SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) OF ADIPONECTIN GENE AND ITS ASSOCIATION WITH SERUM ADIPONECTIN CONCENTRATION AND METABOLIC SYNDROME RISK FACTORS AMONG MALAY ADULTS

by

NUR FIRDAUS ISA

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LIST OF ABBREVIATIONS

ATP III	National Cholesterol Education Program's Adult Treatment Program III
BAT	Brown adipose tissue
BLAST	Basic Local Alignment Search Tool
BMI	Body mass index
CI	Confidence interval
CV	Coefficient of variability
ddNTPs	Dideoxy nucleoside triphosphates
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EGIR	European Group for the Study of Insulin Resistance
ELISA	Enzyme-linked Immunosorbent Assay
Exo I	*Exonuclease* I
GOD	Glucose oxidase
H_2O_2	Hydrogen peroxide
HDL cholesterol	High-density lipoprotein cholesterol
HMW	High molecular weight
HPLC	High-performance liquid chromatography
HWE	Hardy-Weinberg equilibrium
IDF	International Diabetes Federation
IqR	Interquartile range

LD	Linkage disequilibrium
LDL	Low density lipoprotein
LMW	Low molecular weight
MAF	Minor allele frequency
MMW	Medium molecular weight
MS	Metabolic syndrome
NCBI	National Center for Biotechnology Information
OR	Odds ratios
PCR	Polymerase chain reaction
SAP	Shrimp Alkaline Phosphatase
SAT	Subcutaneous adipose tissue
QTL	Quantitative trait locus
SD	Standard deviation
SNPs	Single nucleotide polymorphisms
T2D	Type 2 diabetes
TBE	Tris-Borate-EDTA
VAT	Visceral adipose tissue
VSR	Visceral subcutaneous ratio
WAT	White adipose tissue
WHO	World Health Organization
WHR	Waist hip ratio

POLIMORFISME NUKLEOTIDA TUNGGAL PADA GEN ADIPONEKTIN DAN PERKAITANNYA DENGAN KEPEKATAN SERUM ADIPONEKTIN DAN FAKTOR-FAKTOR RISIKO SINDROM METABOLIK DALAM KALANGAN ORANG MELAYU DEWASA

ABSTRAK

Sindrom metabolik ialah kelompok faktor risiko yang termasuk keobesan di bahagian pinggang dan perut, hipertrigliseridemia, kolestrol HDL rendah, hipertensi, and hiperglisemia. Bukti-bukti terkumpul menyokong hipotesis bahawa hipoadiponektinemia meningkatkan lagi risiko terhadap penyakit metabolik. Antara yang menarik, sesetengah daripada polimorfisme biasa dalam bahagian *promoter*, *exon* dan *intron 2* pada gen adiponektin manusia mempunyai kaitan dengan faktor-faktor risiko sindrom metabolik. Kajian ini bertujuan untuk menyiasat perkaitan antara beberapa polimorfisme nukleotida tunggal (SNPs) pada gen adiponektin dengan kepekatan adiponektin dan faktor-faktor risiko sindrom metabolik dalam kalangan orang Melayu dewasa. Seramai 298 orang Melayu dewasa yang terlibat di dalam kajian ini. Lilitan pinggang dan tekanan darah telah diukur sebelum darah diambil daripada subjek yang telah berpuasa semalaman. Ujian-ujian biokimia untuk paras trigliserida, kolestrol HDL dan glukosa dalam darah dijalankan dengan menggunakan alatan-alatan komersil. Kepekatan adiponektin plasma diukur menggunakan alatan Human Adiponectin ELISA. Sebanyak lima lokasi polimorfisme nukleotida tunggal pada gen adiponektin (SNPs -11426, -11391 dan -11377 pada bahagian *proximal promoter* dan SNPs +276 dan +45 pada bahagian

exon 2) telah disaring menggunakan kaedah minipenjujukan. Dapatan kajian ini menunjukkan bahawa kepekatan adiponektin pada subjek yang mempunyai sindrom metabolik lebih rendah berbanding dengan subjek yang tidak mempunyai sindrom metabolik ($p < 0.05$). Kepekatan adiponektin juga berkait rapat dengan hipertrigliseridemia ($p < 0.001$) dan paras rendah kolestrol HDL ($p < 0.001$). Tidak ada polimorfisme nukleotida tunggal atau haplotip yang menunjukkan perkaitan dengan kepekatan adiponektin. Hanya SNP-11426 yang mempunyai kaitan yang signifikan dengan sindrom metabolik ($p < 0.05$), manakala SNP+276 berkait rapat dengan hipertrigliseridemia ($p < 0.05$) dan haplotip -11426/-11377 mempunyai perkaitan dengan hiperglisemia ($p < 0.05$). Secara keseluruhannya, tidak ada interaksi yang signifikan dari segi statistic di antara status sindrom metabolik dan polimorfisme nukleotida tunggal atau haplotip dengan kepekatan adiponektin. Walau bagaimanapun, terdapat perkaitan yang signifikan antara kepekatan adiponektin dan status paras rendah kolestrol HDL dengan SNP-11426 ($p < 0.05$). Di samping itu, terdapat juga perkaitan yang signifikan antara kepekatan adiponektin dan haplotip -11426/+45 dengan hipertrigliseridemia ($p < 0.05$). Kesimpulannya, hipoadiponektin dan polimorfisme nukleotida tunggal dan haplotip pada gen adiponektin boleh menyumbang kepada perkembangan sindrom metabolik dan faktor-faktor risiko melalui mekanisma yang masih lagi tidak diketahui di dalam populasi Melayu.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) OF ADIPONECTIN GENE AND ITS ASSOCIATION WITH SERUM ADIPONECTIN CONCENTRATION AND METABOLIC SYNDROME RISK FACTORS AMONG MALAY ADULTS

ABSTRACT

Metabolic syndrome is a cluster of risk factors that include central obesity, hypertriglyceridaemia, reduced HDL cholesterol, hypertension, and hyperglycemia. Accumulating evidences support the hypothesis that hypoadiponectinemia, a type of adipokine, confer increased risk for metabolic diseases. Of interest, some of the common polymorphisms in the promoter region, exon and intron 2 of the human adiponectin gene are associated with risk factors of metabolic syndrome. The present study aims to investigate the association of several single nucleotide polymorphisms in the adiponectin gene with serum adiponectin concentration and metabolic syndrome risk factors among Malay adults. A total of 298 Malay adults were recruited in this study. Measurements for waist circumference and blood pressure were taken before drawing an overnight fasting blood. Biochemical tests for triglycerides, HDL cholesterol and glucose were carried out by using commercially available kits. Plasma adiponectin concentration was measured using Human Adiponectin ELISA kit. A total of five sites of single nucleotide polymorphisms in adiponectin gene (SNPs -11426, -11391 and -11377 at proximal promoter and SNPs +276 and +45 at exon 2 regions) were screened using minisequencing method. Findings from this study showed that the adiponectin concentration in the subjects

with MS was significantly lower than those without MS ($p < 0.05$). The adiponectin concentration was also significantly associated with only hypertriglyceridemia ($p < 0.001$) and reduced HDL cholesterol ($p < 0.001$). None of the studied SNPs or haplotypes showed any significant association with the adiponectin concentration. Moreover, only SNP-11426 was significantly associated with MS ($p < 0.05$), while SNP+276 was associated with hypertriglyceridemia ($p < 0.05$), and haplotype -11426/-11377 was associated with hyperglycemia ($p < 0.05$). Overall, there was no statistically significant interaction between the status of MS and SNPs or haplotypes with respect to the adiponectin concentration. However, there was a significant association between the adiponectin concentration and status of reduced HDL cholesterol with SNP-11426 ($p < 0.05$). Besides, a significant association was also observed in the adiponectin concentration and hypertriglyceridemia with haplotype -11426/+45 ($p < 0.05$). In conclusion, hypoadiponectinemia and SNPs and haplotypes of adiponectin gene may contribute to the development of metabolic syndrome and its risk factors, via unknown mechanisms in Malay population.

CHAPTER ONE: INTRODUCTION

1.1 General Introduction

Metabolic syndrome (MS) is an emerging public health problem throughout the world due to increase in the prevalence of the individual metabolic abnormalities *i.e* central obesity, hypertriglyceridaemia (the elevated serum triglyceride), reduced high-density lipoprotein cholesterol (HDL cholesterol), hypertension, and hyperglycemia (the elevated blood glucose levels) (Alberti *et al.*, 2005). The rise of MS cases was shown to be in parallel with the prevalence of obesity (Grundy, 2008). A comprehensive overview on MS pandemic in the America, Europe and India indicates more than 20 % of the adult populations as having MS (Grundy, 2008). The prevalence of MS recorded was 22.2 % in Italy, 23.9 % in Portugal, 46 % in the Netherlands, 41.8 % in Greece, 37 % in Finland and Asians especially India was 41.1 %, Thailand was 12.8 %, China was 13.2 % and Japan was 14.9 % (Grundy, 2008). Across the Southeast Asia, there are several countries such as Singapore and Thailand that are actively involved in evaluating the MS. Tan *et al.* (2004) reported that in Singapore, the Asian Indian had the highest prevalence of MS followed by the Malays and Chinese (Tan *et al.*, 2004). In contrast, a different study in Singapore suggested that Malay women were more likely to develop hypertension in association with insulin resistance, as compared to Chinese and Asian Indian (Ang *et al.*, 2005). Moreover, hyperuricemia and elevated levels of liver enzymes, which were claimed to have association with MS, have been reported among Thai adults (Lohsoonthorn *et al.*, 2006; Perera *et al.*, 2008). Although Malaysia is still lacking of

the representative data on MS, but the neighboring countries somehow reflect the condition of the syndrome herein as we govern the same major ethnics groups *i.e* Malays, Asian Indians and Chinese.

The mechanisms underlying MS are still not fully understood; however, accumulating studies have proposed an association between adiponectin; a type of adipokines with MS risk factors. Several studies have demonstrated low adiponectin concentration (hypoadiponectinemia) as a common denominator for the MS risk factors. The negative correlation between visceral adiposity and adiponectin concentration suggests that hypoadiponectinemia is related to central obesity (Matsuzawa, 2010; Ryo *et al.*, 2004). Clinical reports have pointed out earlier that there are positive associations between the circulating adiponectin concentration with the HDL cholesterol and inverse association with triglycerides (Kazumi *et al.*, 2004; Matsubara *et al.*, 2002). In addition, lower concentrations of adiponectin were observed in individuals with hypertension (Adamczak *et al.*, 2003; Iwashima *et al.*, 2004) and type 2 diabetes (T2D) (Hotta *et al.*, 2000; Lindsay *et al.*, 2002). From these evidences, it can be seen that adiponectin is a potential biomarker for the MS and its risk factors. Moreover, researchers have suggested that adiponectin can be a promising therapeutic agent for MS and its risk factors. It was proposed that by increasing circulating adiponectin concentration or enhancing adiponectin signaling through its receptors could promisingly tackle the root that cause the MS (Zhu *et al.*, 2008). Although the claims excited many researchers, a lot of future investigations are required to support the matter.

Despite the regulation of adiponectin expression in targeting MS which remains ambiguous, there has been growing interest in adiponectin gene that is deemed as potential genetic contributor to MS. Several studies have recently reported the genetic polymorphisms and linkages between adiponectin gene and MS. For instance, a genotype screening that was performed on 811 Hispanic individuals has demonstrated an association between genetic variations or single nucleotide polymorphisms (SNPs) in the adiponectin gene particularly in the promoter region with obesity, especially the visceral obesity (Sutton *et al.*, 2005). However, the genetic variants of adiponectin and hypertriglyceridaemia and reduced HDL cholesterol were less explored and scantly reported (Yang & Chuang, 2006). A study to determine the possible effects of variation in adiponectin gene has shown that the mutant allele T of SNP+276 was associated with the elevated diastolic blood pressure whereas the wild-type allele T of SNP+45 demonstrated its protective role as it was associated with high HDL cholesterol levels (Mousavinasab *et al.*, 2006). On the other hand, Zacharova *et al.* (2005) which aimed to investigate the selected SNPs of the adiponectin gene and T2D reported that mutant allele G of SNP+45 was a predictor for T2D (Zacharova *et al.*, 2005).

Taking together, hypoadiponectinemia and genetic variants of adiponectin gene play an important role in the pathogenesis of MS. Studies on the influence of the different ethnic groups is crucial to promote in-depth understanding on the regulation of MS, adiponectin and adiponectin gene. In this context, this present study was designed to investigate the association of several SNPs in adiponectin gene with serum adiponectin concentration and MS risk factors among Malay adults.

1.2 Literature Reviews

1.2.1 Metabolic Syndrome

The MS has become one of the major public-health challenges worldwide. Grundy (2008) mentioned in his review that MS has a long history as early as 1923 when scientists began to notice the cluster of insulin resistance, hyperglycaemia, hypertension, low HDL cholesterol, and raised triglycerides as common risk factors for cardiovascular disease (Grundy, 2008). Since then, several names appeared to describe the cluster. Waine (2005) has reviewed previous studies on MS and summarized the names that have been used over the decades, such as the insulin resistance syndrome, plurimetabolic syndrome, dysmetabolic syndrome, and the deadly quartet (Waine, 2005). The term MS was first used by the World Health Organization (WHO) in 1998 which proposed a unifying definition for the syndrome by taking abnormal glucose tolerance as a core factor. A year later, European Group for the Study of Insulin Resistance (EGIR) came out with a modified version of the WHO recommendation (Zimmet *et al.*, 2005). The National Cholesterol Education Program's Adult Treatment Program III (ATP III) in 2001 then clinically defined MS by proposing abdominal obesity, dyslipidemia, hypertension, insulin resistance and prothrombotic and inflammatory states as the key components (Gable *et al.*, 2007). This was followed by the International Diabetes Federation (IDF) recommendations on 2005 (Zimmet *et al.*, 2005) which prioritized ethnicity-specific waist circumference cut-off points as a measure for central obesity apart from other known risk factors. The IDF definition was constructed in such a way that it would rapidly

identify individuals at risk without eliminating the ethnic factor. Due to its simplicity and appropriateness to our population, the IDF definition was used throughout the present study. Table 1 listed the criteria used for diagnosing MS by IDF definition. Note that throughout the thesis, hyperglycemia (excessive amount of glucose circulates in the blood plasma) and T2D (characterized by hyperglycemia and caused by insufficient insulin production to regulate the circulating glucose) (Sheng & Yang, 2008) were used interchangeably.

Moreover, Lahiry *et al,* (2008) has described that the concept of MS helped to emphasize on the risk of vascular disease related to central obesity and previously overlooked biomarkers such as serum triglycerides (Lahiry *et al.*, 2008). Intense studies devoted to understand the MS risk factors especially the central obesity, adipose tissue and adipokines have eventually lead the scientists to the discovery of the adiponectin; a type of adipose-specific serum protein.

Table 1 International Diabetes Federation definition of the metabolic syndrome (Zimmet *et al.*, 2005)

No	Risk Factors	Measurement	Sex	Readings
1.	Central obesity	Waist circumference[1]	Men	≥ 90 cm
			Women	≥ 80 cm
		Plus any 2 of the following		
2.	Hypertension	Systolic blood pressure / Diastolic blood pressure (or received treatment of previously diagnosed hypertension)	Both	≥ 130/85 mmHg
3.	Hyperglycemia	Fasting plasma glucose (or previously diagnosed type 2 diabetes)	Both	≥ 5.6 mmol/L (100 mg/dL)
4.	Reduced HDL cholesterol[2]	HDL cholesterol (or specific treatment for this lipid abnormality)	Men	<1.0 mmol/L (40 mg/dL)
			Women	<1.3 mmol/L (50 mg/dL)
5.	Hypertriglyceridemia	Triglycerides (or specific treatment for this lipid abnormality)	Both	≥ 1.7 mmol/L (150 mg/dL)

[1]Following the ethnic-specific measurement of South Asian; [2]HDL cholesterol: high-density lipoprotein cholesterol.

1.2.2 Adipocytokines

Adipose tissue has been considered as an active endocrine due to the production of vital hormones called adipocytokines (also known as adipokines) which significantly involved in the regulation of body's homeostasis (Koerner *et al.*, 2005). There are two types of adipose tissue *i.e* the brown adipose tissue (BAT) and white adipose tissue (WAT) (Gu, 2009). BAT mainly involves in generating the heat by triggering the combustion of lipids and glucose in the tissue (Cannon & Nedergaard, 2004). On the other hand, WAT is of interest due to its role as the primary site in producing the most important adipocytokines such as adiponectin, leptin, zinc-α2-glycoprotein (ZAG), various interleukins, transforming growth factor-β (TGF-β), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), tumor necrosis factor-α (TNF-α), and plasminogen activator inhibitor-1 (PAI-1) (Figure 1) (Trayhurn *et al.*, 2006). Leptin, for instance, has been investigated for its role in energy homeostasis, glucose and lipid metabolism, and immune and neuroendocrine function (Ahima, 2006), while the interleukins have been emphasized as markers for inflammation (Trayhurn *et al.*, 2006).

The WAT is mainly located in the subcutaneous region and viscera, hence closely associated with the pathogenesis of obesity-related disorders (Ahima, 2006). The adiponectin that is abundantly secreted by WAT has received much attention due to its protective role against the obesity-related diseases (Gu, 2009; Koerner *et al.*, 2005).

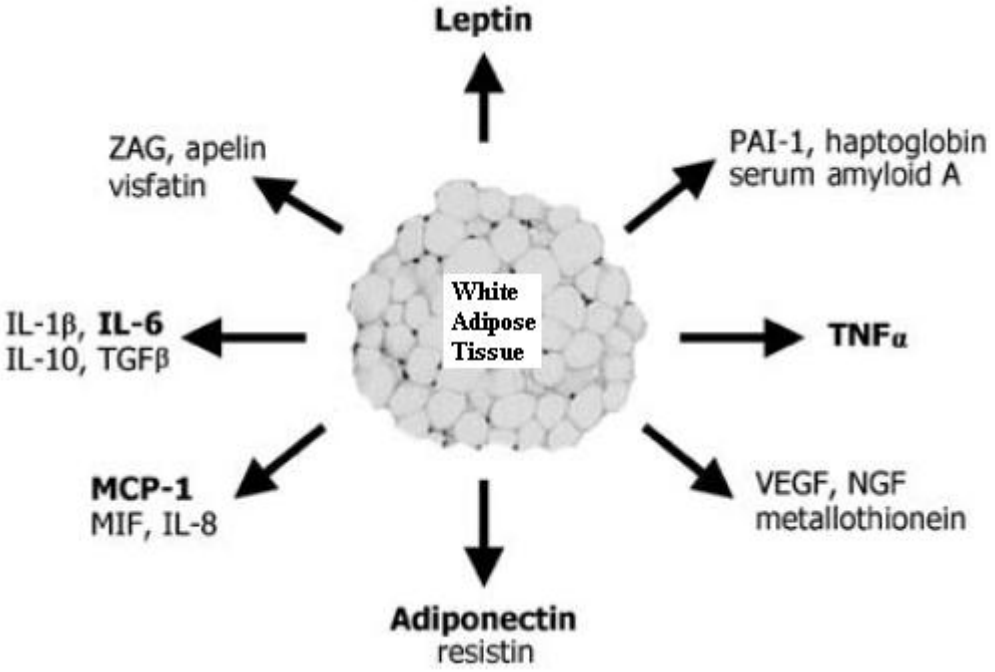


Figure 1 Major adipokines secreted from white adipose tissue (Trayhurn *et al.*, 2006)

Note:

IL-1β	Interleukins 1β
IL-6	Interleukins 6
IL-8	Interleukins 8
IL-10	Interleukins 10
MCP-1	Monocyte chemoattractant protein-1
MIF	Macrophage migration inhibitory factor
NGF	Nerve growth factor
PAI-1	Plasminogen activator inhibitor-1
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
VEGF	Vascular endothelial growth factor
ZAG	Zinc-α2-glycoprotein

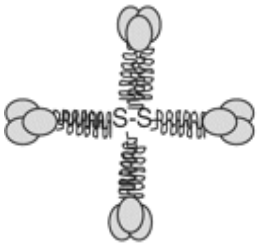
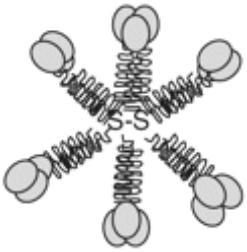
1.2.3 Adiponectin

Various adipocyte-derived secretory proteins have been increasingly linked to MS. Adiponectin, another type of adipokines recently attracts much attention among scientists. It is a hormone extensively secreted by adipocytes, expressed inversely to total fat (Comuzzie *et al.*, 2001) and acted as anti-diabetic, anti-inflammatory and anti-atherogenic agents (Broedl *et al.*, 2006). Adiponectin belongs to the complement 1q family and comprises four domains; an amino-terminal collagen-like sequence, a variable region, a collagenous domain and a carboxy-terminal globular domain (Garaulet *et al.*, 2007; Kadowaki & Yamauchi, 2005). This protein is constructed by 244 amino acids with a molecular weight of 26,414 Da and typically exists in three forms; low molecular weight (LMW) hexamers, medium molecular weight (MMW) and high molecular weight (HMW) multimeric structures (Gu, 2009; Kadowaki & Yamauchi, 2005). Adiponectin can be found extensively in serum ranging from 5 to 30 $\mu g ml^{-1}$ (Garaulet *et al.*, 2007). Table 2 represents the variety of multimeric forms of adiponectin.

This hormone was reported to function in two ways; 1) anti-atherosclerotic actions and 2) insulin-sensitizing actions, through a number of mechanisms. As shown in Figure 2, Wiecek *et al.* (2007) in a review paper on adiponectin has summarized the anti-atherogenic actions of adiponectin that suppresses the production of tumour necrosis factor-α (TNF- α), modulates biological actions of growth factors by binding with platelet-derived growth factor BB (PDGF-BB), basic fibroblast growth factor (FGF), and heparin-binding epidermal growth factor-like

growth factor (HB EGF), reduces accumulation of lipids in human monocyte-derived macrophages, inhibits transformation of macrophages into foam cells by down-regulating scavenger receptors, suppresses superoxide generation, decreases the expression of adhesion molecules (VCAM-1; ICAM-1, E-selectin), increases expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) in infiltrating macrophages, and last but not least increases activity of endothelial nitric oxide (NO) synthase (Wiecek *et al.*, 2007). Figure 2 summarizes the insulin-sensitizing action of adiponectin which stimulates glucose utilization and fatty acid oxidation in skeletal muscles and in the liver, enhances insulin signalling in skeletal muscle, facilitates glucose uptake and suppresses gluconeogenesis in the liver (Wiecek *et al.*, 2007). This mechanism of actions of adiponectin ultimately results in increasing insulin sensitivity and suppression of atherosclerosis (Kadowaki & Yamauchi, 2005). Since this type of protein characterizes a number of metabolic derangements, it is crucial to have insights into its molecular level.

Table 2 Multimeric forms of adiponectin (Wiecek *et al.*, 2007)

Structures	Multimeric Forms	Molecular Weight
	Globular C-terminal domain fragments	None
	Monomer	
	Trimer	Low Molecular Weight (LMW)
	Hexamer	Medium Molecular Weight (MMW)
	12-mers	High Molecular Weight (HMW)
	18-mers	

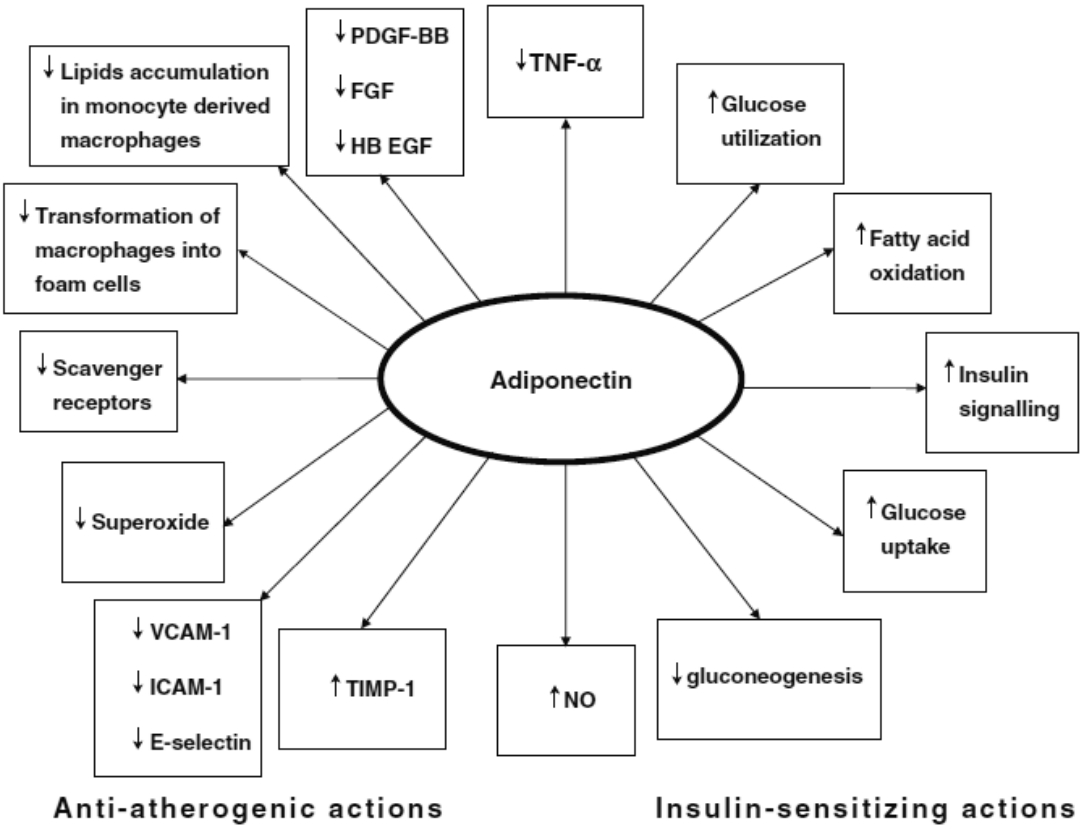


Figure 2 Anti-atherogenic and of insulin-sensitizing actions of adiponectin (Wiecek *et al.*, 2007)

Note:

FGF	Fibroblast growth factor
HB EGF	Heparin-binding epidermal growth factor
ICAM-1	Intercellular adhesion molecule-1
NO	Nitric oxide
PDGF-BB	Platelet-derived growth factor BB
TIMP-1	Tissue inhibitor of metalloproteinase-1
TNF- α	Tumour necrosis factor-α
VCAM-1	Vascular cell adhesion molecule-1

1.2.4 Adiponectin Gene

Despite of extensive studies correlating environmental factors such as high fat diet and physical inactivity with the pathogenesis of MS, there is also strong evidence showing that the syndrome is possibly inheritable (Lyssenko *et al.*, 2008). Groop (2000) listed possible genes which are associated with fat and glucose metabolism such as genes for leptin/leptin receptor, β_2- and β_3- adrenergic receptors, lipases, TNF-α, PPAR-γ, glycoprotein PC-1, IRS-1 and lycogen synthase (Groop, 2000). Apart from these genes, there is an increase of interest among scientists to focus more onto adiponectin gene recently.

The 16-kb structural gene, which has been mapped to chromosome 3 (3q27) in the human *APM1/ACDC/ADIPOQ* gene, encodes the protein product adiponectin (Comuzzie *et al.*, 2001). This particular region of chromosome 3 (Accession ID D45371) has also been found to contain a quantitative trait locus (QTL) with a strong influence on phenotypes of the MS (Kissebah *et al.*, 2000). The adiponectin gene contains three exons and two introns (Gable *et al.*, 2006; Gable *et al.*, 2007; Kadowaki & Yamauchi, 2005). Exon 1 and 2 are 76 and 222 bp, respectively, with 10.3 kb of intron 1 lies in between them while exon 3 is approximately 4.28 kb. The translation starts at exon 2 and ends at exon 3, leaving exon 1 and part of exon 3 untranslated (Takahashi *et al.*, 2000). Figure 3 and 4 show the ideogram and genomic organization of the adiponectin gene, respectively.

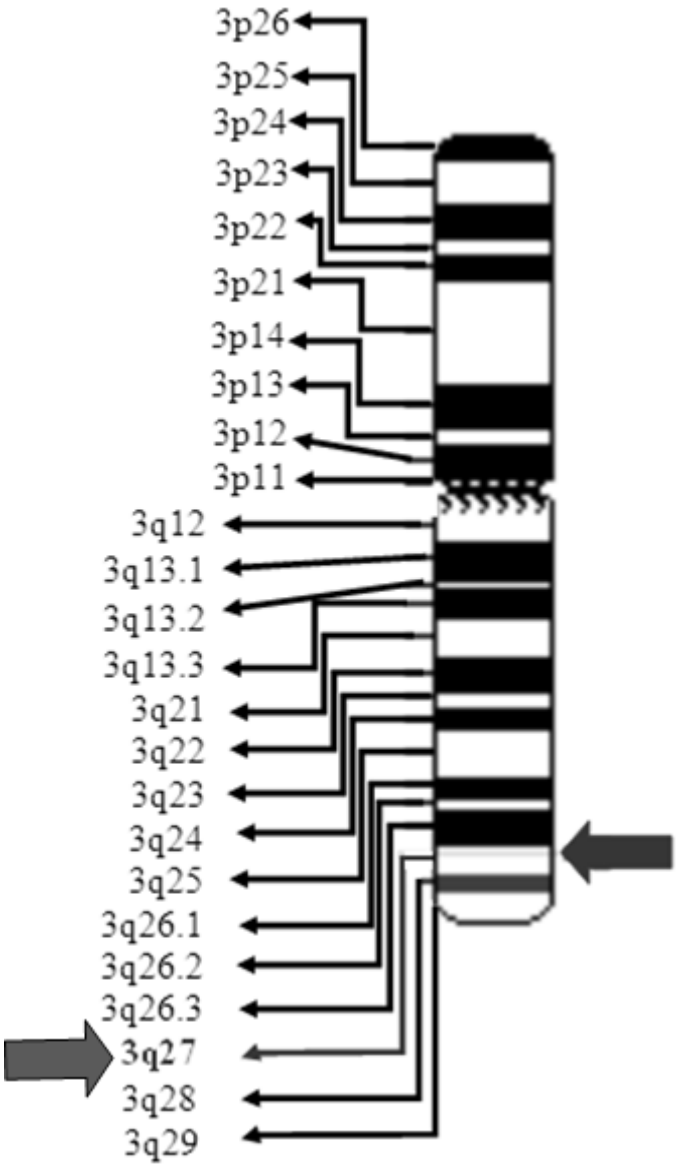


Figure 3 Ideogram of chromosome 3 where adiponectin spanned on 3q27 (National Center for Biotechnology Information, 2008)

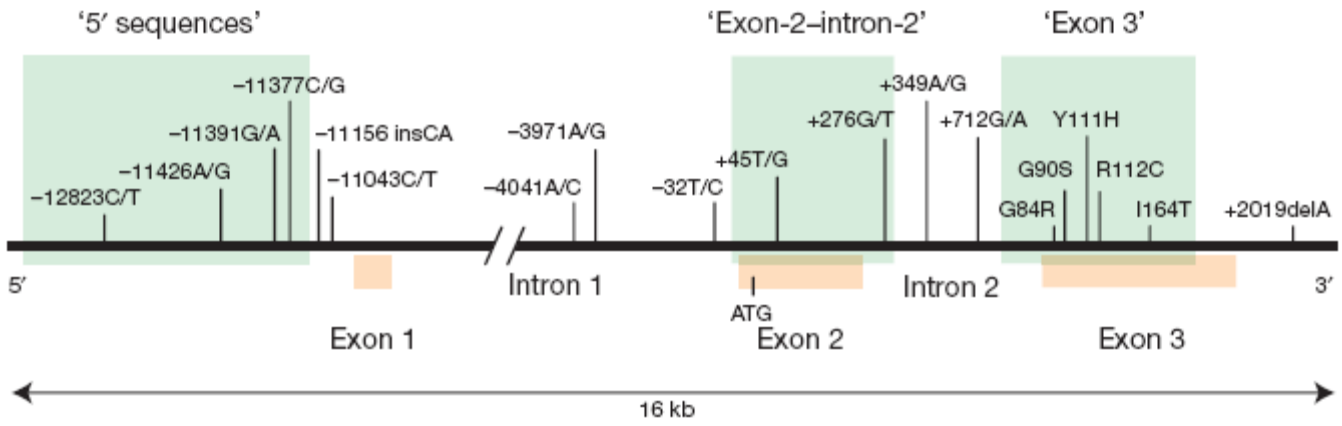


Figure 4 Organization of the adiponectin gene on chromosome 3 (Vasseur *et al.*, 2006)

1.2.5 Association of Adiponectin, Adiponectin Gene and Metabolic Syndrome

The discovery of adiponectin back in 1995 (Scherer *et al.*, 1995) did not receive major attention in the scientific community for the next few years until its markedly protective role in the pathogenesis of obesity-related disorders was acknowledged in the new millennium. Yang and Chuang (2006) in their review article on human genetics of adiponectin in the MS claimed that this adipose-derived serum protein is an important biomarker for MS.

The human genetic studies not only allow scientists to have an insight into various SNPs and genetic make-up of adiponectin, but also provide information and strong evidence to support the fact that this gene is one of the contributing factors to MS (Yang *et al.*, 2007). Studies demonstrated that serum adiponectin concentration is significantly low in subjects with obesity, insulin resistance, MS, T2D, and coronary heart disease (Broedl *et al.*, 2006; Yang & Chuang, 2006; Yang *et al.*, 2007). Scientists also found a number of variants of SNPs and missense mutations in European, North American and Japanese populations that are closely linked to the MS risk factors (Broedl *et al.*, 2006; Gable *et al.*, 2006). However, the association of adiponectin genetic variations with dyslipidemia and blood pressure was poorly explored (Yang & Chuang, 2006).

Three regions that are closely linked to MS have been identified on the highly polymorphic adiponectin gene; 1) the 5' sequences; 2) the intron 2-exon 2 region, and 3) exon 3 (Vasseur *et al.*, 2006). This is supported by Gable *et al.* (2007) which claimed that the promoter variants -11391G>A and -11377C>G, +45T>G variant of

exon 2 and +276G>T variant of intron 2 are the most widely studied; all mentioned variants were selected in this present study. Yang and Chuang (2006) also agreed with the fact that promoter region, exon and intron 2, and the rare non-synonymous mutations in exon 3 harbor a number of common polymorphisms across different ethnic population (Yang & Chuang, 2006). When these polymorphisms were translated, all the mentioned SNPs excluding the -11391G>A introduced changes to the amino acid (Table 3).

Various studies demonstrated that SNP+276 is associated with increased risk of T2D, higher insulin resistance index, lower adiponectin concentration, cardiovascular disease risk, higher BMI, higher glucose level and obesity (Filippi *et al.*, 2004; Hara *et al.*, 2002; Jang *et al.*, 2006; Ukkola *et al.*, 2003; Xita *et al.*, 2005; Yang *et al.*, 2007). Kadowaki and Yamauchi (2005) addressed that SNP+276 as one of the factors in reducing adiponectin concentration and in turn promote the development of insulin resistance, MS and atherosclerosis (Kadowaki & Yamauchi, 2005).

Haplotype of a variant with other variants perhaps doubled the effect on MS susceptibility. The haplotype +276/+45 was the most common haplotype studied that linked with higher body weight, waist circumference, blood pressure, HDL cholesterol/total cholesterol ratio, lower serum adiponectin and higher risk of T2D (Gable *et al.*, 2006). SNP+45, located in exon 2 is a silent mutation which could possibly alter a putative enhancer or silencer of splicing (Vasseur *et al.*, 2003). On the other hand, SNP+276 is located in intronic region (intron 2) which is well

documented as non-coding region. However, studies have proposed that unknown mechanism(s) of these intronic SNPs perhaps involved in the expression level of the adiponectin gene that ultimately cause the development of MS (Hara *et al.*, 2002; Jang *et al.*, 2006).

Moreover, a study on French Caucasian population disclosed association between SNP-11391 and SNP-11377 with serum adiponectin concentration and diabetic status (Vasseur *et al.*, 2003). The SNP-11391 was also associated with hypoadiponectinemia in Amish (people devoted to Christian living in Canada or United States) and Swedish population while SNP-11377 was associated with T2D in Japanese population. SNP-11426 has been shown to be associated with variation in insulin sensitivity in French and Swedish population (Gable *et al.*, 2006). Gu (2009) has summarized SNPs of adiponectin gene that have been associated with MS risk factors in various ethnic populations as shown in Table 4 (Gu, 2009).

Although the genetic variation in 5' sequences has not yet been defined to cause the development of MS, but these variants were shown to be associated with adverse metabolic features. Furthermore, SNPs in adiponectin gene have been demonstrated to contribute to hypoadiponectinemia, decreased insulin sensitivity and T2D in several populations. Therefore, these SNPs are thought to either modulate the expression of the adiponectin gene or in linkage disequilibrium with functional variants to modulate the expression of the gene (Vimaleswaran *et al.*, 2008). There are several other variants contributing to the MS, with some probably still unknown. Thus, in depth investigations are crucial to precisely determine which variant(s) leads

a significant role to the risk of MS. Taken all the consideration from previous studies as a whole, therefore the presence of SNPs +45, +276, -11426, -11391 and -11377 were shown to be worthy predictors to indicate the characteristics of MS in Malay population.

Table 3 Amino acid changes introduced by respective SNPs in adiponectin gene

SNP	Nucleotide	Amino Acid Sequence
+45T>G	T	91 TTG CTG GGA GCT GTT CTA CTG CTA TTA GCT CTG CCC GG**T** CAT GAC 135 31 Leu Leu Gly Ala Val Leu Leu Leu Leu Ala Leu Pro **Gly** His Asp 45 136 CAG GAA ACC ACG ACT CAA GGG CCC GGA GTC CTG CTT CCC CTG CCC 180 46 Gln Glu Thr Thr Thr Gln Gly Pro Gly Val Leu Leu Pro Leu Pro 60
	G	91 TTG CTG GGA GCT GTT CTA CTG CTA TTA GCT CTG CCC GG**G** CAT GAC 135 31 Leu Leu Gly Ala Val Leu Leu Leu Leu Ala Leu Pro **Gly** His Asp 45 136 CAG GAA ACC ACG ACT CAA GGG CCC GGA GTC CTG CTT CCC CTG CCC 180 46 Gln Glu Thr Thr Thr Gln Gly Pro Gly Val Leu Leu Pro Leu Pro 60
	Note: The single nucleotide change from T to G at SNP+45 does not alter the amino acid in the protein chain.	
+276G>T	G	586 ATG AAG GAT GTG AAG **G**TC AGC CTC TTC AAG AAG GAC AAG GCT ATG 630 196 Met Lys Asp Val Lys **Val** Ser Leu Phe Lys Lys Asp Lys Ala Met 210 631 CTC TTC ACC TAT GAT CAG TAC CAG GAA AAT AAT GTG GAC CAG GCC 675 211 Leu Phe Thr Tyr Asp Gln Tyr Gln Glu Asn Asn Val Asp Gln Ala 225
	T	586 ATG AAG GAT GTG AAG **T**TC AGC CTC TTC AAG AAG GAC AAG GCT ATG 630 196 Met Lys Asp Val Lys **Phe** Ser Leu Phe Lys Lys Asp Lys Ala Met 210 631 CTC TTC ACC TAT GAT CAG TAC CAG GAA AAT AAT GTG GAC CAG GCC 675 211 Leu Phe Thr Tyr Asp Gln Tyr Gln Glu Asn Asn Val Asp Gln Ala 225
	Note: The single nucleotide change from G to T at SNP+276 alters the amino acid from valine to phenylalanine in the protein chain.	

+11426A>G	A	2746 TAG TAA AGA CAG GGT TTC ACC ATA TTG GCC AGG CTG GTC TCG A**A**C 2790 916 End End Arg Gln Gly Phe Thr Ile Leu Ala Arg Leu Val Ser **Asn** 930 2791 TCC TGA CCT TGT GAT CTG CCC GCC TCC ATT TTT GTT GTT ATT TTT 2835 931 Ser End Pro Cys Asp Leu Pro Ala Ser Ile Phe Val Val Ile Phe 945
	G	2746 TAG TAA AGA CAG GGT TTC ACC ATA TTG GCC AGG CTG GTC TCG A**G**C 2790 916 End End Arg Gln Gly Phe Thr Ile Leu Ala Arg Leu Val Ser **Ser** 930 2791 TCC TGA CCT TGT GAT CTG CCC GCC TCC ATT TTT GTT GTT ATT TTT 2835 931 Ser End Pro Cys Asp Leu Pro Ala Ser Ile Phe Val Val Ile Phe 945
	Note: The single nucleotide change from A to G at SNP+11426 alters the amino acid from asparagine to serine in the protein chain.	
-11391G>A	G	3151 GTT TCC CTC CCG ATA TCA AAA AGA CTG TGG CCT GCC CAG CTC TC**G** 3195 1051 Val Ser Leu Pro Ile Ser Lys Arg Leu Trp Pro Ala Gln Leu **Ser** 1065 3196 TAT CCC CAA GCC ACA CCA TCT GGC TAA ATG GAC ATC ATG TTT TCT 3240 1066 Tyr Pro Gln Ala Thr Pro Ser Gly End Met Asp Ile Met Phe Ser 1080
	A	3151 GTT TCC CTC CCG ATA TCA AAA AGA CTG TGG CCT GCC CAG CTC TC**A** 3195 1051 Val Ser Leu Pro Ile Ser Lys Arg Leu Trp Pro Ala Gln Leu **Ser** 1065 3196 TAT CCC CAA GCC ACA CCA TCT GGC TAA ATG GAC ATC ATG TTT TCT 3240 1066 Tyr Pro Gln Ala Thr Pro Ser Gly End Met Asp Ile Met Phe Ser 1080
	Note: The single nucleotide change from G to A at SNP-11391 does not alter the amino acid in the protein chain.	

<table>
<tr><td rowspan="3">-11377C>G</td><td>C</td><td>1396 ACG TCC TGT CTT GGA AGG ACT ACT ACT CAA TGG CCC CTG <u>C</u>AC TAC 1440
466 Thr Ser Cys Leu Gly Arg Thr Thr Thr Gln Trp Pro Leu <u>His</u> Tyr 480

1441 TCT ACT TCC TCT TAC CTA TGT CCC TTC TCA TGC CTT TCC CTC CAA 1485
481 Ser Thr Ser Ser Tyr Leu Cys Pro Phe Ser Cys Leu Ser Leu Gln 495</td></tr>
<tr><td>G</td><td>1396 ACG TCC TGT CTT GGA AGG ACT ACT ACT CAA TGG CCC CTG <u>G</u>AC TAC 1440
466 Thr Ser Cys Leu Gly Arg Thr Thr Thr Gln Trp Pro Leu <u>Asp</u> Tyr 480

1441 TCT ACT TCC TCT TAC CTA TGT CCC TTC TCA TGC CTT TCC CTC CAA 1485
481 Ser Thr Ser Ser Tyr Leu Cys Pro Phe Ser Cys Leu Ser Leu Gln 495</td></tr>
<tr><td colspan="2">Note: The single nucleotide change from C to G at SNP-11377 alters the amino acid from histidine to aspartic acid in the protein chain.</td></tr>
</table>

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Table 4 The genetic variants and adiponectin or MS risk factors with the respective ethnic group that has been studied (Gu, 2009)

SNP	Adiponectin or MS Risk Factors	Ethnic Group
+45	Adiponectin concentration	Japanese, Chinese
	Type 2 diabetes	Korean, Italian, Quebec family study
	Obesity	Swedish, Finnish
+276	Adiponectin concentration	European Caucasians
	Type 2 diabetes	Japanese, Italian, German
	Obesity	Chinese, Korean
-11377	Adiponectin concentration	French Caucasians
	Type 2 diabetes	Swedish, Danish
	Obesity	German, Italian
-11391	Adiponectin concentration	French Caucasians
	Type 2 diabetes	UK Caucasian women
	Obesity	German, Italian
-11426	Adiponectin concentration	French Caucasians, Swedish,
	Type 2 diabetes	European Caucasians

1.3 Rationale of the Study

In regards to the gradual increase in prevalence of MS risk factors, Song *et al.* (2006) have pointed out the needs to identify susceptibility genes of MS and its mechanism of actions, which may enable the investigators to design preventive strategies and targeted treatments (Song *et al.*, 2006). To date, there is still lack of nationally representative figures on occurrence and association of adiponectin and MS among Malay adults in Malaysia, let alone their association with the polymorphisms.

Human genetic epidemiological studies on adiponectin and parameters of MS would be helpful to understand the molecular mechanisms involved in regulating metabolism susceptibility hence protect against the development of metabolic diseases. Moreover, metabolic diseases appear to be varied across different population and ethnicity. As the ethnic differences strongly suggest a genetic component in the pathogenesis of metabolic syndrome (Song *et al.*, 2006), therefore, this study perhaps will provide an insight on the underlying causes to prevent or reduce the long-term risks for MS among Malay population.

1.4 Objectives

1.4.1 General Objectives

To investigate the association of the selected SNPs in adiponectin gene with serum adiponectin concentration and MS risk factors among Malay adults.

1.4.2 Specific Objectives

1. To determine the prevalence of SNPs +45, +276, -11426, -11391 and -11377 of the adiponectin gene among Malay adults.

2. To investigate the association between serum adiponectin concentration with the MS and MS risk factors.

3. To investigate the association between the selected SNPs and haplotypes with serum adiponectin concentration.

4. To investigate the association between the selected SNPs and haplotypes with the MS and MS risk factors.