	0.841	98.22 ± 0.92	100.31 ± 2.98	0.509
WHR[#]	0.93 ± 0.13	0.94 ± 0.08	0.658	0.931 ± 0.01	0.954 ± 0.03	0.371
TBF[#] (%)	36.38 ± 5.88	34.39 ± 5.69	0.217	36.76 ± 0.47	35.26 ± 1.52	0.361
SF (%)	30.83 ± 8.09	28.48 ± 8.44	0.257	31.19 ± 0.65	28.51 ± 2.11	0.227
VFL[#] (%)	15.89 ± 5.57	16.77 ± 5.70	0.517	15.80 ± 0.46	17.77 ± 1.49	0.202
RM[#] (kcal)	1507.19 ± 230.74	1602.18 ± 268.86	0.129	1504.28 ± 19.01	1529.46 ± 61.67	0.729
SM[#] (%)	23.58 ± 3.68	24.99 ± 3.74	0.131	23.35 ± 0.30	24.37 ± 0.98	0.326
SBP[#] (mmHg)	144.31 ± 20.61	130.12 ± 16.24	**0.005**	142.72 ± 1.63	132.04 ± 5.27	**0.041**
DBP (mmHg)	84.14 ± 11.59	81.29 ± 10.99	0.334	84.33 ± 0.93	79.55 ± 3.01	0.131
Pulse Rate (bpm)	77.08 ± 14.03	78.04 ± 13.11	0.069	78.85 ± 1.04	69.67 ± 3.36	**0.010**
FBG[#] (mg/dL)	8.04 ± 3.12	5.52 ± 1.05	**0.001**	8.04 ± 0.26	5.49 ± 0.92	**0.004**

Table 4.5 continued.

Variables	Mean ± SD		Unadjusted	Adjusted Mean ± SEM		Adjusted
	Obese with Met-S	Obese without Met-S	*p*	Obese with Met-S	Obese without Met-S	p^{Ψ}
FI[#] (μIU/ml)	14.79 ± 14.53	8.37 ± 3.07	**0.015**	15.48 ± 1.27	8.47 ± 4.27	**0.012**
HOMA-IR[#]	5.50 ± 6.74	2.07 ± 0.90	**<0.001**	5.77 ± 0.59	1.98 ± 1.99	**<0.001**
TC (mmol/L)	4.49 ± 0.86	4.72 ± 1.20	0.365	4.48 ± 0.08	4.69 ± 0.26	0.435
TG[#] (mmol/L)	1.62 ± 1.28	1.42 ± 1.13	0.157	1.61 ± 0.11	1.13 ± 0.38	**0.021**
HDL-C[#](mmol/L)	1.18 ± 0.25	1.21 ± 0.24	0.710	1.16 ± 0.02	1.25 ± 0.07	0.248
LDL-C (mmol/L)	2.58 ± 0.75	2.86 ± 0.88	0.199	2.59 ± 0.07	2.92 ± 0.23	0.174
TC/ HDL-C[#]	3.90 ± 0.81	4.01 ± 0.96	0.708	3.95 ± 0.07	3.83 ± 0.24	0.610
Plasma Adiponectin[#] (ng/ml)	5751.15 ± 4063.32	6416.54 ± 4918.28	0.582	5736.42 ± 362.31	7785.45 ± 1223.97	0.145
Plasma IL-6[#] (pg/ml)	10.10 ± 21.73	6.91 ± 3.51	0.260	10.01 ± 1.89	7.75 ± 6.38	0.569

All values by univariate analysis (General Linear Model), significant at $p < 0.05$ and indicated in bold font.
Unadjusted values presented as mean ± SD (mean ± standard deviation).
Adjusted values presented as adjusted mean ± SEM (estimated marginal means ± standard error of the mean).
$^{\Psi}$ Adjusted to age and ethnicity; [#] Values were log transformed before analysis.
n = obese with Met-S/obese without Met-S for: Anthropometric and BP measurements = 163/17; lipid profile analysis and FBG = 49/231; HOMA-IR, FI, plasma adiponectin and plasma IL-6 = 143/13
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC/HDL-C, total cholesterol to high-density lipoprotein cholesterol ratio; FBG, fasting blood glucose; FI, fasting insulin; IL-6, interleukin 6.

4.4 SNP Genotyping of Subjects

Figure 4.1 depicts the gel image of genomic DNA extracted from the blood samples of subjects. The high quality genomic DNA samples were observed with a band size of more than 10 kb. The genomic DNA samples were amplified via PCR and subsequently the SNP genotyping of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C variants was determined via RFLP analysis.

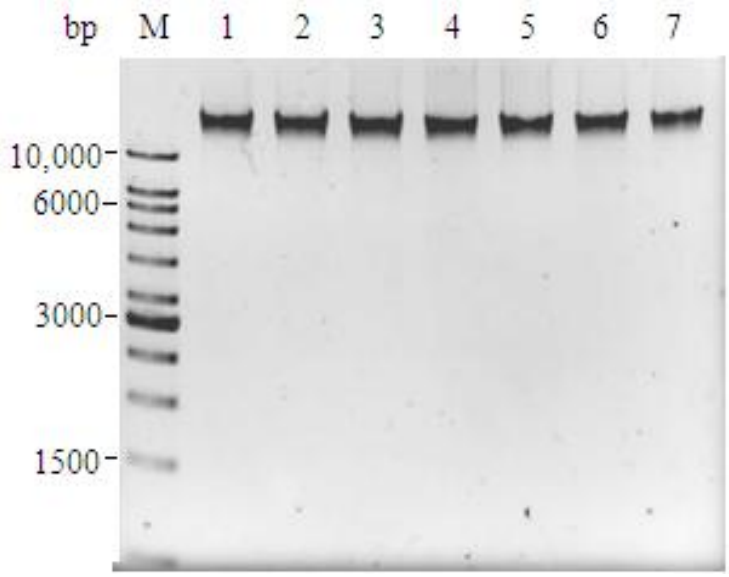


Figure 4.1: Genomic DNA samples on 1 % agarose gel
M- 1 kb DNA ladder (Fermentas); Lane 1 to 7- Genomic DNA extracted from seven subjects.

4.4.1 Genotyping of *PPARα* L162V SNP

Figure 4.2 depicts a 2.5 % agarose gel with PCR product and SNP variants of *PPARα* L162V. PCR product with band size 234 bp is indicated in Lane 1. The homozygous wild-type L162L (Lane 3) was digested with a band showing 206 bp. The shorter band with 28 bp was not visible on the gel as it

might have migrated out of the gel. Heterozygous L162V (Lane 4) was detected as 2 bands with 234 bp and 206 bp. The digested fragment of 28 bp was not visible on the gel. The non-digested homozygous V162V (Lane 5) was detected as 234 bp.

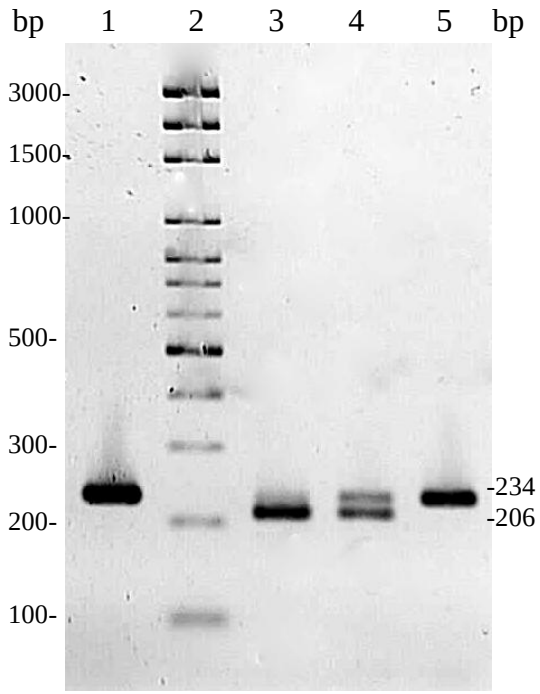


Figure 4.2: PCR product and SNP of *PPARα* L162V on 2.5 % agarose gel
Lane 1- PCR product of *PPARα* L162V; Lane 2- 100 bp Ladder DNA marker (Axygen); Lane 3- Homozygous wild-type L162L; Lane 4- Heterozygous L162V; Lane 4- Homozygous V162V.

4.4.2 Genotyping of *PPARγ2* C161T

Figure 4.3 shows the PCR product and genotypes of *PPARγ2* C161T electrophoresed on a 2.5 % agarose gel. The PCR product of *PPARγ2* C161T (Lane 1) was shown as 197 bp. Homozygous wild-type C161C (Lane 3) was cleaved into 2 bands with the sizes of 120 bp and 77 bp. Heterozygous C161T (Lane 4) was indicated as 3 bands at 197 bp, 120 bp and 77 bp. Homozygous T161T (Lane 5) remained undigested at 197 bp.

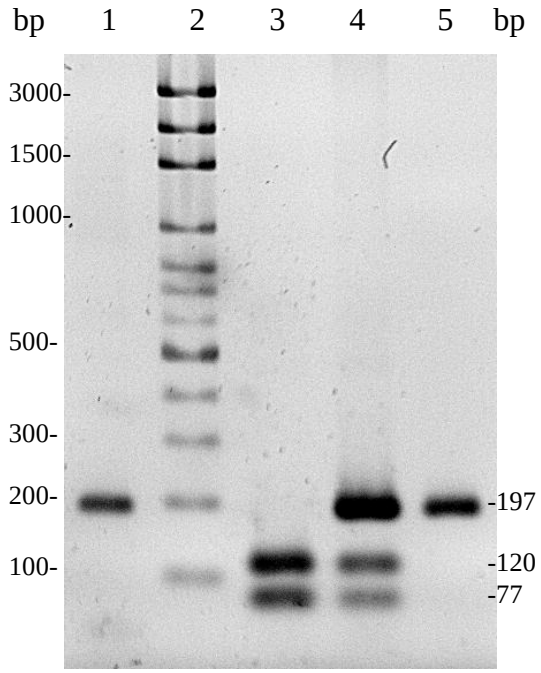


Figure 4.3: PCR product and SNP of *PPARγ2* C161T on 2.5% agarose gel
Lane 1- PCR product of *PPARγ2* C161T; Lane 2- 100 bp Ladder DNA marker (Axygen); Lane 3- Homozygous wild-type C161C; Lane 4- Heterozygous C161T; Lane 4- Homozygous T161T.

4.4.3 Genotyping of *PPARδ* T294C

Figure 4.4 illustrates the PCR product and SNP of *PPARδ* T294C on 2.5 % agarose gel. The PCR product with 269 bp was shown on Lane 1. Homozygous C294C (lane 3) was digested with 2 bands showing 167 bp and 102 bp. Heterozygous T294C (lane 4) was confirmed with 3 bands with 269 bp as T294 allele; 167 bp and 102 bp as C294 allele. Homozygous wild-type T294T (lane 5) was detected at 269 bp.

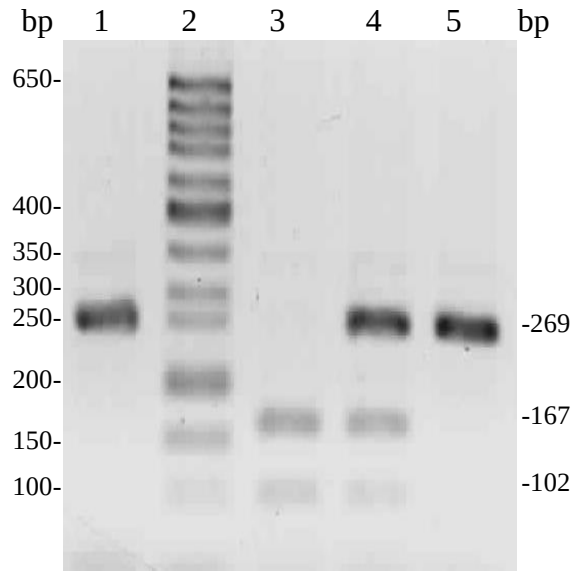


Figure 4.4: PCR product and SNP of *PPARδ* T294C on 2.5% agarose gel
Lane 1- PCR product of *PPARδ* T294C; Lane 2- Mini Sizer 50bp Ladder DNA marker (Norgen); Lane 3- Homozygous C294C; Lane 4- Heterozygous T294C; Lane 5- Homozygous wild-type T294T.

4.4.4 DNA Sequence Analysis

To confirm the results obtained via RFLP analysis, the DNA sample of each *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C variants were sent for DNA sequencing. Subsequently, the DNA sequence of all the variants was proofread via the Basic Local Alignment Search Tool (BLAST). Figure 4.5 illustrates the electropherograms with the correct sequences of the respective polymorphic sites for confirmation of DNA sequence for each PPAR variants.

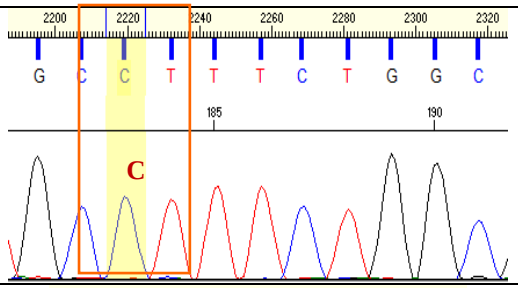
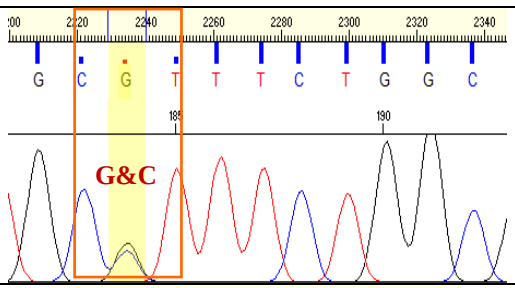
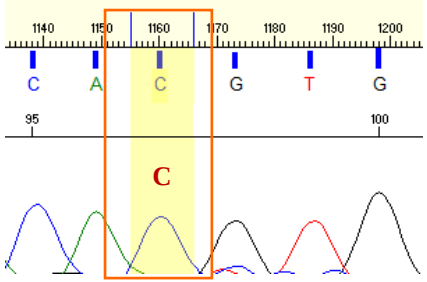
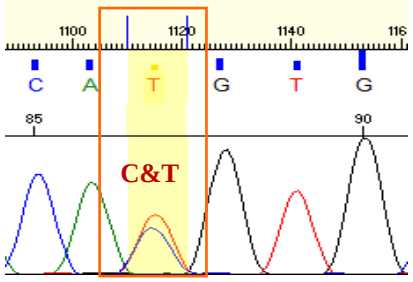
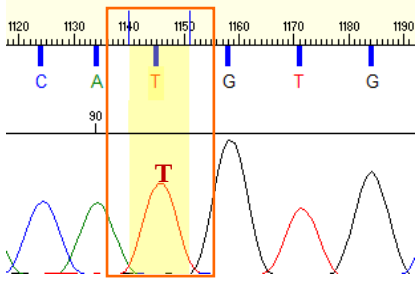
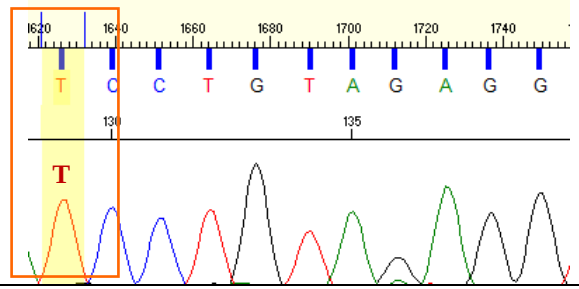
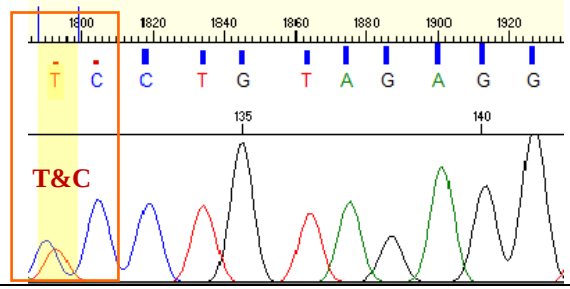
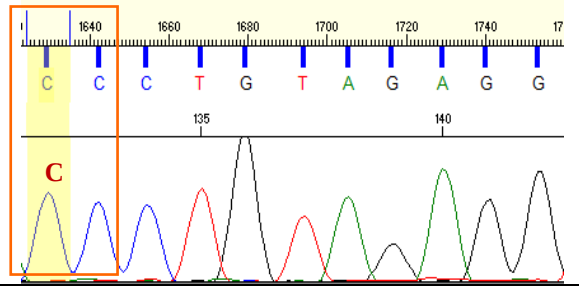
PPAR SNP	Homozygous Wild Type	Heterozygous Variant Type	Homozygous Variant Type
α L162V			Genotype not detected
γ2 C161T			
δ T294C			

Figure 4.5: Electropherograms of PPAR gene variants

4.5 Overall Variant Allele Frequency and Hardy-Weinberg Equilibrium of PPAR SNP

Table 4.6 shows the variant allele frequency of the three PPAR gene variants. Hardy-Weinberg equilibrium for the three PPAR gene variants was calculated by the χ^2 test. All the three PPAR SNP gene variants were found to be in Hardy-Weinberg equilibrium as they did not differ significantly with those expected for a population (df = 2, significant at $p < 0.05$).

Table 4.6: Variant allele frequency and Hardy-Weinberg equilibrium analysis of of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C

Genotypes	Allele	Allele frequency	Hardy-Weinberg equilibrium	
			χ^2	p
PPARα L162V	L162	0.92	2.378	0.3044
	V162	0.08		
PPARγ2 C161T	C161	0.78	1.544	0.4620
	T161	0.22		
PPARδ T294C	T294	0.70	0.051	0.9746
	C294	0.30		

All p values of Hardy-Weinberg equilibrium analysis by χ^2 test, significant at $p < 0.05$.

4.6 Distribution of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C SNPs and Allele Frequencies in Obese, Met-S and Obese with Met-S Groups

As shown in Table 4.7, the frequencies of *PPARα* L162L homozygotes were higher than *PPARα* L162V heterozygotes in all groups of obese (non-obese/obese), Met-S (non-Met-S/Met-S) and obese with Met-S (obese without/with Met-S) with percentage reaching more than 80.0 %. There was no subject with *PPARα* V162V sampled in this study. The frequencies of L162 allele were high with percentage more than 90.0 % in all groups. Pearson's Chi-square test showed no difference in the distribution of genotype and allele frequencies between the groups of obese (non-obese and obese) and Met-S (non-Met-S and Met-S) subjects. Fisher's Exact test employed on the small sample size obese with Met-S group (obese with/without Met-S) showed no significant difference in the distribution of genotype and allele frequencies as well.

The frequencies of *PPARγ2* C161C homozygotes were slightly higher than *PPARγ2* T161 variant carriers (C161T / T161T) in all groups except for the non-Met-S subjects with equal percentage (50.0 %). The allele frequencies of C161 were higher than the variant allele T161 in all groups. Pearson's Chi-Square test indicated no association for the genotype and allele frequencies of *PPARγ2* C161T with obesity, Met-S and both obesity with Met-S.

The frequencies of *PPARδ* T294T homozygotes and *PPARδ* C294 variant carriers (T294C / C294C) had similar distribution. In particular, the percentage of *PPARδ* C294 variant carriers (T294C / C294C) was slightly higher in non-obese, non-Met-S and Met-S subjects. The allele frequencies of T294 were higher than the variant allele C294 in all groups. According to Pearson's Chi-Square analysis, the genotype distribution and allele frequencies were not associated with obesity, Met-S and both obesity with Met-S.

Table 4.7: Genotype and allele frequencies of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C in obese, Met-S and obese with Met-S groups

SNP	Genotypes and Alleles	Obesity			Met-S			Obese With Met-S		
		Non-obese *n* (%)	Obese *n* (%)	χ^2; *p*	Absent *n* (%)	Present *n* (%)	χ^2; *p*	Absent *n* (%)	Present *n* (%)	χ^2; *p*
PPARα	L162L	107 (84.3)	152 (84.4)	0.002; 0.964	47 (81.0)	212 (85.1)	0.601; 0.438	15 (88.2)	137 (84.0)	1.000[ω]
L162V	L162V	20 (15.7)	28 (15.6)		11 (19.0)	37 (14.9)		2 (11.8)	26 (16.0)	
	L162	234 (92.1)	332 (92.2)	0.002; 0.965	105 (90.5)	461 (92.6)	0.550; 0.458	32 (94.1)	300 (92.0)	1.000 [ω]
	V162	20 (7.9)	28 (7.8)		11 (9.5)	37 (7.4)		2 (5.9)	26 (8.0)	
PPARγ2	C161C	71 (55.9)	110 (61.1)	0.834; 0.361	29 (50.0)	152 (61.0)	2.371; 0.124	9 (52.9)	101 (62.0)	0.527; 0.468
C161T	C161T / T161T	51 /5 (44.1)	63/7 (38.9)		26/3 (50.0)	88/9 (38.9)		7/1 (47.1)	56 /6 (38.1)	
	C161	193 (76.0)	283 (78.6)	0.590; 0.443	84 (72.4)	392 (78.7)	2.144; 0.143	25 (73.5)	258 (79.1)	0.577; 0.448
	T161	61 (24.0)	77 (21.4)		32 (27.6)	106 (21.3)		9 (26.5)	68 (20.9)	
PPARδ	T294T	57 (44.9)	95 (52.8)	1.857; 0.173	28 (48.3)	124 (49.8)	0.044; 0.834	9 (52.9)	86 (52.8)	0.001; 0.989
T294C	T294C / C294C	60/10 (55.1)	67/18 (47.2)		24/6 (51.7)	103/22 (50.2)		5/3 (47.1)	62 /15 (47.2)	
	T294	174 (68.5)	257 (71.4)	0.592; 0.441	80 (69.0)	351 (70.5)	0.103; 0.748	23 (67.6)	234 (71.8)	0.257; 0.612
	C294	80 (31.5)	103 (28.6)		36 (31.0)	147 (29.5)		11 (32.4)	92 (28.2)	

p values by Pearson's Chi-Square Test

[ω] *p* values by Fisher's Exact Test

All *p* values significant at $p < 0.05$.

( ) - % within obesity/ Met-S/ obese with Met-S group.

4.7 Distribution of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C SNPs and Allele Frequencies among Different Ethnicities

According to Malays/Chinese/Indians ethnicities, the MAF of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C were 0.07/0.08/0.08; 0.21/0.27/0.13; 0.31/0.32/0.22, respectively. As shown in Table 4.8, the *PPARα* L162L homozygotes had higher prevalence than *PPARα* L162V heterozygotes among all the three ethnicities with no association was found using Chi-square analysis. There was also no association found between allele frequency and ethnicity. Generally, the frequency of L162 allele was similar with high percentage (> 90 %) among the three ethnicities.

Nonetheless, the frequency of *PPARγ2* C161C homozygotes was higher than *PPARγ2* T161 variant carriers (C161T / T161T). The frequency of C161 allele was therefore higher than T161. Based on the Chi-square analysis as shown in Table 4.8, significant association was found between *PPARγ2* C161T gene variants and allele frequency with ethnicity (χ^2 = 8.538, p = 0.014; χ^2 = 10.183, p = 0.006). Further post hoc Chi-square analysis showed that the frequencies of T161 allele genotype carriers (C161T/T161T) and alleles were significantly lower among Indians as compared to Chinese (χ^2 = 8.283, p = 0.004 ; χ^2 = 9.371, p = 0.002).

The prevalence of *PPARδ* C294 variant carriers (T294C / C294C) was found to be slightly higher in both the Malays and Chinese ethnic groups except for the Indians. The allele frequency of T294 was higher than C294. No

association was found between genotype *PPARδ* T294C gene variants and allele frequency with ethnicity.

Table 4.8: The prevalence of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C genotypes and alleles among different ethnic groups

SNP	Genotypes and Alleles	Ethnicity			χ^2; *p*
		Malays	Chinese	Indians	
		n (%)	*n* (%)	*n* (%)	
PPARα L162V	L162L	84 (86.6)	129 (83.2)	46 (83.6)	0.54 1; 0.763
	L162V	13 (13.4)	26 (16.8)	9 (16.4)	
	L162	181 (93.3)	284 (91.6)	101 (91.8)	0.495 ; 0.781
	V162	13 (6.7)	26 (8.4)	9 (8.2)	
PPARγ2 C161T	C161C	59 (60.8)	81 (52.3)	41 (74.5)	8.538 ; **0.014**
	C161T / T161T	36/2 (39.2)	64/10 (47.7)	14/0 (25.5)	
	C161	154 (79.4)	226 (72.9)	96 (87.3)	10.183; **0.006**
	T161	40 (20.6)	84 (27.1)	14 (12.7)	
PPARδ T294C	T294T	47 (48.5)	73 (47.1)	32 (58.2)	2.059 ; 0.357
	T294C / C294C	39/11 (51.5)	66 /16 (52.9)	22/1 (41.8)	
	T294	133 (68.6)	212 (68.4)	86 (78.2)	4.087 ; 0.13
	C294	61 (31.4)	98 (31.6)	24 (21.8)	

All values by Chi-Square Test, significant at $p < 0.05$ and indicated in bold font.
( ) - % within ethnic group.

4.8 Distribution of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C SNPs and Allele Frequencies by Gender

As shown in Table 4.9, Chi-square analysis revealed no association between genotype and allele frequencies of *PPARα* L162V and *PPARγ2* C161T with gender. The genotype distribution of *PPARδ* T294C was found to be associated with gender ($\chi^2 = 4.776$, $p = 0.029$) despite no association found between the frequency of *PPARδ* T294C alleles with gender.

Higher prevalence of *PPARα* L162L homozygotes and L162 allele was found in both male and female subjects. Besides, both genders also had a higher prevalence of *PPARγ2* C161C homozygotes and C161 allele. Unlike female subjects with higher prevalence of *PPARδ* T294T homozygotes, male subjects were reported to have higher prevalence of *PPARδ* C294 variant carriers (T294C / C294C). Despite this discrepancy, both genders had higher prevalence of T294 allele than C294 allele.

Table 4.9: The prevalence of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C SNPs and alleles by gender

SNP	Genotypes and Alleles	Gender		χ^2; *p*
		Male *n* (%)	Female *n* (%)	
PPARα L162V	L162L	101 (81.5)	158 (86.3)	1.338 ; 0.247
	L162V	23 (18.5)	25 (13.7)	
	L162	225 (90.7)	341 (93.2)	1.225 ; 0.268
	V162	23 (9.3)	25 (6.8)	
PPARγ2 C161T	C161C	69 (55.6)	112 (61.2)	0.943 ; 0.331
	C161T / T161T	48 / 7 (44.4)	66 / 5 (38.8)	
	C161	186 (75.0)	290 (79.2)	1.522 ; 0.217
	T161	62 (25.0)	76 (20.8)	
PPARδ T294C	T294T	52 (41.9)	100 (54.6)	4.776 ; **0.029**
	T294C / C294C	62 / 10 (58.1)	65 / 18 (45.4)	
	T294	166 (66.9)	265 (72.4)	2.113 ; 0.146
	C294	82 (33.1)	101 (27.6)	

p values by Chi-Square Test, significant at $p < 0.05$ and indicated in bold font.
( ) - % within gender group

4.9 Stratified Analysis of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C Allele Frequencies Based on Each Ethnic Groups

As significant association was found between *PPARγ2* C161T genotypes and allele frequencies and ethnic groups in Section 4.7, a separate analysis was performed to further investigate the association of all the PPAR allele frequencies with obese, Met-S, obese-Met-S categories and gender within each ethnic group.

As presented in Table 4.10, no association was observed via Chi-square/ Fisher's Exact test on the separate analysis of all the PPAR allele frequencies within each ethnic group ($p > 0.05$).

Table 4.10: Separate analysis for association of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C allele frequencies with obesity, Met-S, obesity with Met-S groups and gender in Malays

Single Nucleotide Polymorphism (SNP)	*PPARα* L162V		*PPARγ2* C161T		*PPARδ* T294C	
Allele	**L162**	**V162**	**C161**	**T161**	**T294**	**C294**
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
			Malays[a]			
Obesity						
Non-obese (*n* = 25)	49 (98.0)	1 (2.0)	39 (78.0)	11 (2.0)	31(62.0)	19 (38.0)
Obese (*n* = 72)	132 (91.7)	12 (8.3)	115 (79.9)	29 (20.1)	102 (70.8)	42 (29.2)
χ^2; *p*	0.190[ω]		0.079; 0.779		1.343; 0.246	
Met-S						
Absent (*n* = 20)	19 (95.0)	1 (5.0)	16 (80.0)	4 (20.0)	12 (60.0)	8 (40.0)
Present (*n* = 87)	162 (93.1)	12 (6.9)	138 (79.3)	36 (20.7)	121 (69.5)	53 (30.5)
χ^2; *p*	1.000[ω]		1.000[ω]		0.757; 0.384	
Obese with Met-S						
Absent (*n* = 6)	11 (91.7)	1 (8.3)	9 (75.0)	3 (25.0)	7 (58.3)	5 (41.7)
Present (*n* = 66)	121(91.7)	11(8.3)	106 (80.3)	26 (19.7)	95 (72.0)	37 (28.0)
χ^2; *p*	1.000[ω]		0.708[ω]		0.332[ω]	
Gender						
Male (*n* = 37)	67 (90.5)	7 (9.5)	57 (77.0)	17 (23.0)	47 (63.5)	27 (36.5)
Female (*n* = 60)	114 (95.0)	6 (5.0)	97 (80.8)	23 (19.2)	86 (71.7)	34 (28.3)
χ^2; *p*	0.249[ω]		0.405; 0.524		1.412; 0.235	

[a]Power of sample size: 34 % (obesity) and 36 % (Met-S) for *PPARα* L162V; 69 % (obesity) and 71 % (Met-S) for *PPARγ2* C161T; 80 % (obesity) and 81 % (met-S) for *PPARδ* T294C

[ω] *p* values by Fisher's Exact test, significant at $p < 0.05$; ( )-% within the obesity/ Met-S/ obese with Met-S group.

Table 4.11: Separate analysis for association of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C allele frequencies with obesity, Met-S, obesity with Met-S groups and gender in Chinese

Single Nucleotide Polymorphism (SNP)	*PPARα* L162V		*PPARγ2* C161T		*PPARδ* T294C	
Allele	**L162**	**V162**	**C161**	**T161**	**T294**	**C294**
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
			Chinese[b]			
Obesity						
Non-obese (*n* = 82)	149 (90.9)	15 (9.1)	121 (73.8)	43 (26.2)	114 (69.5)	50 (30.5)
Obese (*n* = 73)	135 (92.5)	11 (7.5)	105 (71.9)	41 (28.1)	98 (67.1)	48 (32.9)
χ^2; *p*	0.261; 0.609		0.136; 0.713		0.204; 0.652	
Met-S						
Absent (*n* = 37)	67 (90.5)	7 (9.5)	51 (68.9)	23 (31.1)	51 (68.9)	23 (31.1)
Present (*n* = 118)	217 (91.9)	19 (8.1)	175 (74.2)	61 (25.8)	161 (68.2)	75 (31.8)
χ^2; *p*	0.145; 0.703		0.781; 0.377		0.013; 0.910	
Obese with Met-S						
Absent (*n* = 8)	15 (93.8)	1 (6.2)	11 (68.8)	5 (31.2)	11 (68.8)	5 (31.2)
Present (*n* = 65)	120 (92.3)	10 (7.7)	94 (72.3)	36 (27.7)	87 (66.9)	43 (33.1)
χ^2; *p*	1.000 [ω]		0.772 [ω]		0.022; 0.883	
Gender						
Male (*n* = 71)	128 (90.1)	14 (9.9)	101 (71.1)	41 (28.9)	95 (66.9)	47 (33.1)
Female (*n* = 84)	156 (92.9)	12 (7.1)	125 (74.4)	43 (25.6)	117 (69.6)	51 (30.4)
χ^2; *p*	0.739; 0.390		0.419; 0.518		0.268; 0.605	

[b]Power of sample size: 55 % (obesity) and 57 % (Met-S) for *PPARα* L162V; 92 % (obesity) and 93 % (Met-S) for *PPARγ2* C161T; 95 % (obesity) and 95 % (met-S) for *PPARδ* T294C

[ω] *p* values by Fisher's Exact test, significant at $p < 0.05$; ( )-% within the obesity/ Met-S/ obese with Met-S group.

Table 4.12: Separate analysis for association of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C allele frequencies with obesity, Met-S, obesity with Met-S groups and gender in Indians

Single Nucleotide Polymorphism (SNP)	*PPARα* L162V		*PPARγ2* C161T		*PPARδ* T294C	
Allele	**L162**	**V162**	**C161**	**T161**	**T294**	**C294**
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
			Indians[c]			
Obesity						
Non-obese (*n* = 20)	36 (90.0)	4 (10.0)	33 (82.5)	7 (17.5)	29 (72.5)	11 (27.5)
Obese (*n* = 35)	65 (92.9)	5 (7.1)	63 (90.0)	7 (10.0)	57 (81.4)	13 (18.6)
χ^2; *p*	0.721 [ω]		1.289; 0.256		1.190; 0.275	
Met-S						
Non-Metabolic Syndrome (*n* = 11)	19 (86.4)	3 (13.6)	17 (77.3)	5 (22.7)	17 (77.3)	5 (22.7)
Metabolic Syndrome (*n* = 44)	82 (93.2)	6 (6.8)	79 (89.8)	9 (10.2)	69 (78.4)	19 (21.6)
χ^2; *p*	0.380 [ω]		0.150 [ω]		1.000 [ω]	
Obese with Met-S						
Absent (*n* =3)	6 (100.0)	0 (0.0)	5 (83.3)	1 (16.7)	5 (83.3)	1 (16.7)
Present (*n* =32)	59 (92.2)	5 (7.8)	58 (90.6)	6 (9.4)	52 (81.2)	12 (18.8)
χ^2; *p*	1.000 [ω]		0.482 [ω]		1.000 [ω]	
Gender						
Male (*n* = 16)	30 (93.8)	2 (6.2)	28 (87.5)	4 (12.5)	24 (75.0)	8 (25.0)
Female (*n* =39)	71 (91.0)	7 (9.0)	68 (87.2)	10 (12.8)	62 (79.5)	16 (20.5)
χ^2; *p*	1.000 [ω]		1.000 [ω]		0.268; 0.605	

[c]Power of sample size: 24 % (obesity) and 25 % (Met-S) for *PPARα* L162V; 34 % (obesity) and 35 % (Met-S) for *PPARγ2* C161T; 55 % (obesity) and 56 % (met-S) for *PPARδ* T294C

[ω] *p* values by Fisher's Exact test, significant at $p < 0.05$; ( )-% within the obesity/ Met-S/ obese with Met-S group.

4.10 Logistic Regression Analysis to Associate Obesity, Met-S and Obese with Met-S with the *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C Genotypes and Alleles

Logistic regression (enter method) was performed with adjustment to the age, gender and ethnicity to further evaluate association between obesity, Met-S and obese with Met-S with the *PPARα* L162V, *PPARγ* C161T and *PPARδ* T294C SNPs and alleles. The results (all *p* values > 0.05) showed that subjects carrying the variant genotypes (L162V for *PPARα* L162V; C161T / T161T for *PPARγ2* C161T; T294C / C294C for *PPARδ* T294C) and variant alleles (V162, T161 and C294) did not have an increased risk of obesity and Met-S than those with wild-type genotypes and alleles. Likewise, obese subjects carrying the variants genotypes and variant alleles did not show an increased risk to have Met-S. Generally, the Odds Ratio (OR) with value more than 1.000 indicates higher tendency to be affected. Despite no statistical difference, the results suggested that obese carriers of V allele may have slightly higher tendency to develop Met-S. Carriers of T161 allele had a lower tendency to develop obesity and Met-S, meanwhile carriers of C294 allele might have a slight tendency for obesity.

Table 4.13: Logistic regression analysis of the *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C genotypes associated with obesity, Met-S and obese with Met-S

Gene Variants	Genotypes	Obesity			Met-S			Obese with Met-S		
	and allele	OR	p^{Ψ}	95% CI	OR	p^{Ψ}	95% CI	OR	p^{Ψ}	95% CI
PPARα L162V	L162L	1.000	-	-	1.000	-	-	1.000	-	-
	L162V	0.977	0.942	0.519, 1.838	0.944	0.897	0.396, 2.250	1.732	0.507	0.342, 8.774
	V162	1.021	0.945	0. 559, 1.867	0.950	0.903	0.417, 2.163	1.647	0.530	0.348, 7.804
PPARγ2 C161T	C161C	1.000	-	-	1.000	-	-	1.000	-	-
	C161T / T161T	0.783	0.303	0.491, 1.248	0.625	0.165	0.322, 1.214	0.775	0.638	0.269, 2.239
	T161	0.845	0.391	0.574, 1.243	0.693	0.178	0.407, 1.182	0.764	0.534	0.327 , 1.785
PPARδ T294C	T294T	1.000	-	-	1.000	-	-	1.000	-	-
	T294C / C294C	0.714	0.155	0.448, 1.136	1.109	0.761	0.571, 2.153	1.120	0.834	0.387, 3.244
	C294	0.850	0.369	0.596, 1.211	1.008	0.976	0.607, 1.672	0.910	0.816	0.409, 2.022

All values by Logistic Regression Analysis.
$^{\Psi}$ Adjusted for age, gender and ethnicity.

4.11 Association *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C Genotypes and Alleles with Anthropometric and Clinical Measurements

4.11.1 Association *PPARα* L162V Genotypes and Alleles with Anthropometric and Clinical Measurements

Table 4.14 presents the results of association of *PPARα* L162V genotypes and alleles with continuous variables. Plasma IL-6 was found to be significantly higher among subjects with heterozygous L162V compared to homozygous L162L (p = 0.033) and L162 allele carriers compared to V162 allele carriers (p = 0.041). The rest of the anthropometric and clinical variables were found to have no association with *PPARα* L162V genotypes and alleles.

Table 4.14: Means of anthropometric and clinical measurements according to *PPARα* L162V genotypes and alleles

Variables	Genotypes			Alleles		
	L162L	**L162V**	***p***	**L162**	**V162**	***p***
Weight[#] (kg)	67.36 ± 0.89	68.07 ± 2.07	0.815	67.33 ± 0.60	68.07 ± 2.05	0.796
BMI[#] (kg/m^2)	26.87 ± 0.34	26.86 ± 0.80	0.885	26.87 ± 0.23	26.86 ± 0.80	0.890
WC (cm)	91.90 ± 0.79	91.65 ± 1.83	0.899	91.88 ± 0.53	91.65 ± 1.83	0.903
WHR[#]	0.91 ± 0.01	0.90 ± 0.02	0.342	0.91 ± 0.01	0.90 ± 0.02	0.362
TBF[#] (%)	33.12 ± 0.45	32.54 ± 1.06	0.807	33.07 ± 0.31	32.54 ± 1.05	0.815
SF (%)	27.42 ± 0.53	25.70 ± 1.24	0.202	27.27 ± 0.36	25.70 ± 1.23	0.221
VFL[#] (%)	12.05 ± 0.41	12.03 ± 0.94	0.961	12.05 ± 0.27	12.03 ± 0.94	0.962
RM[#] (kcal)	1413.58 ± 14.91	1450.38 ± 34.67	0.335	1416.70 ± 10.06	1450.34 ± 34.57	0.355
SM[#] (%)	24.78 ± 0.25	25.25 ± 0.59	0.374	24.82 ± 0.17	25.25 ± 0.59	0.394
Systolic BP[#] (mmHg)	140.95 ± 1.28	139.45 ±2.98	0.573	140.82 ± 0.87	139.45 ± 2.97	0.589
Diastolic BP (mmHg)	81.35 ± 0.78	82.13 ± 1.81	0.691	81.41 ± 0.52	82.13 ± 1.80	0.703
Pulse Rate (bpm)	77.41 ± 0.83	78.90 ± 1.94	0.481	77.53 ± 0.56	78.89 ± 1.93	0.499
FBG[#] (mg/dL)	7.79 ± 0.21	7.10 ± 0.50	0.300	7.73 ± 0.14	7.10 ± 0.50	0.318
FI[#](μIU/ml)	12.56 ± 0.97	13.64 ± 2.33	0.612	12.65 ± 0.66	13.63 ± 2.31	0.627
HOMA-IR[#]	4.57 ± 0.04	4.32 ± 0.98	0.997	4.55 ± 0.28	4.33 ± 0.98	0.997
TC (mmol/L)	4.45 ± 0.06	4.21 ± 0.15	0.124	4.44 ± 0.04	4.21 ± 0.15	0.139
TG[#] (mmol/L)	1.49 ± 0.07	1.46 ± 0.18	0.633	1.49 ± 0.05	1.46 ± 0.17	0.646
HDL-C[#] (mmol/L)	1.21 ± 0.02	1.21 ± 0.05	0.428	1.21 ± 0.01	1.21 ± 0.05	0.445
LDL-C (mmol/L)	2.57 ± 0.05	2.35 ± 0.12	0.094	2.55 ± 0.03	2.35 ± 0.12	0.107
TC/HDL-C[#]	3.77 ± 0.06	3.66 ± 0.14	0.412	3.76 ± 0.04	3.66 ± 0.14	0.429
Plasma Adiponectin[#] (ng/ml)	6423.75 ± 319.99	5234.96 ± 764.57	0.105	6326.88 ± 216.39	5240.79 ± 761.52	0.120
Plasma IL-6[#] (pg/ml)	8.75 ± 1.16	10.60 ± 2.76	**0.033**	8.90 ± 0.78	10.59 ± 2.75	**0.041**

[#] Values were log transformed before analysis; values are presented as adjusted mean ±SEM (estimated marginal means ± standard error of the mean) by univariate analysis of variance (General Linear Model), adjusted for co-variates:age and ethnicity, $p < 0.05$ and indicated in bold font. *n* = L162L/L162V/L162/V162 for: Anthropometric and BP measurements = 259/48/566/48; lipid profile analysis and FBG = 240/40/520/40; HOMA-IR, FI, plasma adiponectin and plasma IL-6 = 221/39/481/39

4.11.2 Association *PPARγ2* C161T Genotypes and Alleles with Anthropometric and Clinical Measurements

As shown in Table 4.13, FI level was found to be significantly higher among *PPARγ2* C161T genotype, further analysis using ANOVA Bonferroni adjustment for multiple comparisons (Table 4.15) indicated that C161C genotype had higher FI than T161T genotype. Besides, HOMA-IR was significantly different among *PPARγ2* C161T genotypes and alleles, with C161C / C161T genotype more than T161T genotype and C161 allele more than T161 allele. No further association was found between other continuous variables and *PPARγ2* C161T SNP and alleles.

Table 4.15: Means of anthropometric and clinical measurements according *PPARγ2* C161T genotypes and alleles

Variables	Genotypes				Alleles		
	C161C	C161T	T161T	*p*	C161	T161	*p*
Weight[#] (kg)	68.22 ± 1.07	66.41 ± 1.35	66.23 ± 4.14	0.533	67.67 ± 0.65	66.38 ± 1.21	0.363
BMI[#] (kg/m^2)	27.20 ± 0.41	26.49 ± 0.52	25.47 ± 1.59	0.425	27.03 ± 0.25	26.31 ± 0.47	0.207
WC (cm)	92.04 ± 0.94	91.87 ± 1.19	88.97 ± 3.66	0.719	92.00 ± 0.58	91.37 ± 1.08	0.606
WHR[#]	0.90 ± 0.01	0.92 ± 0.01	0.90 ± 0.03	0.510	0.91 ± 0.01	0.92 ± 0.01	0.448
TBF[#] (%)	33.35 ± 0.54	32.96 ± 0.68	28.77 ± 2.10	0.150	33.26 ± 0.33	32.23 ± 0.62	0.194
SF (%)	27.65 ± 0.64	26.86 ± 0.80	22.29 ± 2.46	0.100	27.46 ± 0.39	26.06 ± 0.73	0.091
VFL[#] (%)	12.30 ± 0.48	11.70 ± 0.61	11.48 ± 1.88	0.586	12.16 ± 0.30	11.66 ± 0.55	0.396
RM[#] (kcal)	1430.92 ± 17.86	1396.99 ± 22.51	1456.78 ± 69.30	0.324	1422.78 ± 10.98	1407.45 ± 20.40	0.493
SM[#] (%)	24.88 ± 0.30	24.65 ± 0.38	26.38 ± 1.17	0.345	24.82 ± 0.19	24.96 ± 0.35	0.630
Systolic BP[#] (mmHg)	142.25 ± 1.53	138.47 ± 1.93	139.00 ± 5.95	0.318	141.34 ± 0.94	138.56 ± 1.75	0.198
Diastolic BP (mmHg)	82.21 ± 0.93	80.04 ± 1.17	83.94 ± 3.60	0.275	81.69 ± 0.57	80.72 ± 1.06	0.424
Pulse Rate (bpm)	77.19 ± 0.99	79.01 ± 1.25	71.42 ± 3.85	0.136	77.63 ± 0.61	77.69 ± 1.14	0.964
FBG[#] (mg/dL)	7.73 ± 0.25	7.79 ± 0.32	6.25 ± 0.92	0.287	7.74 ± 0.15	7.49 ± 0.28	0.267
FI[#] (μIU/ml)	13.46 ± 1.15	12.18 ± 1.53	7.01 ± 4.17	**0.040**	13.17 ± 0.72	11.09 ± 1.35	0.088
HOMA-IR[#]	4.91 ± 0.49	4.20 ± 0.64	1.99 ± 1.76	**0.018**	4.75 ± 0.30	3.74 ± 0.57	**0.042**
TC (mmol/L)	4.42 ± 0.07	4.40 ± 0.09	4.52 ± 0.27	0.911	4.42 ± 0.05	4.42 ± 0.08	0.954
TG[#] (mmol/L)	1.44 ± 0.09	1.56 ± 0.11	1.38 ± 0.32	0.992	1.47 ± 0.05	1.53 ± 0.10	0.900
HDL-C[#] (mmol/L)	1.20 ± 0.02	1.20 ± 0.03	1.44 ± 0.08	0.101	1.20 ± 0.01	1.25 ± 0.03	0.223
LDL-C (mmol/L)	2.57 ± 0.06	2.49 ± 0.08	2.46 ± 0.22	0.705	2.55 ± 0.04	2.49 ± 0.07	0.420
TC/HDL-C[#]	3.78 ± 0.07	3.75 ± 0.09	3.40 ± 0.25	0.322	3.78 ± 0.04	3.69 ± 0.08	0.297
Plasma Adiponectin[#] (ng/ml)	6097.11 ± 379.87	6292.92 ± 503.80	7842.48 ± 1376.56	0.543	6140.54 ± 235.79	6618.95 ± 445.36	0.342
Plasma IL-6[#] (pg/ml)	9.52 ± 1.37	7.77 ± 1.82	11.88 ± 4.96	0.223	9.13 ± 0.85	8.64 ± 1.61	0.615

[#] Values were log transformed before analysis; values are presented as adjusted mean ±SEM (estimated marginal means ± standard error of the mean) by univariate analysis of variance (General Linear Model), adjusted for co-variates:age and ethnicity, $p < 0.05$ and indicated in bold font.
n = C161C/C161T/T161T/C161/T161 for: Anthropometric and BP measurements = 181/114/12/476/138; lipid profile analysis and FBG = 167/101/12/435/125; HOMA-IR, FI, plasma adiponectin and plasma IL-6 = 158/90/12/406/114

Table 4.16: Mean difference of the FI and HOMA-IR in *PPARγ2* C161T genotypes (ANOVA Bonferroni adjustment for multiple comparisons)

Dependent Variable	Genotype (I)	Genotype (J)	Mean Difference (I-J)	SE	*p*	95% CI
FI[§#] (μIU/ml)	C161C	C161T	0.01	0.03	1.000	-0.74, 0.09
		T161T	0.20	0.08	**0.031**	0.01, 0.39
	C161T	T161T	0.19	0.08	0.053	-0.01, 0.39
HOMA-IR[§#]	C161C	C161T	0.02	0.04	1.000	-0.08, 0.12
		T161T	0.28	0.10	**0.013**	0.05, 0.51
	C161T	T161T	0.26	0.10	**0.029**	0.02, 0.49

All values by ANOVA Bonferroni adjustment for multiple comparisons, significant at $p < 0.05$ and indicated in bold font.
[§] Values shown are log units.
[#] Values were log transformed before analysis.

4.11.3 Association *PPARδ* T294C Genotypes and Alleles with Anthropometric and Clinical Measurements

By referring to the mean values of continuous variables presented in Table 4.17, no significant difference was observed via the univariate analysis of variance (General Linear Model), adjusted for age and ethnicity with *PPARδ* T294C genotypes and alleles, respectively.

Table 4.17: Means of anthropometric and clinical measurements according *PPARδ* T294C genotypes and alleles

Variables	Genotypes				Alleles		
	T294T	**T294C**	**C294C**	***p***	**T294**	**C294**	***p***
Weight[#] (kg)	67.48 ± 1.17	67.32 ± 1.28	68.08 ± 2.73	0.927	67.32 ± 0.68	67.54 ± 1.05	0.781
BMI[#] (kg/m^2)	27.17 ± 0.45	26.43 ± 0.49	27.20 ± 1.05	0.340	26.95 ± 0.27	26.66 ± 0.41	0.540
WC (cm)	91.76 ± 1.03	91.77 ± 1.13	92.82 ± 2.42	0.916	91.76 ± 0.61	92.09 ± 0.94	0.769
WHR[#]	0.91 ± 0.01	0.91 ± 0.01	0.90 ± 0.02	0.950	0.91 ± 0.01	0.91 ± 0.01	0.954
TBF[#] (%)	33.72 ± 0.59	32.07 ± 0.65	33.66 ± 1.39	0.186	33.23 ± 0.35	32.56 ± 0.54	0.546
SF (%)	28.13 ± 0.70	25.92 ± 0.76	27.42 ± 1.62	0.098	27.48 ± 0.41	26.38 ± 0.63	0.146
VFL[#] (%)	12.21 ± 0.53	11.83 ± 0.58	12.14 ± 1.24	0.656	12.10 ± 0.31	11.92 ± 0.48	0.691
RM[#] (kcal)	1410.79 ± 19.54	1429.89 ± 21.35	1417.81 ± 45.78	0.811	1416.41 ± 11.55	1426.21 ± 17.74	0.658
SM[#] (%)	24.43 ± 0.33	25.41 ± 0.36	24.62 ± 0.77	0.145	24.72 ± 0.20	25.17 ± 0.30	0.171
Systolic BP[#] (mmHg)	141.73 ± 1.68	138.96 ± 1.83	143.12 ± 3.92	0.516	140.92 ± 0.99	140.22 ± 1.52	0.811
Diastolic BP (mmHg)	82.00 ± 1.01	80.83 ± 1.11	81.47 ± 2.38	0.739	81.66 ± 0.60	81.03 ± 0.92	0.567
Pulse Rate (bpm)	78.84 ± 1.08	75.96 ± 1.19	78.75 ± 2.54	0.181	77.99 ± 0.64	76.81 ± 0.99	0.316
FBG[#] (mg/dL)	7.81 ± 0.27	7.63 ± 0.29	7.23 ± 0.67	0.863	7.76 ± 0.16	7.52 ± 0.25	0.597
FI[#] (μIU/ml)	13.77 ± 1.27	11.76 ± 1.39	11.21 ± 3.17	0.742	13.17 ± 0.75	11.61 ± 1.18	0.700
HOMA-IR[#]	4.93 ± 0.54	4.26 ± 0.59	3.43 ± 1.33	0.729	4.73 ± 0.32	4.04 ± 0.50	0.487
TC (mmol/L)	4.42 ± 0.08	4.42 ± 0.09	4.38 ± 0.20	0.980	4.42 ± 0.05	4.41 ± 0.07	0.889
TG[#] (mmol/L)	1.54 ± 0.09	1.42 ± 0.10	1.45 ± 0.23	0.695	1.51 ± 0.06	1.43 ± 0.09	0.845
HDL-C[#] (mmol/L)	1.24 ± 0.03	1.19 ± 0.03	1.18 ± 0.06	0.336	1.22 ± 0.01	1.19 ± 0.02	0.168
LDL-C (mmol/L)	2.49 ± 0.07	2.59 ± 0.07	2.54 ± 0.16	0.594	2.52 ± 0.04	2.58 ± 0.06	0.430
TC/HDL-C[#]	3.69 ± 0.07	3.81 ± 0.08	3.87 ± 0.18	0.421	3.73 ± 0.04	3.83 ± 0.07	0.228
Plasma Adiponectin[#] (ng/ml)	6366.33 ± 420.48	6210.39 ± 458.33	5678.85 ± 1046.39	0.672	6319.63 ± 247.68	6064.10 ± 387.83	0.458
Plasma IL-6[#] (pg/ml)	10.88 ± 1.51	7.18 ± 1.64	7.14 ± 3.75	0.410	7.76 ± 0.16	7.52 ± 0.25	0.597

[#] Values were log transformed before analysis; values are presented as adjusted mean ±SEM (estimated marginal means ± standard error of the mean) by univariate analysis of variance (General Linear Model), adjusted for co-variates:age and ethnicity, $p < 0.05$ and indicated in bold font.

n = T294T/T294C/C294C/T294/T161/C294 for: Anthropometric and BP measurements 152/127/28/431/183; lipid profile analysis and FBG = 138/119/23/395/165; HOMA-IR, FI, plasma adiponectin and plasma IL-6 = 130/109/21/369/151

4.12 Association of Plasma IL-6, Plasma Adiponectin, HOMA-IR and TC/HDL-C with Gender

Figure 4.6 shows the mean values of plasma IL-6 between males and females. The mean of plasma IL-6 was found to be higher in female subjects despite showing no significant difference (p = 0.225).

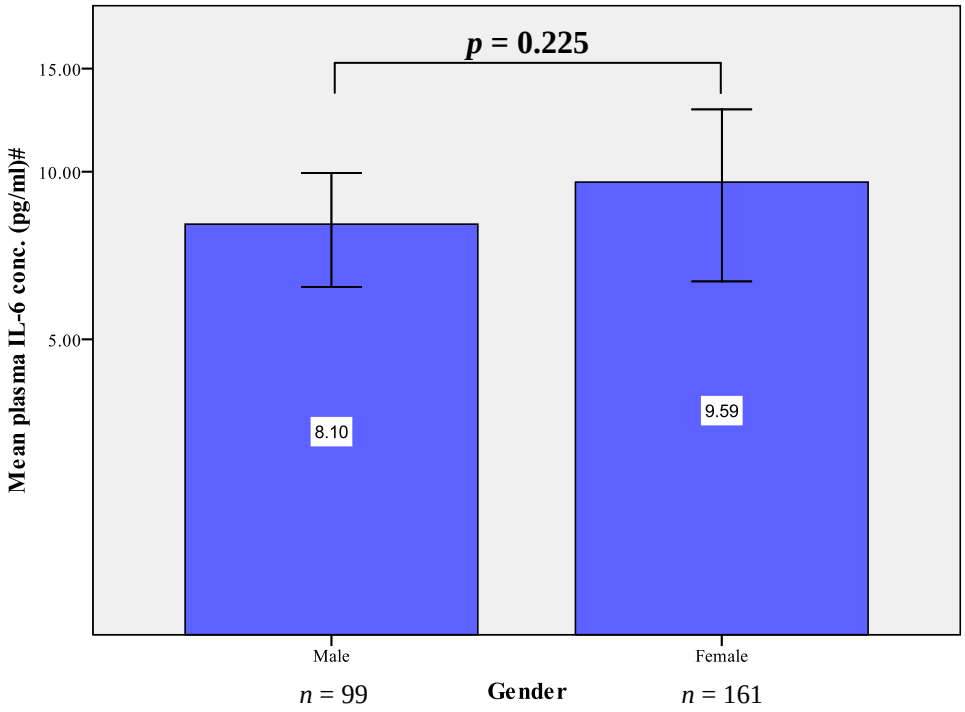


Figure 4.6: Mean plasma IL-6 between males and females
p value by Student's t-test
Values were log transformed before analysis.
Error bars represent 95 % CI.

Figure 4.7 illustrates the mean plasma adiponectin between males and females. Plasma adiponectin was found to be significantly different between gender (p = 0.003). The mean plasma adiponectin of female subjects was significantly higher than that of male subjects with mean difference ± SE of 1833.25 ± 555.98 ng/ml.

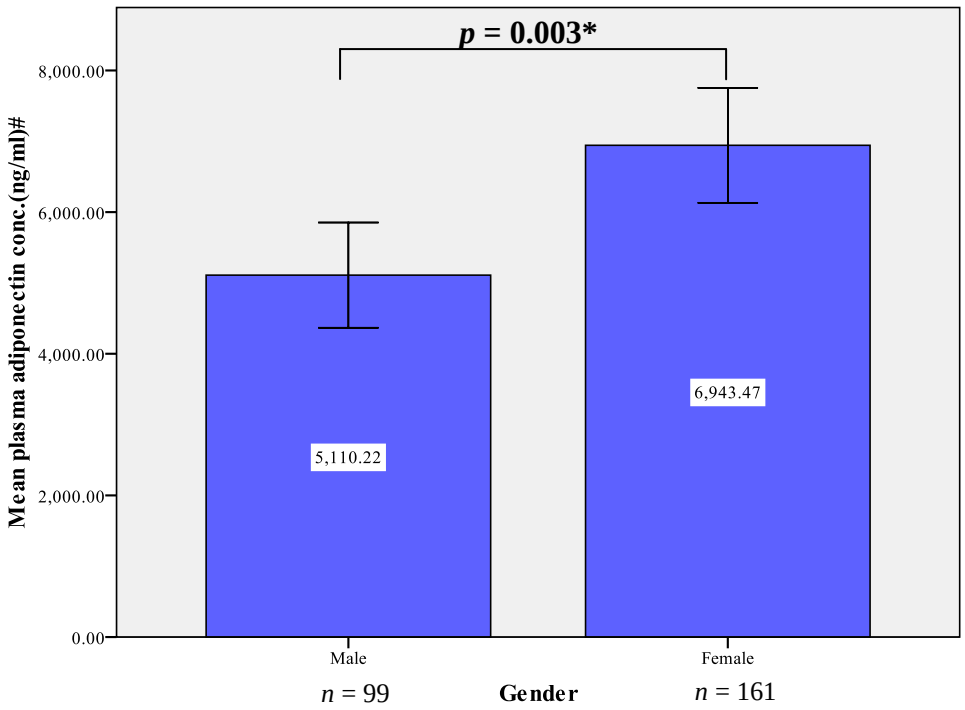


Figure 4.7: Mean plasma adiponectin between males and females
p value by Student's *t*-test
* Values significant between groups
# Values were log transformed before analysis.
Error bars represent 95 % CI.

Figure 4.8 displays the mean HOMA-IR between males and females. No significant association was found between HOMA-IR and gender (p = 0.135) as both female and male subjects showed similar HOMA-IR.

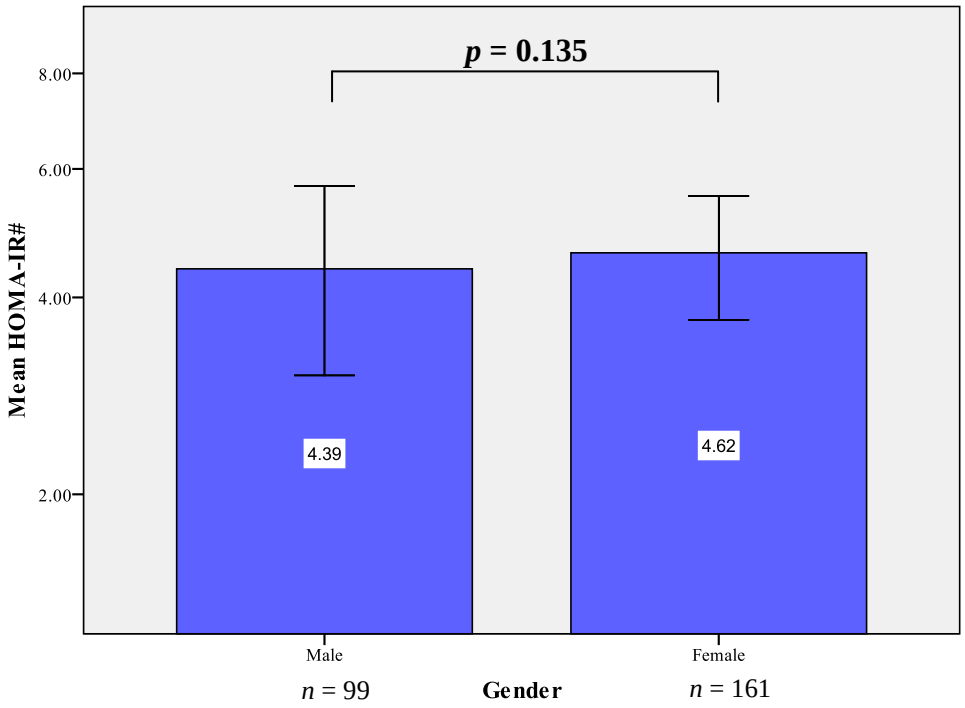


Figure 4.8: Mean HOMA-IR between males and females
p value by Student's *t*-test
[#] Values were log transformed before analysis.
Error bars represent 95 % CI.

Figure 4.9 presents the mean TC/HDL-C of males and females participants. The mean TC/HDL-C of males was slightly higher than females. Student's *t*-test indicated no significant difference between TC/HDL-C and gender ($p = 0.093$).

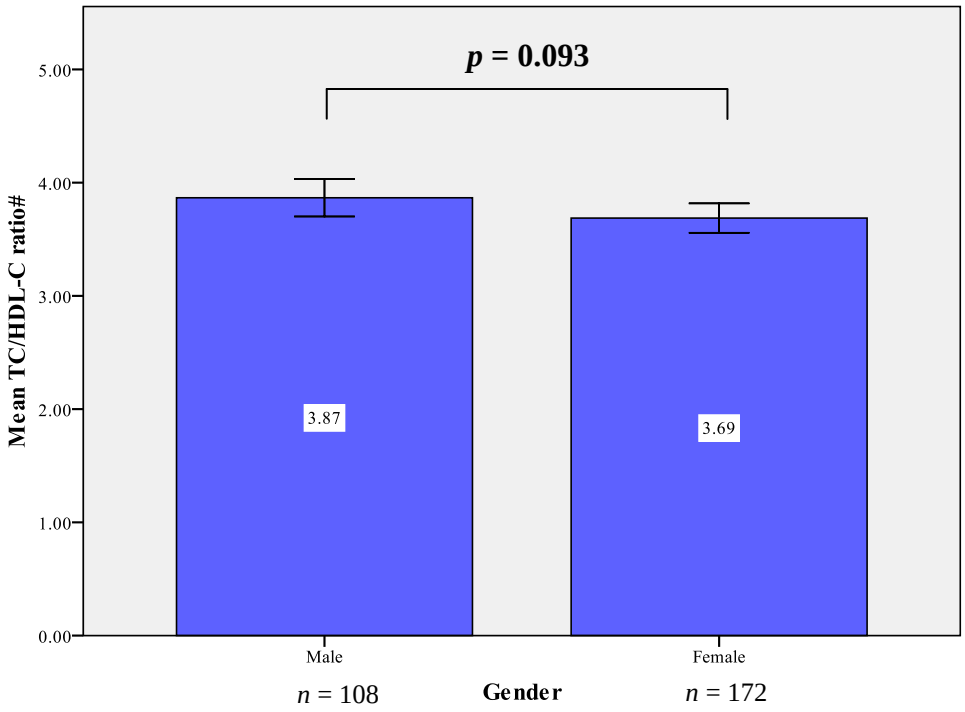


Figure 4.9: Mean TC/HDL-C ratio between males and females
p value by Student's *t*-test
[#] Values were log transformed before analysis.
Error bars represent 95 % CI.

4.13 Association of Plasma IL-6, Plasma Adiponectin, HOMA-IR and TC/HDL-C with Ethnicities

Figure 4.10 presents significant association between mean plasma IL-6 with ethnicity. Malays had highest mean plasma IL-6 as compared to Chinese and Indians with mean difference ± SE of 4.69 ± 2.42 pg/ml and 3.44 ± 3.19 pg/ml, respectively. The mean difference ± SE between Indians and Chinese was 1.25 ± 2.94 pg/ml. Further analysis by ANOVA Bonferroni post-hoc test indicated significant higher mean plasma IL-6 among Malays with 0.10 ± 0.04 log units than Chinese (p = 0.008). Chinese was also found to have significant lower plasma IL-6 than Indians with 0.11 ± 0.04 log unit (p = 0.018).

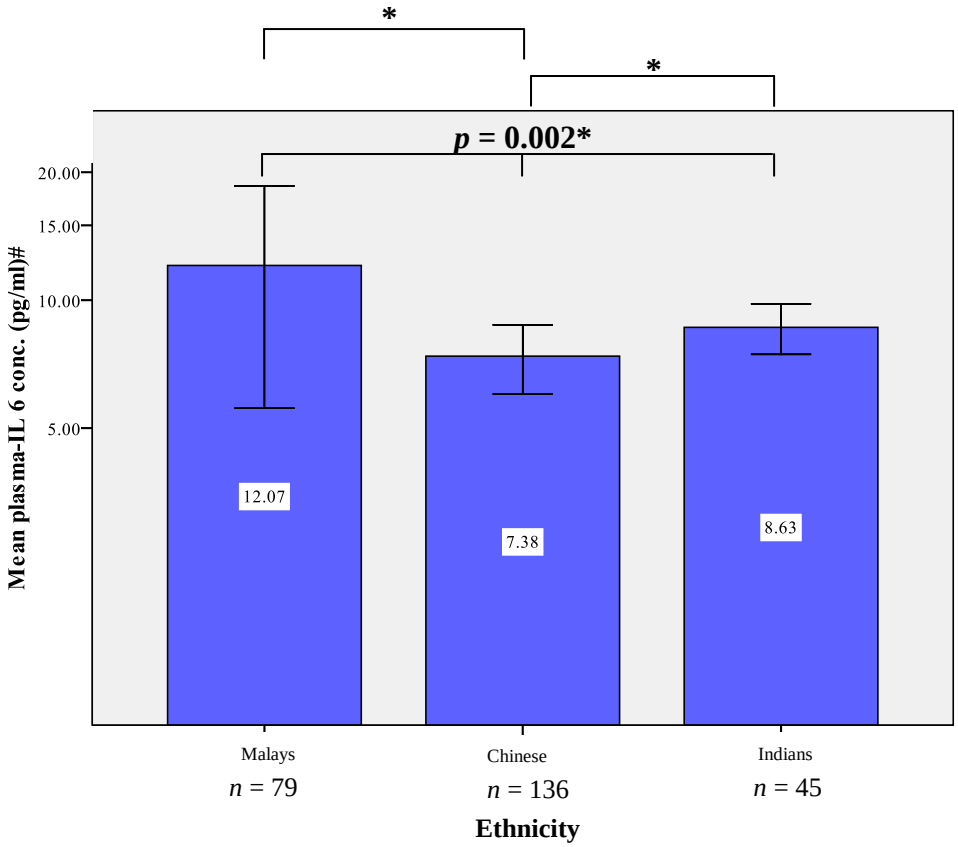


Figure 4.10: Mean plasma IL-6 among ethnicities
p value by one-way ANOVA.
[#] Values were log transformed before analysis.
*Values significant between groups by ANOVA Bonferroni adjustment for multiple comparisons
Error bars represent 95 % CI.

Figure 4.11 shows the mean plasma adiponectin among the three recruited ethnic groups with the same number of subjects as plasma IL-6 measurement. The *p* value of 0.586 showed no significant different between the mean plasma adiponectin among ethnicities.

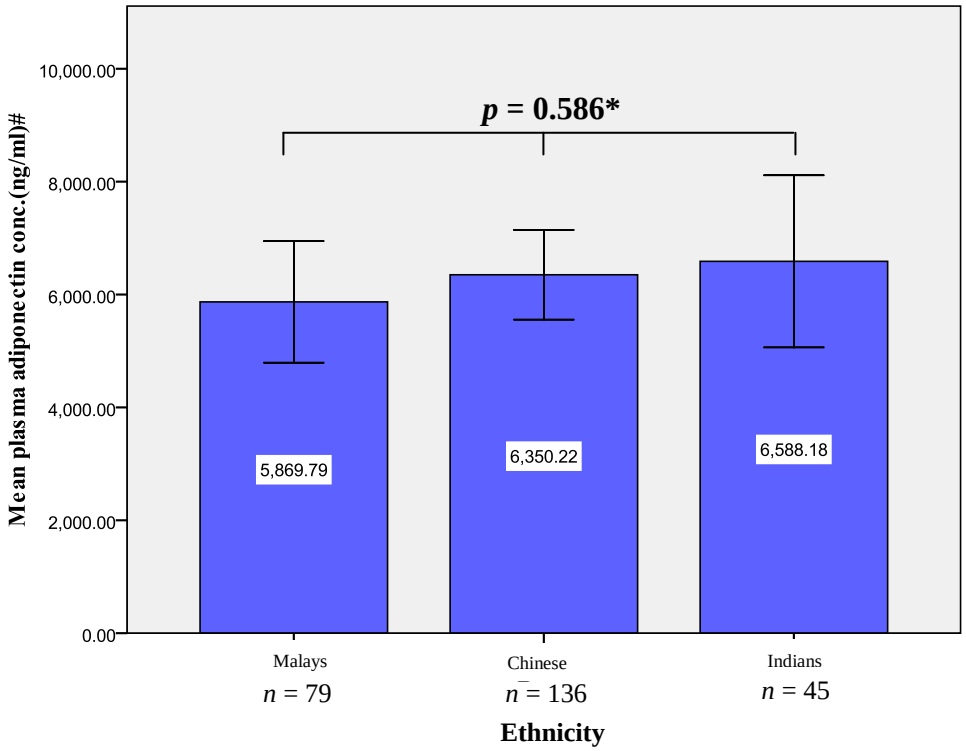


Figure 4.11: Mean plasma adiponectin among ethnicities
p value by one-way ANOVA.
[#] Values were log transformed before analysis.
Error bars represent 95 % CI.

The mean of HOMA-IR among ethnicities was observed with *p* value of 0.053, approaching significant level as shown in Figure 4.12. The mean of HOMA-IR was highest among Indians, followed by Malays and Chinese.

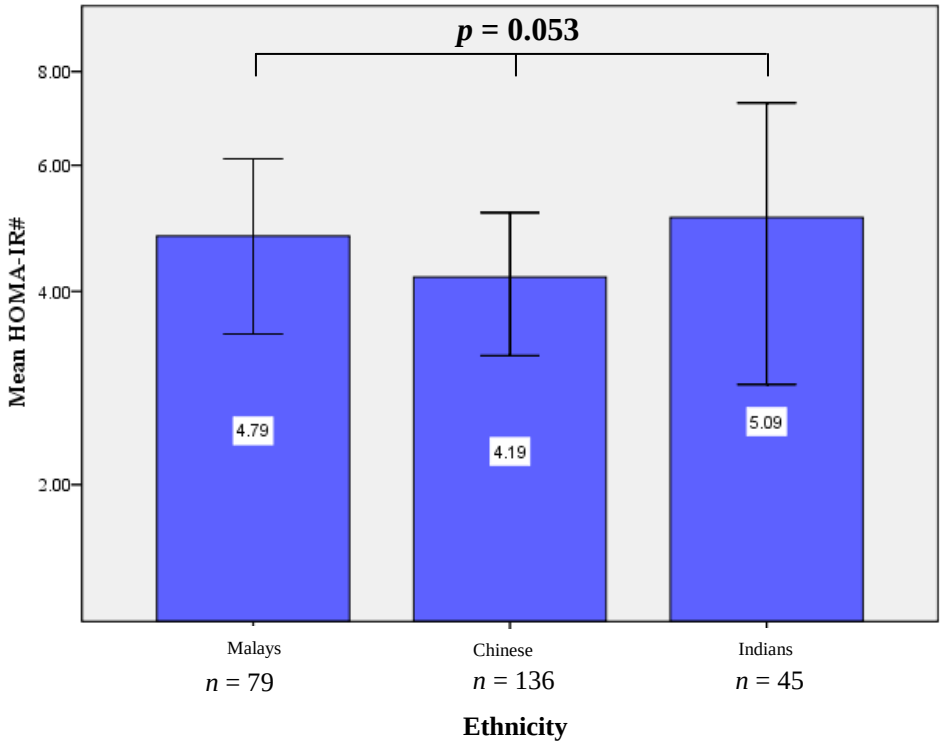


Figure 4.12: Mean HOMA-IR among ethnicities
p value by one-way ANOVA.
[#] Values were log transformed before analysis.
Error bars represent 95 % CI.

As illustrated in Figure 4.13, significant difference of mean of TC/HDL-C was observed among ethnicities ($p < 0.001$). The mean of TC/HDL-C was significantly lower in Chinese subjects as compared to both Malays and Indians subjects with mean difference ± SE of 0.31 ± 0.12 and 0.43 ± 0.14, respectively. Chinese participants showed significant lower 0.04 ± 0.01 log unit of TC/HDL-C as compared to Malays ($p = 0.011$) and 0.06 ± 0.02 log unit of TC/HDL-C as compared to Indians ($p = 0.001$).

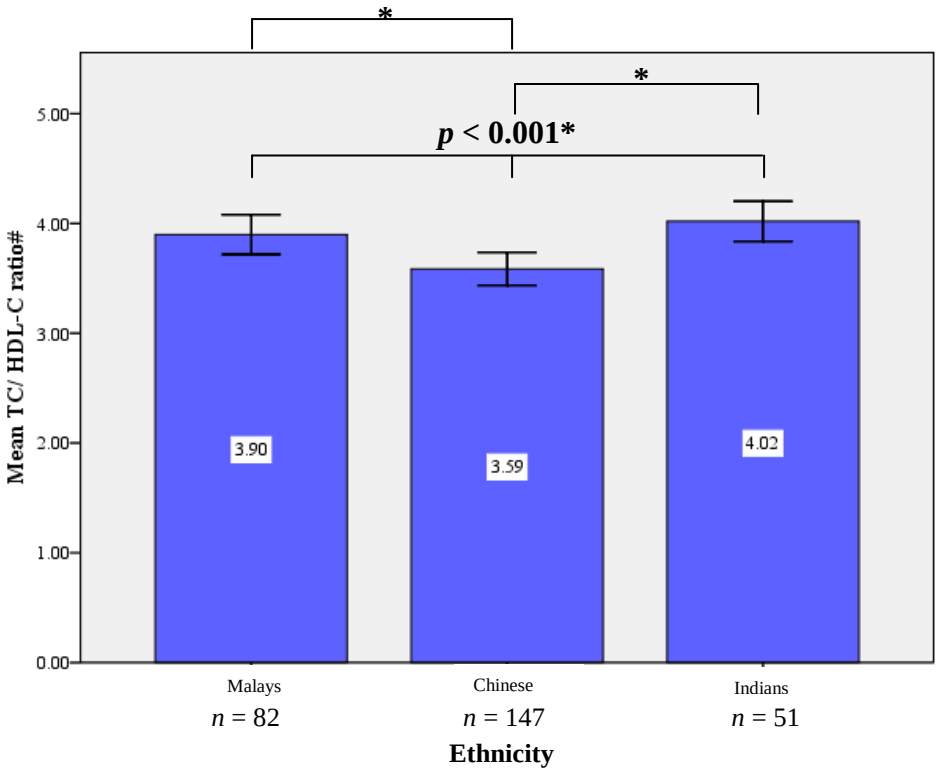


Figure 4.13: Mean TC/HDL-C among ethnicities
p value by one-way ANOVA.
Values were log transformed before analysis.
*Values significant between groups by ANOVA Bonferroni adjustment for multiple comparisons.
Error bars represent 95 % CI.

4.14 Dietary Habits and Lifestyle Factors

4.14.1 Dietary Habits and Lifestyle Factors in Obese, Met-S and Obese with Met-S Groups

Table 4.16 displays the differences of dietary habits and lifestyle factors among obese, Met-S and obese with Met-S participants. The studied population was found to show no significance difference for all the self-reported dietary habits and lifestyle factors with the three groups. According to the survey, the prevalence of salty food preference, strict vegetarian practice, daily coffee intake and weekly fast food intake was lower in all groups.

Majority of the participants did not have an active lifestyle. The survey also suggested a higher prevalence of physical inactivity among the affected groups despite not significantly difference. This finding supports the importance of regular exercise to reduce the obesity and Met-S. Besides, majority of the subjects were non-smokers. In particular, affected groups (obese or metabolic syndrome subjects) were showing higher prevalence of smoking-habit. Thus, the hazard of smoking is undeniable. There were 3 Chinese and 3 Indians participants found to be drinker. As Muslims (all the Malays) in Malaysia are prohibited to consume alcohol and so the only drinker of this ethnic group was *Orang Asli*. There were more drinkers observed in affected subjects. All the four obese subjects with Met-S were found to be frequent drinkers.

Table 4.16: Prevalence of dietary habits and lifestyle factors in obese, Met-S and obese with Met-S groups

Dietary Habits/ Lifestyle Factors	Obesity			Met-S			Obese With Met-S		
	Non-obese *n* (%)	Obese *n* (%)	χ^2; *p*	Absent *n* (%)	Present *n* (%)	χ^2; *p*	Absent *n* (%)	Present *n* (%)	χ^2; *p*
Salty Food Preference									
Yes	59 (46.5)	79 (43.9)	0.198; 0.656	23 (39.7)	115 (46.2)	0.811; 0.368	4 (23.5)	75 (46.0)	3.160; 0.075
No	68 (53.5)	101 (59.1)		35 (60.3)	134 (53.8)		13 (76.5)	88 (54.0)	
Strict Vegetarian Practice	8 (6.3)	12 (6.7)	0.017; 0.898	2 (3.4)	18 (7.2)	0.387$^{\omega}$	0 (0.0)	12 (7.4)	0.608$^{\omega}$
Yes									
No	119 (93.7)	168 (93.3)		56 (96.6)	231 (92.8)		17 (100.0)	151 (92.6)	
Coffee Intake									
Yes	43 (33.9)	51 (28.3)	1.070; 0.301	16 (27.6)	78 (31.3)	0.310; 0.578	6 (35.3)	45 (27.6)	0.573$^{\omega}$
No/ Occasionally	84 (66.1)	129 (71.7)		42 (72.4)	171 (68.7)		11 (64.7)	118 (72.4)	
Fast Food Intake (weekly)	11 (8.7)	15 (8.3)	0.010; 0.919	9 (15.5)	17 (6.8)	0.062$^{\omega}$	2 (11.8)	13 (8.0)	0.638$^{\omega}$
Yes									
No	116 (91.3)	165 (91.7)		49 (84.5)	232 (93.2)		15 (88.2)	150 (92.0)	
Physical Activity									
Yes	44 (34.6)	53 (29.4)	0.932; 0.334	17 (29.3)	80 (32.1)	0.173; 0.678	2 (11.8)	51 (31.3)	2.825; 0.093
No	83 (65.4)	127 (70.6)		41 (70.7)	169 (67.9)		15 (88.2)	112 (68.7)	
Smoking									
Yes	15 (11.8)	19 (10.6)	0.119; 0.730	8 (13.8)	26 (10.4)	0.536; 0.464	3 (17.6)	16 (9.8)	0.396$^{\omega}$
No	112 (88.2)	161 (89.4)		50 (86.2)	223 (89.6)		14 (82.4)	147 (90.2)	
Alcohol Consumption									
Yes	3 (2.4)	4 (2.2)	1.000$^{\omega}$	1 (1.7)	6 (2.4)	1.000$^{\omega}$	0 (0.0)	4 (2.5)	1.000$^{\omega}$
No	124 (97.6)	176 (97.8)		57 (98.3)	243 (97.6)		17 (100.0)	159 (97.5)	

p value by Chi-Square Test, significant at $p < 0.05$; $^{\omega}$ *p* value by Fisher's Exact Test.
% within the obese/ Met-S/ obese with Met-S subjects.

4.14.2 Dietary Habits and Lifestyle Factors with Plasma IL-6, Plasma adiponectin, HOMA-IR and TC/HDL-C

As displayed in Table 4.17, the univariate analysis (General Linear Model) of plasma IL-6 and TC/HDL-C with dietary habits and lifestyle factors which were adjusted for ethnicity did not show any significant association. In addition, there was no significant association found between all the dietary habits and lifestyle factors with the unadjusted variable, HOMA-IR.

Plasma adiponectin was found to be associated with smoking habits after adjusting for the gender. Smokers was reported with significantly higher adiponectin level compared to non-smokers ($p = 0.020$). The rest of the dietary habits and lifestyle variables were not associated with plasma adiponectin.

Table 4.17: Mean plasma IL-6, plasma adiponectin, HOMA-IR and TC/HDL-C in subjects based on their dietary habits and lifestyle factors

Dietary Habits/ Lifestyle Factors	IL-6[#] (pg/ml) Adjusted mean ± SEM	p^{Ψ}	Adiponectin[#] (ng/ml) Adjusted mean ± SEM	p^{ω}	HOMA-IR[#] Adjusted mean ± SEM	p	TC/ HDL-C[#] Adjusted mean ± SEM	p^{Ψ}
Salty Food Preference								
Yes (n = 122[a]/131[b])	11.26 ± 1.72	0.253	5657.02 ± 432.28	0.411	4.77 ± 0.55	0.813	3.79 ± 0.08	0.581
No (n = 138[a]/149[b])	7.67 ± 1.58		6344.20 ± 414.21		4.32 ± 0.52		3.87 ± 0.08	
Strict Vegetarian Practice								
Yes (n = 13[a]/18[b])	7.41 ± 5.61	0.964	7008.24 ± 1412.15	0.529	3.30 ± 1.69	0.545	3.63 ± 0.22	0.310
No (n = 247[a]/262[b])	9.44 ± 1.21		5964.74 ± 307.35		4.60 ± 0.39		3.85 ± 0.06	
Coffee Intake								
Yes (n = 82[a]/90[b])	9.52 ± 2.08	0.831	5520.46 ± 519.65	0.101	4.66 ± 0.67	0.869	3.83 ± 0.10	0.946
No (n = 178[a]/190[b])	9.30 ± 1.43		6224.96 ± 376.27		4.47 ± 0.46		3.84 ± 0.07	
Fast Food Intake (weekly)								
Yes (n = 21[a]/22[b])	8.52 ± 4.08	0.646	5342.31 ± 1030.81	0.642	6.11 ± 1.33	0.157	3.84 ± 0.19	0.974
No (n = 239[a]/258[b])	9.42 ± 1.23		6094.41 ± 315.66		4.39 ± 0.39		3.83 ± 0.06	
Physical Activity								
Yes (n = 88[a]/93[b])	7.81 ± 2.02	0.308	6415.48 ± 505.81	0.280	4.33 ± 0.65	0.739	3.77 ± 0.10	0.522
No (n = 172[a]/187[b])	10.05 ± 1.44		5799.33 ± 377.10		4.63 ± 0.47		3.87 ± 0.07	
Smoking Habit								
Yes (n = 30[a]/31[b])	7.36 ± 4.23	0.346	3064.75 ± 1711.16	**0.020**	4.36 ± 1.11	0.771	4.07 ± 0.21	0.183
No (n = 230[a]/249[b])	9.46 ± 1.23		6325.43 ± 333.70		4.55 ± 0.41		3.80 ± 0.06	
Alcohol Consumption								
Yes (n = 4[ab])	6.76 ± 9.91	0.760	5115.02 ± 2726.09	0.923	2.74 ± 3.05	0.657	3.98 ± 0.49	0.521
No (n = 256[a]/276[b])	9.39 ± 1.18		6047.08 ± 304.79		4.56 ± 0.38		3.83 ± 0.06	

All values by univariate analysis (General Linear Model), significant at $p < 0.05$ and indicated in bold font. Values presented as adjusted mean ± SEM (estimated marginal means ± standard error of the mean); $^{\Psi}$ Adjusted for ethnicity; $^{\omega}$ Adjusted for gender; $^{\#}$ Values were log transformed before analysis; [a] Sample size of plasma IL-6, plasma adiponectin and HOMA-IR; [b] Sample size of TC/HDL-C

4.15 Correlation between Plasma IL-6, Plasma Adiponectin, HOMA-IR, TC/HDL-C and Baseline and Biochemical Variables

Pearson's Correlation test was performed to estimate the association of plasma IL-6, plasma adiponectin, HOMA-IR, TC/HDL-C with baseline and biochemical variables. Age was not included in the covariate as it was found that plasma IL-6, plasma adiponectin, HOMA-IR and TC/HDL did not correlate with age in the present study (data not shown). Partial correlation was conducted for plasma IL-6 and TC/HDL-C after controlling for ethnicity. Partial correlation for plasma adiponectin was analyzed by controlling the gender. Association of HOMA-IR with baseline variables was tested via Bivariate Pearson correlation.

As shown in Figure 4.14, plasma IL-6 was positively correlated with weight, BMI, WC, TBF, SF, VFL, pulse rate and plasma adiponectin. The relationship of the mentioned variables with plasma IL-6 was weak as the correlation coefficient (r) was far more less than 1. Meanwhile, SM was found to be negatively correlated with plasma IL-6.

Plasma adiponectin was negatively correlated with weight, BMI, WC, RM, DBP, FBG, FI, HOMA-IR, TG and TC/HDL-C. Positive correlation was found on HDL-C with plasma adiponectin.

Furthermore, HOMA-IR was found to be positively correlated with weight, BMI, WC, TBF, SF, VFL, RM, pulse rate, FBG, FI, TC, TG and

TC/HDL-C. Strong relationship with high r value (0.584 and 0883, respectively) was shown by FBG and FI as they are the derivative parameter of HOMA-IR. Negative correlation was observed between HOMA-IR and SM, HDL-C and plasma adiponectin.

TC/HDL-C was positively correlated with weight, BMI, WC, WHR, TBF, SF, VFL, RM, SBP, DBP, FBG, FI, HOMA-IR, TC, TG and LDL-C. Negative correlation was shown between TC/HDL-C with HDL-C and plasma adiponectin.

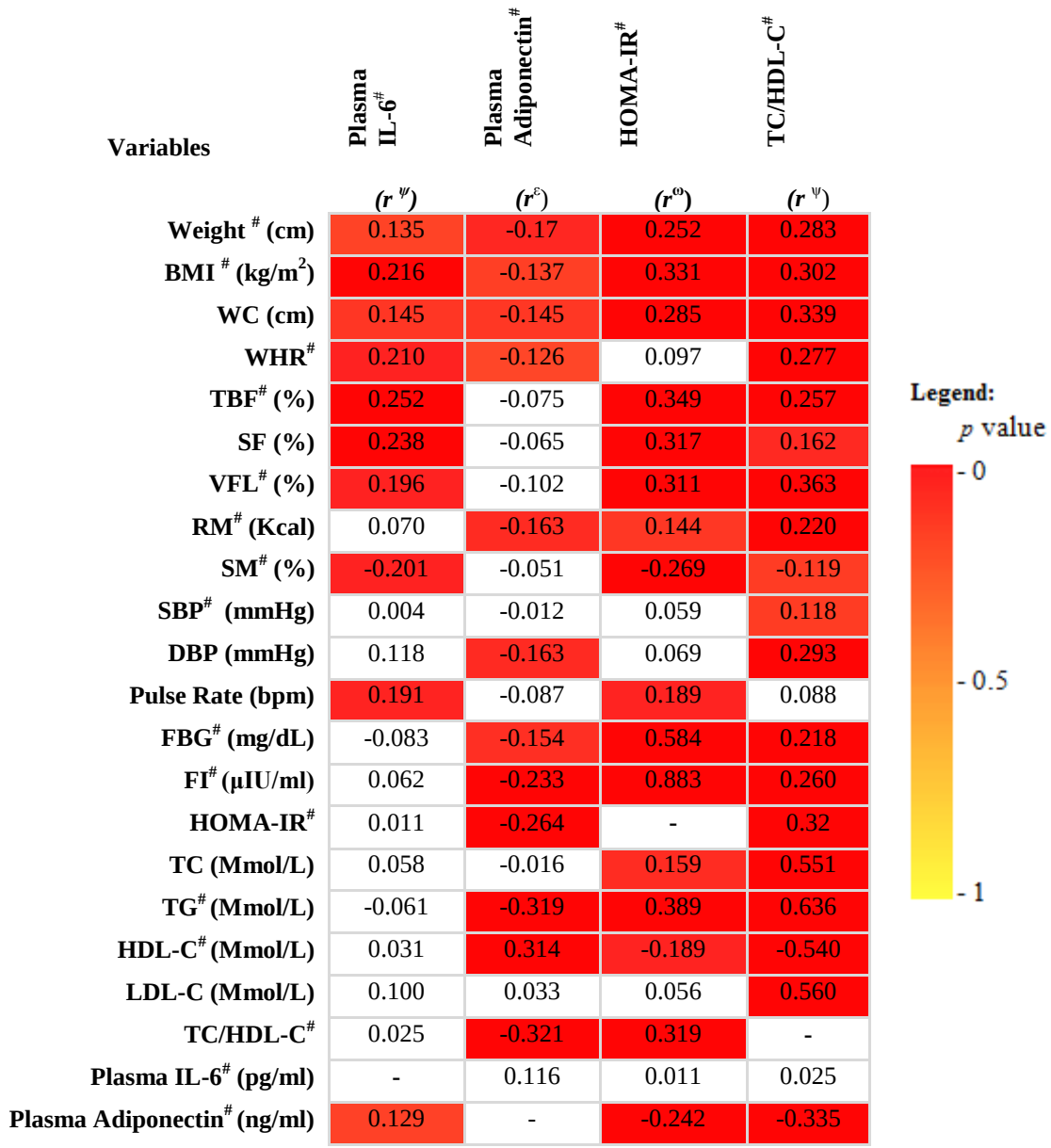


Figure 4.14: Heat-map of correlation between plasma IL-6, plasma adiponectin, HOMA-IR, TC/HDL and continuous (baseline and biochemical) variables

Ψ Values presented as Partial Correlation after controlling for ethnicity.

ε Values presented as Partial Correlation after controlling for gender.

ω Values presented as Bivariate Pearson Correlation.

Values/Variables were log-transformed before analysis.

Non-significant values are not coloured

4.16 Relationship between Genotype and Allele Combinations with Anthropometric and Biochemical Variables

Anthropometric and biochemical variables were compared with the genotype combinations as shown in Table 4.18 via univariate analysis of variance (General Linear Model). As an overall, the results show no significant association between the anthropometric and biochemical variables with subjects with homozygous wild type (*PPARα* L162L, *PPARγ2* C161C and *PPARδ* T294T) and without homozygous wild type.

General Linear Model by univariate analysis of variance was performed to compare the different allele combination of the three PPAR SNPs (allele combination in the sequence of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C) with anthropometric and clinical measurements as shown in Table 4.19. HDL-C measurement was found to have significant difference with VTT combination showing the highest whereas VCT combination showing the lowest. Meanwhile, there was no significant association found between different allele combination of the three PPAR SNPs and other anthropometric and clinical measurements.

Table 4.18: Relationship between genotype combinations with anthropometric and biochemical variables

Variable	Subjects with homozygous wild type for all three SNPs[a]	Subjects without homozygous wild type for all three SNPs	*p*
Weight # (kg)	66.21 ± 1.68	67.87 ± 0.94	0.438
BMI # (kg/m^2)	26.80 ± 0.65	26.88 ± 0.36	0.977
WC (cm)	90.03 ± 1.48	92.44 ± 0.83	0.157
WHR#	0.90 ± 0.01	0.91 ± 0.01	0.479
TBF# (%)	33.51 ± 0.86	32.88 ± 0.48	0.632
SF (%)	28.52 ± 1.00	26.71 ± 0.56	0.117
VFL# (%)	11.64 ± 0.76	12.17 ± 0.43	0.927
RM# (kcal)	1388.32 ± 28.04	1429.18 ± 15.73	0.269
SM# (%)	24.46 ± 0.48	24.98 ± 0.27	0.278
SBP# (mmHg)	141.97 ± 2.41	140.32 ± 1.35	0.698
DBP (mmHg)	81.27 ± 1.46	81.53 ± 0.82	0.874
Pulse Rate (bpm)	78.41 ± 1.57	77.40 ± 0.88	0.573
FBG# (mg/dL)	7.79 ± 0.38	7.65 ± 0.22	0.581
FI# (μIU/ml)	13.02 ± 1.78	12.61 ± 1.04	0.839
HOMA-IR#	4.81 ± 0.75	4.44 ± 0.44	0.957
TC (mmol/L)	4.42 ± 0.11	4.42 ± 0.07	0.987
TG# (mmol/L)	1.38 ± 0.13	1.52 ± 0.08	0.566
HDL-C# (mmol/L)	1.24 ± 0.04	1.20 ± 0.02	0.144
LDL-C (mmol/L)	2.56 ± 0.09	2.53 ± 0.54	0.809
TC/HDL-C #	3.65 ± 0.11	3.79 ± 0.06	0.237
Plasma adiponectin# (ng/ml)	6414.12 ± 586.59	6186.87 ± 344.19	0.913
Plasma IL-6# (pg/ml)	11.83 ± 2.10	8.05 ± 1.23	0.318

#Values were log transformed before analysis; values are presented as adjusted mean ± SEM (estimated marginal means ± standard error of the mean) by univariate analysis of variance (General Linear Model), adjusted for co-variates: age and ethnicity

[a]Subjects with homozygous mutated genotypes for all three PPAR SNPs: VV for *PPARα* L162V, TT for *PPARγ2* C161T and CC for *PPARδ* T294C

The total number of subjects with homozygous mutated genotypes for all variables was 74/307, except for FBP, TC, TG, HDL-C, LDL-C, TC/HDL-C (70/280) and FI, HOMA-IR, IL-6 (67/260).

Table 4.19: Means of anthropometric and clinical measurements according PPARs allele combination

Variable	Allele combination								*p*
	LCT	**LCC**	**LTT**	**LTC**	**VCT**	**VCC**	**VTT**	**VTC**	
Weight[#] (kg)	67.25 ± 0.74	68.71 ± 1.32	68.40 ± 1.76	64.36 ± 1.87	70.99 ± 3.54	70.61 ± 4.36	64.61 ± 3.82	62.79 ± 4.35	0.453
BMI[#] (kg/m^2)	26.85 ± 0.30	27.23 ± 0.53	27.41 ± 0.70	25.52 ± 0.75	27.78 ± 1.42	28.98 ± 1.74	25.78 ± 1.53	25.13 ± 1.74	0.431
WC (cm)	91.56 ± 0.68	93.01 ± 1.21	93.15 ± 1.62	90.35 ± 1.72	94.80 ± 3.26	93.28 ± 4.01	88.48 ± 3.51	88.51 ± 4.00	0.651
WHR[#]	0.91 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.92 ± 0.01	0.91 ± 0.03	0.87 ± 0.03	0.89 ± 0.03	0.89 ± 0.03	0.852
TBF[#] (%)	32.98 ± 0.31	33.39 ± 0.54	33.31 ± 0.73	32.06 ± 0.77	33.81 ± 1.47	34.70 ± 1.80	32.70 ± 1.58	31.91 ± 1.80	0.902
SF (%)	27.26 ± 0.33	27.45 ± 0.58	27.54 ± 0.79	25.78 ± 0.83	25.75 ± 1.57	29.89 ± 1.93	26.43 ± 1.69	25.42 ± 1.93	0.413
VFL[#] (%)	12.05 ± 0.34	12.48 ± 0.61	12.63 ± 0.81	10.68 ± 0.87	13.58 ± 1.64	11.84 ± 2.01	10.83 ± 1.77	10.33 ± 2.01	0.746
RM[#] (kcal)	1418.99 ± 10.73	1436.21 ± 19.00	1424.73 ± 25.35	1373.64 ± 26.99	1516.96 ± 51.08	1435.72 ± 62.81	1371.99 ± 55.07	1359.65 ± 62.70	0.275
SM[#] (%)	24.89 ± 0.15	25.03 ± 0.27	24.49 ± 0.36	24.72 ± 0.39	24.78 ± 0.73	23.93 ± 0.90	25.02 ± 0.79	25.27 ± 0.89	0.897
Systolic BP[#] (mmHg)	140.98 ± 1.11	143.15 ± 1.97	140.02 ± 2.63	136.21 ± 2.80	145.38 ± 5.30	129.69 ± 6.52	140.40 ± 5.71	138.00 ± 6.50	0.335
Diastolic BP (mmHg)	81.40 ± 0.68	82.25 ± 1.20	82.40 ± 1.60	78.85 ± 1.70	85.18 ± 3.22	81.40 ± 3.95	82.24 ± 3.47	77.36 ± 3.95	0.584
Pulse Rate (bpm)	77.84 ± 0.71	75.88 ± 1.26	78.22 ± 1.69	77.82 ± 1.80	77.88 ± 3.40	85.96 ± 4.18	77.92 ± 3.66	76.48 ± 4.17	0.517
FBG[#] (mg/dL)	7.76 ± 0.18	7.75 ± 0.32	7.82 ± 0.42	7.35 ± 0.45	7.08 ± 0.91	6.90 ± 1.05	7.22 ± 0.95	7.56 ± 1.11	0.935
FI[#] (μIU/ml)	13.16 ± 0.84	12.37 ± 1.52	12.24 ± 2.01	10.21 ± 2.20	21.85 ± 4.36	11.64 ± 4.83	9.64 ± 4.37	9.91 ± 5.12	0.515
HOMA-IR[#]	4.77 ± 0.36	4.55 ± 0.64	4.37 ± 0.85	3.20 ± 0.93	6.83 ± 1.84	3.56 ± 2.04	3.26 ± 1.85	3.36 ± 2.16	0.554
TC (mmol/L)	4.41 ± 0.05	4.47 ± 0.09	4.52 ± 0.12	4.42 ± 0.13	4.03 ± 0.27	4.41 ± 0.31	4.53 ± 0.28	3.89 ± 0.33	0.563
TG[#] (mmol/L)	1.47 ± 0.06	1.48 ± 0.11	1.73 ± 0.15	1.33 ± 0.16	1.56 ± 0.32	1.37 ± 0.37	1.37 ± 0.33	1.58 ± 0.39	0.941
HDL-C[#] (mmol/L)	1.21 ± 0.02	1.19 ± 0.03	1.26 ± 0.04	1.22 ± 0.04	1.05 ± 0.08	1.16 ± 0.10	1.53 ± 0.09	1.08 ± 0.10	**0.012**
LDL-C (mmol/L)	2.54 ± 0.04	2.61 ± 0.08	2.48 ± 0.10	2.60 ± 0.11	2.27 ± 0.22	2.64 ± 0.26	2.39 ± 0.23	2.11 ± 0.27	0.563
TC/HDL-C[#]	3.74 ± 0.05	3.89 ± 0.09	3.72 ± 0.12	3.71 ± 0.12	3.85 ± 0.25	3.79 ± 0.29	3.32 ± 0.26	3.64 ± 0.31	0.498
Adiponectin[#] (ng/ml)	6195.30 ± 271.79	6227.88 ± 490.32	6920.31 ± 647.67	6568.49 ± 711.72	3979.05 ± 1408.66	4699.32 ± 1557.96	7974.05 ± 1410.87	4648.87 ± 1651.80	0.444
IL-6[#] (pg/ml)	9.64 ± 1.00	7.06 ± 1.81	9.90 ± 2.39	6.31 ± 2.62	9.01 ± 5.19	12.28 ± 5.74	12.52 ± 5.20	8.94 ± 6.08	0.227

[#] Values were log transformed before analysis; values are presented as adjusted mean ±SEM (estimated marginal means ± standard error of the mean) by univariate analysis of variance (General Linear Model), adjusted for co-variates:age, gender and ethnicity, $p < 0.05$ and indicated in bold font. Allele combination in the sequence of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C; *n* = LCT/LCC/LTT/LTC/VCT/VCC/VTT/VTC for: Anthropometric and BP measurements = 342/109/61/54/15/10/13/10; lipid profile analysis and FBG = 316/98/56/50/12/9/11/8; HOMA-IR, FI, plasma adiponectin and plasma IL-6 = 295/91/52/43/11/9/11/8

CHAPTER 5

DISCUSSION

5.1 Prevalence of Obesity and Met-S in Malaysia

A systematic analysis of Global Burden of Disease Study 2013 has reported a very high rate (more than 40 %) of overweight/obese (BMI ≥ 25 kg/m^2) in Malaysia in year 2013, with a prevalence of 43.8 % in men and 48.6 % in women (Ng et al. 2014). Likewise, The Malaysian National Health and Morbidity Survey (NHMS) IV showed that the prevalence of obesity and overweight had increased more than two-fold within two and a half decade, with the prevalence of overweight/obese (BMI ≥ 25 kg/m^2) at 44.5 % in 2011 as compared to 20.6 % in 1996 (NHMS II). In addition, a cross-sectional national study which involved five different regions in Peninsular and East Malaysia between the years of 2007-2008 (Mohamud et al., 2011b) has documented a prevalence of 53.1 % overweight/obese adults (BMI ≥ 25.0 kg/m^2). A latter study (Mohamud et al., 2011a) indicated that the prevalence of Met-S to be 34.4 % in accordance to NCEP-ATP III guidelines, consistent with an earlier study which found the prevalence at 36.1 % between the years 2005-2006 (Tan et al., 2011).

In this study, 58.6 % was found to be obese (BMI ≥ 25 kg/m^2), whereas 81.1 % was found to have fulfilled the diagnosis of Met-S. The high prevalence of Met-S was due to the selection bias as the recruited subjects

were from health clinic based-population. Among the obese subjects, 9.4 % was found to be metabolically healthy, whereas the rest was diagnosed with Met-S. The higher percentage of obese subjects and especially Met-S subjects recruited for the study was explainable as the majority of the subjects were patrons of the clinic who underwent routine medical check-ups. Thus, distribution of the obesity and Met-S is only relevant to the Kampar Health clinic and is neither meant to be representative of the whole Kampar district population nor to be compared with their prevalence at the national level.

In this study, Malays were the ethnicity with highest prevalence of obesity, followed by Indians and Chinese, which is similar to the Malaysian Adults Nutrition Survey (MOH 2008). Meanwhile, majority of the Met-S subjects were Chinese, followed by Indians and Malays. The high prevalence of Met-S in Chinese in this study was indeed as expected as the Chinese is the major population of Kampar (Department of Statistics, Malaysia 2010). Previous nationwide studies indicated that Indians had the highest prevalence of Met-S, followed by Malays and Chinese (Mohamud et al., 2011a; Tan et al., 2011)

5.2 Socio-demographic Characteristics, Dietary Habits and Lifestyle Factors in relation to Obesity and Met-S

According to the report of The 2010 Population and Housing Census of Malaysia which is conducted once in every 10 years, Kampar district council of Perak state is populated by 88,638 Malaysian citizens (Census

2010). District Kampar is a suburban town under the administration of the Kampar district council. This suburban town is populated by approximately 15 thousands of residents, comprising mainly of Chinese followed by Malays and Indians. Majority of the residents are made up of age group 50-59 and followed by 40-49. They sustain the economy of the area by involving mainly in the commercial and industrials sectors. The economy is further driven by establishment of universities such as Universiti Tunku Abdul Rahman (UTAR) and Kolej Universiti Tunku Abdul Rahman (KTAR). Kampar town is generally found to be populated by far more retirees/old folks with age group more than 60 years old as compared to young adults (20-29 and 30-39). This scenario is most probably due to the drift of young adults to urban areas with higher chances of job opportunity. This is supported by the evidence of the increase percentage of the employed workers in urban areas from all states as shown in the report on economic characteristics of census 2010. Besides, female residents are found to slightly outnumber male residents in Kampar. As for health care access, there are six government health clinics (Malim Mawar, Kampar, Gopeng, Tanjung Tualang, Kota Bharu and Tronoh) and a government hospital serving the residents within the Kampar district council.

Overall, the socio-demographic characteristics of the 307 recruited subjects were found to be nicely matched with above-mentioned characteristics reported in census 2010, suggesting a well representation of Kampar population among the recruited subjects in aspects of ethnicity, gender and age groups. Majority of the recruited subjects were retired/not working, followed by self-employed and blue-collar workers. The finding of

high retirees among the recruited subjects is self-explanatory by examining the age groups distribution among the recruited subjects, given also the fact that elder subjects are more frequent attendee of most health clinics for the purpose of medical check-up.

Although the rate of obesity is rising both in developed and developing countries, there is a slight different in the trend pertaining to different socio-economic status. In countries with lower income, wealthier and well-educated people are more likely to be overweight than people with lower incomes or less schooling. However, in higher income countries, wealthier people have lower rates of obesity than the poor. Meanwhile, in lower-middle and middle-income countries, obesity rates are higher or growing more rapidly among the poor as compared to the rich (Popkin, 2011). This scenario is also portrayed in Malaysia as a country with middle income, even in the suburban area such as Kampar. As the income scale of the population starts to move up, the obesity rates also increases.

In this study, highest percentage of the subjects was observed with monthly household income ranging from RM 1001-3000, which was in agreement with the monthly household income of RM 2809 reported for the Perak state in year 2009 (Census 2010). The monthly household income and the occupation of the subjects may be attributed to the attainment of education levels. Literate subjects were found to be more economic productive and in general had a higher salary income. Unless retired, most of the elder financial independent subjects were reported to be self-employed in the questionnaire.

Majority of the elder senior citizens were found to be illiterate as they received no schooling during the time when education was not a priority. There is increasing improvement in the trend of number and levels of educational attainment from the younger senior citizens to middle-age and younger adults. Among the recruited subjects, there was a tendency for better educated individuals to stay healthy, indicating the importance of education in promoting healthy lifestyle and raising better awareness in health care.

Besides, obese subjects were found to have more positive family history which is in concordant with other studies (Allison et al., 1996; Mo-suwan and Geater, 1996; Cummings and Schwartz, 2003; Mohan et al., 2003). This also suggests the genetic predisposition to obesity. Family history is a good predictor to identify the increased susceptibility to obesity and Met-S because of an interaction between genetic traits, environmental, social, behavioural and cultural factors, which are shared to a larger extent among the family than among the general population. Nonetheless, adaptation of healthier lifestyle can modify the risks of having obesity and Met-S.

Obesity and Met-S are accentuated by urbanization and modernization as a result of poorer dietary habits and sedentary lifestyles. Excessive food intake, poor nutrition intake includes instant foods, fried foods, snacking, salty or sweet foods, high meat, alcohol and smoking habits are among the poor dietary habits and unhealthy lifestyle behaviors that attributed to the growing obesity and Met-S (Schrauwen and Westerterp, 2000; Zhu et al., 2004; Hayes

et al., 2006; Astrup et al., 2008). Urbanization and modernization has result in society-wide declines in physical activity in the workplace, transportation, household chores and leisure. The shift of workforces from active jobs like farming and mining to less active jobs like manufacturing and service industries, use of automated appliances, elevators, cars and leisure pursuits such as internet and television had promote physical inactivity in daily life. Overall, the excessive energy intake and reduced energy expenditure have results in the energy imbalance that exacerbate the obesity and Met-S (Anderssen et al., 2007; Goris and Westerterp, 2008; Hill et al., 2012).

Nonetheless, current study was unable to detect the association of three categorical groups (obese, Met-S and Obese with Met-S) with all the dietary habits and lifestyle factors in the survey. Due to the practical difficulties during the survey, the questionnaire of current study had assessed the dietary habits and lifestyle factors in a qualitatively way, thus did not collect sufficient details to reveal significant observation in the analysis. Being essential components in contributing to both co-morbidities of obesity and Met-S, the assessment of dietary habits and lifestyle factors is necessary. It requires the compliance of the respondents in keeping track on record of daily food intake and activity as well as the employment of more comprehensive methods for dietary and activity assessment by the investigator depending on the purpose of the study.

5.3 Anthropometrics and Biochemical Measurements in relation to Obesity and Met-S

As a commonly used index to measure relative weight, BMI is proven to be closely related to the TBF (Gray and Fujioka, 1991). Beside presented with high TBF, obese individual is also seen with distinct regional fat distribution within the body, particularly at the abdominal region. Such abdominal obesity is commonly evaluated using WC and WHR.

Obesity impairs the normal functions of adipose tissue. Excessive TG accumulation within adipocytes causes adipocyte hyperthrophy and adipokines dysregulation, leading to imbalance secretions of pro- and anti-inflammatory adipokines such as IL-6 and adiponectin. Growing evidences have now shown that increased IL-6 and decreased adiponectin link obesity and Met-S with insulin resistance (Kadowaki et al., 2006; Suzuki et al., 2011). In this study, subjects with obesity and Met-S were showing all the unfavourable readings for the anthropometric and clinical measurements which include higher anthropometric measurements, BP, lipid profile, IR and IL-6 as well as lower plasma adiponectin.

In the midst of rising rate of obesity pandemic and its related health complications, there is a subset of obese individuals who appeared to have a low-burden of adiposity-related metabolic abnormalities compared with ‘at risk’ obese individuals. These apparently healthy obese subjects are the so-called ‘metabolically healthy obese’ (MHO) phenotype, which have been

classified as obese-non-Met-S in this study. These subjects showed a favourable metabolic profile which is characterized by high levels of insulin sensitivity, absence of hypertension, and a favourable lipid and inflammation profile (Hinnouho et al., 2014).

Consistently, obese-Met-S subjects displayed significantly higher SBP, FBG, FI and HOMA-IR than obese-non-Met-S subjects. Pulse rate and TG were significantly higher in obese-Met-S subjects only after adjustment for age and ethnicity. Notably, the glucose related indices (FBG, FI and HOMA-IR) appeared to be strongly associated with Met-S and non-Met-S among obese subjects in this study. The strong association of HOMA-IR with Met-S has implied the causal link of insulin resistance to Met-S, indicating that most individuals meeting ATP III criteria will be insulin resistant. Thus, these findings also suggest the feasibility to monitor HOMA-IR among obese individuals to predict their tendency to develop Met-S in the future.

Obesity has been known to have a strong link with body fat distribution, lipids and glucose metabolism as well as energy homeostasis. Obese subjects tend to utilize more lipids for energy expenditure as compared to non-obese subjects (Golay et al., 1984). This larger part of energy metabolism taken by lipids is a consequence of increased fat stores, resulting in a decrease in carbohydrate metabolism. Subsequently, the increase in lipid metabolism may result in negative changes of normal circulating plasma lipid profiles to lipids disorders state or dyslipidemia, which include elevation of TG, reduction of HDL-C and increase in LDL-C. The alteration in

carbohydrate metabolism also results in insulin resistance which is characterised by alteration in glucose tolerance, delayed glucose storage and oxidation (Ageeva et al., 2002).

Till date, the complex etiological implication of obesity to Met-S and various diseases such type II diabetes mellitus and cardiovascular diseases is still obscure. One of the possible underlying molecular mechanisms to link such relationship is through inflammation. Obesity is now known as a state of low-grade inflammation (Rajala and Scherer, 2003). Besides serving as storage reservoir of fat, adipose tissue is an active endocrine organ which secretes cytokines also known as adipokines. These include adipokines such as leptin, adiponectin, IL-6, tumor necrosis factor alpha (TNF-α), procoagulant substances such as plasminogen activator inhibitor-1, vasoactive substances such as leptin, angiotensinogen and endothelin, and molecules that may contribute to insulin resistance such free fatty acid, TNF-α and resistin (Wang and Nakayama, 2010). Among these numerous adipokines, adiponectin and IL-6 were the two adipokines of interest in present study.

IL-6 is known to play important roles in acute phase reactions, inflammation, bone metabolism, hematopoiesis and cancer progression. In particular, its roles in inflammation and energy homeostasis regulation have been implicated in obesity and Met-S. It suppresses lipoprotein lipase activity, controls appetite and energy intake at hypothalamic level (Stenlof et al., 2003). IL-6 is involved in the transition from acute inflammation to chronic inflammation disease, such as obesity, insulin resistance and Met-S (Naugler

and Karin, 2008). In line with this study, several studies had also reported higher circulating levels of IL-6 with both fat mass and BMI in obesity as well as in Met -S (Bastard et al., 2000; Kern et al., 2001; Vozarova et al., 2001; Simao et al., 2012).

Adiponectin is an adipokine that regulates lipid and glucose metabolism, increases insulin sensitivity, regulates food intake and body weight, and protects against chronic inflammation. Weight loss has been shown to increase adiponectin levels (Liu and Liu, 2010; Prakash et al., 2012). In agreement with our study, there are more evidences confirming that adiponectin is negatively associated with obesity (Arita et al., 1999; Abbasi et al., 2004; Nayak et al., 2010; Ouchi et al., 2003) and Met-S (Kadowaki et al., 2006; Ryo et al., 2004). Thus, such negative association of adiponectin as seen in obese and/or Met-S individuals suggest that individuals with obesity and Met-S might have impaired synthesis and secretion of adiponectin by adipocytes, or interaction with specific adiponectin receptors.

5.4 *PPARα, γ and δ* Genes in Obesity and Met-S

5.4.1 *PPARα* L162V (rs1800206)

5.4.1.1 Prevalence of *PPARα* L162V

This study revealed that the V162 allele was a less common allele with overall frequency of 0.08 in multi-ethnic Kampar population. Similar to Yiew et al. (2010) from Malaysia and some previous studies (Skoczynska et al., 2005; Uthurralt et al., 2007; Domenici et al., 2013) from other population,

PPARα V162V genotype was found to be absent in this study. The MAF of each ethnicity in Malaysia in current study and other populations is summarized in Table 5.1.

Table 5.1: MAF of *PPARα* L162V in various populations

Population	**MAF**	**Reference**
Chinese Han	0.13 - 0.16	Luo et al. (2013); Gu et al. (2014)
Caucasians	0.06 - 0.08	Domenici et al. (2013); Volcik et al. (2008); Uthurralt et al. (2007), Sparso et al. (2007); Raalte et al. (2004); Tai et al. (2002); Evans et al. (2001)
Brazilian	0.05	Mazzotti et al. (2011)
General Mediterranean	0.04	Manresa et al. (2006)
Turkish	0.02	Koytak et al. (2008)
Singaporean Malays/Chinese/ Indians	0.005/0.004/0.02	Chan et al. (2006)
African Senegalese black	0	Lopez-Sall et al. (2008)
Malaysian Malays/Chinese/ Indians	0.07/0.08/0.08	Current study

The MAF of all the three ethnic groups in this study were found to be similar to Caucasians. Despite similar demography, Chan et al. (2006) from Singapore reported much lower MAF among the three ethnicities of Malays, Chinese and Indians from nationwide sampling with a total of 4248 subjects. Nonetheless, Luo et al. (2013) and Gu et al. (2014) reported higher MAF among Chinese Han population with sampling size of 820 and 647 respectively. Such discrepancies may be due to the difference in the sample size as well as the sampling technique, geographical coverage and target group of the study. Thus, large-scale nationwide study encompassing more parts of Malaysia is required to confirm to the current finding with the confined geographical area in Kampar, Perak.

In this study, there was no significant association between the distribution of V162 allele genotype carriers and allele frequencies with ethnicities and gender, respectively. Further stratified analysis of the SNP by ethnicity with obesity, Met-S and obese with/without Met also showed no significant association.

5.4.1.2 *PPARα* L162V in Obesity

Overall, this study found no association between the V162 allele genotype carriers and allele frequencies with obesity and the obesity related parameters (BMI, WHR, TBF, SF, and VFL). In agreement with the study, no association was found between L162V polymorphism with obesity in other studies from other populations include Chinese Han population (Luo et al.,

2013; Gu et al., 2014), Brazilian population (Domenici et al., 2013) and Caucasians (Silbernagel et al., 2009). Other studies also reported no significant difference between BMI and WC between L162L and L162V/V162V genotypes (Vohl et al., 2000; Robitaille et al., 2004; Skoczynska et al., 2005; Aberle et al., 2006a; Haworth et al., 2008).

However, Uthurralt et al. (2007) reported that V162 allele was associated with higher BMI and greater baseline SF in Caucasian men, whereas Evans et al. (2001) and Bosse et al. (2003a) found that BMI and TBF were significantly lower in V162 allele carriers comparing to L162 allele carriers in overweight and type II diabetes women.

5.4.1.3 *PPARα* L162V in Met-S and Obese with/without Met-S

There was no association detected between the *PPARα* L162V SNP with Met-S. There were not many studies directly reported on the association of Met-S with the L162V polymorphism. In a study conducted by Robitaille et al. (2004) to compare the frequency of L162V polymorphism in a sample of single gender (632 men) with and without Met-S as defined by the NCEP-ATPIII guidelines, no association was reported between the frequencies of V162 allele with Met-S. This study is the first to examine the association of *PPARα* L162V SNP and obese with/without Met-S. Apparently, there was no association detected between the *PPARα* L162V SNP and obese with/without Met-S.

5.4.1.4 *PPARα* L162V in relation to Metabolic Parameters

In this study, plasma IL-6 was the only metabolic parameter with significant association with *PPARα* L162V SNP, being significantly higher among V162 allele carriers. Carriers of *PPARα* V162 variants may exhibit lower anti-inflammatory activity, resulting in higher level of IL-6. Meanwhile, there was no association detected between the *PPARα* L162V SNP and the other metabolic parameters which include total lipid profile parameters (TC, TG, HDL-C, LDL-C, TC/HDL-C), FBG, HOMA-IR and plasma adiponectin level.

Findings of the association of *PPARα* L162V SNP on various metabolic parameters were inconclusive. V162 allele carriers have been previously related to variation in lipid profile. It has been found to be associated with higher concentrations of TG (Robitaille et al., 2004; Uthurralt et al., 2007; Shin et al., 2008), TC (Tai et al., 2002) and LDL-C (Vohl et al., 2000; Tai et al., 2002) and lower HDL-C (Uthurralt et al., 2007) in healthy subjects. However, the significant findings on lipid variations reported by Tai et al. (2002) and Uthurralt et al. (2007) were limited to men but not women, indicating the possible gender specific effect in the modulating of the *PPARα* gene polymorphism on various metabolic traits.

Similar to this study, many other studies also found no significant association between *PPARα* L162V SNP and TC (Vohl et al., 2000; Robitaille et al., 2004; Aberle et al., 2006a; Domenici et al., 2013), TG (Vohl et al.,

2000; Skoczynska et al., 2005; Aberle et al., 2006a; Domenici et al., 2013), HDL-C (Vohl et al., 2000; Robitaille et al., 2004; Skoczynska et al., 2005; Aberle et al., 2006a; Domenici et al., 2013), LDL-C (Robitaille et al., 2004; Skoczynska et al., 2005; Alberle et al., 2006; Domenici et al., 2013), FBG, (Robitaille et al., 2004), HOMA-IR (Domenici et al., 2013), BP (Robitaille et al., 2004) and adiponectin (Domenici et al., 2013). In type II diabetic studies, four independent studies had reported no significant difference in the V162 allele frequency between subjects with and without type II diabetes, indicating that *PPARα* L162V SNP may not directly affect the glucose metabolism which can result in the development of type II diabetes (Lacquemant et al., 2000; Vohl et al., 2000; Evans et al., 2001; Bosse et al., 2003b).

There are not many studies investigating the influence of *PPARα* L162V on plasma/serum IL-6 level. The only one study found so far by Skoczynska et al. (2005) has reported a contradictory finding with current study. In that study, there was significantly lower serum IL-6 level in V162 allele carriers than L162 allele carriers among subjects with/ without coronary atherosclerosis. Noteworthy that population and sampling differences may result in such discrepancy. In addition, the decreased serum IL-6 level in men with angiographically confirmed atherosclerosis may also be due to persistent treatment with drugs, especially simvastatin and/or acetylsalicylic acid.

PPARα has been implicated in the regulation of inflammation (Plutzky, 2003, Lefebvre et al., 2006). Activation of *PPARα* has been shown to inhibit

the production of pro-inflammatory cytokines which include IL-6 (Staels et al., 1998, Sethi et al., 2002). Nevertheless, studies have shown that there are differences in the rates of transcriptional activity of the *PPARα* L162 and *PPARα* V162 variants. According to Sapone et al. (2000), the basal transactivation activity of variant (*PPARα* V162) was less than one-half as active as the wild-type receptor (*PPARα* L162) in a transfection assay in Hepa-1 cells. *PPARα* V162 variant may not bind to PPREs as efficiently as the wild-type receptor. However, a low activation found in the absence of ligand or with low doses can be overcome by a hyper responsiveness at high doses. High doses of ligand could produce a rearrangement in the helix 12 of *PPARα* to trigger a critical change in activity. It appears that the V162-PPAR*α* has the potential to reach comparable transcription rates as L162-PPAR*α* with doses of ligands (Sapone et al., 2000; Rudkowska et al., 2009).

5.4.2 *PPARγ*2 C161T (rs 3856806)

5.4.2.1 Prevalence of *PPARγ*2 C161T

The present study reported an overall MAF of T161 as 0.22 in multi-ethnic Kampar population. In this study, statistically significant differences in the prevalence of the *PPARγ2* C161T variants by ethnic group were noted, with Indians showing the lowest MAF and Chinese showing the highest. This finding is also shown in a previous study from Singapore (Tai et al., 2004). The MAF of Malaysian Chinese was similar with Han Chinese Taiwan and China (Liu et al., 2008; Chen et al., 2011). The MAF of Malaysian Indians was found to be more similar to South Indians population (Haseeb et al., 2009)

and Caucasians population (Meirhaeghe et al., 1998; Doney et al., 2002; Doney et al., 2004; Meirhaeghe et al., 2005; Jaziri et al., 2006) compared to other ethnicities in Malaysia. The MAF of *PPARγ2* C161T of current and various populations is shown in Table 5.2.

Table 5.2: MAF of *PPARγ2* C161T in various populations

Population	MAF	Reference
Chinese Han (Beijing/Taiwan)	0.21 - 0.25	Liu et al. (2008); Chen et al. (2011)
Korean	0.20	Rhee et al. (2011)
Tunisia	0.18	Youssef et al. (2014)
Japanese	0.15 - 0.17	Hishida et al. (2013); Sanada et al. (2011)
Caucasians	0.12 - 0.14	Meirhaeghe et al. (1998); Meirhaeghe et al. (2005); Doney et al. (2002); Doney et al. (2004); Jaziri et al. (2006)
South/North Indians	0.14 - 0.15	Haseeb et al. (2009), Prakash et al. (2012)
Iran	0.07	Rooki et al. (2013)
Singaporean Malays/Chinese/ Indians	0.22/0.25/ 0.17	Tai et al. (2004)
Malaysian Malays/Chinese/ Indians	0.21/0.27/ 0.13	Current study

5.4.2.2 *PPARγ*2 C161T in Obesity

The distribution of T161 allele genotype carriers (C161T/T161T) and allele frequencies was not associated with obese/non-obese, Met-S/non-Met-S and obese with/without Met-S. Besides, the distribution of T161 allele genotype carriers and allele frequencies did not show significant association with gender. Stratified analysis of *PPARγ*2 C161T allele frequencies with obesity, Met-S and obese with/without Met-S as well as gender within each ethnicity did not differ significantly.

Similar to this study, majority of the published literature reported no significant association between *PPARγ2* C161T SNP and obesity (Meirhaeghe et al., 1998; Cecil et al., 2007; Costa et al., 2009; Haseeb et al., 2009; Chen et al., 2011; Sanada et al., 2011; Prakash et al., 2012; Wang et al., 1999; Liu et al., 2008; Luo et al., 2013). There is no literature reporting on the significant association between obesity/nonobese group and *PPARγ2* C161T SNP. However, one study by Valve et al. (1999) reported that obese women with T161T genotype had higher mean BMI and WC than C161C/C161T genotypes.

5.4.2.3 *PPARγ*2 C161T in Met-S and Obese with/without Met-S

This study found no significant association between *PPARγ2* C161T SNP and Met-S, which was also seen in many previous studies (Meirhaeghe et al., 2005; Rhee et al., 2006; Morini et al., 2008; Liu et al., 2008; Chen et al.,

2011; Shi et al., 2012; Liu et al., 2008). However, some studies have suggested that this SNP may alter the risk of Met-S by allelic linkage disequilibrium with *PPARγ2* Pro12Ala (rs 1801282) (Yang et al., 2009; Youssef et al., 2014). In addition, this study was the first to show no association between *PPARγ2* C161T SNP and obese with/without Met-S.

5.4.2.4 *PPARγ*2 C161T in relation to Metabolic Parameters

In this study, a significant lower HOMA-IR was present in T161 allele carriers and a trend towards lower mean FI when compared to C161 allele carriers, although not showing significant association with Met-S. This finding is supported by Liu et al. (2008) from Beijing, China, who have also reported a significant lower HOMA-IR among T161 allele carriers and no significant association between *PPARγ*2 C161T SNP and Met-S. Of note, another similarity is that the target subjects of both studies (current and Liu et al. 2008) were recruited from the clinic or hospital based patients who underwent routine medical checkup. Earlier, Antoine et al. (2007) have also reported that carriers of T161 allele had a significantly lower HOMA-IR in healthy women. Jaziri et al. (2006) reported that the normoglycemic carriers of the T allele were less prone to develop fasting hyperglycemia during a six-year follow-up in contrast to the subjects with the *PPARγ*2 C161C genotype. Taken together, these findings have implied the protective role of T161 allele in enhancing insulin sensitivity and protecting against type II diabetes.

On the other hand, *PPARγ*2 C161T was not associated with other metabolic parameters which include glucose and lipid-related variables, IL-6 and adiponectin in this study. This finding is in agreement with previous studies in other populations (Meirhaeghe et al., 1998; Valve et al., 1999; Wang et al., 1999; Doney et al., 2002; Poulsen et al., 2003; Tai et al., 2004; Rhee et al., 2006; Morini et al., 2008; Shi et al., 2012). However, Gu et al. (2013) and Yilmaz-Aydogan et al. (2011) have reported that the T161 allele carriers had higher serum TG but Tavares et al. (2005) has reported a contradictory finding with lower serum TG among T161 allele carriers.

A few more recent studies which found similar findings with this study are discussed here. Study conducted by Shi et al. (2012) from Nanjing reported no association between *PPARγ*2 C161T SNP and BP, BMI, WC, HDL-C, LDL-C TC, TG, FBG and serum adiponectin among Han Chinese. Another study by Morini et al. (2008) from Italy reported no association between *PPARγ*2 C161T SNP and BMI, WC, HOMA-IR, FBG, FI, TG and HDL-C among non-diabetic white subjects. Rhee et al. (2006) reported no association between *PPARγ*2 C161T SNP and BMI, percentage of body fat, WC, FBS, HOMA, BP, TC, TG, HDL-C and LDL-C among Korean women. Meanwhile, Tai et al. (2004) reported no significant association between *PPARγ*2 C161T analysis and TC, TG, HDL-C, LDL-C, FBG, FI and impaired glucose tolerance among multi-ethnic Singaporean, with similar ethnicity composition like current study.

As a silent mutation, the functional effect of *PPARγ2* C161T SNPs is still unknown. The functionality of PPARγ2 C161T is related to linkage disequilibrium with other SNPs, although the findings are controversial. *PPARγ2* C161T SNP have been reported to be in linkage disequilibrium with *PPARγ2* Pro12Ala (rs1801282) (Valve et al., 1999; Meirhaeghe et al., 2005; Doney et al., 2002; Tai et al., 2004; Haseeb et al., 2009) and *PPARγ2* P-689T (Meirhaeghe et al., 2005). Doney et al. (2002) reported an opposing association with body weight between both *PPARγ2* C161T and *PPARγ2* P12A, but the finding was not supported by Tai et al. (2004). In particular, Doney et al. (2002) demonstrated that increased body mass was associated with the Pro-T161 haplotype, whereas the Ala-C161 haplotype was associated with a lower body mass when compared to the common Pro-C161 haplotype.

Meanwhile, Meirhaeghe et al. (2005) showed that subjects carrying rare alleles Ala12 and P-689T with *PPARγ2* C161C genotype background had increased risk of developing Met-S. However, Haseeb et al. (2009) indicated that both the *PPARγ2* Pro12Ala and C161T variants did not exhibit any association with obesity and Met-S. Taken together, the inconsistencies of the findings may be due the discrepancy in the allele frequency that varies in different populations and the level of linkage disequilibrium of another functional polymorphism in the *PPARγ* gene or nearby gene.

5.4.3 *PPARδ* T294C (rs2016520)

5.4.3.1 Prevalence of *PPARδ* T294C

This study reported an overall MAF of 0.30 in multi-ethnic Kampar population for *PPARδ* T294C. The prevalence difference between gender and *PPARδ* T294C genotypes was noted. Male subjects were found to have higher C294 allele genotype (T294C/C294C) as compared to female subjects. Table 5.3 shows the prevalence of *PPARδ* T294C SNP across difference populations.

Table 5.3: MAF of *PPARδ* T294C in various populations

Population	MAF	Reference
Israeli Caucasians	0.35	Eynon et al. (2009)
China Chinese Han	0.30	Gu et al. (2013); Luo et al. (2013)
African-Americans	0.28	Hautala et al. (2007)
Caucasians	0.16 - 0.25	Hautala et al. (2007); Robitaille et al. (2007); Aberle et al. (2006a); Aberle et al. (2006b); Gouni-Berthold et al. (2005); Skogsberg et al. (2003a); Skogsberg et al. (2003b)
Malaysian Malays/Chinese/ Indians	0.31/0.32/0.22	Current study

The MAF of *PPARδ* T294C SNP for Malaysian Chinese was similar with Han Chinese from China (Gu et al., 2013; Luo et al., 2013). The MAF of Malaysian Indians was found to be more similar to Caucasians population (Skogsberg et al., 2003a; Skogsberg et al., 2003b; Gouni-Berthold et al., 2005; Aberle et al., 2006a; Aberle et al., 2006b; Hautala et al., 2007; Robitaille et al., 2007) compared to other ethnicities in Malaysia.

5.4.3.2 *PPARδ* T294C in Obesity

This study observed no significant association between *PPARδ* T294C SNP and obesity. Several previous studies from other populations have also reported the lack of association between *PPARδ* T294C SNP and obesity (Gouni-Berthold et al., 2005; Robitaille et al., 2007; Grarup et al., 2007; Lagou et al., 2008; Yin et al., 2012b). However, the minor variants of *PPARδ* T294C SNP have been reported to be associated with lower BMI (Aberle et al., 2006b; Luo et al., 2013) and significant lower risk to develop obesity (Luo et al., 2013).

5.4.3.3 *PPARδ* T294C in Met-S and Obese with/without Met-S

There was no association found between *PPARδ* T294C SNP and Met-S as shown in many studies (Gouni-Berthold et al., 2005; Yan et al., 2005, Grarup et al., 2007; Robitaille et al., 2007). A previous study by Robitaille et al., 2007 reported that carriers of the minor C294 allele may be at lower risk of developing the Met-S, particularly when consuming a diet low in fat.

However, since this study did not take the dietary intervention into account, it is therefore not possible to make direct comparison. Besides, this study appeared to be the first to study the association between *PPARδ* T294C SNP and obese with/without Met-S where no association was observed.

5.4.3.4 *PPARδ* T294C in relation to Metabolic Parameters

For all the metabolic parameters assessed in this study, no significant association was detected with *PPARδ* T294C SNP. Such observation were in agreement with many studies in search of the association between *PPARδ* T294C SNP and BP (Grarup et al., 2007; Robitaille et al., 2007; Yin et al., 2012a), glucose related variables (Robitaille et al., 2007) and lipid related variables (Aberle et al., 2006a; Gouni-Berthold et al., 2005; Grarup et al., 2007; Hu et al., 2006; Jguirim-Souissi et al., 2010; Wei et al., 2011) and adiponectin (Burch et al., 2010). So far, the study which documented on the association of *PPARδ* T294C SNP with plasma IL-6 and adiponectin is yet to be found. The minor C294 allele has been inconsistently associated with decreased risk of abdominal obesity (Ding et al., 2012b), increased fasting level plasma LDL-C (Skogsberg et al., 2003a), decreased fasting level plasma HDL-C (Skosberg et al., 2003b; Aberle et al., 2006b), higher baseline cholesterol (Gallicchio et al., 2008), increased fasting plasma glucose concentrations (Hu et al., 2006; Shin et al., 2004) and lower insulin sensitivity (Hu et al., 2006).

Skogsberg et al. (2003a) have shown that C294 allele of *PPARδ* T294C SNP had a higher basal transcriptional activity than the common T294 allele in transient transfection assay, by inducing a binding site for Sp-1 transcription factor. However, studies in human have indicated an unresolved observation which is contradictory, with lower HDL and a trend towards higher LDL levels among C294 allele carriers (Skogsberg et al., 2003a; Aberle et al., 2006b). Meanwhile, some studies attempted to link the functionality of *PPARδ* T294C SNP by linkage disequilibrium in *PPARδ* (Shin et al., 2004) and interaction with other SNP of lipid related-genes (Yin et al., 2012b). Skogsberg et al. (2003a) found an interactive effect between the polymorphisms on plasma LDL and triglycerides but failed to replicate in their following study (Skogsberg et al., 2003b). Aberle et al. (2006b) detected a significant effect of a gene-to-gene interplay between both *PPARδ* T294C SNP and *PPARα* L162V SNP on body weight and BMI. However, Gouni-Berthold et al. (2005) reported that there was no gene-gene interaction between *PPARδ* T294C SNP and *PPARα* L162V observed regarding lipoprotein concentrations and atherosclerotic disease in type II diabetes/non-diabetes subjects. In view of such discrepancies, more studies are needed to validate this finding.

5.5 Correlation Analysis of Plasma IL-6, Plasma Adiponectin, HOMA-IR and TC/HDL-C

This study has observed a gender difference in the plasma adiponectin level but not seen in the plasma IL-6, HOMA-IR and TC/HDL-C levels. In

line with many previous reports, the mean plasma adiponectin level of females was significantly higher than males (Hotta et al., 2000; Nishizawa et al., 2002; Yamamoto et al., 2002; Bottner et al., 2004; Onat et al., 2008; Eglit et al., 2013a; Song et al., 2014). Such difference may be attributed by the body fat distribution and sex hormones difference between genders. Body fat distribution is known to differ between genders, with men having more visceral and less subcutaneous fat (Ludescher et al., 2007). Generally, men store more visceral fat and this result in the central obesity and increased WC. Meanwhile, women store more SF, especially in the lower gluteal-regions (Lemieux et al., 1993; Kotani et al., 1994). These findings are also well reflected in current study. It was found that the mean VFL of men was to be significantly higher than women (13.90 ± 6.56 % in men, 10.79 ± 6.44 % in women; $p < 0.001$) whereas the SF of men were significantly lower than women (19.96 ± 5.51 % in men, 31.98 ± 6.64 % in women, $p < 0.001$). On the other hand, adiponectin has been shown to be secreted from adipocytes mainly synthesized in SF (Fain et al., 2004), which is higher in women. The inhibitory effects of androgens on adiponectin levels may also contribute to considerably lower plasma adiponectin level in men than women (Nishizawa et al., 2002).

In Kampar population, Chinese was seen to have significantly lower TC/HDL-C ratio as compared to both Malays and Indians. This difference may partly due to the dietary habits and methods of food preparation as each ethnicity has their own culture, social mannerism and etiquette. In addition, Chinese was observed with significantly lower plasma IL-6 as compared to Malays (highest) and Indians. The findings of highest plasma IL-6 in Malays,

followed by Indians and Chinese was in concordance with the prevalence of obesity among ethnicity in this study.

In addition, General Linear Model analysis had revealed that plasma adiponectin level was significantly lower in current smoker than non-smoker even after adjusted for the gender as a confounding factor, this finding is also supported by several previous studies (Miyazaki et al., 2003; Iwashima et al., 2005; Thamer et al., 2005; Tsukinoki et al., 2005; Takefuji et al., 2007). The exact mechanism on the influence of smoking on the adiponectin level remains unclear. A few possible speculations are discussed in accordance to Takefuji et al. (2007). Firstly, smoking may influence the adiponectin level (Staiger et al., 2003) via alterations of body fat distribution (Canoy et al., 2005; Cnop et al., 2003). In population-based cohort studies, current smokers had higher WHR, or pattern of central adiposity than non-smokers (Barrett-Connor and Khaw, 1989; Canoy et al., 2005). The number of cigarettes smoked per day was positively associated with WHR in both genders (Bamia et al., 2004).

Secondly, nicotine found in cigarette smoke is reported to cause various endocrine changes in the hypothalamic-pituitary-adrenal (HPA) axis in humans. Chronic nicotine exposure alters basal HPA axis activity and stimulates circulating levels of adrenocorticotropic hormone and cortisol in smokers (Rohleder and Kirschbaum, 2006). These changes in the activity of HPA axis have been associated with visceral obesity, which may lower the adiponectin levels in smokers (Fasshauer et al., 2002; Halleux et al., 2001).

Thirdly, smoking may promote pro-inflammatory cytokines such as TNF-α and IL-6 that can inhibit adiponectin promoter activity, suppress adiponectin production (Bruun et al., 2003; Fernandez-Real et al., 2003; Matsuzawa et al., 2004; Helmersson et al., 2005), via nicotine binding to its functional nicotinic acetylcholine receptor (Liu et al., 2004). In addition, increased oxidative stress (Soares et al., 2005), anti-estrogenic effect (Toth et al., 2000) and sympathetic nerve activation (Nowak et al., 2005) occurring in smokers have also been speculated.

As mentioned earlier in this chapter, HOMA-IR and TC/HDL-C ratio were significantly higher in obese subjects and Met-S subjects in present study. In addition, data from present study have shown that plasma IL-6 was positively regulated whereas adiponectin was negatively regulated both in obesity and Met-S. Obese subjects with Met-S also showed higher plasma IL-6 and lower plasma adiponectin than obese subjects without Met-S.

In this study, HOMA-IR was significantly positive correlated with obesity and body fat indices, other glucose related indices, TC, TG and TC/HDL-C; negatively correlated with SM and HDL-C. Meanwhile, TC/HDL-C ratio was found to be positively correlated with all the measurements includes obesity indices, BP, glucose related indices, TC, TG, LDL-C; negatively correlated with SM, HDL-C and adiponectin after adjusted for ethnicity. Overall, these findings were well iterated by previous studies

(Grundy, 2004; Vega et al., 2006; Grundy et al., 2008; Povel et al., 2011; Wedin et al., 2012; Grundy et al., 2013; Singh et al., 2013).

Correlation analysis had found that IL-6 shown significant positive correlation with obesity indices and negatively correlated with SM after adjustment for ethnicity. However, current study did not observe any significant correlation between IL-6 and BP, glucose and lipid parameters. Its significant correlation with plasma adiponectin (ethnicity-adjusted) was found to be diminished after gender-adjustment. Both abdominal subcutaneous and visceral depots are important in IL-6 secretion. Adipose tissue has been found to produce 10-35 % of IL-6 in individual at rest, and this production is elevated with increased adiposity (Mohamed-Ali et al., 1997). However, IL-6 secretion is found to be higher in visceral adipose tissue than subcutaneous adipose tissue (Fain et al., 2004; Fried et al., 1998). IL-6 has strong association with insulin resistance and provides a possible link between obesity and Met-S. IL-6 is known to increase hepatic triglyceride secretion, decrease lipoprotein lipase activity and promote lipolysis, indicating that it may contribute to insulin resistance via an increase in circulating free fatty acids (Kern et al., 2001).

Correlation analysis of adiponectin has revealed that it is statistically significant negative correlation with obesity indices, DBP, glucose related indices, TG and TC/HDL-C ratio except with HDL-C which is positively correlated after adjusted for gender. Plasma adiponectin level is reported to be negatively associated with characteristic features of obesity and Met-S such as

obesity indices, DBP, adiposity, insulin resistance and other inflammatory cytokines in many previous studies (Arita et al., 1999; Matsuzawa et al., 1999; Kern et al., 2001; Yang et al., 2001; Bruun et al., 2003; Gable et al., 2006; Hung et al., 2008). Obesity induced insulin resistance has been attributed to hyperinsulinemia, release of inflammatory cytokines and decrease in the levels of circulating adiponectin (Kadowaki et al., 2006; Prakash et al., 2012). In many previous studies, adiponectin levels has been reported to be negatively correlated with several anthropometric measurements which include BMI (Hotta et al., 2000; Matsubara et al., 2002b; Ruige et al., 2005; Yamamoto et al., 2002), RM (Ruige et al., 2005), TBF (Ruige et al., 2005), SF, (Ruige et al., 2005),VFL (Ruige et al., 2005) and BP (Yamamoto et al., 2002). As for glucose measurement parameters, negative correlation was found in many other studies such as FBG (Eglit et al., 2013b; Hotta et al., 2000; Yamamoto et al., 2002) and FI (Abbasi et al., 2004; Hotta et al., 2000; Matsubara et al., 2002b; Yamamoto et al., 2002; Yamauchi et al., 2001). Other than that, HOMA-IR (Abbasi et al., 2004; Eglit et al., 2013b; Matsubara et al., 2002b; Ruige et al., 2005; Yamamoto et al., 2002; Yamauchi et al., 2001) and IL-6 (Hung et al., 2008) were negatively correlated in some studies. In addition, there are also negative correlation between adiponectin and several parameters of lipid profile such as TC (Yamamoto et al., 2002), TG (Eglit et al., 2013b; Hotta et al., 2000; Matsubara et al., 2002a; Ruige et al., 2005; Yamamoto et al., 2002), LDL-C (Yamamoto et al., 2002), but positively with plasma HDL-C (Hotta et al., 2000; Matsubara et al., 2002a).

5.6 Relationship between Genotype and Allele Combinations with Anthropometric and Biochemical Variables

When subjects with homozygous wild type for all the three SNPs were clustered together to compared with those with at least one mutant allele, no association was observed for the anthropometric and biochemical measurements. These findings had reflected the polygenic paradigm of obesity and Met-S, and complex modulation of gene-gene interaction and gene-environment interactions, ruling out the biases such as sampling standards, different phenotypic definition and ethnic differences.

The analysis of allele combination of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C with HDL-C level was found to have significant highest mean value for VTT allele combination and significant lowest level for VCT allele combination. This result suggests that singly PPARs mutant allele may not exert any dominant observable effect when standing alone, as in the case of mutant allele *PPARα* V162 on the HDL-C level, but may achieve suggestive observation under synergistic or additive effect when present in combination. However, due to the norm of relatively low MAF for *PPARα* V162 allele in this study population and other general populations as discussed earlier, the power needed to detect additional variants in combination with the presence of *PPARα* V162 allele will therefore be reduced. Further studies are thus required to address this concern in order to rule out the possibility of chance finding.

5.7 Limitations and Future Studies

The search of for SNPs of candidate genes implicated in obesity and Met-S has always been challenging. The overall sample size of this study is deemed adequate via the Raosoft® Sample Size Calculator Software calculated based on the whole Kampar population. However, the power of sample size was apparently insufficient especially for the *PPARα* L162V SNP (MAF<0.1) in the attempt to study the possible ethnicity effects on the influence of association between the three PPAR SNPs and obesity and Met-S. As such, the finding of the results on the stratified analysis by ethnicity had to be interpreted with caution and was therefore cannot be further elaborated.

As our subjects were recruited from health clinic, the findings on the SNPs prevalence may not be fully representative of the general population. Besides, the increased awareness of nutrition issues and concern over body weight among the respondents may result in the consciously or sub-consciously mis-reporting of their dietary habits and physical activity.

In addition, this study did not investigate into details the dietary habit (dietary fat and drugs intake) of the recruited subjects which may interfere with results of current study. For instance, the effect of *PPARα* L162V SNP has been suggested to be modulated via gene-drug and gene nutrient interactions (Bosse et al., 2002; Brisson et al., 2002; Fu et al., 2003; Tai et al., 2005). As the dietary PUFA intake and drug prescription (hypolipidemic fibrates) investigation were not included in the evaluation of this study, their

possible interaction with the SNPs in influencing the phenotypic outcome of obesity and Met-S is still currently unknown.

Overall, it is noteworthy that lifestyle, drugs, and dietary modifications may influence the activation of the *PPARs* gene, and diet can have long-lasting effects on PPAR gene expression as in epigenetic effects through parental exposures (Contreras et al., 2013), which are however beyond the scope of current study and thus may be considered in future studies.

CHAPTER 6

CONCLUSIONS

The present study revealed that obesity and Met-S are indeed public health challenges of our nations, with prevalence as high as 58.6 % and 81.1 %, respectively in current study though with subjects recruited in one of the sub-urban area of Perak state, Kampar. From the recruited subjects whose socio-demographic characteristics were well matched with that of the 2010 Population and Housing Census of Malaysia, the prevalence of obesity was found to be highest in Malays followed by Indians and Chinese whereas the reverse trend was found for Met-S. As the survey of all the dietary habits and lifestyle factors did not appear to show direct association with obesity and Met-S, thus they were not included as confounding variables in adjusted analysis. Thus, these have once again indicated the complex etiology of both the obesity and Met-S, referred to as multi-factorial traits, instead of an isolated factor that solely decides their development, whilst these can be further complicated by gene modifying factors, so called the gene-environmental and gene-gene interactions.

Overall, *PPARα* L162V, PPAR*γ2* C161T and *PPARδ* T294C SNPs are not the major genetic determinants of obesity and Met-S in the multi-ethnic Malaysian population. For the first time, this study had assessed the association of obese with/without Met-S with the three PPAR SNPs. Lack of association was found between the three SNPs and obesity, Met-S and obese

with Met-S status when subjects were analyzed as a whole study population or stratified by ethnic groups. Logistic regression analysis also revealed that subjects with the minor alleles for the three *PPAR* SNPs did not have a significantly higher odds ratio to develop obesity, Met-S or obesity with Met-S.

The MAF of all the three SNPs of current study were comparable with some populations. The MAF of *PPARα* L162V, *PPARγ2* C161T and *PPARδ* T294C were 0.08, 0.22 and 0.30, respectively, and did not deviate from the Hardy-Weinberg equilibrium. The allele distribution of all the SNPs was also not significantly different among gender and ethnicities, except for the allele distribution of *PPARγ2* C161T, with significantly lower MAF among Indians observed. Even though the elaboration on the stratified analysis of the association of the three PPAR SNPs with obese, Met-S and obese with Met-S groups was omitted due to insufficient power of sample size, the obtained data may however help in providing clues for future studies on the insight of the possible pattern of ethnicity differences in allelic frequencies and their contribution to the obesity and Met-S.

The means for anthropometric and clinical measurements were not significantly different among the alleles of the three PPAR SNPs, except for IL-6 and HOMA-IR, which were significantly higher in subjects with *PPARα* V162 allele and *PPARγ2* C161 allele, respectively. The significantly higher plasma IL-6 level among *PPARα* V162 allele carriers suggests exhibition of lower anti-inflammatory activity for *PPARα* V162 variants. Meanwhile

subjects with *PPARγ2* T161 allele may confer some protective effect on insulin sensitivity, rendering significantly lower HOMA-IR.

The current study demonstrates that all the three PPARs SNPs genotype and allele in combinations did not pose major effects on the anthropometric and biochemical measurements related to the obesity and Met-S as a whole, suggesting no obvious synergistic effects of the three PPARs SNPs on most of the parameters except for HDL-C level. It is important to note that the polygenic paradigm of obesity and Met-S rely upon dissection of many small effects of multiple genetic variants or SNPs. Through non-exhaustive investigation and continued refinement studies via stratified analysis involving as many possible factors, the underlying genetic and molecular basis of obesity and Met-S can be better understood, allowing better prognosis and therapeutic intervention of the patients.

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APPENDIX A

(i) Approval letter from the Medical Research and Ethics Committee

PEJABAT TIMBALAN KETUA PENGARAH KESIHATAN
OFFICE OF THE DEPUTY DIRECTOR-GENERAL OF HEALTH
(PENYELIDIKAN & SOKONGAN TEKNIKAL)
(RESEARCH & TECHNICAL SUPPORT)
KEMENTERIAN KESIHATAN MALAYSIA
MINISTRY OF HEALTH MALAYSIA
Aras 12, Blok E7, Parsel E, Presint 1
Level 12, Block E7, Parcel E, Precinct 1
Pusat Pentadbiran Kerajaan Persekutuan
Federal Government Administrative Centre
62590 PUTRAJAYA

Tel : 03 88832543
Faks : 03 88895184

JAWATANKUASA ETIKA & PENYELIDIKAN PERUBATAN
KEMENTERIAN KESIHATAN MALAYSIA
d/a Institut Pengurusan Kesihatan
Jalan Rumah Sakit, Bangsar
59000 Kuala Lumpur

Ruj. Kami : (2) dlm.KKM/NIHSEC/08/0804/P09-

Tarikh : 31 Disember 2009

Dr Say Yee How
Jabatan Sains
Universiti Tunku Abdul Rahman Kuala Lumpur

Tuan,

NMRR-09-826-4686
Association Study of Common Gene Polymorphisms affecting Food Intake, Energy Balance and Adipogenesis with Metabolic Syndrome and Obesity among Malaysians

Lokasi projek : Klinik Kesihatan Kampar/ Klinik Kesihatan Malim Nawar/ Klinik Kesihatan Gunung Rapat

Dengan hormatnya perkara di atas adalah dirujuk.

2. Jawatankuasa Etika & Penyelidikan Perubatan (JEPP), Kementerian Kesihatan Malaysia (KKM) mengambil maklum bahawa projek tersebut adalah untuk memenuhi keperluan akademik Ijazah Sarjana Sains, Universiti Tunku Abdul Rahman.

3. Sehubungan dengan ini, dimaklumkan bahawa pihak JEPP, KKM tiada halangan, dari segi etika, ke atas pelaksanaan projek ini. JEPP mengambil maklum bahawa kajian tersebut tidak mempunyai intervensi klinikal ke atas subjek dan segala prosedur pengambilan darah sebanyak 5ml adalah berisiko rendah ke atas subjek serta menggunakan borang soal selidik untuk mengumpul data kajian. Segala rekod dan data subjek adalah SULIT dan hanya digunakan untuk tujuan kajian dan semua isu serta prosedur mengenai *data confidentiality* mesti dipatuhi. Kebenaran daripada Pengarah hospital di mana kajian akan dijalankan mesti diperolehi terlebih dahulu sebelum kajian dijalankan. Tuan perlu akur dan mematuhi keputusan tersebut.

4. Laporan tamat kajian dan sebarang penerbitan dari kajian ini hendaklah dikemukakan kepada Jawatankuasa Etika & Penyelidikan Perubatan selepas tamatnya kajian ini.

Sekian terima kasih.

BERKHIDMAT UNTUK NEGARA

Saya yang menurut perintah,

(DATO' DR CHANG KIAN MENG)
Pengerusi
Jawatankuasa Etika & Penyelidikan Perubatan
Kementerian Kesihatan Malaysia

(ii) Permission letter from the Kinta Health District Office

PEJABAT KESIHATAN KINTA
JALAN AMAN
31000 BATU GAJAH
PERAK DARUL RIDZUAN

Tel : **05-3652077**
05-3652062
: **05-3668070**
05-3668075 – Peg.Kes. Daerah
Fax : **05-3668073**
Email : **pkkinta@po.jaring.my**

Ruj. Kami : Bil. (29) dlm. PK. Kta. 001/109 Jld. 1

Tarikh : 7 APRIL, 2010

Pegawai Perubatan Pentadbir y/m
Klinik Kesihatan **KAMPAR**

Pegawai Perubatan dan Kesihatan y/m
Klinik Kesihatan **GOPENG**

Tuan

PERMOHONAN KEBENARAN UNTUK MENJALANKAN KAJIAN SOAL SELIDIK DAN PENGUMPULAN SAMPEL DARAH

Dengan segala hormatnya merujuk kepada perkara tersebut di atas.

2. Bersama-sama ini dikepilkan surat permohonan menjalankan kajian, serta surat kelulusan daripada Jawatankuasa Etika & Penyelidikan Perubatan (JEPP) dan jadual pengambilan darah dan soalselidik.

3. Pejabat ini tiada halangan untuk membenarkan kajian ini dijalankan dan pengambilan darah seperti jadual. Kerjasama di pohon daripada tuan/puan untuk memberi sokongan kepada projek ini tanpa menganggu kerja harian di klinik.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

"PENYAYANG, PROFESIONALISMA DAN BEKERJA BERPASUKAN ADALAH BUDAYA KERJA KITA"

Saya yang menurut perintah,

(DR. S. ELANGOVAN AMN, AMP)
Pakar Perubatan (Kesihatan Awam)
Pegawai Kesihatan UD52
b.p Pegawai Kesihatan Daerah
Pejabat Kesihatan Kinta
Batu Gajah

s.k : i) Dr. Say Yee How
Penolong Professor
UTAR KualaLumpur

ii) Cik Fan Sook Ha
Penyelidik Jurusan Sarjana Sains,
UTAR Kampar

(Sila catatkan rujukan jabatan ini apabila berhubung)

(iii) Approval of extension of study by IRB/IEC Medical Research Ethnics Committee (MREC)

Submission Status & History

NMRR ID	NMRR-09-826-4686
Research ID	4686
Approval Authority	IRB/IEC Medical Research Ethics Committee (MREC)
Research Title	Association Study of Common Gene Polymorphisms Affecting Food Intake, Energy Balance and Adipogenesis with Metabolic Syndrome and Obesity Among Malaysians.
Research Title Abbreviation	Genetic Markers for Obesity and Metabolic Syndrome Among Malaysians
Correspondence Person	Say Yee How

Submission Status & History List (from 10 April 2010 onwards)- IRB/IEC Medical Research Ethics Committee (MREC)

No.	Audit Date	Submission Status Date	Submission Status	Decision Date	Decision Status	Submission No	Revision No	Amendment No	Amendment Decision Date	Amendment Decision	Renewal Decision Date	Renewal Decision	Closure Decision Date	Closure Decision	Remarks
1		11-01-2010 16:07:26	Has final decision	31-12-2009	Approve, unconditional (with exemption from MREC full board review)	S1	R0								
2		08-02-2011 10:19:55	Amendment request	31-12-2009	Approved research (with exemption from MREC full board review); Pending amendment	S1	R0	A0							
3		10-02-2011 09:07:43	Amendment need completion; Request by CP	31-12-2009	Approved research (with exemption from MREC full board review); Pending amendment	S1	R0	A0							
4		14-02-2011 14:20:51	Has final decision	31-12-2009	Approve, unconditional (with exemption from MREC full board review)	S1	R0	A0							
5	01-03-2015 04:48:06			31-12-2009	Approve, unconditional (with exemption from MREC full board review)	S1	R0	A0							System Update - 01/03/2015 04:48

Decision History List (before 10 April 2010)

Approval Authority	Decision Date	Decision Status
IRB/IEC Medical Research Ethics Committee (MREC)	31-12-2009 00:00:00	
IRB/IEC Medical Research Ethics Committee (MREC)	31-12-2009 00:00:00	Approve, unconditional (with exemption from MREC full board review)
Research Registration	30-10-2009 11:30:19	
Research Registration	30-10-2009 11:30:19	Registered

Submission History List (before 10 April 2010)

Approval Authority	Date Submitted	Submission By
IRB/IEC Medical Research Ethics Committee (MREC)	30-10-2009 12:03:21	Say Yee How
Research Registration	30-10-2009 11:30:19	Say Yee How

Re-submission History

Re-submission	☐
Re-submission Reason	Extension of study period from 1st March 2011-31st December 2011
Date Request Re-submission	08-02-2011 10:19:55
Re-submission By	Say Yee How
Approve Re-submission Date	10-02-2011 09:07:43
Approve Re-submission By	MRECSEC

APPENDIX B

(i) Research information and consent form (English version)

Research Information and Consent Form

Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Barat
31900 Kampar, Perak.

Subject ID Number

DRAWING BLOOD FOR THE RESEARCH STUDY OF GENETIC MARKERS FOR OBESITY

PURPOSE:	We would like your permission to enroll you as a participant in a research study to identify **potential markers for obesity among Malaysians**. Scientists have found that there are genetics markers for obesity in our body which control how much food we take in, how much energy we use and how much fat we have in our body. These genetic markers may have been inherited from your parents and can be passed down to your children. This study involves the detection of these genetic markers of obesity in your body by analyzing your blood sample.
PROCEDURE:	**You will have to be fasted overnight in order to take part in this study**. A brief medical history will be taken in order to identify whether you are suitable as a subject or not in our research. After that, you will have to answer a series of questions in a questionnaire to assess your lifestyle and dietary habits. We will then take your body measurements, which include your height, weight, waist and hip circumferences. You will then be asked to step on a scale which will measure your Body Mass Index, Body Fat Percentage, Subcutaneous Fat Percentage, Visceral Fat Percentage, Resting Metabolism Rate and Skeletal Muscle Percentage. After you have rested for 5 minutes, we will then take your blood pressure. Finally, a small amount of blood will be taken from your vein by inserting a sterile needle and withdrawing up to 5ml of blood. We will make sure you are feeling well after your blood is drawn.
PAYMENT:	You will not be paid for your time and for providing your blood sample. Your blood sample may be used to create new tests or treatments that could have commercial value. Researchers or the university may benefit financially if this happens. You will not be paid or benefit financially of this happens.
RISKS:	You may feel a little pain when your blood is withdrawn. In addition, a temporary bruise or 'black and blue mark' may develop. In *very* rare cases, the vein in which the needle has been inserted may become inflamed or infected, which can be treated.
BENEFITS:	Collection of your blood will not provide any direct benefit to you. However, you will be informed and get to keep all the data of body measurements as mentioned above, which may be indicative of your health and fitness status.
CONFIDENTIALITY:	Information obtained from this research study will be stored in the researcher's files and will be identified only by a number. Your name or other information that could be used to identify you will not be recorded with or linked to the sample or health information. In short, all the information provided by you and all the results obtained will be kept confidential and anonymous by the interviewer and the researcher, and will be destroyed after the end of the study.
ALTERNATIVES:	This research study does not involve any treatment or diagnosis. The alternative to participation is not to participate.
STUDY CONTACTS:	You can contact **Dr. Say Yee How (016-5342882)** or **Ms. Chia Phee Phee (012-8926559)** if you have any queries.
SIGNATURE:	I have read this consent form and understood the purpose of this research, the study procedures, possible risks and discomforts, as well as potential benefit and alternatives. My signature below indicates my willingness to participate in this study.
	Name: Date: Signature:

(ii) Research information and consent form (Malay version)

Maklumat Kajian dan Borang Pengizinan

Faculti Sains
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Barat
31900 Kampar, Perak.

Nombor ID Subjek

PENGUMPULAN SAMPEL DARAH UNTUK KAJIAN PETANDA GENETIK UNTUK OBESITI

TUJUAN:	Kami ingin memohon kebenaran anda untuk menjemput anda sebagai peserta dalam satu kajian untuk menentukan petanda-petanda genetik untuk obesiti (kegemukan) di kalangan rakyat Malaysia. Saintis telah medapati bahawa petanda-petanda genetik untuk obesity dalam badan kita menentukan berapa banyakkah kita makan, berapa tenaga yang kita guna dan berapa banyakkah lemak di dalam kita. Petanda-petanda genetik ini mungkin telah diperturunkan daripada ibubapa anda, dan boleh diwariskan kepada anak-anak anda. Kajian ini melibatkan penentuan petanda-petanda genetik untuk obesiti di dalam badan anda dengan mengkaji sampel darah anda.
ATURCARA:	**Anda mesti perlu telah berpuasa semalaman untuk mengambil bahagian dalam kajian ini.** Sejarah perubatan ringkas anda juga akan diambil untuk menentukan sama ada anda sesuai sebagai subjek atau tidak dalam kajian ini. Selepas itu, anda perlu menjawab satu siri soalan dalam borang soal-selidik untuk menilai cara hidup dan pemakanan anda. Kami juga akan mengambil ukuran badan anda, termasuk tinggi, berat, lilitan pinggang dan pinggul anda. Kemudian, anda akan dipanggil untuk berdiri di atas satu penimbang yang akan mengukur Index Jisim Tubuh, Peratusan Jumlah Lemak, Peratusan Lemak Bawah Kulit, Peratusan Lemak Dalam Badan, Kadar Metabolisma Asas dan Peratusan Otot Rangka. Selepas berehat selama 5 minit, kami akan mengukur tekanan darah anda. Akhirnya, kami akan mengambil sedikit sampel darah anda dengan mencucuk satu jarum yang bersih dan menyedut sehingga 5ml darah anda. Kami akan memastikan anda berasa sihat selesa selepas darah anda diambil.
BAYARAN:	Anda tidak akan dibayar untuk masa dan sampel darah anda. Sampel darah anda mungkin diguna untuk mencipta ujian atau rawatan baru yang mungkin mempunyai nilai komersial. Pengkaji atau Universiti mungkin memperoleh faedah kewangan sekiranya ia berlaku. Anda tidak akan dibayar atau memperoleh faedah kewangan sekiranya ia berlaku.
RISIKO:	Anda mungkin akan merasa kesakitan yang amat kecil apabila darah akan disedut. Juga, satu lembam atau tanda "hitam dan biru" mungkin juga akan timbul. Dalam keadaan yang *amat* jarang, saluran darah di mana jarum itu dicucuk, mungkin menjadi benjol atau bernanah, di mana ianya boleh dirawati.
FAEDAH:	Pengumpulan sampel darah anda tidak akan membawa sebarang faedah secara langsung kepada anda. Namun begitu, anda akan dimaklumkan dan anda boleh menyimpan maklumat ukuran badan anda seperti di atas. Maklumat ini mungkin memberi indikasi status kesihatan anda.
KERAHSIAAN:	Maklumat yang diperolehi dari kajian ini akan disimpan dalam fail pengkaji dan hanya akan dikenalpasti secara nombor. Nama anda atau sebarang maklumat yang boleh digunakan untuk mengenalpasti anda tidak akan dicatatkan atau dikaitkan dengan sampel atau maklumat kesihatan. Pendek kata, segala maklumat yang diberi anda dan keputusan yang diperolehi akan dirahsiakan dan dihilangkan identitinya oleh penemuramah dan pengkaji, dan akan dimusnahkan selepas kajian ini tamat.
ALTERNATIF:	Kajian ini tidak melibatkan sebarang rawatan atau diagnosis. Alternatif untuk penyertaan adalah untuk tidak menyertai.
HUBUNGI:	Anda boleh menghubungi **Dr. Say Yee How (016-5342882)** atau **Ms. Chia Phee Phee (012-8926559)** sekiranya anda mempunyai sebarang pertanyaan.
TANDATANGAN:	Saya telah membaca borang pengizinan dan telah memahami tujuan kajian ini, aturcara kajian ini, risiko berpotensi dan ketidakselesaan, juga faedah berkemungkinan dan alternatif. Tandatangan saya di bawah menandakan kesudian saya untuk menyertai kajian ini.
	Nama: Tarikh: Tandatangan:

(iii) Research information and consent form (Chinese version)

研究资料和同意书

Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Barat
31900 Kampar, Perak.

参与者登记号码

制定促进肥胖的遗传标记物研究性学习的抽血活动

目的：	我们希望您允许被注册作为一项以确定**马来西亚人肥胖的潜在标记**研究性学习的参与者。科学家们发现我们身体内的肥胖遗传标记能控制我们的食物摄入量，能源使用和脂肪水平。这些遗传标记可能是遗传自父母，也可传给您的孩子。这项研究涉及到通过您的血液样本分析而检测您体内的肥胖遗传标志物。
程序：	**你将必须隔夜禁食，以参与这项研究。**我们将采取您的简要病历以确认您是否适合我们的研究。之后，你必须回答问卷中的一系列问题，以评估你的生活方式和饮食习惯。然后，我们将采取你的身体测量，其中包括你的身高，体重，腰围和臀围。你将被要求站在衡量器上，测量你的身体质量指数，身体脂肪，皮下脂肪百分比，内脏脂肪百分比，静止代谢率和骨骼肌的百分比。当你休息 5 分钟后，我们将会为您量血压。最后，我们会从你的静脉抽取少量的血液。我们会插入消毒针头， 抽取 5 毫升血液。我们将会确保你在被抽取血液之后没有感觉不适。
付款方式：	您将不会受支付于您所提供的时间和血液样本。您的血液样本可能会被用于创建可能具有商业价值的新测验和治疗。如果发生这种情况，该大学或研究人员可能会得经济利益。于这种情况，您将无法受支付或得经济利益。
风险：	在抽血的过程中，你可能会觉得有点痛。此外，可能会出现临时瘀伤或'黑色和蓝色的标记'。在极少数情况下，被插入了针的静脉可能会发炎或受感染，这是可以被治疗的。
优势：	对您进行采血将不会提供任何好处给您。但是，您将会被告知和存有如上所述所有人体测量的有关数据。 这些数据可能是您的健康和健身状况的指示。
保密：	从这项研究取得的资料将被存储在研究人员的文件及将只能一个数字号码辨识。您的姓名或其他可用于识别您身份的信息将不会和该样本或健康信息一起记录。总之，所有您的资料和所有所取得的成果将会被采访者和研究人员保密，并将于研究结束后被销毁。
备选方案：	本研究不涉及任何治疗或诊断。备选方案是可以选择不参加。
研究联系人：	如果您有任何疑问，您可以联系 **Dr. Say Yee How（016-5342882）**或**Ms. Chia Phee Phee (012-8926559).**
签名：	我已阅读本同意书，了解了本研究的目的、研究程序、可能的风险和不适，以及潜在的利益和选择，以下是我的签名，以表示我愿意参加这项研究。
	姓名: 日期: 签名:

(iv) Brief medical history for the eligibity in this study

Research Information and Consent Form

Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Barat
31900 Kampar, Perak.

Subject ID Number

BRIEF MEDICAL HISTORY FOR THE ELIGIBITY IN THIS STUDY

Please mark 'X' to your answer:			
Do you have any of the following conditions:		*Yes*	*No*
1	Triglycerides (TGs) level >1.7mM		
2	High-density lipoprotein cholesterol (HDL-C) <1.0 mM for men and <1.3 mM for women		
3	Blood pressure ≥130/85 mmHg		
4	Currently being treated for hypertension		
5	Elevated fasting glucose >6.1 mM		
6	Currently being treated for diabetes		
7	Waist circumference >90cm for men and >80cm for women		
8	Hyperthyroidism		
9	Pituitary diseases		
10	Chronic liver disease		
11	Chronic renal disease		
12	Acute infection		
13	Haematologic diseases		
14	Taking glucocorticoids or other medications affecting glucose metabolism		

Maklumat Kajian dan Borang Pengizinan

Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Barat
31900 Kampar, Perak.

Nombor ID Subjek

SEJARAH PERUBATAN RINGKAS UNTUK PENENTUAN KESESUAIAN DALAM KAJIAN INI

Sila tanda 'X' untuk jawapan anda:			
Adakah anda mempunyai mana-mana keadaan berikut:		*Ya*	*Tidak*
1	Paras Triglycerides (TGs) >1.7mM		
2	Kolesterol 'High-density lipoprotein' (HDL-C) <1.0 mM untuk lelaki dan <1.3 mM untuk wanita		
3	Tekanan darah ≥130/85 mmHg		
4	Menjalani perubatan untuk tekanan darah tinggi		
5	Paras gula berpuasa >6.1 mM		
6	Menjalani perubatan untuk kencing manis		
7	Lilitan pinggang >90sm untuk lelaki dan >80sm untuk wanita		
8	'Hyperthyroidism'		
9	Penyakit kelenjar pituitary		
10	Penyakit hati berpanjangan		
11	Penyakit buah pinggang berpanjangan		
12	Jangkitan semasa		
13	Penyakit-penyakit darah		
14	Mengambil 'glucocorticoids' atau sebarang ubat yang boleh mengganggu metabolisma gula.		

研究资料和同意书

Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Barat
31900 Kampar, Perak.

参与者登记号码

通过这研究资格限额的简介医疗史

请于您的答案标记'X'：			
请问你有没有下列情况：		*是*	*否*
1	甘油三脂水平 >1.7mM		
2	高密度脂蛋白胆固醇<1.0mM（男性）和 <1.3mM（女性）		
3	血压≥130/85mmHg		
4	目前正在治疗的高血压患者		
5	高空腹血糖(>6.1mM)		
6	目前正在接受治疗的糖尿病		
7	腰围）90 厘米（男性）和）80 厘米（女性）		
8	甲状腺功能亢进症		
9	垂体疾病		
10	慢性肝病		
11	慢性肾脏疾病		
12	急性感染		
13	血液病		
14	摄取糖皮质激素或其他影响糖代谢的药物		

APPENDIX C

(i) Questionnaire (English version)

PART A: SOCIO-DEMOGRAPHIC DATA
Instruction: Please complete this part. Fill in the particulars or circle only one most relevant answer.

Please do not fill this part (reserved for data analysis)

1. **Name:**_____
2. **Age :**_____
3. **Sex:** [1] Male [2] Female
4. **Race:** [1] Malay [2] Chinese [3] Indian [4] Others
5. **Marital status:** [1] Single [2] Married [3] Divorced [4] Widowed
6. **Occupation:**
   [1] Professional [2] White-collar [3] Blue-collar [4] Retired/ not working [5] Own/Others [6] Student
7. **Monthly household income:**
   [1] below RM 1000 [2] RM 1001-3000 [3] RM 3001- 5000 [4] above RM 5000
8. **Educational status:**
   [1] No formal education [2] Primary level [3] Lower Secondary level
   [4] Upper secondary level [5] Pre-university level [6] University level
9. **Are any of your family members overweight or obese?** [1] Yes [2] No

PART B: DIETARY HABITS
Instruction: Please circle/tick the most relevant answer

10. **Do you like to take salty food?** [1] Yes [2] No
11. **Are you a strict vegetarian? (do not consume meat/animal origins)**
    [1] Yes [2] No
12. **Caffeine (coffee) intake**
    A. Do you drink coffee? [1] Yes [2] No/ Occasionally – **GO TO QUESTION 13**
    B. In average, how many cups of coffee do you drink in a day?
    [1] 1-2 cups [2] 3-4 cups [3] **≥ 5 cups**
13. **Do you eat fast food on a weekly basis?** [1] Yes [2] No

PART C: PHYSICAL ACTIVITY
Instruction: Please circle/tick the most relevant answer.

14. **Do you exercise? (excluding occupational and household physical movements):**
    [1] Yes [2] No/ Occasionally – **GO TO QUESTION 16**
15. **How frequent do you exercise in a week?**
    [1] 2-3 days a week [2] ≥ 3 days a week [3] Everyday

2.
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15.

PART D: SMOKING AND DRINKING HABITS

Instruction: Please circle/tick the most relevant answer and fill in the particulars.

16. Do you currently smoke cigarettes?

[1] Yes **[2]** Quitted/ Smoked before – **GO TO QUESTION 17**

[3] No – **GO TO QUESTION 19**

17. How long (years) have/had you been smoking cigarettes? _____

18. In average, how many sticks do/did you smoke in a day? _____

19. Do you currently drink alcohol?

[1] Yes **[2]** Stopped – **GO TO QUESTION 20**

[3] No **[4]** Social drinker – **GO TO QUESTION 22**

20. How long (years) have/had you been drinking? _____

21. In average, how many alcoholic drinks do/did you drink in a day? _____

16.
17.
18.
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21.

PART E: ANTHROPOMETRIC MEASUREMENTS

Instruction: To be filled in based on measurements taken.

22.

Measurement	1st reading	2nd reading	Average
SBP (mmHg)			
DBP (mmHg)			
Pulse rate (bpm)			
Waist circumference (cm)			
Hip circumference (cm)			
Height (cm)			
Weight (kg)			
BMI (kg/m^2)			
TBF (%)			
SF (%)			
VFL (%)			
RM (kcal)			
SM (%)			

END OF QUESTIONNAIRE. THANK YOU VERY MUCH FOR YOUR CO-OPERATION

(ii) Questionnaire (Malay version)

BAHAGIAN A: DATA SOSIODEMOGRAFIK

Arahan: Sila lengkapkan bahagian ini. Bulatkan jawapan yang paling sesuai atau isi tempat kosong

Jangan isi bahagian ini (dikhaskan untuk analisa data).

1. **Nama:**_____
2. **Umur:**_____
3. **Jantina:** [1] Lelaki [2] Perempuan
4. **Bangsa:** [1] Melayu [2] Cina [3] India [4] Lain-lain
5. **Taraf perkahwinan:** [1] Bujang [2] Berkahwin [3] Bercerai [4] Duda/ Balu
6. **Pekerjaan:**

   [1] Profesional [2] Kolar putih [3] Kolar biru [4] Tidak bekerja/bersara

   [5] Sendiri/Lain-lain [6] Pelajar
7. **Pendapatan isirumah sebulan:**

   [1] bawah RM 1000 [2] RM 1001-3000 [3] RM 3001- 5000 [4] melebihi RM 5000
8. **Taraf pendidikan**

   [1] Tiada pendidikan formal [2] Peringkat darjah [3] Tingkatan rendah

   [4] Tingkatan atas [5] Pra-universiti [6] Peringkat universiti
9. **Adakah mana-mana ahli keluarga anda ada masalah kegemukan?** [1]Ya [2]Tidak

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3.
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8.
9.

BAHAGIAN B: AMALAN PEMAKANAN

Arahan: Sila bulatkan jawapan yang paling sesuai

10. **Adakah anda suka mengambil makanan masin ?** [1] Ya [2] Tidak
11. **Adakah anda pengamal vegetarian? (tidak memakan daging/sumber haiwan)**

    [1] Ya [2] Tidak
12. **Pengambilan kaffein (kopi)**

A. Adakah anda meminum kopi? [1] Ya [2] Tidak/Sekali-sekala **–SILA KE SOALAN 13**

B. Secara purata, berapakah cawan kopi yang anda minum dalam sehari?

[1] 1-2 cawan [2] 3-4 cawan [3] ≥ 5 cawan

13. **Adakah anda memakan di restoran makanan segera setiap minggu?** [1] Ya [2] Tidak

10.
11.
12.
13.

BAHAGIAN C: AKTIVITI FIZIKAL

Arahan: Sila bulatkan jawapan yang paling sesuai.

14. **Adakah anda bersenam? (tidak termasuk pergerakan pekerjaan dan rumahtangga) :**

    [1] Ya [2] Tidak/Sekali-kala **– SILA KE SOALAN 16**

15. **Berapa kerapkah anda bersenam ?**

    [1] 2-3 hari seminggu [2] lebih daripada 3 hari seminggu [3] Setiap hari

14.
15.

BAHAGIAN D: AMALAN MEROKOK DAN MEMINUM ALKOHOL

Arahan: Sila bulatkan jawapan yang paling sesuai dan isi tempat kosong.

16.
17.
18.
19.
20.
21.

16. Adakah anda merokok sekarang ?

[1]Ya **[2]** Pernah sebelum ini **– SILA KE SOALAN 17**

[3] Tidak pernah **– SILA KE SOALAN 19**

17. Berapa lamakan (tahun) yang anda telah merokok? _____

18. Secara purata, berapakah batang rokok yang anda hisap sehari? _____

19. Adakah anda meminum alkohol sekarang ?

[1] Ya **[2]** Berhenti **– SILA KE SOALAN 20**

[3] Tidak pernah **[4]** Peminum sosial **– SILA KE SOALAN 22**

20. Berapa lamakah anda telah minum alkohol? _____tahun

21. Secara purata, berapakah minuman alkohol yang anda minum dalam sehari ?

_____

BAHAGIAN E: UKURAN ANTROPOMETRI

Arahan : Ukuran akan dibuat dan nilai-nilai akan diisi dalam bahagian ini

22.

Ukuran	Bacaan pertama	Bacaan kedua	Purata
SBP (mmHg)			
DBP (mmHg)			
Denyutan nadi (bpm)			
Lilitan pinggang (cm)			
Lilitan pinggul (cm)			
Tinggi (cm)			
Berat (kg)			
BMI (kg/m^2)			
TBF (%)			
SF (%)			
VFL (%)			
RM (kcal)			
SM (%)			

BORANG KAJI SELIDIK TAMAT. RIBUAN TERIMA KASIH DI ATAS KERJASAMA ANDA.

(iii) Questionnaire (Chinese version)

A 部分：社会人口统计资料
指示: 请完成这个部分, 将资料填写或圈出一个最关联的答案。

请别填写这部分（保留作资料分析用途）

1. **姓名：** _____
2. **年龄：** _____
3. **性别：** [1] 男 [2] 女
4. **种族：** [1] 马来人 [2] 华人 [3] 印度人 [4] 其他
5. **婚姻状况：** [1] 单身 [2] 已婚 [3] 离婚 [4] 寡居
6. **职业：** [1] 专业 [2] 白领 [3] 蓝领 [4] 退休/无工作 [5] 自己/其他 [6] 学生
7. **家庭月入：** [1] RM 1000 以下 [2] RM 1001 至 3000 [3] RM 3001 至 5000 [4] RM 5000 以上
8. **教育状况：**
   [1] 无正统教育 [2] 小学教育 [3] 初中教育 [4] 高中教育 [5] 大学先修课程 [6] 大学教育
9. **您的家族是否有肥胖历史?** [1] 是 [2] 否

2.
3.
4.
5
6.
7.
8.
9.

B 部分：饮食习惯
指示:请圈出一个最关联的答案

10. **您是否爱吃咸食?** [1] 是 [2] 否
11. **您是否是一个全素食者? (不食用肉类或动物性食品)** [1] 是 [2] 否
12. **咖啡因（咖啡）摄取量**
    A. **您是否喝咖啡?** [1] 是 [2] 否/偶尔 - **请回答第十三道问题**
    B. **您一天平均喝几杯咖啡?** [1] 一至两杯 [2] 三至四杯 [3] 超过五杯
13. **您一个星期是否会吃起码一次的快餐?** [1] 是 [2] 否

10.
11.
12.
13.

C 部分：体能活动
指示:请圈出一个最关联的答案。

14 **您是否有运动? (职业性与家务性质的活动除外):**
[1] 是 [2] 否/偶尔 - **请回答第十六道问题**

15. **您运动的频率是?** [1] 一个星期二至三次 [2] 一个星期超过三次 [3] 每天

14.
15.

D 部分: 吸烟习惯并且酒精摄取量

指示: 请圈出一个最关联的答案及将资料填写。

16.
17.
18.
19.
20.
21.

16. 您是否有吸烟的习惯?

[1] 是　　[2] 戒了/曾经吸过　**-请回答第十七道问题**

[3] 否　　**-请回答第十九道问题**

17. 您吸烟几年了? _____年

18. 您一天平均抽几枝烟?_____

19. 您是否有喝酒?

[1] 是　[2] 戒了　**- 请回答第二十道问题**

[3] 否　[4] 偶尔应酬才喝　**- 请回答第二十二道问题**

20. 您喝酒几年了? _____年

21. 您一天喝几杯酒? _____

E 部：人体测量

说明：要按照采取的测量填写。

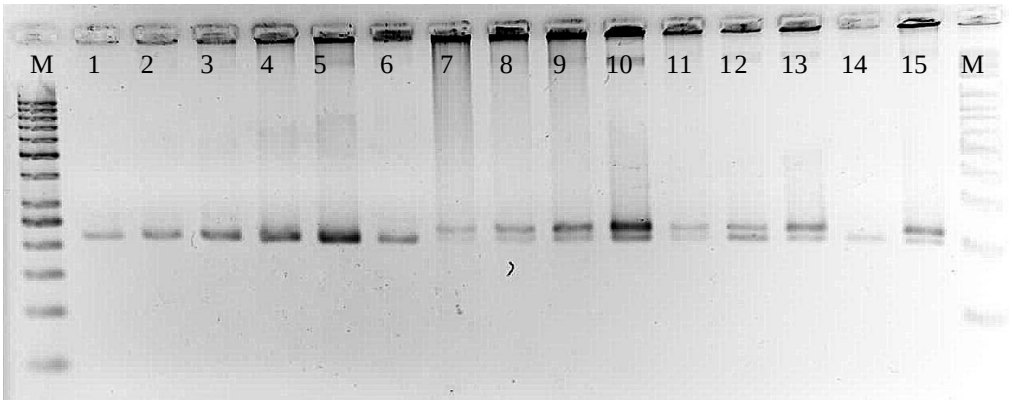
22.

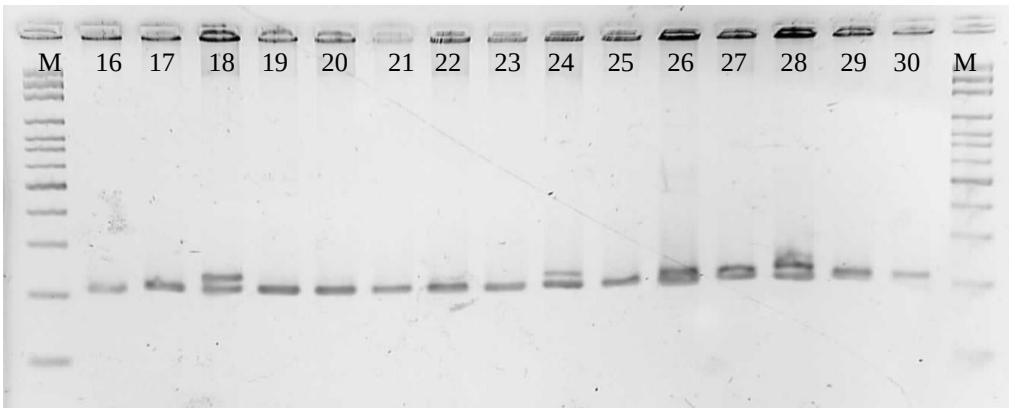
测量	数据 1	数据 2	平均
收缩压（毫米汞柱）			
舒张压（毫米汞柱）			
脉冲率（每分钟心跳）			
腰围（厘米）			
臀围（厘米）			
身高（厘米）			
重量(公斤)			
体重指数（公斤/平方米）			
全身脂肪（%）			
皮下脂肪(%)			
内脏脂肪 (%)			
静息代谢(千卡)			
骨骼肌 (%)			

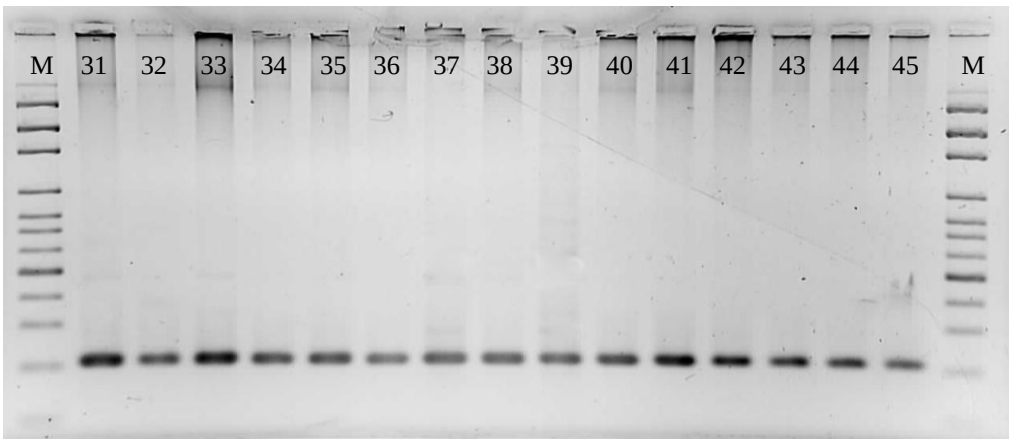
问卷完毕。非常感谢您配合

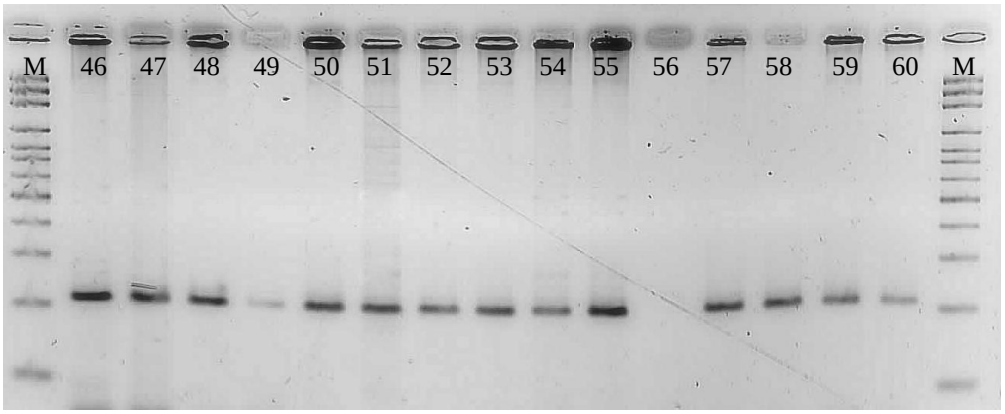
APPENDIX D

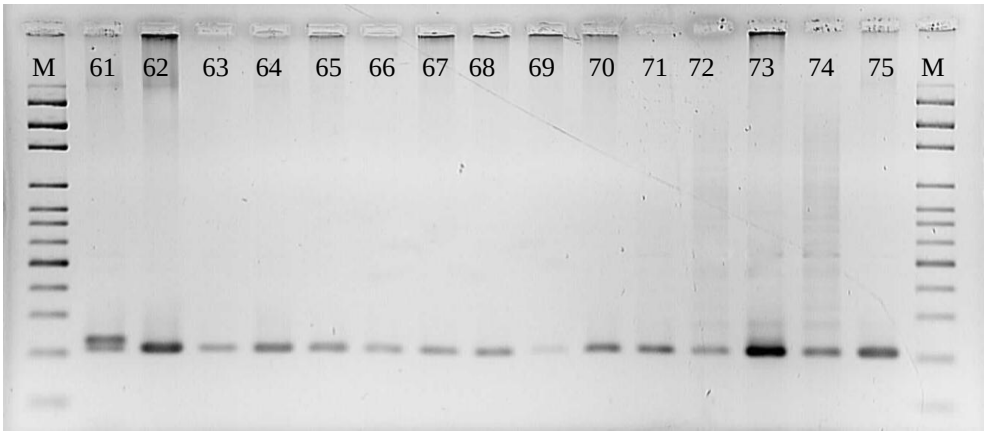
(i) RFLP products for *PPARα* L162V SNP

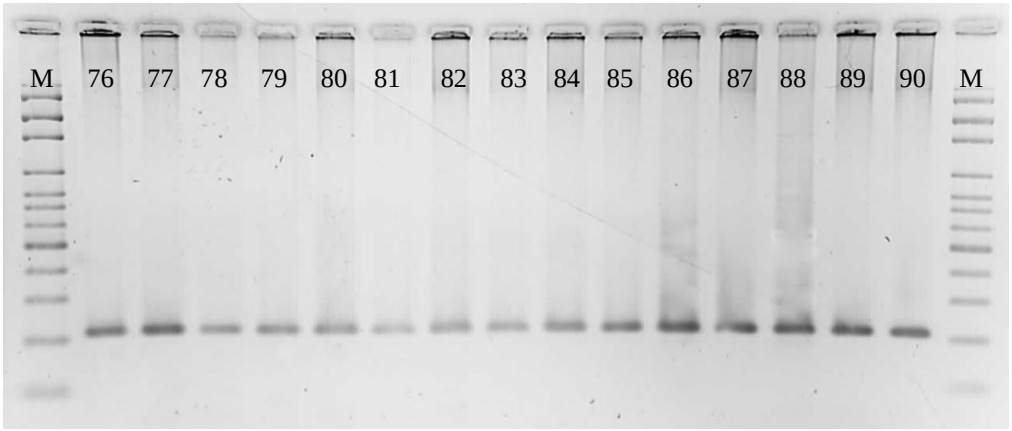


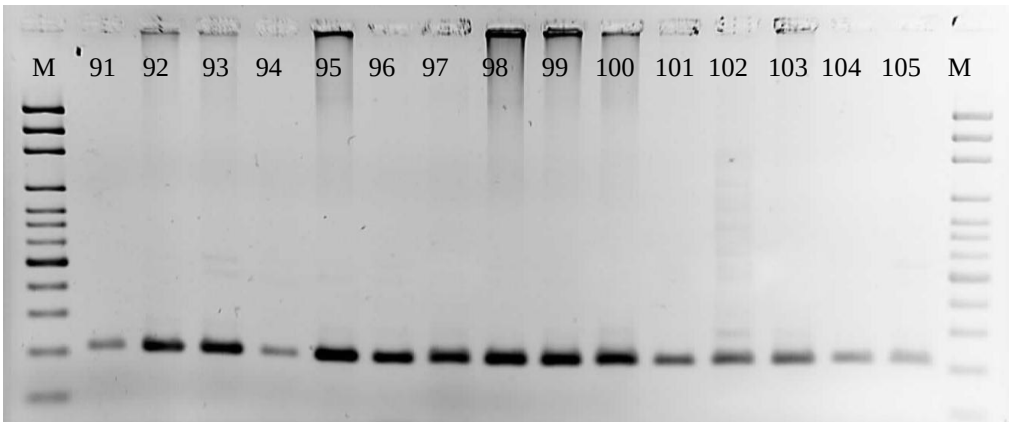


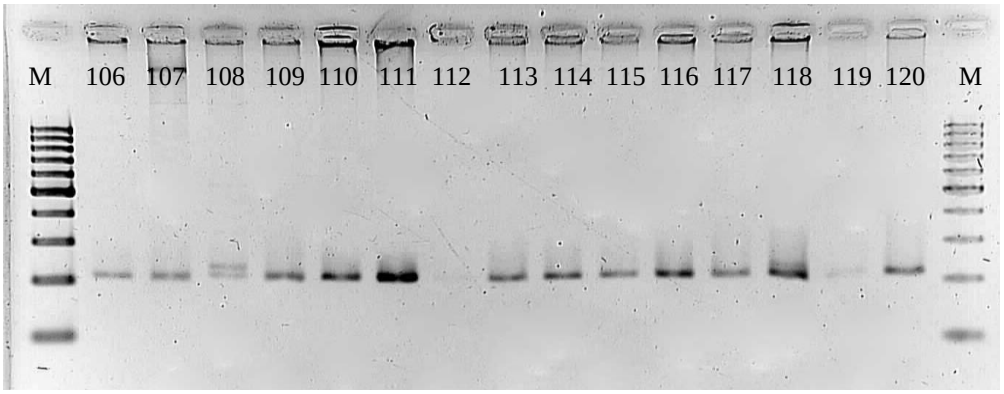


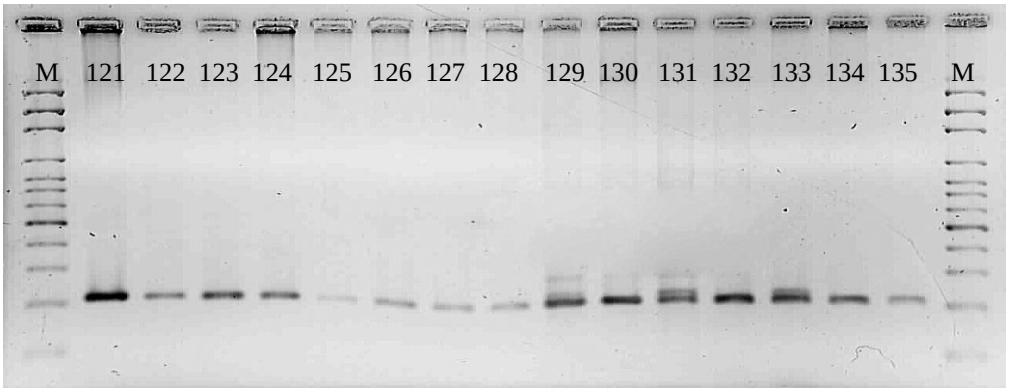

M 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 M

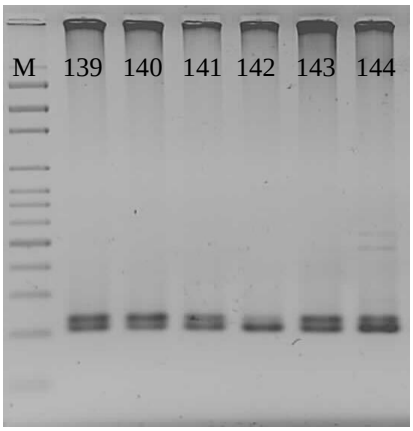

M 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 M

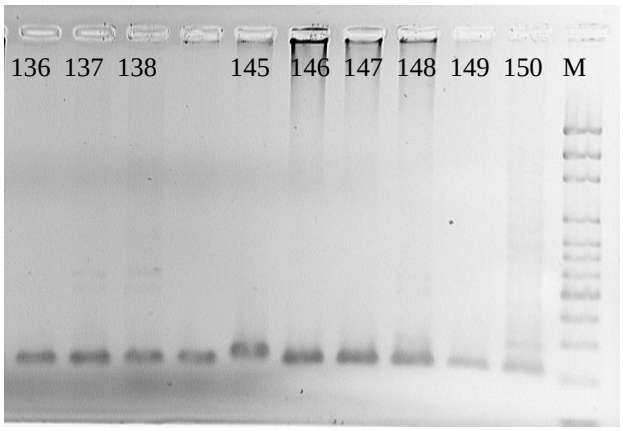

M 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 M

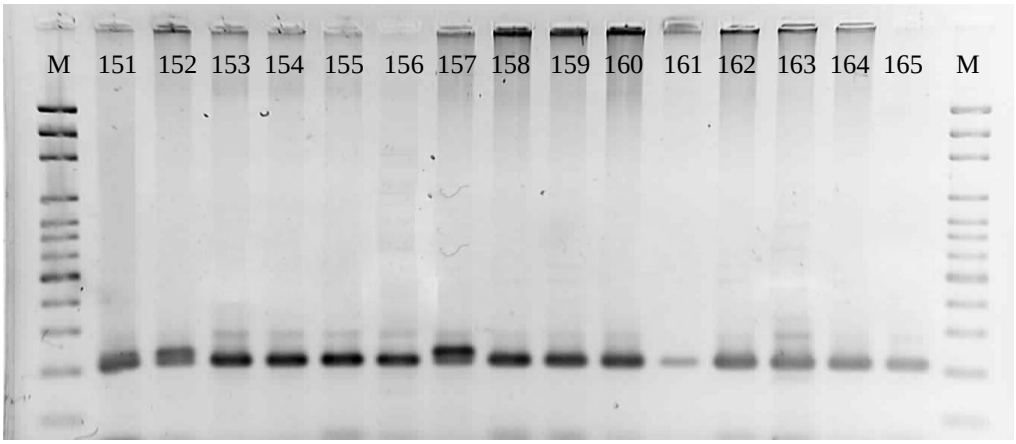

M 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 M

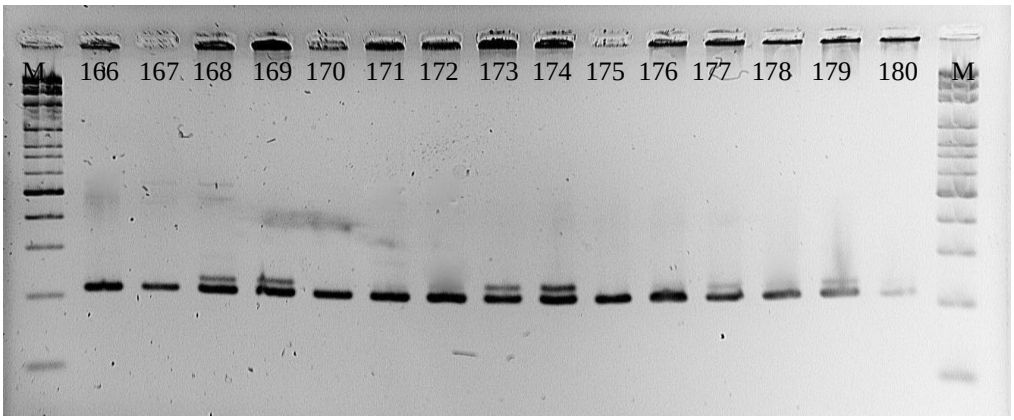

M 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 M

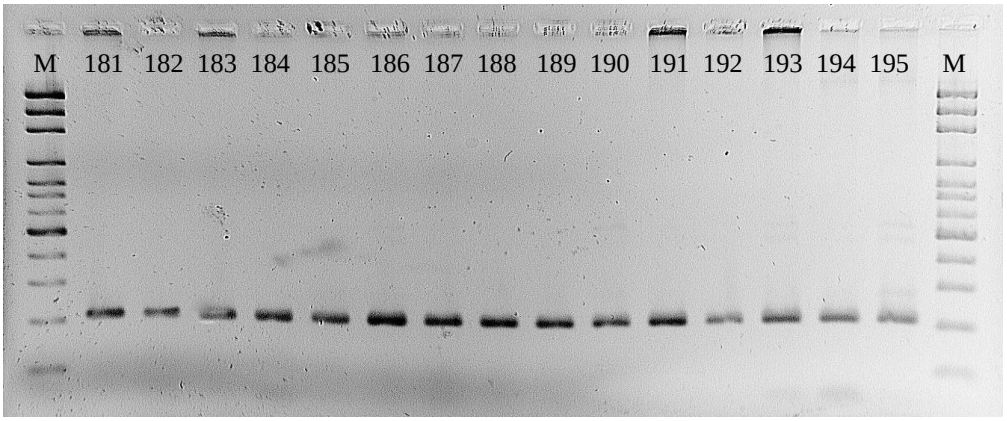

M 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 M


M 139 140 141 142 143 144

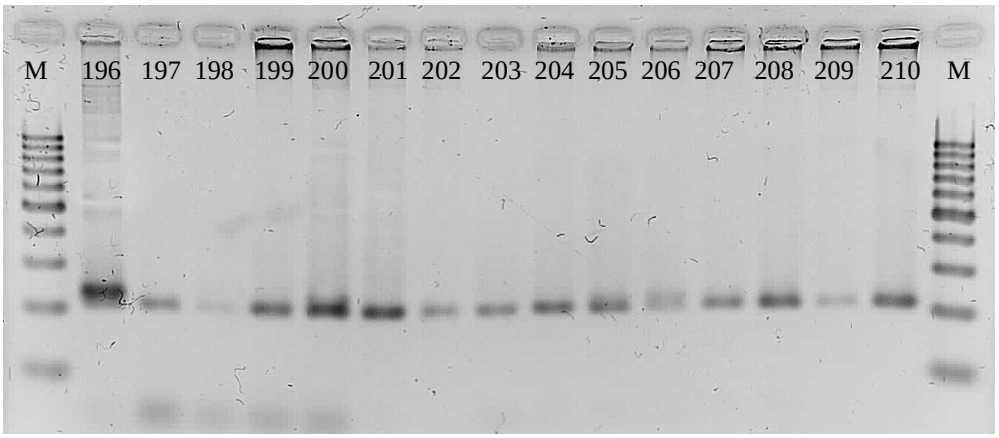

136 137 138 145 146 147 148 149 150 M

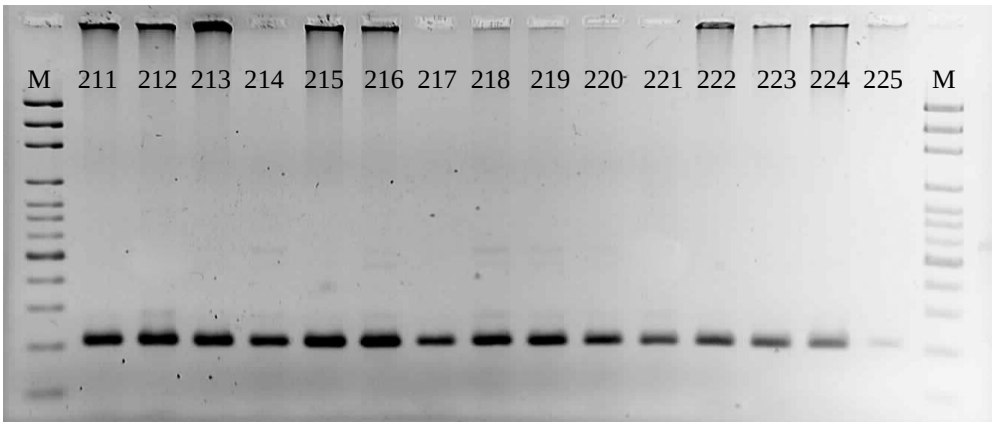

M 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 M

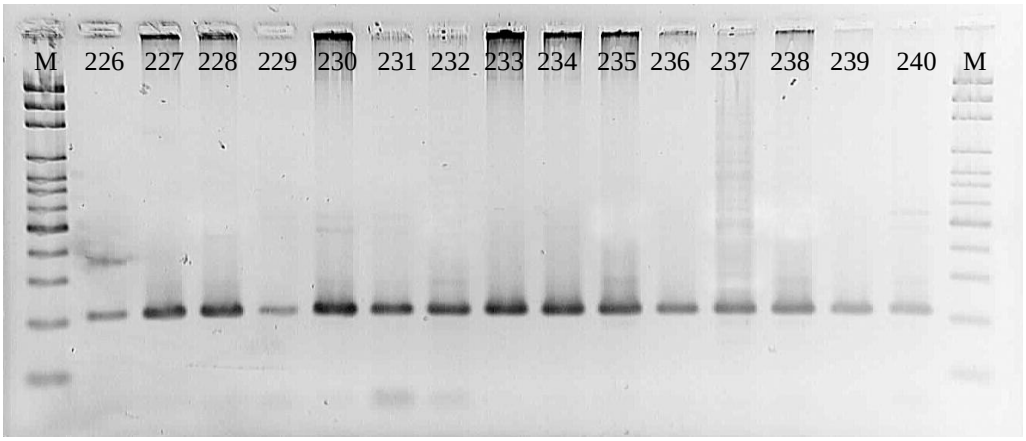

M 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 M

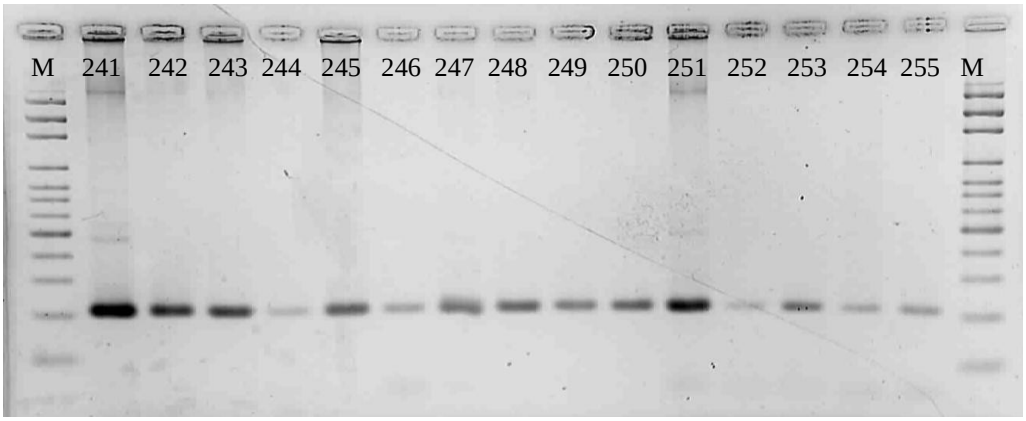

M 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 M

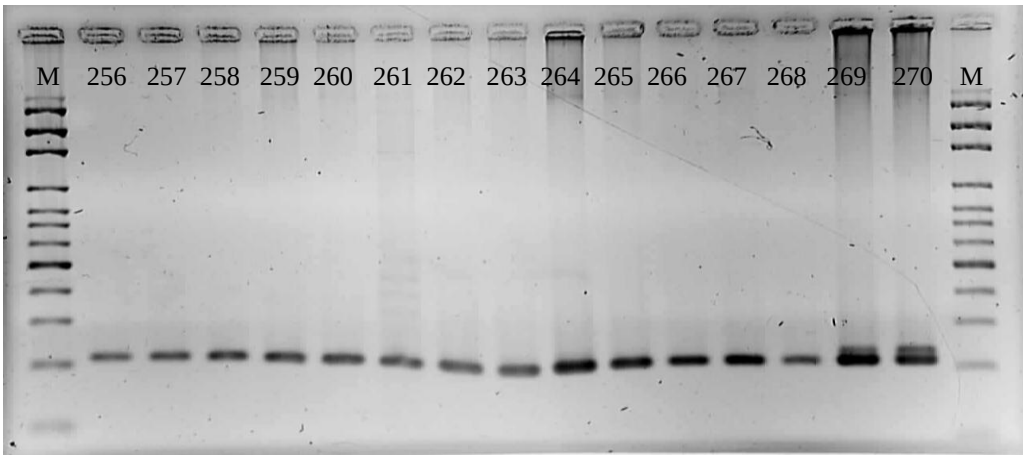
\

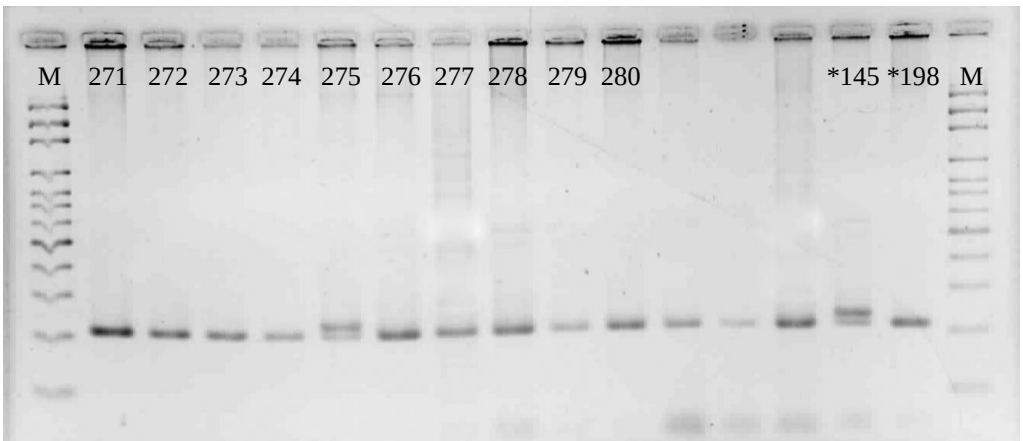

M 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 M

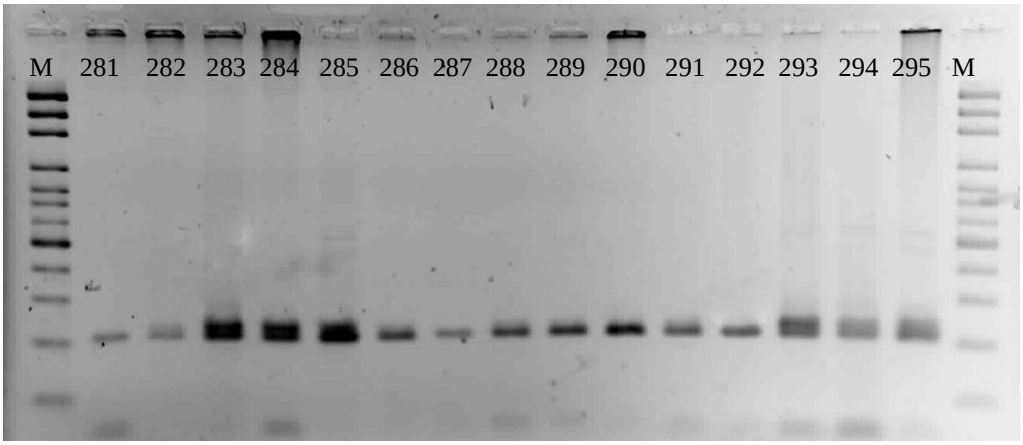

M 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 M

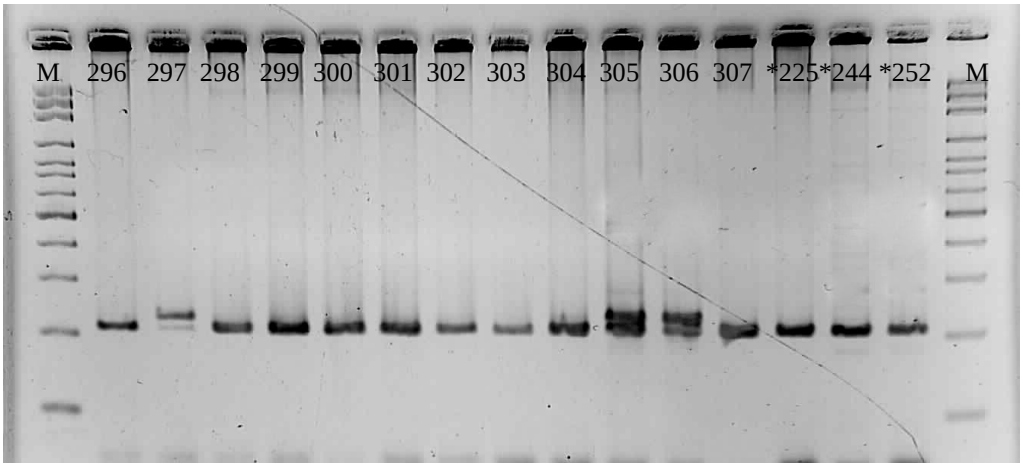

M 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 M

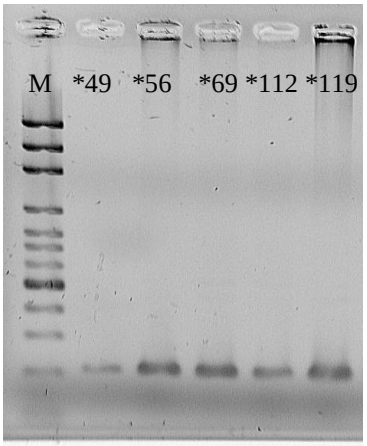

M 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 M


M 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 M


M 271 272 273 274 275 276 277 278 279 280 *145 *198 M







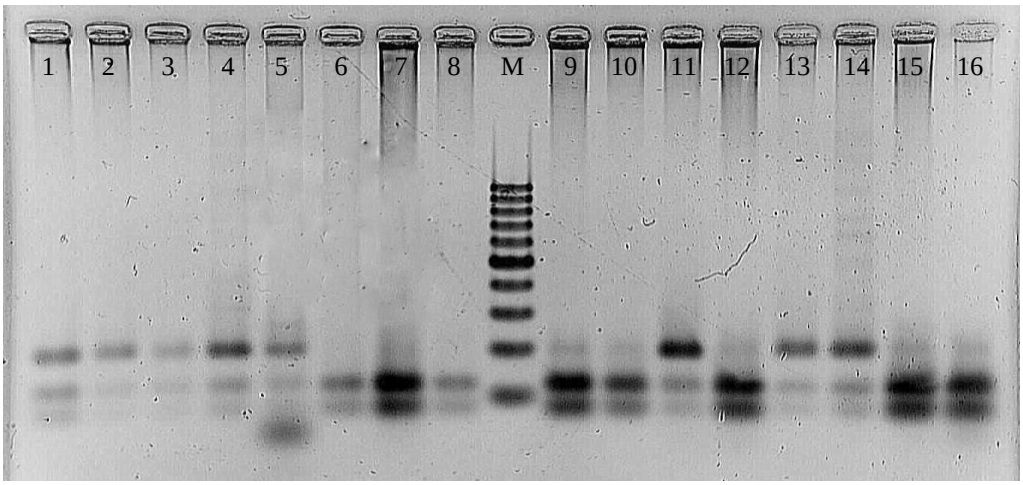
Legend:
M: Marker (100bp DNA ladder)
* repeat

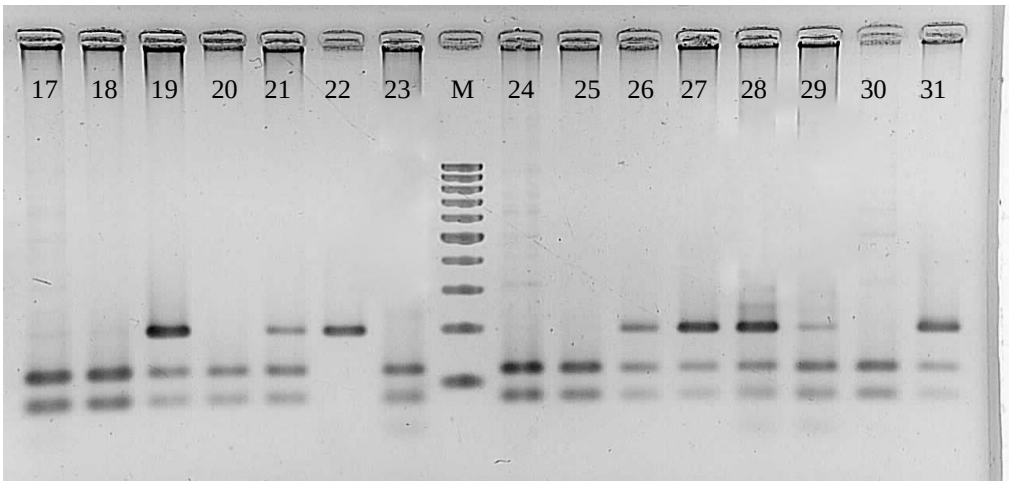
Band size:
LL (homozygous wild-type) ~ 206 bp
LV (heterozygous variant) ~ 234 & 206 bp

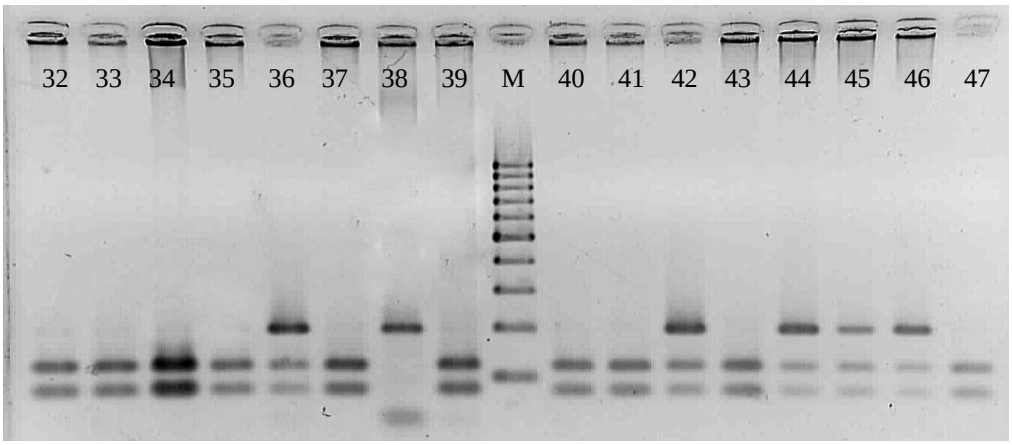
Sample No	*PPARα L162V*	Sample No	*PPARα L162V*	Sample No	*PPARα L162V*	Sample No	*PPARα L162V*	Sample No	*PPARα L162V*
1	LL	**46**	LL	**91**	LL	**136**	LL	**181**	LL
2	LL	**47**	LL	**92**	LL	**137**	LL	**182**	LL
3	LL	**48**	LL	**93**	LL	**138**	LL	**183**	LL
4	LL	**49**	LL	**94**	LL	**139**	LV	**184**	LL
5	LL	**50**	LL	**95**	LL	**140**	LV	**185**	LL
6	LL	**51**	LL	**96**	LL	**141**	LV	**186**	LL
7	LV	**52**	LL	**97**	LL	**142**	LL	**187**	LL
8	LV	**53**	LL	**98**	LL	**143**	LV	**188**	LL
9	LV	**54**	LL	**99**	LL	**144**	LV	**189**	LL
10	LV	**55**	LL	**100**	LL	**145**	LV	**190**	LL
11	LV	**56**	LL	**101**	LL	**146**	LL	**191**	LL
12	LV	**57**	LL	**102**	LL	**147**	LL	**192**	LL
13	LV	**58**	LL	**103**	LL	**148**	LL	**193**	LL
14	LL	**59**	LL	**104**	LL	**149**	LL	**194**	LL
15	LV	**60**	LL	**105**	LL	**150**	LL	**195**	LL
16	LL	**61**	LV	**106**	LL	**151**	LV	**196**	LV
17	LL	**62**	LL	**107**	LL	**152**	LV	**197**	LL
18	LV	**63**	LL	**108**	LV	**153**	LL	**198**	LL
19	LL	**64**	LL	**109**	LL	**154**	LL	**199**	LL
20	LL	**65**	LL	**110**	LL	**155**	LL	**200**	LL
21	LL	**66**	LL	**111**	LL	**156**	LL	**201**	LL
22	LL	**67**	LL	**112**	LL	**157**	LV	**202**	LL
23	LL	**68**	LL	**113**	LL	**158**	LL	**203**	LL
24	LV	**69**	LL	**114**	LL	**159**	LL	**204**	LL
25	LL	**70**	LL	**115**	LL	**160**	LL	**205**	LL
26	LV	**71**	LL	**116**	LL	**161**	LL	**206**	LV
27	LV	**72**	LL	**117**	LL	**162**	LL	**207**	LL
28	LV	**73**	LL	**118**	LV	**163**	LL	**208**	LL
29	LL	**74**	LL	**119**	LL	**164**	LL	**209**	LL
30	LL	**75**	LL	**120**	LL	**165**	LL	**210**	LL
31	LL	**76**	LL	**121**	LL	**166**	LL	**211**	LL
32	LL	**77**	LL	**122**	LL	**167**	LL	**212**	LL
33	LL	**78**	LL	**123**	LL	**168**	LV	**213**	LL
34	LL	**79**	LL	**124**	LL	**169**	LV	**214**	LL
35	LL	**80**	LL	**125**	LL	**170**	LL	**215**	LL
36	LL	**81**	LL	**126**	LL	**171**	LL	**216**	LL
37	LL	**82**	LL	**127**	LL	**172**	LL	**217**	LL
38	LL	**83**	LL	**128**	LL	**173**	LV	**218**	LL
39	LL	**84**	LL	**129**	LV	**174**	LV	**219**	LL
40	LL	**85**	LL	**130**	LL	**175**	LL	**220**	LL
41	LL	**86**	LL	**131**	LV	**176**	LL	**221**	LL
42	LL	**87**	LL	**132**	LL	**177**	LV	**222**	LL
43	LL	**88**	LL	**133**	LV	**178**	LL	**223**	LL
44	LL	**89**	LL	**134**	LL	**179**	LV	**224**	LL
45	LL	**90**	LL	**135**	LL	**180**	LL	**225**	LL

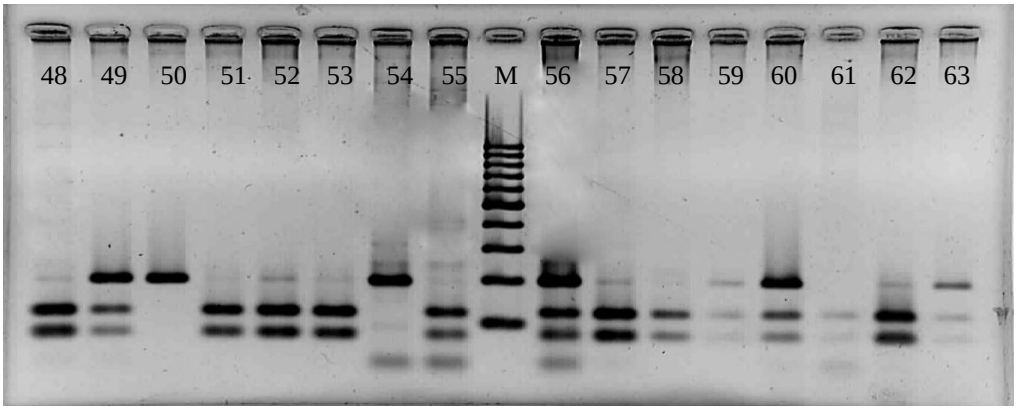
Sample No	*PPARα L162V*	Sample No	*PPARα L162V*
226	LL	**271**	LL
227	LL	**272**	LL
228	LL	**273**	LL
229	LL	**274**	LL
230	LL	**275**	LV
231	LL	**276**	LL
232	LL	**277**	LL
233	LL	**278**	LL
234	LL	**279**	LL
235	LL	**280**	LL
236	LL	**281**	LL
237	LL	**282**	LL
238	LL	**283**	LV
239	LL	**284**	LV
240	LL	**285**	LV
241	LL	**286**	LL
242	LL	**287**	LL
243	LL	**288**	LL
244	LL	**289**	LL
245	LL	**290**	LL
246	LL	**291**	LL
247	LL	**292**	LL
248	LL	**293**	LV
249	LL	**294**	LV
250	LL	**295**	LV
251	LL	**296**	LL
252	LL	**297**	LV
253	LL	**298**	LL
254	LL	**299**	LL
255	LL	**300**	LL
256	LL	**301**	LL
257	LL	**302**	LL
258	LL	**303**	LL
259	LL	**304**	LL
260	LL	**305**	LV
261	LL	**306**	LV
262	LL	**307**	LL
263	LL		
264	LL		
265	LL		
266	LL		
267	LL		
268	LL		
269	LV		
270	LV		

(ii) RFLP products for *PPARγ2* C161T SNP





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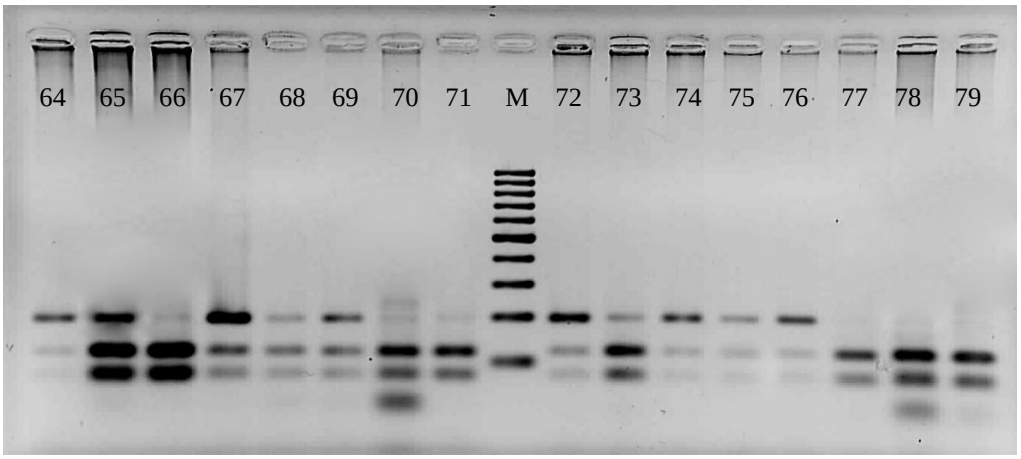
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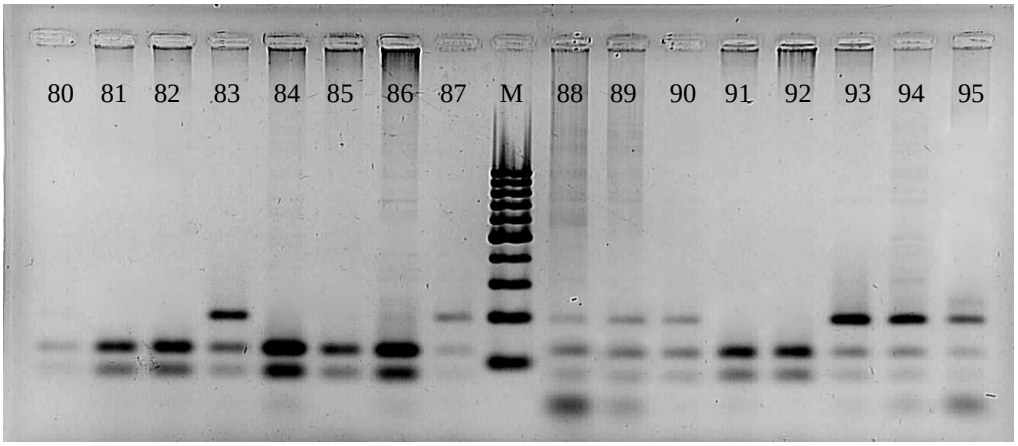
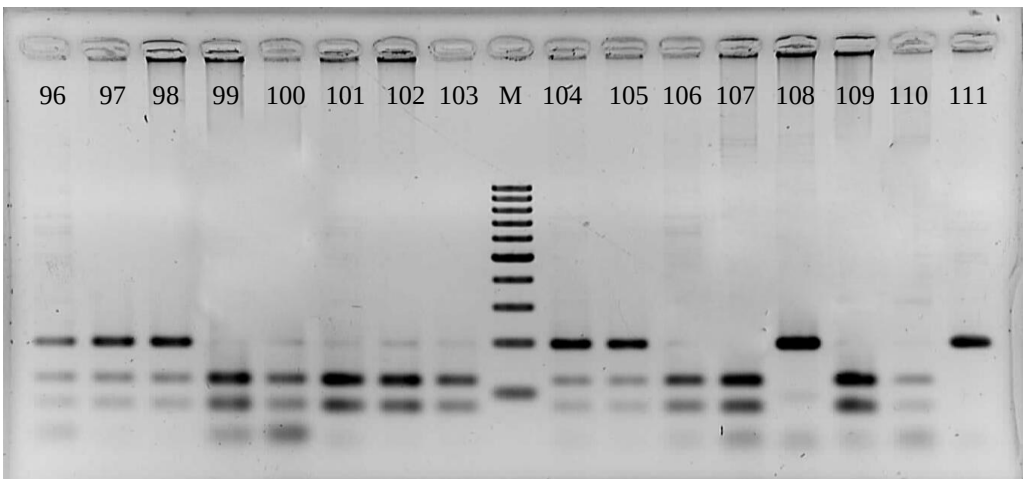
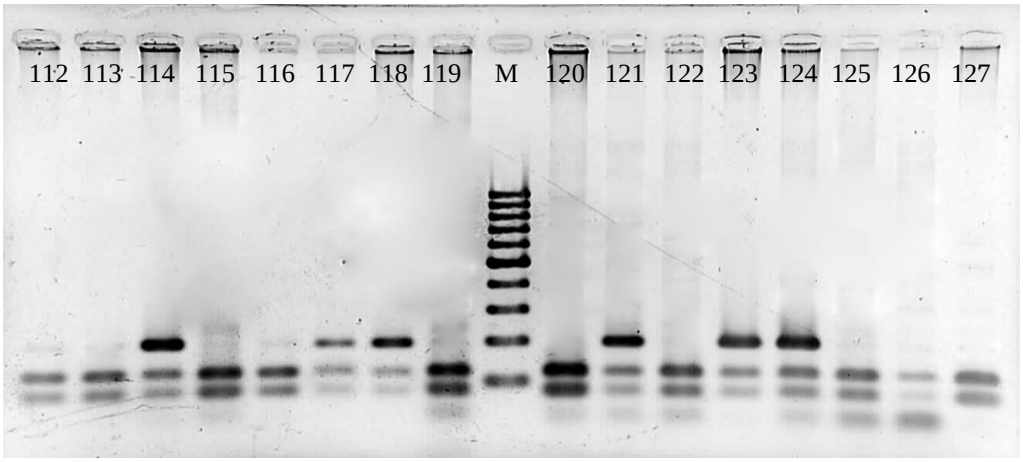
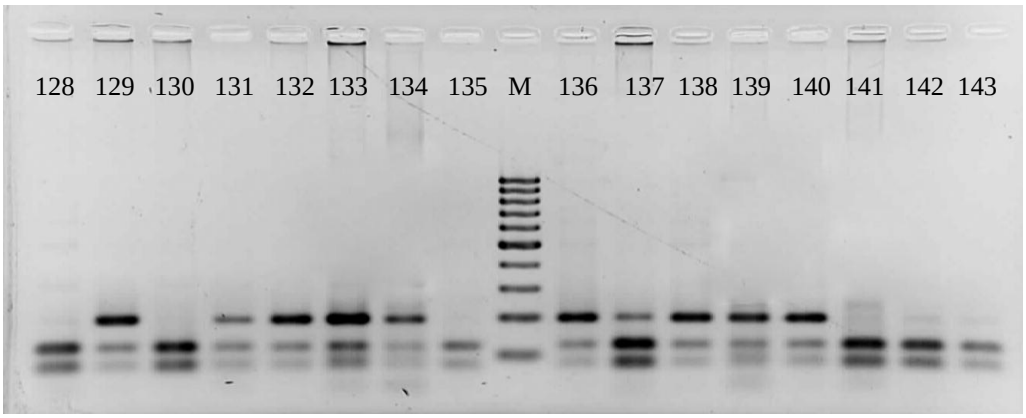
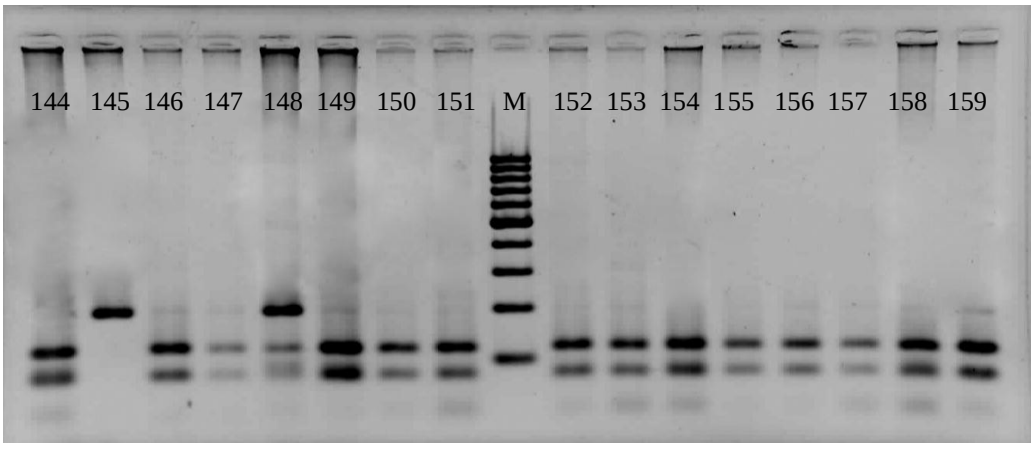
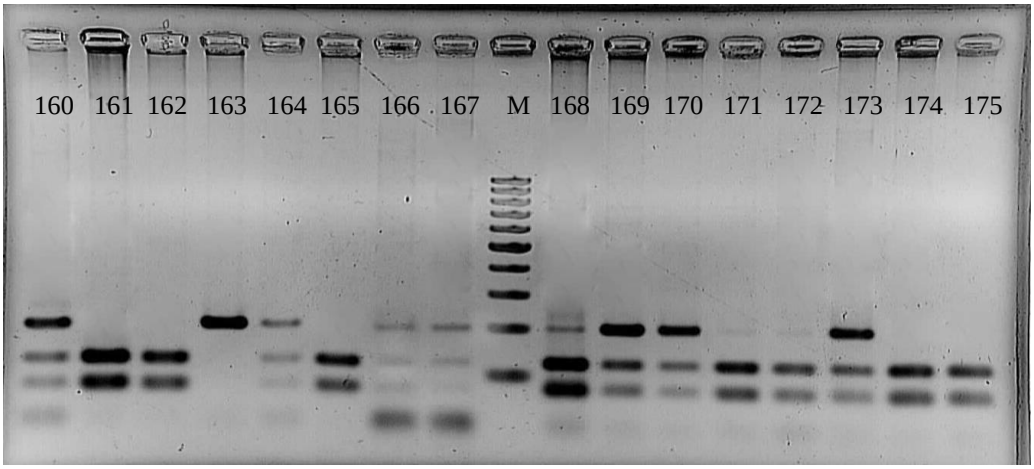

80 81 82 83 84 85 86 87 M 88 89 90 91 92 93 94 95

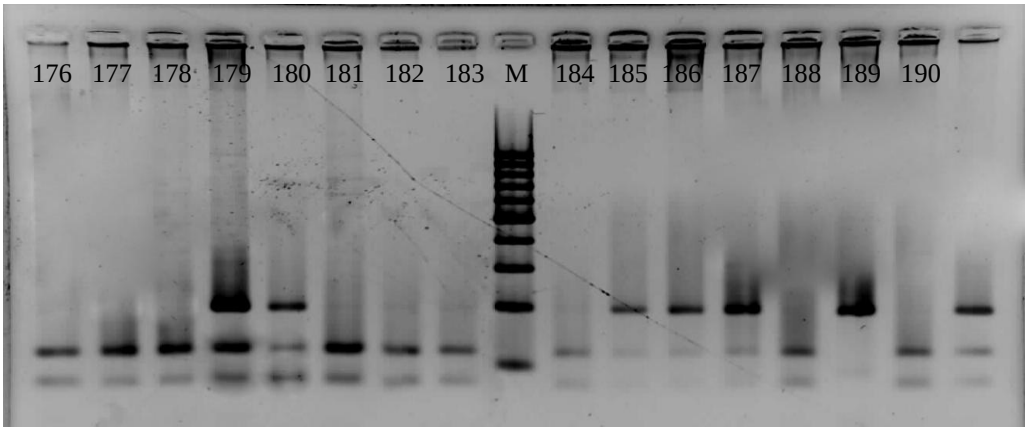

96 97 98 99 100 101 102 103 M 104 105 106 107 108 109 110 111

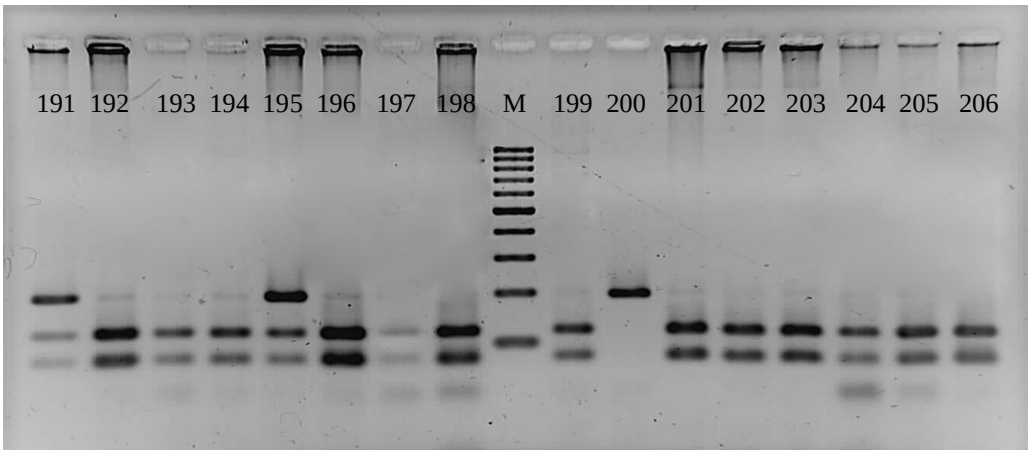

112 113 114 115 116 117 118 119 M 120 121 122 123 124 125 126 127

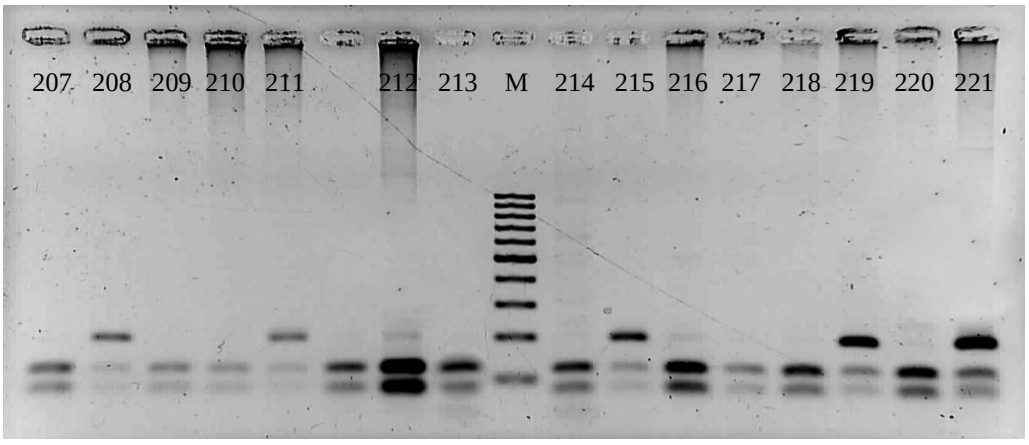

128 129 130 131 132 133 134 135 M 136 137 138 139 140 141 142 143


144 145 146 147 148 149 150 151 M 152 153 154 155 156 157 158 159


160 161 162 163 164 165 166 167 M 168 169 170 171 172 173 174 175


176 177 178 179 180 181 182 183 M 184 185 186 187 188 189 190


191 192 193 194 195 196 197 198 M 199 200 201 202 203 204 205 206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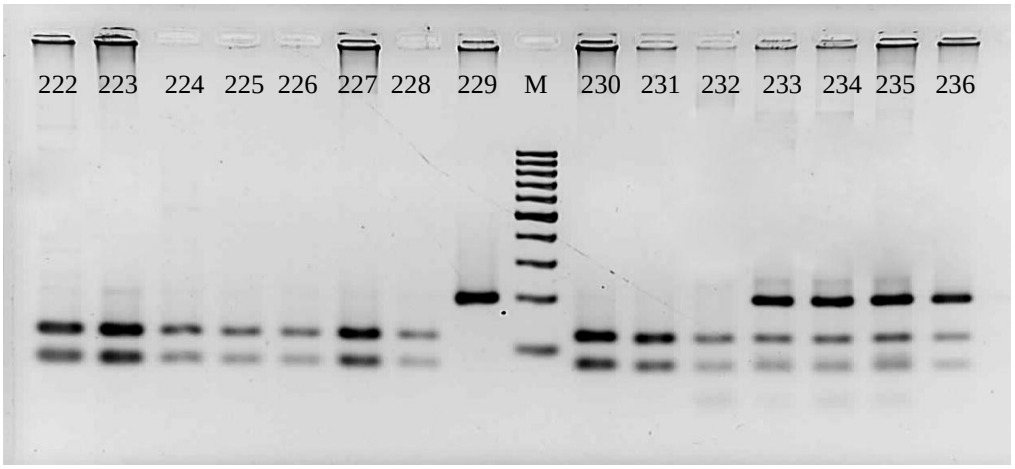
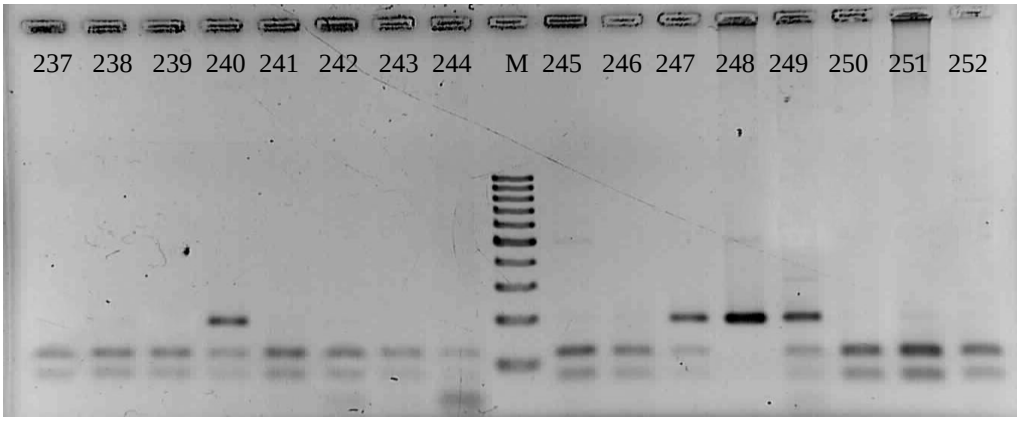
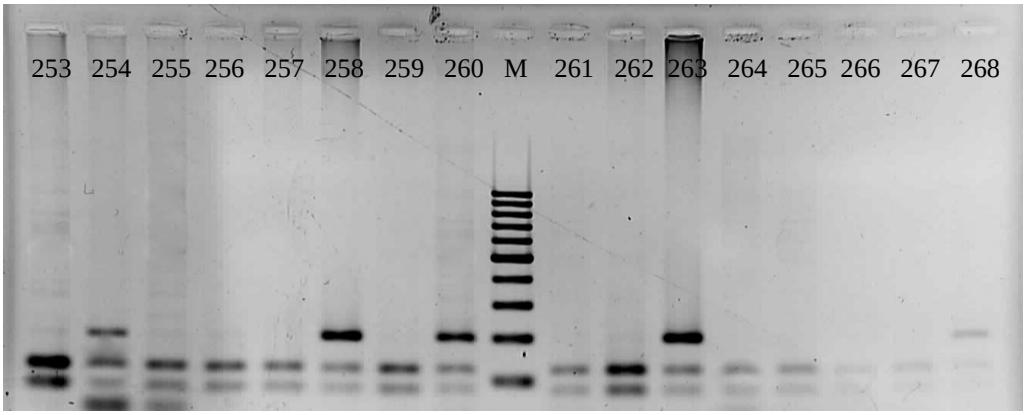
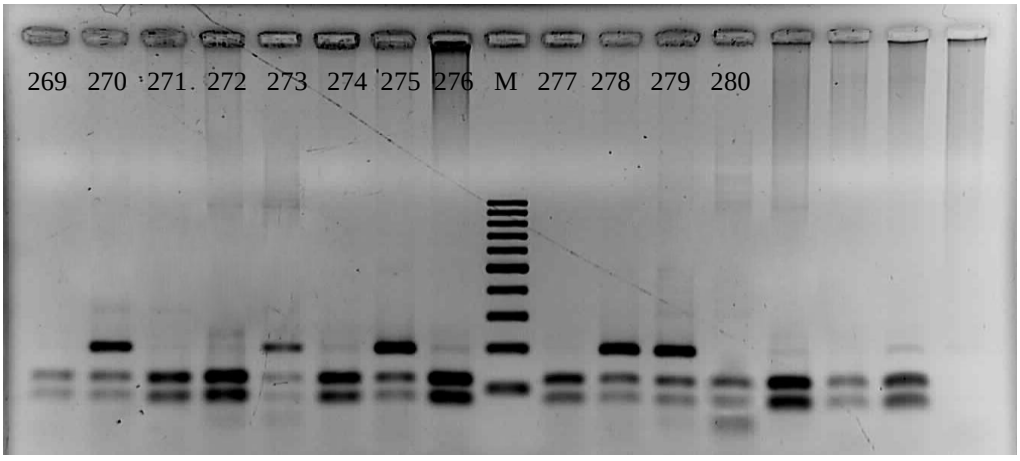
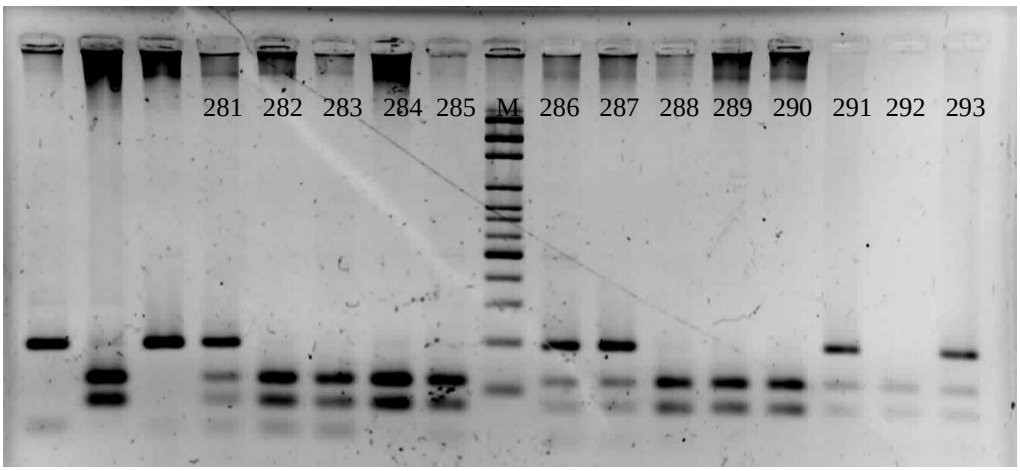
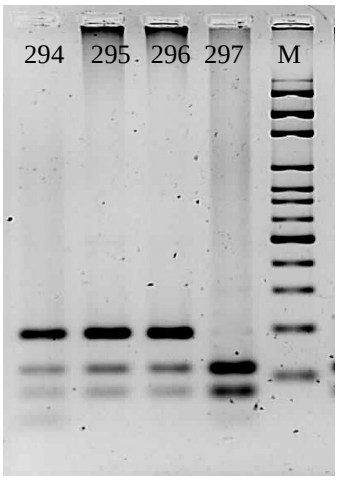

222 223 224 225 226 227 228 229 M 230 231 232 233 234 235 236

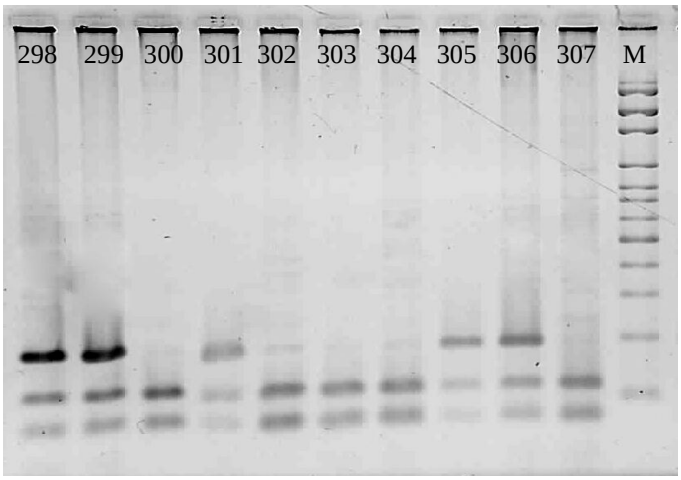

237 238 239 240 241 242 243 244 M 245 246 247 248 249 250 251 252

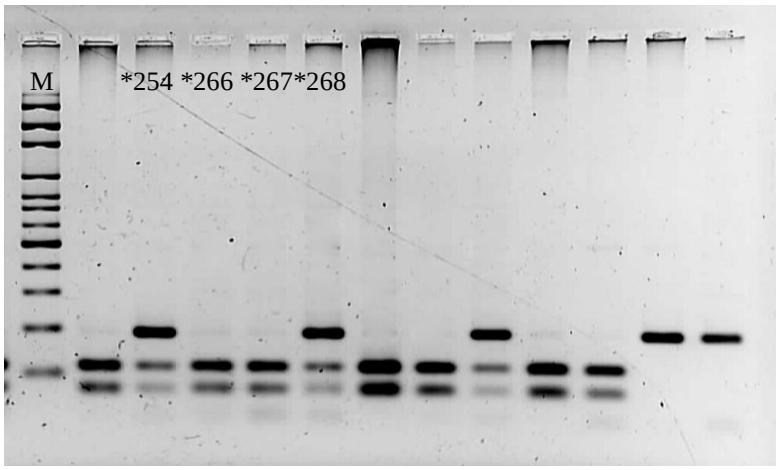

253 254 255 256 257 258 259 260 M 261 262 263 264 265 266 267 268


269 270 271 272 273 274 275 276 M 277 278 279 280


281 282 283 284 285 M 286 287 288 289 290 291 292 293







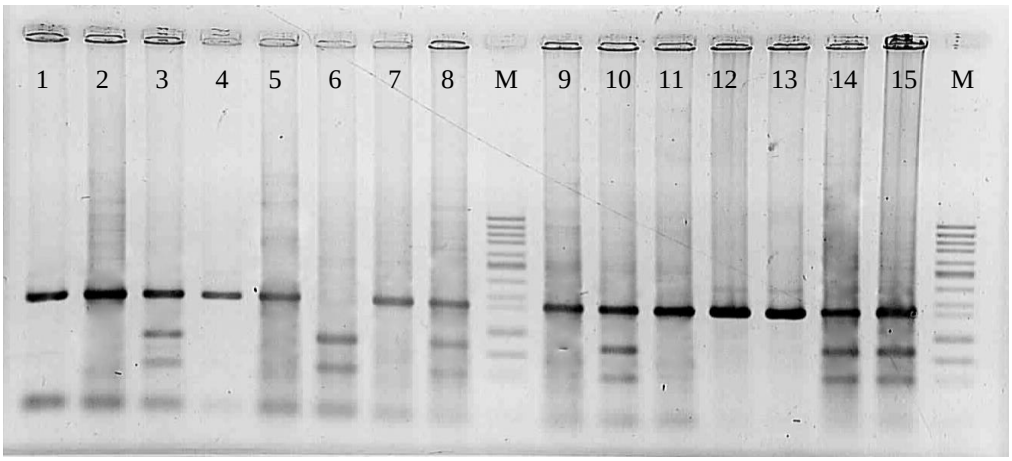
Legend:
M: Marker (100 bp DNA ladder)
* repeat

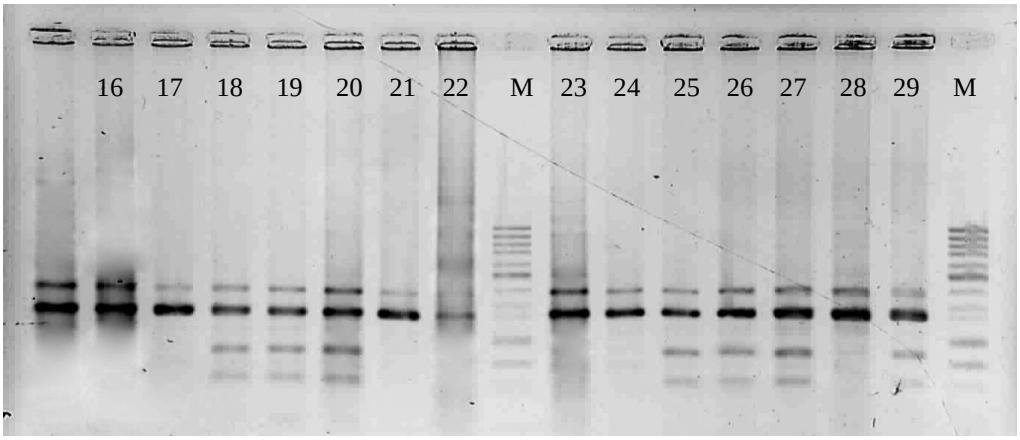
Band size:
CC (homozygous wild-type) ~ 120 & 97 bp
CT (heterozygous variant) ~ 197, 120 & 97 bp
RR (homozygous variant) ~ 197 bp

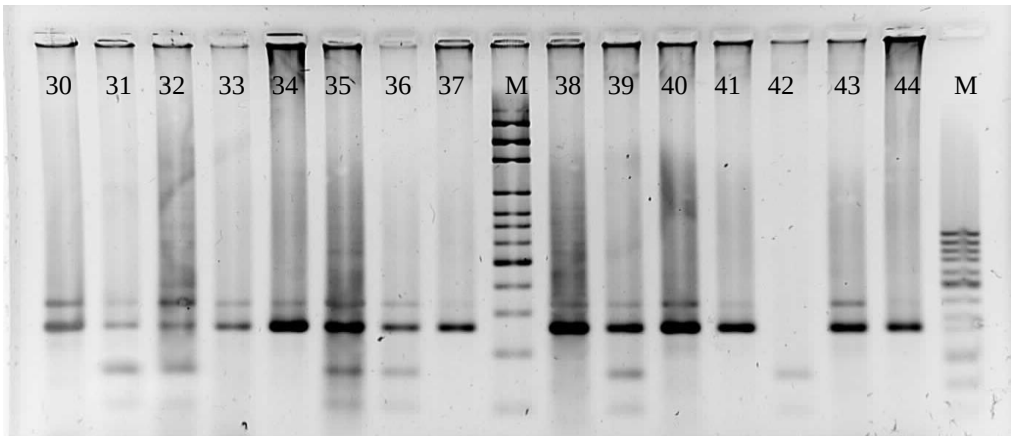
Sample No	*PPAR γ2 C161T*	Sample No	*PPAR γ2 C161T*	Sample No	*PPAR γ2 C161T*	Sample No	*PPAR γ2 C161T*	Sample No	*PPAR γ2 C161T*
1	CT	**46**	CT	**91**	CC	**136**	CT	**181**	CC
2	CT	**47**	CC	**92**	CC	**137**	CT	**182**	CC
3	CT	**48**	CC	**93**	CT	**138**	CT	**183**	CC
4	CT	**49**	CT	**94**	CT	**139**	CT	**184**	CC
5	CT	**50**	TT	**95**	CT	**140**	CT	**185**	CT
6	CC	**51**	CC	**96**	CT	**141**	CC	**186**	CT
7	CC	**52**	CC	**97**	CT	**142**	CC	**187**	CT
8	CC	**53**	CC	**98**	CT	**143**	CC	**188**	CC
9	CC	**54**	TT	**99**	CC	**144**	CC	**189**	TT
10	CC	**55**	CC	**100**	CC	**145**	TT	**190**	CC
11	CT	**56**	CT	**101**	CC	**146**	CC	**191**	CT
12	CC	**57**	CC	**102**	CC	**147**	CC	**192**	CC
13	CT	**58**	CC	**103**	CC	**148**	CT	**193**	CC
14	CT	**59**	CT	**104**	CT	**149**	CC	**194**	CC
15	CC	**60**	CT	**105**	CT	**150**	CC	**195**	CT
16	CC	**61**	CC	**106**	CC	**151**	CC	**196**	CC
17	CC	**62**	CC	**107**	CC	**152**	CC	**197**	CC
18	CC	**63**	CT	**108**	TT	**153**	CC	**198**	CC
19	CT	**64**	CT	**109**	CC	**154**	CC	**199**	CC
20	CC	**65**	CT	**110**	CC	**155**	CC	**200**	TT
21	CT	**66**	CC	**111**	TT	**156**	CC	**201**	CC
22	TT	**67**	CT	**112**	CC	**157**	CC	**202**	CC
23	CC	**68**	CT	**113**	CC	**158**	CC	**203**	CC
24	CC	**69**	CT	**114**	CT	**159**	CC	**204**	CC
25	CC	**70**	CC	**115**	CC	**160**	CT	**205**	CC
26	CT	**71**	CC	**116**	CC	**161**	CC	**206**	CC
27	CT	**72**	CT	**117**	CT	**162**	CC	**207**	CC
28	CT	**73**	CT	**118**	CT	**163**	TT	**208**	CT
29	CT	**74**	CT	**119**	CC	**164**	CT	**209**	CC
30	CC	**75**	CT	**120**	CC	**165**	CC	**210**	CC
31	CT	**76**	CT	**121**	CT	**166**	CT	**211**	CT
32	CC	**77**	CC	**122**	CC	**167**	CT	**212**	CC
33	CC	**78**	CC	**123**	CT	**168**	CC	**213**	CC
34	CC	**79**	CC	**124**	CT	**169**	CT	**214**	CC
35	CC	**80**	CC	**125**	CC	**170**	CT	**215**	CT
36	CT	**81**	CC	**126**	CC	**171**	CC	**216**	CC
37	CC	**82**	CC	**127**	CC	**172**	CC	**217**	CC
38	TT	**83**	CT	**128**	CC	**173**	CT	**218**	CC
39	CC	**84**	CC	**129**	CT	**174**	CC	**219**	CT
40	CC	**85**	CC	**130**	CC	**175**	CC	**220**	CC
41	CC	**86**	CC	**131**	CT	**176**	CC	**221**	CT
42	CT	**87**	CT	**132**	CT	**177**	CC	**222**	CC
43	CC	**88**	CT	**133**	CT	**178**	CC	**223**	CC
44	CT	**89**	CT	**134**	CT	**179**	CT	**224**	CC
45	CT	**90**	CT	**135**	CC	**180**	CT	**225**	CC

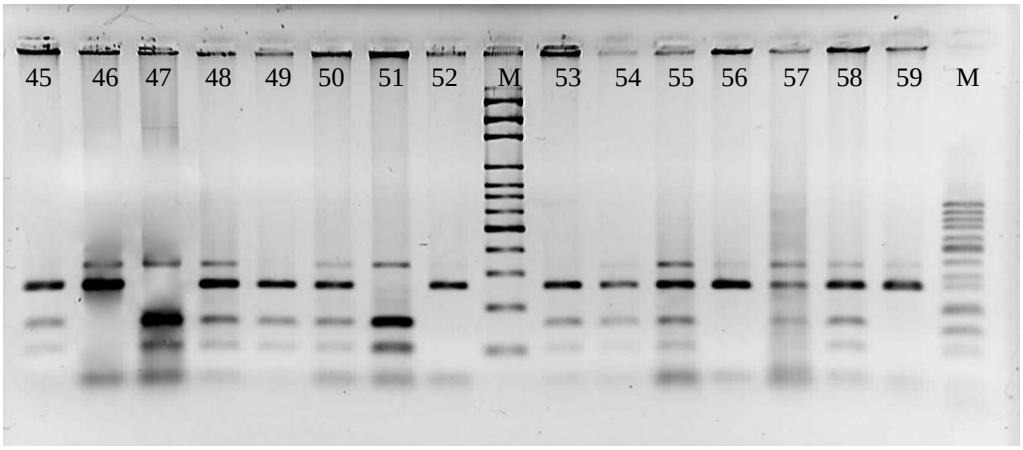
Sample No	*PPAR γ2 C161T*	Sample No	*PPAR γ2 C161T*
226	CC	**271**	CC
227	CC	**272**	CC
228	CC	**273**	CT
229	TT	**274**	CC
230	CC	**275**	CT
231	CC	**276**	CC
232	CC	**277**	CC
233	CT	**278**	CT
234	CT	**279**	CT
235	CT	**280**	CC
236	CT	**281**	CT
237	CC	**282**	CC
238	CC	**283**	CC
239	CC	**284**	CC
240	CT	**285**	CC
241	CC	**286**	CT
242	CC	**287**	CT
243	CC	**288**	CC
244	CC	**289**	CC
245	CC	**290**	CC
246	CC	**291**	CT
247	CT	**292**	CC
248	TT	**293**	CT
249	CT	**294**	CT
250	CC	**295**	CT
251	CC	**296**	CT
252	CC	**297**	CC
253	CC	**298**	CT
254	CT	**299**	CT
255	CC	**300**	CC
256	CC	**301**	CT
257	CC	**302**	CC
258	CT	**303**	CC
259	CC	**304**	CC
260	CT	**305**	CT
261	CC	**306**	CT
262	CC	**307**	CC
263	CT		
264	CC		
265	CC		
266	CC		
267	CC		
268	CT		
269	CC		
270	CT		

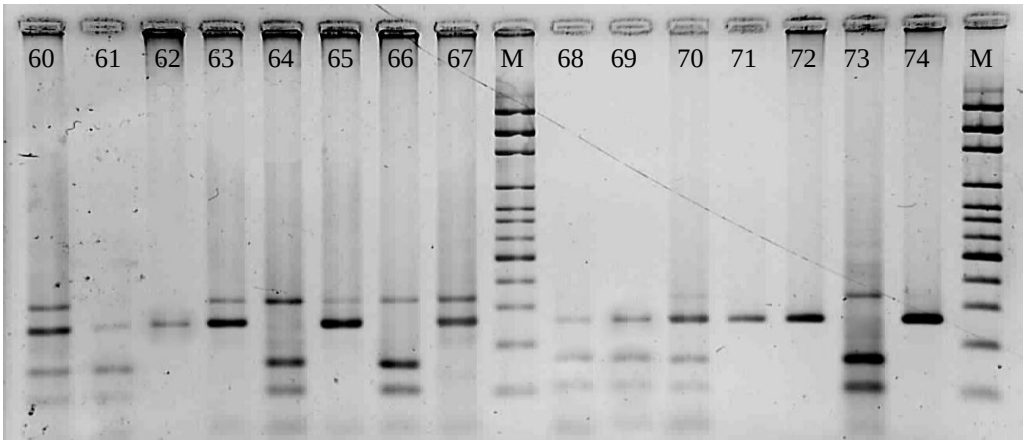
(iii) RFLP products for *PPARδ* T294C SNP

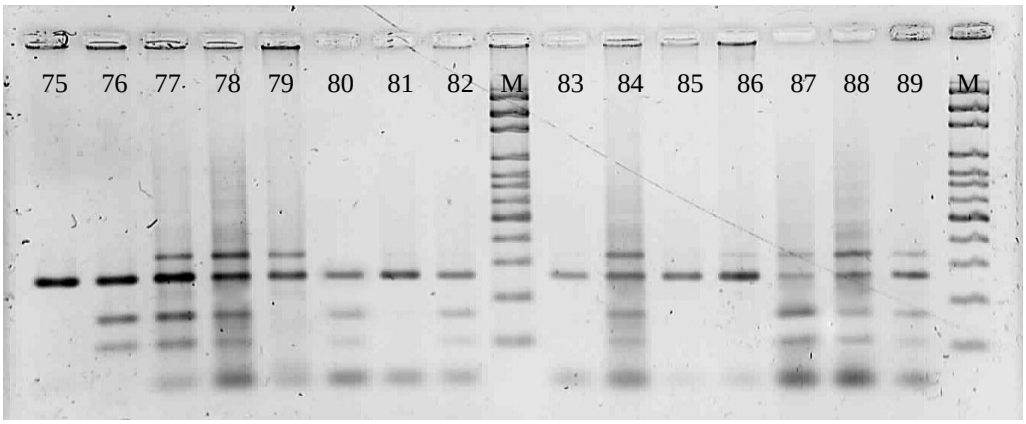







45 46 47 48 49 50 51 52 M 53 54 55 56 57 58 59 M


60 61 62 63 64 65 66 67 M 68 69 70 71 72 73 74 M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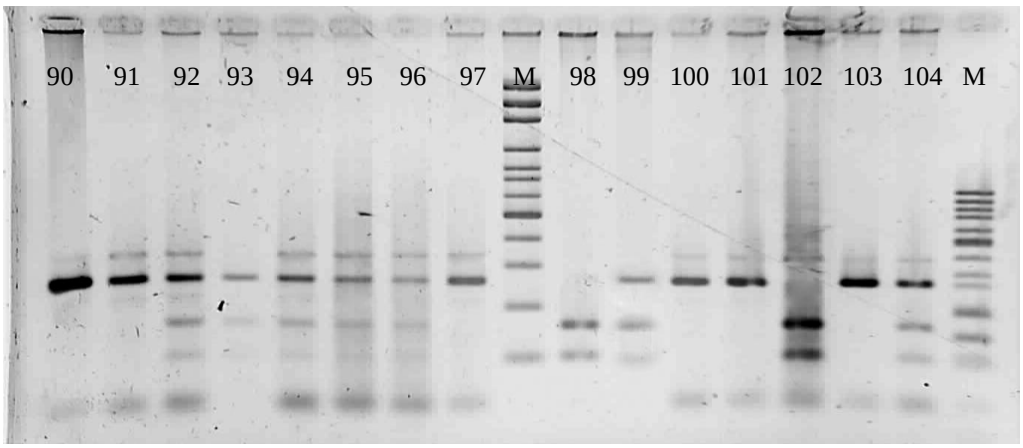
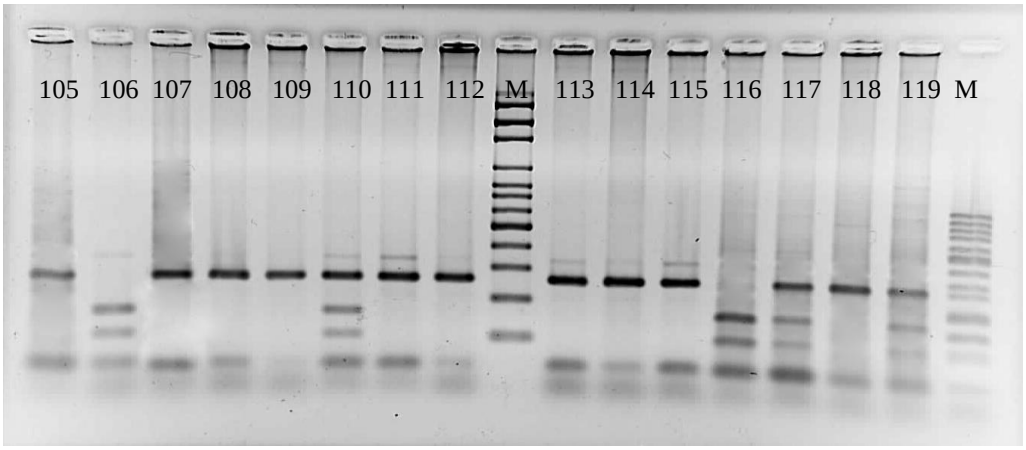

90 91 92 93 94 95 96 97 M 98 99 100 101 102 103 104 M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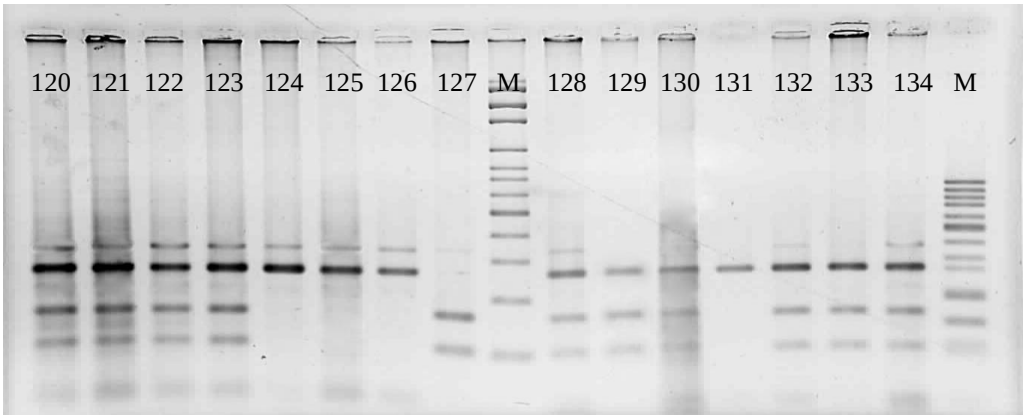
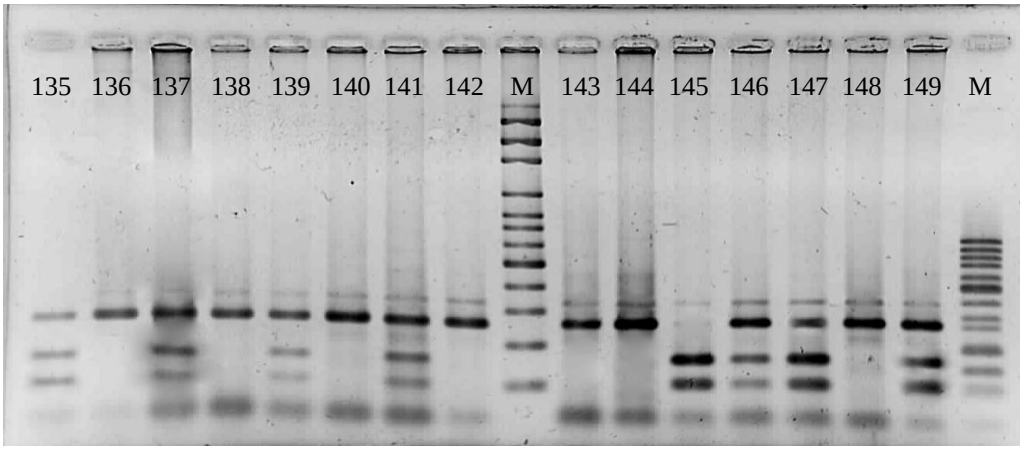
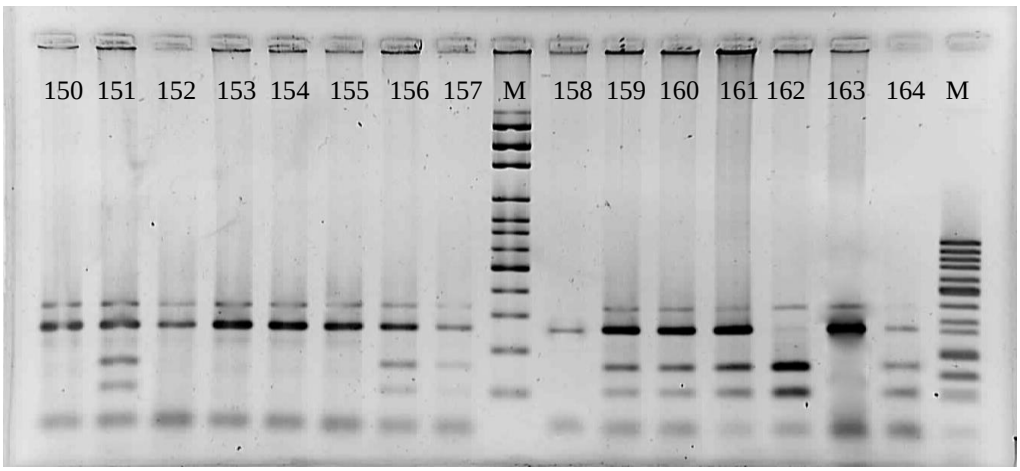

120 121 122 123 124 125 126 127 M 128 129 130 131 132 133 134 M


135 136 137 138 139 140 141 142 M 143 144 145 146 147 148 149 M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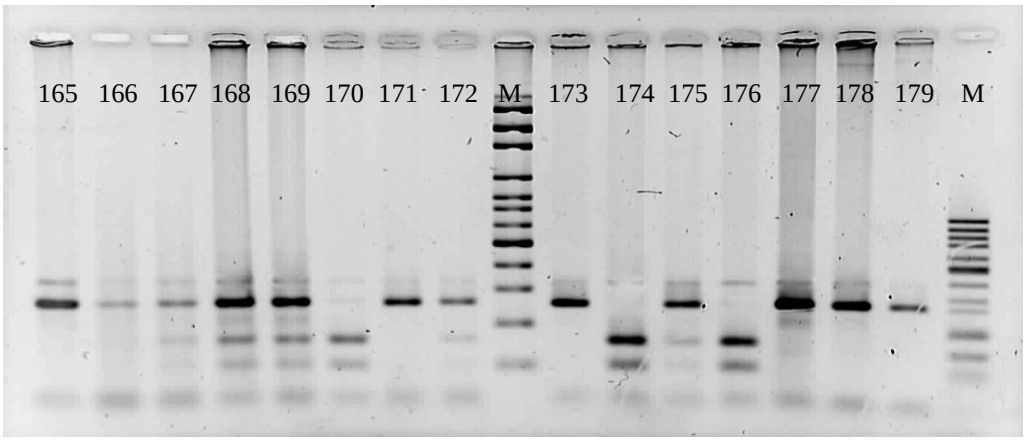
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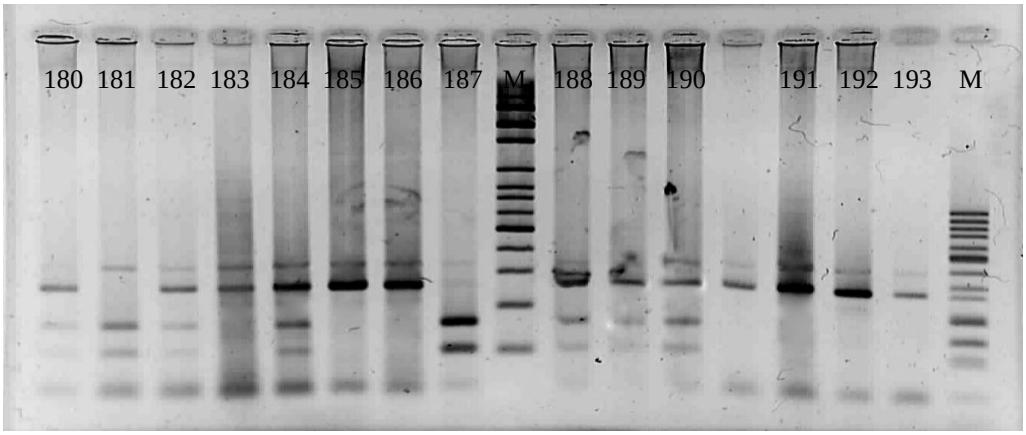
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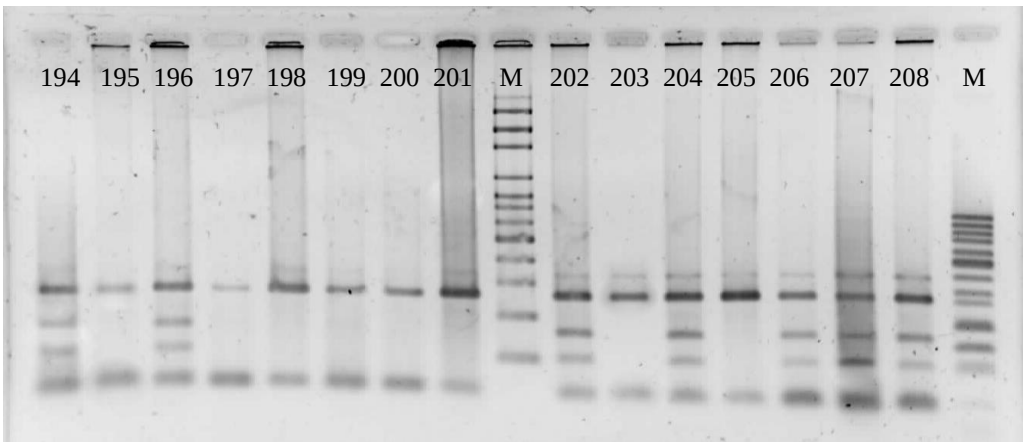
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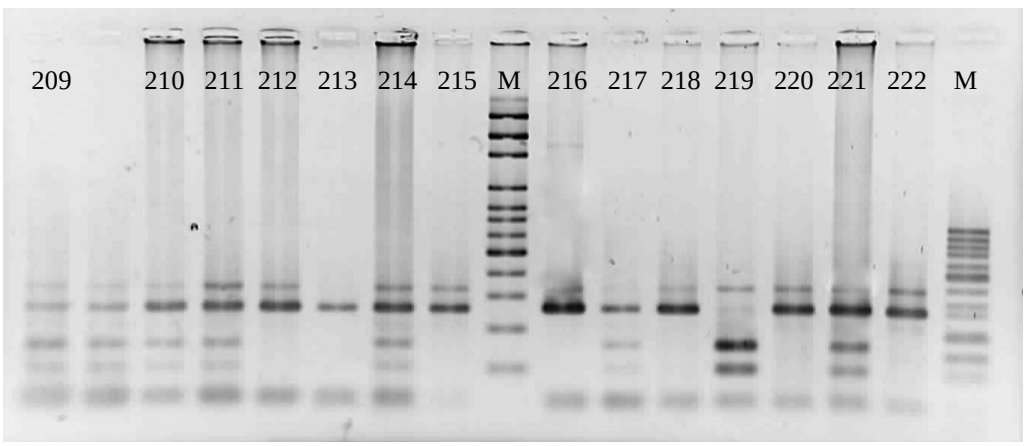
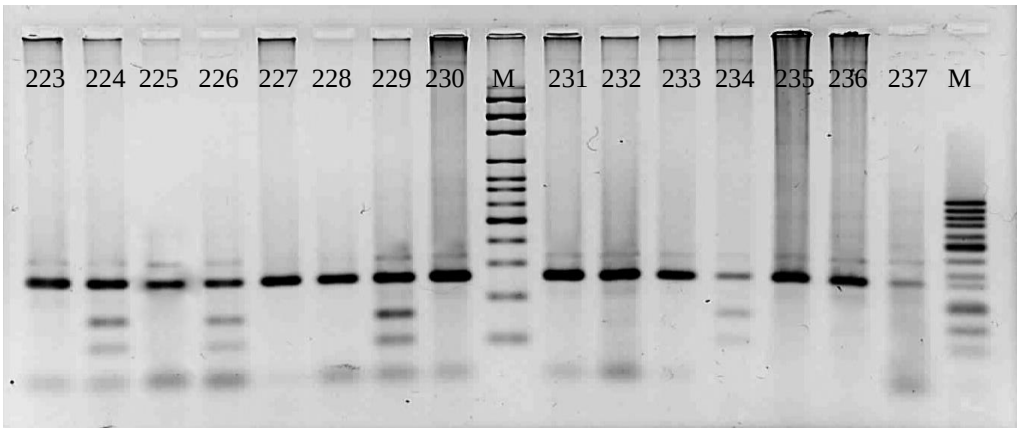
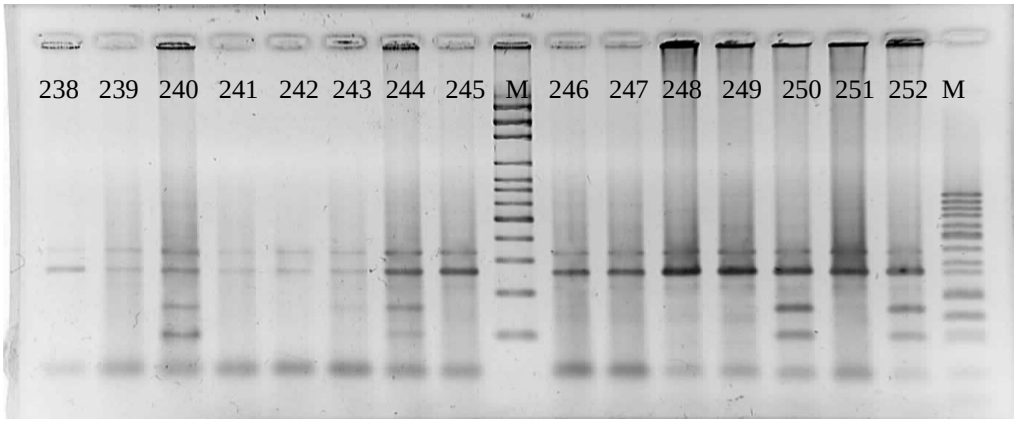

209 210 211 212 213 214 215 M 216 217 218 219 220 221 222 M


223 224 225 226 227 228 229 230 M 231 232 233 234 235 236 237 M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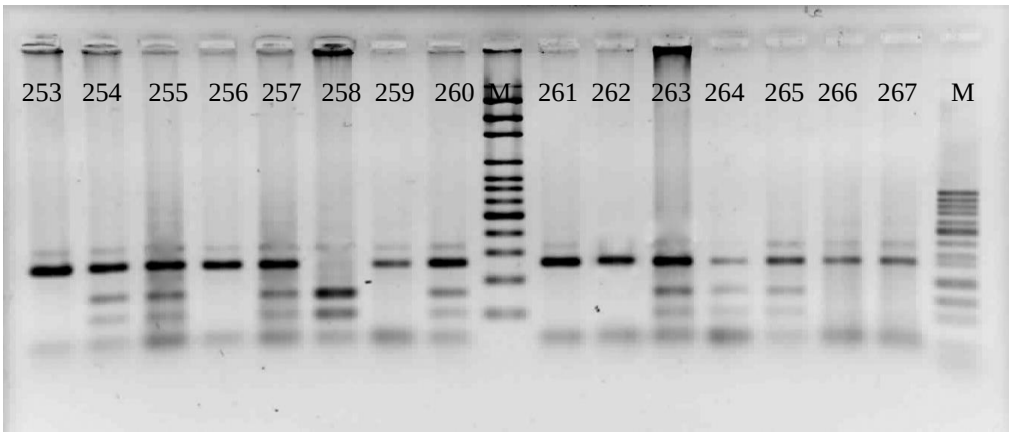
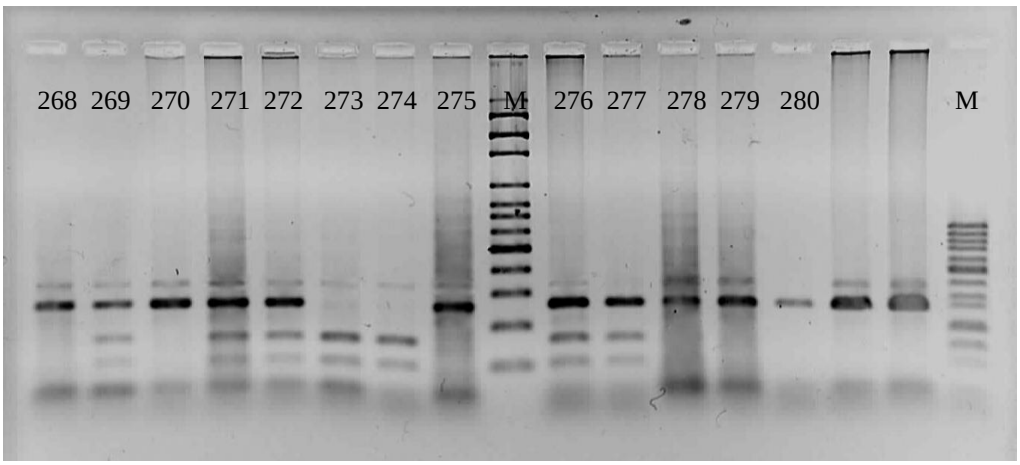
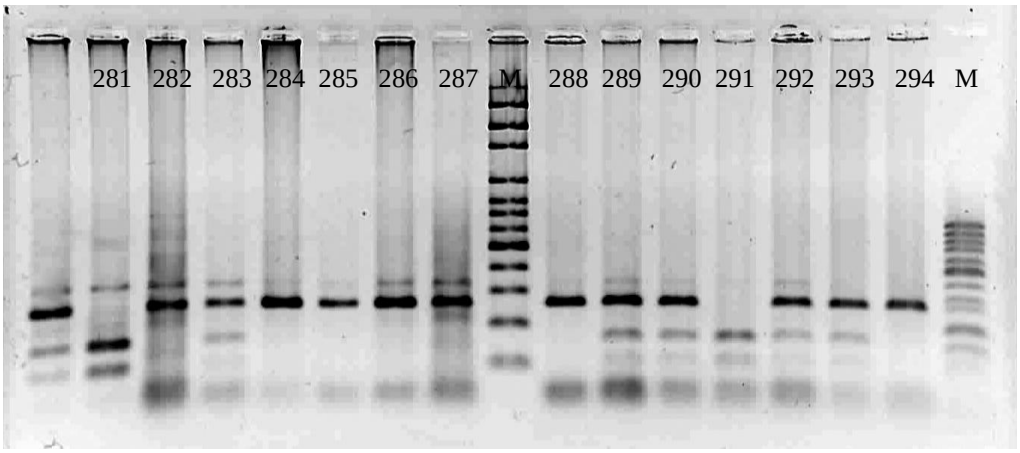
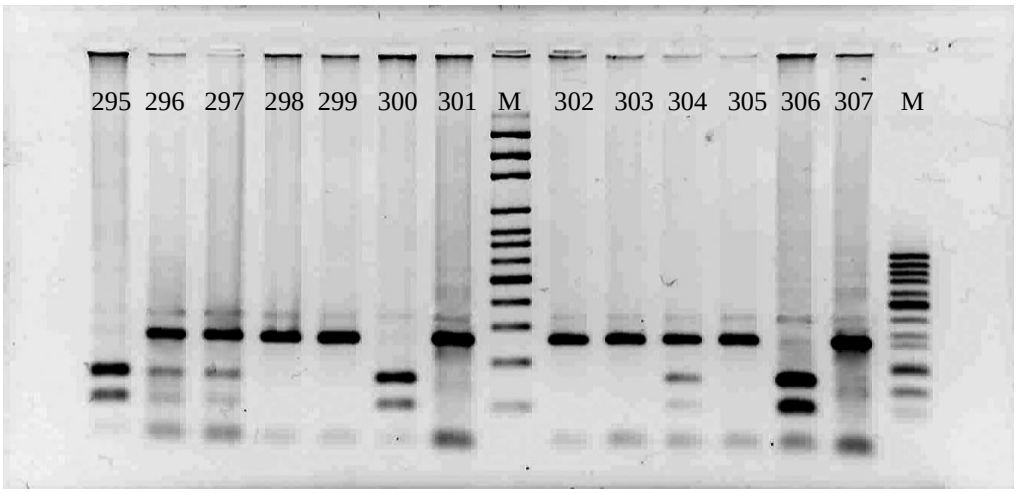

253 254 255 256 257 258 259 260 M 261 262 263 264 265 266 267 M


268 269 270 271 272 273 274 275 M 276 277 278 279 280 M





Legend:
M: Marker (100bp/50bp DNA ladder)
* repeat

Band size:
TT (homozygous wild-type) ~ 269 bp
TC (heterozygous variant) ~ 269, 167 & 102 bp
CC (homozygous variant) ~ 167 & 102 bp
Unspecific band ~ >300 bp

Sample No	*PPARδ T294C*	Sample No	*PPARδ T294C*	Sample No	*PPARδ T294C*	Sample No	*PPARδ T294C*	Sample No	*PPARδ T294C*
1	TT	**46**	TT	**91**	TT	**136**	TT	**181**	CC
2	TT	**47**	CC	**92**	TC	**137**	TC	**182**	TC
3	TC	**48**	TC	**93**	TC	**138**	TT	**183**	TT
4	TT	**49**	TC	**94**	TC	**139**	TC	**184**	TC
5	TT	**50**	TC	**95**	TC	**140**	TT	**185**	TT
6	CC	**51**	CC	**96**	TC	**141**	TC	**186**	TT
7	TT	**52**	TT	**97**	TT	**142**	TT	**187**	CC
8	TC	**53**	TC	**98**	CC	**143**	TT	**188**	TC
9	TT	**54**	TC	**99**	TC	**144**	TT	**189**	TC
10	TC	**55**	TC	**100**	TT	**145**	CC	**190**	TC
11	TT	**56**	TT	**101**	TT	**146**	TC	**191**	TT
12	TT	**57**	TC	**102**	CC	**147**	TC	**192**	TT
13	TT	**58**	TC	**103**	TT	**148**	TT	**193**	TT
14	TC	**59**	TT	**104**	TC	**149**	TC	**194**	TC
15	TC	**60**	TC	**105**	TT	**150**	TT	**195**	TT
16	TT	**61**	TC	**106**	CC	**151**	TC	**196**	TC
17	TT	**62**	TT	**107**	TT	**152**	TT	**197**	TT
18	TC	**63**	TT	**108**	TT	**153**	TT	**198**	TT
19	TC	**64**	CC	**109**	TT	**154**	TT	**199**	TT
20	TC	**65**	TT	**110**	TC	**155**	TT	**200**	TT
21	TT	**66**	CC	**111**	TT	**156**	TC	**201**	TT
22	TT	**67**	TT	**112**	TT	**157**	TC	**202**	TC
23	TT	**68**	TC	**113**	TT	**158**	TT	**203**	TT
24	TT	**69**	TC	**114**	TT	**159**	TC	**204**	TC
25	TC	**70**	TC	**115**	TT	**160**	TC	**205**	TT
26	TC	**71**	TT	**116**	CC	**161**	TC	**206**	TC
27	TC	**72**	TT	**117**	TC	**162**	CC	**207**	TC
28	TT	**73**	CC	**118**	TT	**163**	TT	**208**	TC
29	TC	**74**	TT	**119**	TC	**164**	TC	**209**	TC
30	TT	**75**	TT	**120**	TC	**165**	TT	**210**	TC
31	TC	**76**	TC	**121**	TC	**166**	TT	**211**	TC
32	TC	**77**	TC	**122**	TC	**167**	TC	**212**	TT
33	TT	**78**	TC	**123**	TC	**168**	TC	**213**	TT
34	TT	**79**	TT	**124**	TT	**169**	TC	**214**	TC
35	TC	**80**	TC	**125**	TT	**170**	CC	**215**	TT
36	TC	**81**	TT	**126**	TT	**171**	TT	**216**	TT
37	TT	**82**	TC	**127**	CC	**172**	TC	**217**	TC
38	TT	**83**	TT	**128**	TC	**173**	TT	**218**	TT
39	TC	**84**	TC	**129**	TC	**174**	CC	**219**	CC
40	TT	**85**	TT	**130**	TC	**175**	TC	**220**	TT
41	TT	**86**	TT	**131**	TT	**176**	CC	**221**	TC
42	CC	**87**	TC	**132**	TC	**177**	TT	**222**	TT
43	TT	**88**	TC	**133**	TC	**178**	TT	**223**	TT
44	TT	**89**	TC	**134**	TC	**179**	TT	**224**	TC
45	TC	**90**	TT	**135**	TC	**180**	TC	**225**	TT

Sample No	*PPARδ T294C*	Sample No	*PPARδ T294C*
226	TC	**271**	TC
227	TT	**272**	TC
228	TT	**273**	CC
229	TC	**274**	CC
230	TT	**275**	TT
231	TT	**276**	TC
232	TT	**277**	TC
233	TT	**278**	TT
234	TC	**279**	TT
235	TT	**280**	TT
236	TT	**281**	CC
237	TT	**282**	TT
238	TT	**283**	TC
239	TT	**284**	TT
240	TC	**285**	TT
241	TT	**286**	TT
242	TT	**287**	TT
243	TC	**288**	TT
244	TC	**289**	TC
245	TT	**290**	TC
246	TT	**291**	CC
247	TT	**292**	TC
248	TT	**293**	TC
249	TT	**294**	TT
250	TC	**295**	CC
251	TT	**296**	TC
252	TC	**297**	TC
253	TT	**298**	TT
254	TC	**299**	TT
255	TC	**300**	CC
256	TT	**301**	TT
257	TC	**302**	TT
258	CC	**303**	TT
259	TT	**304**	TC
260	TC	**305**	TT
261	TT	**306**	CC
262	TT	**307**	TT
263	TC		
264	TC		
265	TC		
266	TT		
267	TT		
268	TT		
269	TC		
270	TT		