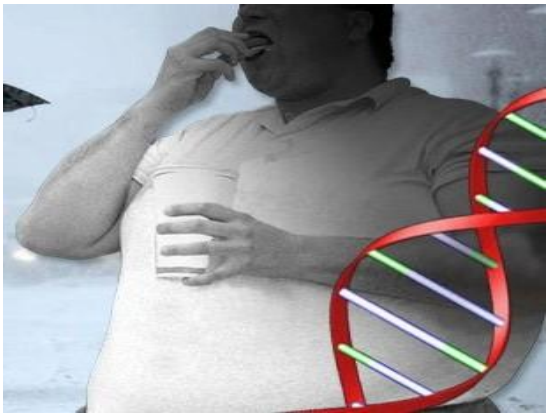
CHAPTER 1

INTRODUCTION



1.1 Introduction

Obesity is a chronic disorder that can increase the risk of developing type 2 diabetes mellitus, coronary heart disease, hypertension, asthma, sleep apnoea, osteoarthritis and certain types of cancer (Loos & Bouchard, 2003). Due to its increasing health burden worldwide, research into obesity has attracted the attention of many researchers. According to the World Health Organization, obesity is one of the top five global health risk factors. WHO projected that more than 700 million adults will be obese by 2015. Obesity is becoming a major problem in Malaysia as it is worldwide. The Malaysian Non Communicable Disease Survey (NCD 2005) reported that 48.6% of adults in Malaysia are obese.

Obesity is strongly linked to cardiovascular risk factors such as diabetes, hyperlipidemia and hypertension but the underlying mechanisms for this remain largely unknown. The Human Genome Project, International HapMap Project and Genome-Wide Association Studies (GWAS) are breakthrough studies that enable researchers to move towards a new era of discoveries of the genetics of human diseases (Loos & Bouchard, 2008; Schrauwen, van Marken Lichtenbelt, Saris, & Westerterp, 1997). Obesity is a multifactorial disease that occurs due to complex interactions between genetic and environmental factors. Many studies have shown that genetic susceptibility to obesity varies among different ethnic groups (Markison & Foster, 2006; Yanagiya et al., 2007). Understanding the physiological mechanisms together with the underlying genetic basis of human obesity is necessary in order to address this complex disorder. One of possible way to understand the full mechanism is by studying obesity genetic markers such as single-nucleotide polymorphisms (SNPs) and biomarkers. This will aid in early detection, prevention and also prediction of cardiovascular risk factors in susceptible obese subjects. Biomarkers which are important in inflammation and

oxidative stress are extensively studied in humans due to their future clinical impacts. Investigation into the genetic effects of adipokines such as leptin, resistin, adiponectin and visfatin on obesity is growing globally. It is therefore essential to investigate the circulating levels and effects of these peptide hormones in different populations. Further elucidation of bioactive forms and specific mechanisms involved in metabolic pathways, the adipokines especially adiponectin, may hold great discoveries in the diagnosis, risk assessment and pharmacological treatment of obesity and other disorders globally and also in Malaysia. As levels of these adipocytokines are influenced by genetic background, a comprehensive study on genetic and biomarkers may aid in the prevention and management of the rising prevalence of obesity in Malaysia.

Linkage disequilibrium (LD) occurs when one locus is found together with a specific allele at a specific locus more frequently than expected if alleles at the loci were merged independently in a population. Strong linkage may produce an effect in high levels of LD. LD between a marker locus and a disease locus indicates that the two loci are closely linked (Briscoe, Stephens, & O'Brien, 1994; Jorde, 1995; Kaplan, Hill, & Weir, 1995; Plomin, Owen, & McGuffin, 1994). LD testing is important for disease-gene localization as the LD is related to recombination which occurs between disease locus and linked locus. If the multiple loci are strongly linked, LD will be stronger as the recombination fraction is infrequent for many generations (Chapman & Wijsman, 1998).

Haplotyping is a joint analysis of several SNP markers for test of association between phenotype and genotype. A pair of bi-allelic markers is genotyped to determine the distribution of two-locus linkage. Haplotyping from genotype data is very crucial in association studies because analysis based on haplotypes is more powerful due to the application of multi-marker genotypes as compared to single locus test alone (Zhang,

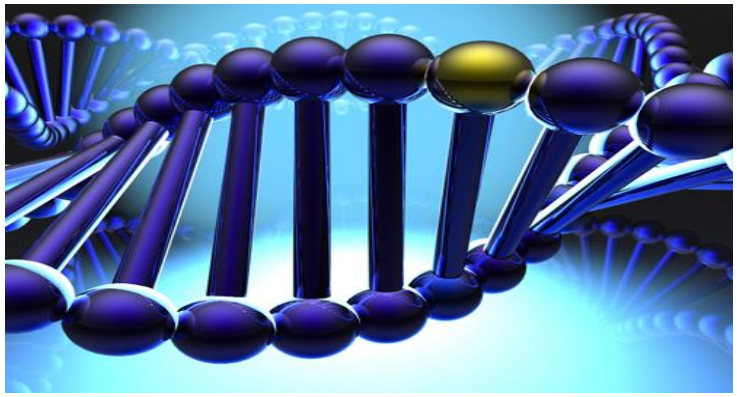
Calabrese, Nordborg, & Sun, 2002). Therefore, analysis of disease-causing mutations with the haplotype is a crucial strategy for association studies. A complete study on the influence of genetics on obesity has not been done in Malaysia and hence goal of this study is to determine the distribution of genetic variations in the obesity related genes and to provide an important genetic resource for genotype-obesity association study in the Malaysian Malay population

1.2 Objectives

1. To determine the association between various single nucleotide polymorphisms (SNPs) of certain candidate genes and obesity phenotypes, such as body weight, BMI, height, hip circumference, waist circumference and WHR in the Malaysian Malay population.
2. To determine the association between the candidate SNPs and the obesity-related parameters such as blood pressure, cholesterol and lipid parameters in the Malaysian Malay population.
3. To determine whether allele and genotype frequencies of the SNPs of the candidate genes differ between obese and non-obese groups.
4. To identify genetic regions associated with obesity based on haplotype analysis by exploring nearby SNPs of candidate genes.
5. To investigate the gene-gene interactions between the FTO and MC4R SNPs.
6. To compare levels of the candidate obesity biomarkers such as leptin, resistin and adiponectin in obese and in non-obese subjects.
7. To examine the correlation between the candidate biomarkers with obesity parameters.
8. To examine the association between genetic variants and obesity biomarkers such as leptin, resistin and adiponectin levels in study subjects.

CHAPTER 2

LITERATURE REVIEW



2.0 Literature review

2.1 Obesity

Obesity is defined as the accumulation of excessive body fat which results from increase in energy input, decrease in energy output or both (Fair & Montgomery, 2009). Obesity happens when there is excess of body weight for height. Obesity is measured on the basis of body mass index (BMI). BMI is calculated based on body weight in kilograms divided by height in square meters. BMI is strongly linked to total body fat. World Health Organization (WHO) defines obesity as BMI of $\geq$ 30 kg/m^2 and overweight as BMI of $\geq$ 25kg/m^2 (Guilbert, 2003).

2.1.1 Definition of obesity

WHO defines that obesity occurs when an individual's BMI is equal to or higher than 30 kg/m^2. WHO standard uses BMI to classify individuals who are placed in the category of underweight, normal, pre-obese, obese, obese class I, obese class II and obese class III.

2.1.2 Classification of obesity

The classification of BMI is shown in Table 2.1. An individual with BMI equal to or less than 18.5 kg/m^2 is classified as underweight. Normal weight subject is one who has BMI within the range of 18.5 kg/m^2 to 24.9 kg/m^2. For overweight or pre-obese individuals, the BMI ranges from 25.0 kg/m^2 to 29.9 kg/m^2. The obese individual has a BMI of more than 30.0 kg/m^2. The obese group is subdivided into 3 categories which are obese class I, obese class II and obese class III which is also known as morbidly obese.

Table 2.1 : Classification of BMI

BMI Classification	
Underweight	**< 18.5**
Normal range	**18.5 - 24.9**
Overweight	**≥ 25.0**
Preobese	**25.0 - 29.9**
Obese	**≥ 30.0**
Obese class I	**30.0 - 34.9**
Obese class II	**35.0 - 39.9**
Obese class II	**≥ 40.0**

Source: (WHO, 2012a)

2.1.3 Abdominal obesity

Abdominal obesity or central obesity is a form of obesity that is linked to metabolic syndrome. Abdominal obesity is measured by waist circumference (WC). International Diabetes Federation (IDF) defines abdominal obesity with ethnic specific value for South Asians (Chinese, Malay and Indian). The gender specific WC cut-offs for male is ≥ 90cm and for female is ≥80cm. The WC correlates directly to abdominal obesity. A recent study highlighted the importance of adjusting hip circumference in central obesity for the assessment of obesity-related risk in Asians. This study reported that hip-adjusted waist circumference was a strong predictor of CVD and of all-cause mortality in both men and women, in South Asians and Africans (Cameron et al., 2012).

2.2 Prevalence of Obesity

According to a report by World Health organization (WHO), obesity has reached epidemic proportions globally. Globally, overweight and obesity represent the fifth leading risk for death. Almost 2.6 million people die each year by being obese or overweight. WHO estimates that more than one in ten people, which is approximately 1.5 billion of the world's adult population was obese in 2008. WHO postulated that

roughly about 2-3 billion will be overweight and more than 700 million will be obese by 2015 (http://www.who.int/mediacentre/factsheets/fs311/en/index.html).

In Asia, prevalence of overweight and obese individuals has increased dramatically with various degrees between the countries. Vietnam and Indonesia are at an early level of progression of obesity while Japan, Singapore, Malaysia and Hong Kong are at more advanced levels of obesity (Ramachandran & Snehalatha, 2010).

There is a rising prevalence of overweight and obesity in Malaysia. The Third National Health and Morbidity Survey (NHMS III) in Malaysia reported evidence of significant rise in the prevalence of obesity and overweight in the adult Malaysian population as compared to the NHMS II. Adult prevalence of overweight has increased from 16.6% to 28.6%. This shows a 1.6 times of rise in overweight within 10 years. NHMS III reported that over the past decade obesity has reached alarming state in which there is a threefold rise in prevalence of obesity, from 4.4% to 14.2% in Malaysia (Suzana et al., 2012). Malaysian women had almost two times higher risk of obesity (13.8%) compared to men (7.4%) (Suzana, et al., 2012).

2.3 Body weight regulation

The control of body weight depends on complex feedback mechanism of food intake, nutrient turnover, thermogenesis and body fat stores (Martinez & Fruhbeck, 1996). Food intake stimulates various sensory signals via distension, local hormones and nutrients (Xi & Mi, 2009). The afferent signals modulate appetite through specific pathways involving various neurotransmitters such as monoamines, amino acids and neuropeptides (Karasawa et al., 2010). The autonomic nervous system and circulating hormones such as insulin, growth hormone and cortisol all act in various ways in response to food intake (Scuteri et al., 2007; Tonjes et al., 2010). The signals induced by food intake result in neuronal and hormonal outputs that elicit adjustment in nutrient

intake, energy and nutrient metabolism. The control of substrate cycling and thermogenesis depends on food supply and specific mechanism influences the fuel mixture oxidized through efferent nervous and endocrine events. This directly influences on fat deposition and food intake (Peeters et al., 2008). The body fat stores are less markedly affected by daily imbalances in energy intake. Nevertheless, the hormone leptin which is involved in lipostatic mechanism modulates fat deposition by triggering efferent nervous and endocrine signals that are mediated by β3-adrenergic receptors. In addition, peptides or hormones affect lipid turnover such as growth hormones, insulin-like growth factor-1, insulin and adrenal steroids (Bray, 1991; Scott, 1996; Xi et al., 2010).

2.4 Obesity and associated comorbidities

Increase in BMI is a risk factor for cardiovascular disease such as heart disease, stroke, diabetes, musculoskeletal disorders and cancers.

2.4.1 Obesity and cardiovascular risk

Obesity is a chronic metabolic disorder linked to cardiovascular risk. Previous studies have suggested that obesity and CVD are dependently linked with the presence of diabetes, hypertension and dyslipidemia while other studies have reported the independent relation between CVD and abdominal obesity (Larder, Cheung, Tung, Yeo, & Coll, 2011; Wu, Saunders, Szkudlarek-Mikho, Serna Ide, & Chin, 2010). Many studies have highlighted that abdominal obesity is strongly related to coronary heart disease (CHD) (Bravard et al., 2011; Larder, et al., 2011). A meta-analysis which includes 900,000 adults reported that for each 5 kg/m^2 increase in BMI, the overall mortality will increase by 30% while cardiovascular mortality will increase by 40% (Whitlock et al., 2009). However, since BMI fails to reflect body fat distribution, use of

BMI as a cardiovascular risk factor is widely under controversial (Reich et al., 2001; Shi & He, 2005). BMI is not a good indicator of visceral fat which is the basis for metabolic disorders that is linked to CVD (Shi & He, 2005). Nevertheless, it was observed that the cardiovascular mortality risk is increased in those with severe obesity (BMI>35kg/m^2) (Ramachandran & Snehalatha, 2010). BMI is correlated to total body fat mass while waist-hip-ratio (WHR) and waist circumference (WC) is correlated to abdominal adiposity. Recently, WC is being widely used as a predictor for metabolic syndrome. The IDF criteria provides ethnic specific cut-offs (James, 2005). The International Diabetes Federation (IDF) has defined abdominal obesity to be ethnic specific, the cutoffs for South Asians (Malay, Chinese and Asian Indian populations) being a waist circumference of >90 cm for men and >80 cm for women.

2.4.2 Obesity and insulin resistance

Insulin resistance manifests when the ability of cells in the body (liver, skeletal muscle and adipose tissue) is impaired and there is resistance to insulin (Lebovitz, 1999). Insulin is a hormone produced by the pancreas for glucose absorption. Previous studies confirmed that an increase in visceral adiposity is the causative reason for insulin resistance in Type 2 DM patients (Banerji et al., 1997; Lebovitz, 1999). Insulin signaling and glucose homeostasis is blunted by obesity-induced fat deposition (Rimm et al., 1995). Abdominal obesity is an important risk factor for imbalance of hepatic glucose homeostasis. Accumulation of abdominal fat leads to very high influx of fatty acids, cytokines and hormones into liver. Insulin resistance suppresses the release of non-esterified fatty acid (NEFA) from adipose tissue and this may be the causative factor for the high risk of obesity-related diseases (Frayn, 2002).

2.4.3 Obesity and diabetes mellitus

The manifestation of diabetes mellitus is mainly due to insulin resistance and reduced secretory function of the pancreatic β-cells (Lillioja et al., 1993; Weyer, Bogardus, Mott, & Pratley, 1999). Increase in adipose tissue mass may play an important role in the pathogenesis of T2DM. Studies reported that most of the diabetic patients are overweight and about 90% of subjects who develop T2DM have BMI higher than 23.0 kg/m^2 (Hu et al., 2001; Martinez & Fruhbeck, 1996; Wannamethee & Shaper, 1999).

2.4.4 Obesity and dyslipidemia

Obesity is linked to low High-Density Lipoprotein (HDL-C) levels. Reduction in HDL-C levels happens due to impairment of lipoprotein lipase activity and enhanced cholesteryl ester transfer protein (CETP)-mediated lipid exchange (Bamba & Rader, 2007; Vinik, 2005). Atherogenic dyslipidemia is described as elevated levels of small dense low-density lipoprotein (sdLDL) particles, increased serum triglycerides (TG) and low HDL-C levels (Vinik, 2005). Dyslipidemia gradually develops as BMI increases more than $21kg/m^2$ and sdLDL is elevated (James, 2005; Vinik, 2005). It has been suggested that these metabolic alterations give rise to CHD risk by 3-6 fold (James, 2005).

2.4.5 Obesity and adipose tissue dysfunction

Obesity manifests due to changes in the secretory function of adipocytes and macrophages that simultaneously low-grade inflammation and increased risk of insulin resistance, diabetes and vascular disease (Iacobellis, Ribaudo, Zappaterreno, Iannucci, & Leonetti, 2005; Ross, 1999). Macrophages are found more abundantly in adipose tissues of obese subjects compared to lean subjects. The quantity of macrophages

directly correlates to insulin resistance (Otto & Lane, 2005). Adipose tissue is known as a passive store of excess calories. Recently, it has been confirmed that adipocytes synthesize and secretes adipokines that are involved in cardiovascular pathophysiology by affecting metabolic function and insulin action. These adipokines include procoagulant proteins such as leptin, adiponectin, resistin, plasminogen activator inhibitor- (PAI-1) and proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1, monocyte chemotactic protein-1 (MCP-1), C-reactive protein and other mediators (Calabro et al., 2008; Chrysohoou et al., 2007; Frayn, 2002; Hotamisligil, Shargill, & Spiegelman, 1993; Montague & O'Rahilly, 2000; Tousoulis, Antoniades, & Stefanadis, 2007; Weisberg et al., 2003).

Adipokines are important in body metabolism homeostasis as well as atherosclerotic process. Alteration in expression of adipokines in obesity might be moderately crucial for the insulin resistance and atherosclerosis in obese subjects. Adiponectin and leptin have major roles in obesity compared to other adipocytokines (Lyon, Law, & Hsueh, 2003; Tousoulis, et al., 2007). Excess adiposity results in increased expression of adipokines in obese humans and animals. Reduction in fat mass is strongly linked to reduction in circulating proinflammatory adipokines levels (Lyon, et al., 2003).

2.4.6 Obesity and cancer

Previous studies have shown a link between obesity and cancer (Donohoe, Pidgeon, Lysaght, & Reynolds, 2010; Percik & Stumvoll, 2009; Siegel et al., 2010; Teucher, Rohrmann, & Kaaks, 2010). Obesity is associated with high risk for manifestation of numerous types of cancer including colon, esophagus, breast, kidney, liver, endometrium, pancreas and gallbladder cancers (Calle, 2007; Calle & Kaaks, 2004; Maccio et al., 2010; Percik & Stumvoll, 2009; Pischon, Nothlings, & Boeing,

2008). Dysfunction of adipose tissue in obesity might lead to the onset and development of cancer (van Kruijsdijk, van der Wall, & Visseren, 2009). This could possibly occur via dysregulated secretion of pro-inflammatory cytokines, adipokines and chemokines such as IL-6, leptin, adiponectin, PAI-1 and TNF-α (Prieto-Hontoria et al., 2011).

2.5 Aetiological factors of obesity

Obesity is a multifactorial disorder. Obesity occurs due to interactions between genetic factors, diet, physical activity, gene-environment interaction, lifestyle and epigenetic factors that affect the energy balance (Grundy, 1998; Rosenbaum, Leibel, & Hirsch, 1997). Epigenetic changes at DNA level could contribute to obesity. Modern living lifestyle may possibly influence methylation pattern of specific genes which eventually results in increased risk of obesity (Haemer, Huang, & Daniels, 2009).

2.5.1 Genetic factors

Genes affect afferent and efferent signals as well as central mechanism involved in body weight regulation (Hirsch & Leibel, 1998). Genetic factors play an important role in the predisposition of obesity. Twin and family studies have revealed that genetics strongly contribute to obesity (Maes, Neale, & Eaves, 1997). Twin and adoption studies have proven that genetic factors play an important role in body weight regulation (Bouchard et al., 1990; Stunkard, Harris, Pedersen, & McClearn, 1990; Stunkard et al., 1986). Single gene mutations studies on leptin, leptin receptor, pro-opiomelanocortin, peroxisome proliferator-activated receptor-γ, melanocortin-4 receptor, protein convertase 1, thyroid hormone receptor β, mendelian syndromes with obesity (Prader-Willi, Wilson-Turner, Bordet-Bield), animal model studies, transgenic, murine, association, case-control and linkage studies (nuclear families, twins, adoption), candidate and genome-wide association studies (GWAS) have reported a huge number

of genetic loci associated with obesity (Al-Attar et al., 2008; Fischer, Koch et al., 2009; Hennig et al., 2009; Hinney et al., 2007; Hunt et al., 2008; Stratigopoulos et al., 2008). The human obesity gene map that was published in 2006 includes >600 loci that have been shown to be linked to obesity-related traits (Rankinen et al., 2006).

2.5.2 Dietary intake

Macronutrient intake, energy expenditure and partitioning in nutrient storage are the important elements of energy balance (Bray & Bouchard, 1997). High-fat diets stimulate an overconsumption of energy that consequently results in obesity (Perusse & Bouchard, 2000). Protein and carbohydrate intake spontaneously induce potent autoregulatory adjustments in protein and carbohydrate oxidation. In contrast, the fat balance is less acutely regulated and more easily interrupted (Flatt, 1995; Schrauwen, et al., 1997; Schutz, 1995). Weight maintenance occurs when the average composition of fuels has been oxidized is equivalent to the energy nutrient distribution in the diet (Burstein et al., 1996; Hirsch & Leibel, 1998; Jequier & Tappy, 1999).

Nutrients and bioactive food compounds can alter the epigenetics and expression of genes at the transcriptional level (Choi & Friso, 2010). Choline, methionine, vitamin B-12, folate, betaine are capable of causing DNA and histone methylation. Water-soluble B vitamins like biotin, niacin and pantothenic acid have important roles in histone modifications (Jin, Kadam, & Pfeifer, 2010).

Nutrition is a most important environmental factor that affects obesity. Dietary components are able to improve, impair or modify the possibility of developing obesity, exerting effect on genetic background (Costa, Casamassimi, & Ciccodicola, 2010). Nutrigenomics is the study of the gene and nutrient interaction which aim to design optimal diets based on the genetic background of an individual (Kaput, 2008; Kaput &

Rodriguez, 2004). There is evidence of gene-diet interaction but the replications studies in many populations are very low. Recently, a study in 3 independent US populations has proven the gene-diet interaction influences body weight and BMI. Polymorphisms in high-density lipoprotein apolipoprotein, *APOA2* and *APOA5* are shown for evidence of gene-diet interaction in these studies (Corella et al., 2007; Corella et al., 2009).

2.5.3 Physical activity

Globally, physical inactivity (lack of physical activity) is the sixth main leading cause of death (WHO, 2012b). Physical activity is defined as bodily movement made by skeletal muscle that need energy expenditure. Physical activity influences the energy expenditure that may influence body weight and composition (Westerterp & Goran, 1997). Rising prevalence of obesity appears to be related to a reduction in physical activity patterns (Martinez, 2000). Reduced levels of daily physical activity and inadequate energy expenditure due to sedentary lifestyles are factors causing energy imbalances leading to high rise of obesity (Saris et al., 2003).

Studies have shown that the Malaysian adult population is commonly relatively inactive. NHMS III reported that 43.7 % Malaysian adults are physically inactive with 35.3 % men and 50.5% women being classified as inactive (IPH, 2008). A recent study reported that the prevalence of sufficient exercise in Malaysian is only 14.2%. The study highlighted that the majority of Malaysian adults are either sedentary (39.7%) or moderately active (47.6%), whereas only a small percentage are active (12.8%). In addition, physical activity is also low among Malaysian adults (Poh BK & Wan Manan WM, 2010). The Malaysian government and the Academy of Medicine, Malaysia (2003), have taken preventive measures for reducing weight in overweight and obese people by having guidelines for increasing energy expenditure by improving physical activity, reduction in energy intake, behavior modification linked with eating habits and

also created awareness activities to parents on feeding their children with healthy food (Ahmad, 2004).

2.6 Heritability of obesity

BMI is a highly heritable phenotype. Heritability studies confirmed that genetic factors with heritable rate of 40-70% are responsible for high risk of obesity (Maes, et al., 1997; Stunkard, Foch, & Hrubec, 1986). Twin, family and adoption studies have proven that strong predictive value of parental BMI is principally affected by genetic rather than environmental factors (Maes, et al., 1997; Stunkard & Sorensen, 1993). Twin studies have revealed that between 60% and 90% of discrepancy in BMI in a population is caused by genetic effects (Stunkard, Sorensen, et al., 1986). From the context of the heritability of obesity, there are two types of obesity, and these are known as monogenic and polygenic obesity (Farooqi & O'Rahilly, 2005).

2.6.1 Monogenic Obesity

Monogenic forms of obesity are caused by monogenes. A monogene refers to a gene with a strong effect on the phenotype and causes close one-to-one association between phenotype and genotype. Monogenic form of obesity is caused by single gene mutations which are very rare, very severe and commonly start in childhood. The monogenic forms of obesity are caused by rare mutations in leptin, leptin receptor (LEPR), proopiomelanocortin (POMC), proconvertase 1 (PC1), melanocortin-4 receptor (MC4R) and neurotrophic tyrosine kinase, receptor type 2 (NTRK2) genes (Clement, 2006; Clement et al., 1998; Farooqi & O'Rahilly, 2005; Jackson et al., 2003; Jackson et al., 1997; Krude et al., 2003; Montague et al., 1997; Strosberg & Issad, 1999; Yeo et al., 2004).

2.6.2 Polygenic obesity

Polygenic forms of obesity involve a number of genetic variants which interact with an ‘at-risk’ environment. The polygenic variants are groups of alleles at different loci that act together to control the inheritance of a quantitative phenotype or change the expression of a qualitative character. For the quantitative traits (examples: BMI and body weight) each allele has a small effect and the allelic effect can be additive and non-additive (Gerken et al., 2007). Hence, obesity can occur in an individual who has many polygenic variants which act collectively to increase the body weight. In polygenic obesity, each susceptibility gene would only have a minor effect on weight (Farooqi & O'Rahilly, 2004; Khoury MJ, 1993). The risk for common obesity is contributed by a large number of loci but it occurs due to low frequency of disease-predisposing alleles (Pritchard, 2001).

Although the obesity risk and predictive value is low in these recent discoveries, these markers are worth examining for discoveries into the pathophysiological role of obesity (Loos, 2009). Genetic studies in different ethnicities will likely explain the discrepancies in body weight and BMI.

2.7 Genetic variation in human disease

The completion of the Human Genome Project and the sequencing of human genome are major advancements which led to the understanding of the human genome and genetic variation in human disease. The Human Genome Project has made significant impact in applying genetic epidemiology technologies in order to understand the more common and complex diseases (Bell, 2004). Mendelian diseases are rare, severe and have clear patterns of inheritance. Mutations involve single-base substitutions such as missense and nonsense mutations, micro-deletions and

trinucleotide repeat sequences are commonly observed in Mendelian disease. Technologies applied for the study of Mendelian disease are widely being used for studying the molecular basis of complex diseases.

Complex diseases such as obesity and inflammatory disease do not follow Mendelian inheritance patterns. Although complex or multifactorial diseases are hard to study, understanding the genetic predisposition of these diseases are very crucial as they are common cause of mortality in humans (Bell, 2004). Study of complex diseases require methods of identifying genetic markers of multiple genes on chromosomes which lead to predisposition of the disease phenotype. Linkage studies, association studies and genome-wide association studies (GWAS) are three broad epidemiological approaches used for gene identification since the last few decades. Linkage studies are performed using large pedigree. Association studies approaches use large numbers of unrelated cases and controls, or family groupings (Nowotny, Kwon, & Goate, 2001). GWAS comprise of larger sample sizes and are better powered as it does not require familial relatedness. GWAS screens across the whole genome with discoveries of more than 300 replicated association for more than 70 common diseases (Loos, 2009). Recent progresses in obesity genetics have been made through GWAS to study many obesity susceptibility loci (Loos et al., 2008; Scuteri, et al., 2007; Speliotes et al., 2010; Thorleifsson et al., 2009; Wang et al., 2011; Willer et al., 2009). BMI is widely used in GWAS as continuous trait as this is a good and simple measurement of adiposity in adults. The genetic variants that have been investigated in genetic epidemiological studies are single nucleotide polymorphisms (SNPs), haplotypes, complex chromosomal arrangements, copy number variants (CNVs) and epigenetic changes (Barber et al., 2005; Bell, 2004; Bird, 1996; Jones, 2005; Stefansson et al., 2005).

2.7.1 Single nucleotide polymorphisms (SNPs)

SNPs contribute to about 90% of genetic variations in humans. A SNP is a genetically stable substitution of a single nucleotide (A, T, G, or C) in the genome sequence. About 15 million SNPs occur throughout the whole genome. It has been estimated that SNPs with minor allele frequencies of at least 1% are found at a rate of 1 in every 200-300 bases in the genome (Kruglyak & Nickerson, 2001; Serre & Hudson, 2006; Stephens et al., 2001). SNP with minor allele frequencies of 5% or more compose of 29% of the total SNPs. These SNPs are useful in understanding the disease phenotype than the rarer variants (Cargill et al., 1999; Halushka et al., 1999).

The SNPs can occur in both coding and non-coding region of the genome. The SNPs which occur in the coding regions could be non-synonymous or synonymous. The non- synonymous SNP modifies an amino acid that can influence the function and structure of the encoded protein. The synonymous SNP could possibly affect the folding and stability of mRNA transcripts. The non-coding SNPs are located in untranslated regions and may influence the processing of stability of the mRNA. The non-coding SNPs are located in introns of genes and can result in alternatively spliced mRNAs (Liljedahl, Fredriksson, Dahlgren, & Syvanen, 2004; Syvanen, Landegren, Isaksson, Gyllensten, & Brookes, 1999).

SNPs are important in linkage and association studies for many reasons. Firstly, a SNP could possibly encode differences in a protein form and expression when it occurs in a functional gene region which consequently causes the disease. Secondly, SNPs could track the presence of other less simply identified genetic variations that cause the disease phenotype. Third, SNPs are useful in the analysis of mutation rates and evolutionary history (Salisbury et al., 2003).

2.7.2 Haplotypes

A haplotype is a set of polymorphic alleles that co-occur on a single chromosome. Studies confirmed that individuals who carry a particular SNP allele at one site are often expected to carry specific allele at other nearby variant site. This new allele combinations are inherited together. This correlation is known as linkage disequilibrium (LD). This set of block of nearby SNPs is termed as haplotype. When there is an excess of haplotypes, it provides evidence for the occurrence of recombination. Recombination is a key process that brings allele onto the chromosome where they previously did not co-occur. This possibly lead to formation of new proteins and different expression and protein combinations (Salisbury, et al., 2003).

Haplotype analysis is essential in identifying LD patterns in different regions and different populations. By this, the history of a population can be assumed and genetic variants underlying complex traits can be identified. LD is influenced by various factors such as population history, recombination rates, gene conversion, age of the variants, natural variants and other factors (Zhao, Pfeiffer, & Gail, 2003). The degree of association between two polymorphisms is commonly measured using the D' and r^2 (Devlin & Risch, 1995). LD is population specific. It has been reported that LD differs in different populations. For example, LD is commonly weaker among Africans compared to other populations (Gabriel et al., 2002; Stephens, et al., 2001). Therefore, studies in various human populations are needed in order to understand the LD in the human genome.

The SNP consortium Allele Frequency Project has highlighted that accuracy and power of association mapping can be improved by grouping SNPs into haplotype blocks (Gabriel, et al., 2002; Zhao, et al., 2003). Many rationale have been proposed for testing for associations between phenotypes and haplotypes, rather than single SNPs, including

that haplotypes analysis is able to capture epistastic interactions between SNPs at a locus and can provide valuable information to calculate approximately whether alleles are identical by descent (IBD). Haplotype analysis reduces the number of tests and therefore lower the Type I error rate, allows informed testing between haplotypes by capturing information from evolutionary history and also provide more power than single SNPs when an allelic series exists at a locus (Bardel, Danjean, Hugot, Darlu, & Genin, 2005; Clark, 2004; Meuwissen & Goddard, 2000; Morris & Kaplan, 2002; Templeton, Boerwinkle, & Sing, 1987; Zhao et al., 2007).

2.7.3 Complex chromosomal arrangements

Complex chromosomal rearrangements possibly happen at low levels in humans. These genetic variations are inversions, insertions, deletions and few others (Stefansson, et al., 2005). Deletion, insertion, inversion or translocation would affect the level of gene expression or disrupt gene functions. Chromosomal changes occur probably due to loss of control of chromosomal numbers and/or replication (Feuk, Carson, & Scherer, 2006).

2.7.4 Copy number variants

It has been suggested that CNV is characterized by a copy number change that involves a DNA fragment that is around 1kb or larger (Feuk, Carson, et al., 2006). CNVs may manifest on human disease by directly influencing the gene dosage and gene expression (Feuk, Marshall, Wintle, & Scherer, 2006). Gene dosage alteration is the reason for evolution that involves the losses and gains that regulate the expression of genes (Albertson, 2006; Knuutila et al., 1999).

2.7.5 Epigenetics

Epigenetics is known as somatically heritable states of gene expression resulting from changes in chromatin structure without alterations in the DNA sequence. These alterations consist of DNA methylation, histone modifications and chromatin modeling (Choi & Friso, 2010).

2.8 Candidate genes in obesity

The recent success of the Human Obesity Gene Map which catalogues all genetic variants and chromosomal loci linked to obesity-related traits has provided valuable knowledge in the field of genetics of obesity. More than 600 genes, markers and chromosomal regions have been identified to be associated with human obesity traits. Candidate genes that predispose to obesity are neuropeptides, receptors, hormones, solute carriers and enzymes (Rankinen, et al., 2006). Association studies involving large numbers of genes and single nucleotide polymorphisms with obesity have been studied in various heterogenous populations worldwide.

Mutations in genes involved in food intake, energy metabolism, energy expenditure, inflammation, lipid and glucose metabolism and adipose tissue metabolism are found to be associated with obesity in humans (Clement, 2006; Loktionov, 2003). Genes that are found to be linked to food intake are leptin (LEP), leptin receptor (LEPR), agouti related peptide (AGRP) and dopamine D2 receptor (DRD2). Genes that are found to be involved in energy metabolism are uncoupling protein 1 (UCP1), Uncoupling protein 2 (UCP2), Uncoupling protein 3 (UCP3), β^3-adrenergic receptor (β3-AR) and G protein beta 3 subunit gene (GNB3). The genes that are involved in adipose tissue metabolism are adiponectin (ADIPOQ), peroxisome proliferator activated receptor γ (PPAR-γ), tumor necrosis factor α (TNF-α), β^2-adrenergic receptor (β2-AR),

interleukin 6 (IL-6), hormone-lipase sensitive (LIPE) and glucocorticoid receptor (NCR3C1). The genes that are associated with lipid and glucose metabolism are insulin (INS) and low density lipoprotein (LDL) receptor. The above-mentioned genes are frequently studied and confirmed for positive association in more than 5 independent studies (Clement, 2006).

GWAS have identified many loci that are associated with BMI such as fat mass and obesity associated gene (FTO), transmembrane protein 18 (TMEM18), Melanocortin-4 receptor gene (MC4R), glucosamine-6-phosphate deaminase 2 (GNPDA2), brain-derived neurotrophic factor (BDNF), neuronal growth regulator 1 (NEGR1), SH2B adaptor protein 1 (SH2B1), ets variant 5 (ETV5), mitochondrial carrier 2 (MTCH2), potassium channel tetramerisation domain containing 15 (KCTD15), G protein-coupled receptor, family C, group 5, member B (GPRC5B), mitogen-activated protein kinase 5 (MAP2K5), glutaminyl-peptide cyclotransferase-like (QPCTL), TNNI3 interacting kinase (TNNI3K), solute carrier family 39 (zinc transporter), member 8 (SLC39A8), transmembrane protein 160 (TMEM160), Fanconi anemia, complementation group L (FANCL), cell adhesion molecule 2 (CADM2), protein kinase D1 (PRKD1), low density lipoprotein receptor-related protein 1B (LRP1B), zinc finger protein 608 (ZNF608), ribosomal protein L27a (RPL27A), nudix (nucleoside diphosphate linked moiety X)-type motif 3 (NUDT3), insulin-induced gene 2 (INSIG2) and catenin beta like 1 (CTNNBL1) (Frayling et al., 2007; Herbert et al., 2006; Liu, Liu et al., 2008; Loos, et al., 2008; Scuteri, et al., 2007; Speliotes, et al., 2010; Thorleifsson, et al., 2009).

There many more genes associated with obesity-related traits that have been tested in association studies across various ethnic populations. These genes are ghrelin (GHRL), syndecan 3 (SDC3), resistin (RETN) and myotubularin related protein 9 (MTMR9) (Chung et al., 2009; Reizes, Benoit, & Clegg, 2008; Ukkola, Kunnari, & Kesaniemi, 2008; Yanagiya, et al., 2007). These genes may provide new insights into human body weight regulation. The genetic variants could provide valuable implication in potential functional roles and pathway analysis.

2.8.1 Fat mass and obesity associated gene (FTO)

The FTO protein belongs to AlkB family of the $Fe^{2+}/^{2-}$ oxoglutarate-dependent oxidative DNA/RNA demethylases (Gerken, et al., 2007; Jia et al., 2008; Trewick, Henshaw, Hausinger, Lindahl, & Sedgwick, 2002). Members of this family play roles in fatty acid metabolism, post-translational modification and DNA repair (Clifton et al., 2006; Ozer & Bruick, 2007). Fat mass and obesity associated gene (FTO) is located on chromosome 16q22.2 (Figure 2.1). The FTO gene encodes for a protein expressed in the hypothalamus, a center of energy balance, and adipose tissue. FTO is located in the cellular nucleus (Gerken, et al., 2007).

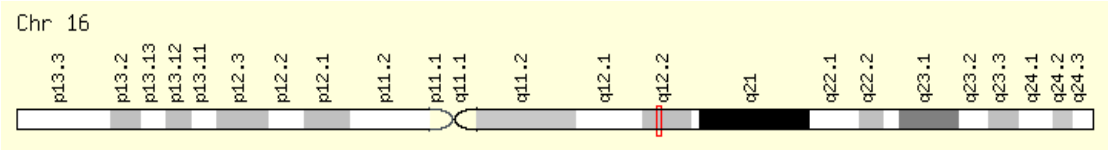


Figure 2.1 : Location of FTO gene on chromosome 16

The true causal function of FTO remains a puzzle. Variants in the human FTO gene may have an effect in up or dysregulation of FTO expression, thereby affecting susceptibility to obesity (Fischer, Koch, et al., 2009). Previous report suggested that FTO expression might lead to increased food intake, thereby causing increased

adiposity (Fischer, Koch, et al., 2009). FTO variants predispose to obesity due to increased appetite or food intake rather than regulation of energy expenditure (Cecil, Tavendale, Watt, Hetherington, & Palmer, 2008; Haupt et al., 2009; Loos & Bouchard, 2008; Sonestedt et al., 2009; Tung et al., 2010; Wardle, Llewellyn, Sanderson, & Plomin, 2009). A few studies have rejected an effect of FTO on energy expenditure (Cecil, et al., 2008; Do et al., 2008; Haupt, et al., 2009; Speakman, Rance, & Johnstone, 2008). In addition FTO mediates the regulation of lipolysis (Wahlen, Sjolin, & Hoffstedt, 2008). Table 2.2 summarizes the effects of FTO gene in human. Although the FTO gene is found to be involved in molecular pathways linked to energy homeostasis, the specific pathway of this gene on adiposity and its true physiological functions is still largely unknown.

Recently, FTO was discovered to be a novel gene and characterized by clusters of 10 SNPs in the first intron. Two independent GWAS in European populations have shown strong association between FTO rs9939609 SNP and obesity. Many studies have shown positive association between the FTO gene in early onset and severe obesity in children and adults (Frayling, et al., 2007; Karasawa S. & 2009; Peeters, et al., 2008; Scuteri, et al., 2007; Xi & Mi, 2009).

Table 2.2 : Summary of effects of FTO variants in human

Effect	FTO variation in human	References
Post-natal death	Association with post-natal mortality. Risk allele carriers showed reduced ability to cope with disease.	(Andreasen, Stender-Petersen et al., 2008; Boissel et al., 2009; Cecil, et al., 2008; Church et al., 2009; Dina et al., 2007; Fischer, Koch, et al., 2009; Frayling, et al., 2007; Grant et al., 2008; Hinney, et al., 2007; Lopez-Bermejo et al., 2008; Wardle, Carnell, Haworth, & Plomin, 2008)
Growth retardation	No association with height.	(Boissel, et al., 2009; Church, et al., 2009; Fischer, Koch, et al., 2009; Frayling, et al., 2007; Song et al., 2008)
Development	No association with development.	(Boissel, et al., 2009; Church, et al., 2009; Fischer, Koch, et al., 2009)

Table 2.2, continued

Effect	FTO variation in human	References
Adipose tissue mass and adipokines	Association caused changes in fat mass. No compelling association with leptin or adiponectin.	(Andreasen, Stender-Petersen, et al., 2008; Bauer et al., 2009; Boissel, et al., 2009; Cecil, et al., 2008; Church, et al., 2009; Fischer, Burnard et al., 2009; Frayling, et al., 2007; Haupt et al., 2008; Kring et al., 2008)
Sex differences	No reported evidence for gender difference in the effect of FTO SNPs.	(Boissel, et al., 2009; Church, et al., 2009; Dina, et al., 2007; Fischer, Koch, et al., 2009; Frayling, et al., 2007; Jacobsson et al., 2008)
Energy intake	Few studies reported no association with food intake, however other studies reported association with energy intake.	(Boissel, et al., 2009; Cecil, et al., 2008; Church, et al., 2009; Do, et al., 2008; Fischer, Burnard, et al., 2009; Franks et al., 2008; Hakanen et al., 2009; Haupt, et al., 2009; Johnson et al., 2009; Sonestedt, et al., 2009; Speakman, et al., 2008; Stutzmann et al., 2009; Tanofsky-Kraff et al., 2009; Wardle et al., 2008; Wardle, et al., 2009)

Table 2.2, continued

Effect	FTO variation in human	References
Energy expenditure	No associations with energy expenditure parameters. No correlation between skeletal muscle or adipose tissue FTO expression with energy expenditure.	(Berentzen et al., 2008; Boissel, et al., 2009; Cecil, et al., 2008; Church, et al., 2009; Do, et al., 2008; Fischer, Koch, et al., 2009; Franks, et al., 2008; Goossens et al., 2009; Grunnet et al., 2009; Haupt, et al., 2009)
Physical activity	No associations with physical activity however there was an evidence of interaction between physical activities and genotypes on BMI.	(Andreasen, Stender-Petersen, et al., 2008; Boissel, et al., 2009; Cauchi et al., 2009; Church, et al., 2009; Fischer, Koch, et al., 2009; Hakanen, et al., 2009; Lappalainen et al., 2009; Rampersaud et al., 2008; Vimaleswaran et al., 2009; Wardle, Carnell, Haworth, Farooqi, et al., 2008; Wardle, et al., 2009)
Lipids	A few studies have reported association with triglycerides and cholesterol levels.	(Boissel, et al., 2009; Church, et al., 2009; Fischer, Koch, et al., 2009; Hertel et al., 2008)

Table 2.2, continued

Effect	FTO variation in human	References
Glucose tolerance and insulin	Convincing reports for lack of association between FTO SNPs and FTO expression with glucose tolerance or insulin sensitivity.	(Al-Attar, et al., 2008; Boissel, et al., 2009; Church, et al., 2009; Fischer, Koch, et al., 2009; Freathy et al., 2008; Grunnet, et al., 2009; Haupt, et al., 2008; Hubacek et al., 2008; Hunt, et al., 2008; Kring, et al., 2008; Loos, et al., 2008; Muller et al., 2008; Ng et al., 2008; Zabena et al., 2009)
FTO expression and function	No reported association between FTO expression and FTO variants. Nonetheless, there are some studies which reported that FTO expression is higher in obese individuals. No reported effects on catalytic activity.	(Boissel, et al., 2009; Church, et al., 2009; Fischer, Koch, et al., 2009; Grunnet, et al., 2009; Kloting et al., 2008; Villalobos-Comparan et al., 2008; Wahlen, et al., 2008; Zabena, et al., 2009)

Adapted from (Fawcett & Barroso, 2010)

2.8.2 Melanocortin-4 receptor gene (MC4R)

Melanocortin-4 receptor (MC4R) is a protein which is composed of 332 amino acids. MC4R is a seven-transmembrane G protein–coupled receptor (GPCR) (Gantz et al., 1993). The MC4R receptor is encoded by a single exon gene. The MC4R gene is located at human chromosome 18*q*22 (Figure 2.2.). MC4R is expressed in the central nervous system.

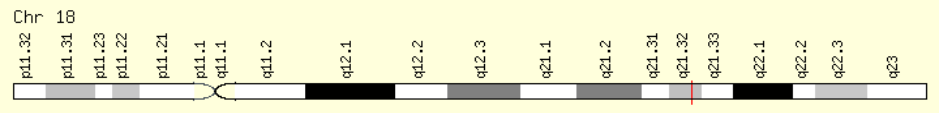


Figure 2.2 : Location of MC4R gene on chromosome 18

The Melanocortin pathway plays a crucial role in the central regulation of energy homeostasis. As a key regulator of appetite, MC4R plays an important role in homeostasis of long-term energy balance in humans (Huszar et al., 1997). In the basal state, leptin maintains the expression of POMC in the arcuate nucleus of the hypothalamus. The POMC-derived peptide ligand α-melanocyte-stimulating hormone (α-MSH) activates MC4R in the paraventricular nucleus of the hypothalamus leading to suppression of food intake. The effect of MC4R on body weight regulation was first observed in targeted disruption of the MC4R gene resulting in hyperphagia, hyperinsulinemia, mature-onset obesity and elevated linear growth in mice (Huszar, et al., 1997). MC4R deficiency is a common form of monogenic obesity. Studies have shown that up to 6% of subjects with severe early-onset obesity exhibit pathogenic mutations in MC4R (Farooqi et al., 2003; Vaisse et al., 2000).

Association study involving MC4R variants have been widely carried out in many ethnicities across the globe. Although many studies have shown that polymorphisms in the MC4R gene are positively associated with obesity, other studies however, showed no association (Carroll, Voisey, & van Daal, 2005; Farooqi & O'Rahilly, 2007; Mutch & Clement, 2006). V103I (rs2229616) and 1251L are the two most common polymorphisms of the MC4R gene. These are non-synonymous variants (Xiang et al., 2006). These polymorphisms of MC4R have been found to be linked with obesity (Heid et al., 2008; Markison S, 2006; Stutzmann et al., 2007).

To date four meta-analyses have been performed by combining data from case-control studies to study the effect of rs2229616 SNP (Loos, 2011). The first meta-analysis on MC4R polymorphisms which included 12 studies reported that the 103I-allele was associated with a 31% decreased risk of obesity risk (Geller et al., 2004). The second meta-analysis which included 29,563 individuals confirmed that rs2229616 was significantly associated with obesity risk (Odds ratio 0.82; 95% confidence interval 0.70-0.96). However, the observed protective effect of 103I-allele on obesity risk was reduced to 18% (Young et al., 2007). The third meta-analysis which included an additional 10,000 individuals showed that 103I-allele was associated with a 20% reduction of obesity risk (Stutzmann, et al., 2007). The latest meta-analysis composed of 55,195 individuals form 37 cohorts consequently confirmed that 103I-allele carriers had 20% reduction of obesity risk (Wang et al., 2010).

A few studies have shown the association of V103I with BMI as a continuous trait. Positive association between V103I with BMI was observed in French subjects. In this study, 103I-allele was significantly associated with a 0.8 Kg/m2 lower BMI (Stutzmann, et al., 2007). Nevertheless, a study on large population which included

7713 subjects showed negative association between the V103I polymorphism and BMI (Geller, et al., 2004).

2.8.3 β_2-adrenoceptor gene (ADRB2)

β_2-adrenoceptor is a protein which belongs to the G-protein-coupled adrenergic receptor family. ADRB2 encodes a 413 amino acid protein. ADRB2 is located in chromosome 5q31-q32 (Figure 2.3). ADRB2 consists of a single exon of 2015 nucleotides (Kobilka et al., 1987; Postma et al., 1995). ADRB2 is highly expressed in bronchial smooth muscle cells, cardiac myocytes and vascular smooth muscles (Litonjua et al., 2010).

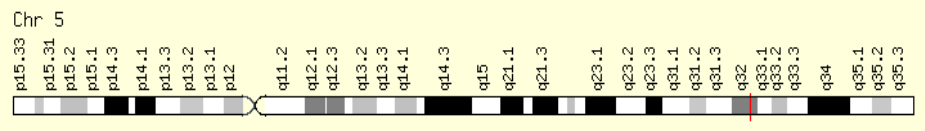


Figure 2.3 : Location of ADRB2 gene on chromosome 5

ADRB2 specifically binds and is activated by catecholamine which includes adrenaline. Catecholamines are central regulators in energy expenditure, both as hormones and neurotransmitters. Genes that affect the catecholamine functions are important in human obesity. This is because catecholamine regulate body fat accumulation and energy expenditure via β2 and β3 adrenergic receptors (Lafontan & Berlan, 1993). Catecholamines regulate lipolysis in human adipose tissue (Lafontan & Berlan, 1993; Reynisdottir et al., 1995; Reynisdottir, Wahrenberg, Carlstrom, Rossner, & Arner, 1994).

Obesity occurs due to increased energy storage and reduction in lipolysis (Lima et al., 2007; Mori et al., 1999). Adrenergic receptors such as β_1, $\beta 2$ and β_3 play essential role in lipolysis (Ehrenborg et al., 2000). Polymorphisms in genes that are involved in

the regulation of catecholamine functions may be the pathogenic factors in human obesity due to their role in energy expenditure (Rosmond, Chagnon, Bouchard, & Bjorntorp, 2001). Polymorphism of the β_2-adrenoceptors (ADRB2) gene has been reported to modify the protein function and promote obesity (Loktionov, 2003).

There are many polymorphisms of the ADRB2 that are associated with obesity (Large et al., 1997). More than 80 polymorphisms have been detected in various populations (Litonjua, et al., 2010). Two of these polymorphisms rs1042713 (Glu27Gln) and rs1042714 (Arg16Gly) are non-synonymous polymorphisms which result in amino acid changes and are widely studied. These SNPs have demonstrated positive association with obesity in many studies while some other studies in different populations failed to replicate these findings (Echwald, Sorensen, Tybjaerg-Hansen, Andersen, & Pedersen, 1998; Galletti et al., 2004; Hayakawa et al., 2000; Kawamura, Egusa, Fujikawa, & Okubo, 2001; Kim et al., 2002; Large, et al., 1997; Lin, Ericsson, Benjafield, & Morris, 2001; Mori, et al., 1999; Rosmond, et al., 2001; Ukkola et al., 2000). The reason for discrepancies among these studies remains unclear but this may be due to genetic variation and the degree of obesity among different populations.

2.8.4 Leptin gene (LEP)

Discovery of leptin and the leptin receptor has led to new era in obesity research. Leptin is a 167 amino acid protein. It is found mainly in adipose tissue in human. Leptin is also expressed in placenta, ovaries, skeletal muscle, stomach, pituitary and liver (Muoio & Lynis Dohm, 2002). The *ob* gene encoding leptin gene (LEP) is located on chromosome 7q31.3 (Figure 2.4) (Friedman, Leibel, Siegel, Walsh, & Bahary, 1991; Geffroy et al., 1995). LEP gene has three exons separated by two introns (Isse et al., 1995). *Ob* gene is expressed only in adipose tissue (Zhang et al., 1994). This gene encodes a protein that is secreted by white adipocytes and plays an important role in

body weight and adipose tissue homeostasis. This gene acts through the leptin receptor which is involved in the signaling pathway that inhibit food intake and activate energy expenditure (Kennedy, 1953).

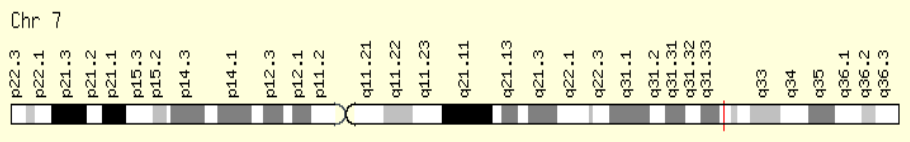


Figure 2.4 : Location of LEP gene on chromosome 7

Leptin upregulates anorexigenic neuropeptide such as alpha-melanocyte stimulating hormone which acts on MC4R to directly affect metabolism and function of peripheral tissues such as adipocytes and skeletal muscles (Houseknecht & Portocarrero, 1998; Jequier, 2002; Paracchini, Pedotti, & Taioli, 2005). Human LEP gene was found to be overexpressed in both subcutaneous and omental adipose tissue of massively obese subjects (Lonnqvist, Arner, Nordfors, & Schalling, 1995). A LEP gene nonsense mutation in codon 105 of the mouse was first discovered by Ingalls and colleagues (Ingalls, Dickie, & Snell, 1950). This mutation resulted in obesity, hyperphagia, hypothermia, extreme insulin resistance and infertility.

There are many LEP polymorphisms that have been studied in different ethnicities (Jiang et al., 2004; Paracchini, et al., 2005). These SNP includes rs1349419, rs12535708, rs12535747, rs7799039 (G-2548A), rs2167270, rs2278815, rs12706832, rs322825 and many other SNPs. G-2548A LEP gene polymorphism have shown positive association in Europeans, Taiwanese aborigines, North Caucasian such Spanish and French while lack of association was seen in the Tunisian and Brazilian populations (Ben Ali et al., 2009; Chapman & Wijsman, 1998; Duarte, Francischetti, Genelhu, Cabello, & Pimentel, 2007; Le Stunff, Le Bihan, Schork, & Bougneres, 2000; Portoles

et al., 2006; Wang, Zhang, Zhang, Pan, & Ma, 2008; Wang et al., 2006; Yiannakouris, Melistas, Yannakoulia, Mungal, & Mantzoros, 2003).

2.8.5 Resistin gene (RETN)

Resistin is a protein which belongs to the cystein-rich C-terminal domain proteins called resistin-like molecules. These proteins are similar to those which occur in inflammatory zone family (Holcomb et al., 2000; Kim, Lee, Moon, & Sul, 2001; Steppan, Brown et al., 2001). Resistin gene (RETN) is located on chromosome 19p13.3 (Figure 2.5).

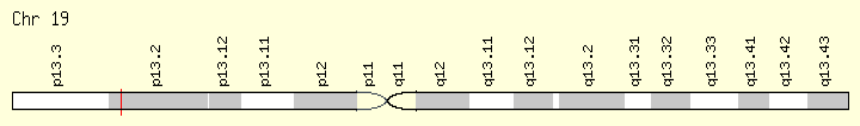


Figure 2.5 : Location of RETN gene on chromosome 19

Most of the SNPs on the RETN gene are located on non-coding regions. Genetic variation in RETN have shown to have a role in the determination of plasma resistin level, dyslipidemia, serum insulin level, obesity, metabolic syndrome, serum triglycerides level, HDL-C level and serum adiponectin level (Asano et al., 2010; Cho et al., 2004; Mattevi, Zembrzuski, & Hutz, 2004; Piestrzeniewicz et al., 2008; Ukkola, et al., 2008). However, few studies failed to confirm these findings (Cao & Hegele, 2001; Pizzuti et al., 2002; Sentinelli et al., 2002; Wang, Chu, Hemphill, & Elbein, 2002).

2.8.6 Insulin-induced gene 2 (INSIG2)

There are two isoforms of Insig proteins, known as Insig-1 and Insig-2 (Sever, Yang, Brown, Goldstein, & DeBose-Boyd, 2003; Sewter et al., 2002). Human Insig-2 protein is composed of 225 amino acids (Yabe, Brown, & Goldstein, 2002). This

protein is deeply embedded in the endoplasmic reticulum (ER) membranes through the presence of six transmembrane helices (Feramisco, Goldstein, & Brown, 2004; Goldstein, DeBose-Boyd, & Brown, 2006). Insigs isoforms are oxysterol-binding proteins (Radhakrishnan, Ikeda, Kwon, Brown, & Goldstein, 2007). Insulin-induced gene 2 (INSIG2) is a protein-coding gene on chromosome 2q14.2 (Figure 2.6).

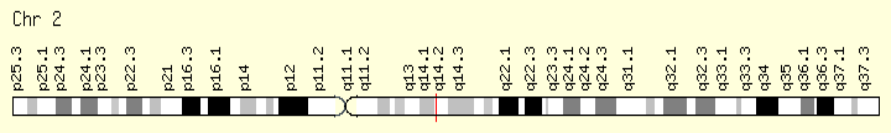


Figure 2.6 : Location of INSIG2 gene on chromosome 2

SREBP cleavage-activating protein (SCAP) and HMG-CoA coordinate regulation of cholesterol levels in mammalian cells (Hampton, 2002). Through their binding to Insig proteins, SCAP and HMG CoA reductase play important roles on cholesterol homeostasis. SCAP directs the activation of SREBPs which improve the transcription of genes involved in cholesterol synthesis and uptake (Brown & Goldstein, 1999). Insig-2 blocks the processing of sterol regulatory element binding proteins (SREBPs), in which it binds to (SCAP) to inhibit it from convoying SREBPs to the Golgi apparatus. Thus, Insig-2 prevents SREBPs from triggering cholesterol synthesis as SREBPs cannot be processed and activated by the Golgi apparatus (Yabe, et al., 2002). As SREBPs are transcription factors that trigger the synthesis of cholesterol and fatty acids in the liver and other organs, the inhibitory role of INSIG2 plays an important role in cholesterol homeostasis (Albu, Murphy, Frager, Johnson, & Pi-Sunyer, 1997; Friedman & Halaas, 1998). In addition, Insig-2 protein is crucial in lipogenesis (Li, Takaishi, Cook, McCorkle, & Unger, 2003). Studies showed that Insig-1 and Insig-2 are found to block proteolytic activation of SCAP, transcription factors

that regulate lipogenic enzymes and adipocyte differentiation (Li, et al., 2003; Sever, Lee, Song, Rawson, & Debose-Boyd, 2004).

Genetic polymorphisms in INSIG2 have shown to be associated with weight gain, obesity, hypercholesterolemia and antipsychotic induced-weight gain (Herbert, et al., 2006; Oki et al., 2009; Tiwari et al., 2010). In the first study, Herbert and colleagues found in a GWAS performed on samples from the Framingham Heart Study that rs7566605 SNP located on the 10kb upstream of the INSIG2 gene was strongly associated with BMI (Herbert, et al., 2006). This significant effect of rs7566605 on BMI was replicated in samples of African-American, Western European ancestry, white and Asian populations and few other studies (Hotta, Nakamura et al., 2008; Lyon et al., 2007; Rosskopf et al., 2007; Yang et al., 2008; Zhang et al., 2008). However, in some studies in Austrians, Afro-Carribean, German, African-American, Mexican-American, Samoans of the Western Pacific, Large Salvonic Caucasian, Western-European cohorts, Korean, Japanese and other studies failed to replicate the positive findings of rs7566605 on obesity (Boes et al., 2008; Bressler et al., 2009; Campa et al., 2010; Cha, Koo, Choi et al., 2009; Deka et al., 2009; Hubacek et al., 2010; Oki, et al., 2009; Wiedmann et al., 2009). A tagging SNP of INSIG2, rs9308762 was found to be associated with BMI in the Samoans of the Western Pacific population (Deka, et al., 2009).

2.8.7 Adiponectin gene (ADIPOQ)

Adiponectin gene encodes for a secreted protein on chromosome 3q27 (Figure 2.7) (Kissebah et al., 2000). Adiponectin belongs to the complement 1q family (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). Adiponectin is abundantly expressed and secreted in adipose tissue (Koerner, Kratzsch, & Kiess, 2005). Serum adiponectin level

is highly heritable (~50%) (Heid et al., 2006; Lange et al., 2005; Lasseka WD, 2008; Lindsay et al., 2003; Litonjua, et al., 2010).

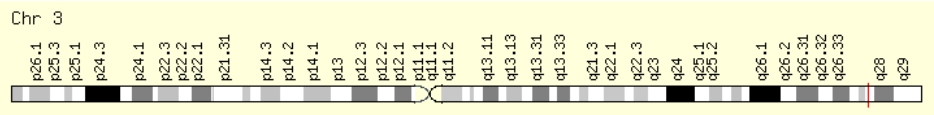


Figure 2.7 : Location of ADIPOQ gene on chromosome 3

The single-nucleotide polymorphisms in the ADIPOQ gene are confirmed to have effects on diabetes, obesity and insulin resistance in different ethnic backgrounds (Gu, 2009). ADIPOQ SNPs have shown to be linked to obesity and insulin resistance in few studies but other studies failed to replicate these findings (Filippi et al., 2004; Stumvoll et al., 2002; Vasseur, Meyre, & Froguel, 2006; Xita et al., 2005; Yang & Chuang, 2006).

2.8.8 Syndecan 3 gene (SDC3)

Syndecan is a protein that belongs to the syndecan proteoglycan family (Berndt, Casaroli-Marano, Vilaro, & Reina, 2001; Carey et al., 1997; Reizes et al., 2001). The syndecan proteoglycan family is involved in cell adhesion, regulation of growth factor and hormone activity and the organization of cell-matrix adhesion (Bernfield et al., 1999; Chen, Gotte, Liu, & Park, 2008). Syndecan 3 gene (SDC3) is located on chromosome 1pter-p22.3 (Figure 2.8).

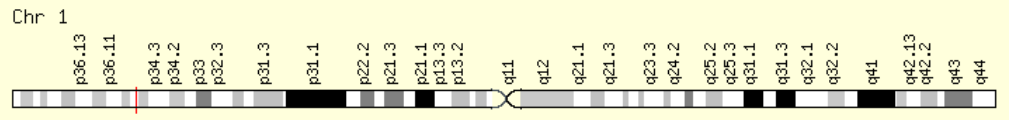


Figure 2.8 : Location of SDC3 gene on chromosome 1

Syndecan-3 is highly expressed in the hypothalamus and plays an important role in the regulation of food intake and body weight regulation mediated by the melanocortin pathway specially MC4R (Reizes, et al., 2008; Reizes, et al., 2001). In addition, SDC3 is expressed in the uterus (Hjelm Cluff, Malmstrom, Tingaker, David, & Ekman-Ordeberg, 2005) and also in the hypothalamic feeding centers. The expression of SDC3 is elevated during food deprivation (Reizes, et al., 2008).

SDC3 rs2491132 is a nonsynonymous SNP. This SNP is strongly associated with obesity in the Korean population (Masuo et al., 2006). However, there was lack of association of the SDC3 rs2491132 with obesity in the European population (Schuring et al., 2009).

2.9 Linkage Disequilibrium

Obesity is a complex disease, the susceptibility of which is controlled by multiple genetic and environmental risk factors. Understanding the LD structure in a genome is very useful in benefiting the association genetics. It is likely to identify regions that are associated with phenotype of interest if the LD is present. This can be done by identifying polymorphisms that occurs in individuals in a particular population (Terwilliger & Weiss, 1998). LD mapping is essential in human gene mapping. LD mapping plays essential role in identifying genetic regions that have etiological effect on predisposition of complex diseases such as obesity.

2.10 Haplotype and Linkage Disequilibrium Coefficient

Haplotype is a set of polymorphic alleles that co-occur on a chromosome. Linkage disequilibrium (LD) of genes depends on analysis of individual or haplotypes of polymorphic markers. LD analysis is a useful indicator of recombination and recurrent mutation as *D'* (LD Coefficient), the distance between each pair of SNPs in a

gene will be measured. Previous studies have shown LD patterns are different across the genomic regions and also across populations (Ardlie, Kruglyak, & Seielstad, 2002; Pritchard & Przeworski, 2001; Przeworski & Wall, 2001; Salisbury, et al., 2003). Studies reported that for the susceptibility SNPs and their collective contribution to genetic variance were highest for SNPs with common MAF from 30-50% and dropped substantially for SNPs with lower allele-frequency (Park et al., 2011). SNPs with minor allele frequencies (MAF) of 5% or greater are more useful then rare markers in order to understand the common phenotype. These SNPs are useful for linkage mapping studies (Lewontin, 1995). Therefore, SNPs which showed high minor allele frequencies and also in Hardy-Weinberg equilibrium in the Malaysian Malay population are further investigated to establish haplotype blocks which are useful for linkage analysis with obesity.

2.11 Adipocytokines

Adipose tissue acts as an endocrine organ as it secretes many peptide hormones or adipokines such as leptin, adiponectin, resistin and retinol binding protein-4 (RBP4), TNF-alpha, plasminogen activator inhibitor-1, interleukin-6 (IL-6), angiotensinogen and cytokines (Ahima & Flier, 2000). Adipocytokines or adipokines regulates energy balance, glucose and lipid homeostasis. These biomarkers are proven to play a role in the pathogenesis of obesity and obesity- related disease (Flier, 2004).

Higher level of adiposity leads to elevation of serum adipokine levels in human and animals (Samad & Loskutoff, 1996; Samad, Yamamoto, Pandey, & Loskutoff, 1997; Yudkin, Stehouwer, Emeis, & Coppack, 1999; Zhang et al., 1996). Adipose tissue undergoes molecular and cellular changes once an individual becomes obese as the adipocytes enlarge possibly leading to disturbances in systemic metabolism. When obesity elevates, numerous proinflammatory factors are produced and macrophage

numbers increased in the adipose tissue (Weisberg, et al., 2003; Xu et al., 2003). Hence, obesity is linked to slow-grade state of inflammation due to the secretion of proinflammatory cytokines by adipocytes (Calabro et al., 2009).

2.11.1 Leptin as a biomarker

Leptin is the first adipocyte hormone that was discovered in obesity biomarker research. Leptin is known as a signal of adipose tissue store. Leptin plays a role as an afferent signal, regulating appetite and weight. Leptin suppresses food intake and stimulates energy expenditure by exerting a direct effect on the hypothalamus (Halaas et al., 1995; Lee et al., 1996). Therefore, leptin acts in the control of body fat stores via regulation of feeding behavior, autonomic nervous system, metabolism and body energy balance. Plasma leptin levels elevates with weight gain and decrease with weight reduction (Havel et al., 1996). Serum leptin levels are significantly higher in females compared to males after correction for total body fat (Havel, Kasim-Karakas, Dubuc, Mueller, & Phinney, 1996). In addition, plasma leptin levels are distinctly reduced by fasting or dieting (Considine et al., 1996; Frederich et al., 1995).

In humans, plasma leptin concentrations are highly correlated with body mass index (BMI) (Maffei et al., 1995). Most of obese subjects have increased circulating levels of leptin because of the high amount of leptin-secreting adipose tissue (Luo, Zhang, & Chen, 2005). Obese subjects are resistant to leptin and maintain high levels of body fat despite an increase in leptin concentrations which should reduce food intake and body fat (Calabro, et al., 2009). The roles of leptin signaling in controlling body weight become more complex once an individual become more obese. A latest study showed that endoplasmic reticulum (ER) stress is a reason for development of obesity-induced-leptin-independent leptin resistance (Ozcan et al., 2009). Pathological stress conditions such as a high-fat diet, increased levels of free fatty acids and cytokines have

been identified to cause the accumulation of improperly folded proteins in the ER lumen and to the activation of the unfolded protein response (UPR) pathway. This complex signaling network is involved in the development of obesity-induced leptin resistance (Zhang & Kaufman, 2004).

2.11.2 Adiponectin as a biomarker

Adiponectin was independently discovered by four groups in 1995/1996 (Hu, Liang, & Spiegelman, 1996; Maeda et al., 1996; Nakano, Tobe, Choi-Miura, Mazda, & Tomita, 1996; Scherer, et al., 1995). Adiponectin is exclusively secreted from adipocytes. It is the most abundant protein secreted by adipose tissue. Adiponectin is found abundantly in plasma, accounting for about 0.01% of total plasma (Weyer et al., 2001). Its circulating level is inversely correlated to BMI (Arita et al., 1999). There is a strong negative correlation between plasma adiponectin concentrations and fat mass in humans (Hu, et al., 1996). Contrary to most other adipokines, adiponectin level is decreased in obesity and increase during weight reduction (Brichard, Delporte, & Lambert, 2003; Matsubara, Maruoka, & Katayose, 2002; Ouchi et al., 1999). The concentration of adiponectin is lower in men than women (Combs et al., 2003). This may occur due to the suppression by androgen as this sexual dimorphism occurs during pubertal development (Bottner et al., 2004; Nishizawa et al., 2002). In addition, women have higher magnitude of high molecular weight adiponectin than men (Nishizawa, et al., 2002). Diurnal and pulsatile secretion patterns of adiponectin are observed in human. Adiponectin peaks in the morning and reduces at night which is in opposition to the secretion pattern of leptin (Gavrila et al., 2003).

Previous studies have shown that adiponectin influences energy homeostasis and glucose and lipid metabolism (Hardie, 2003). Low adiponectin levels in obesity, insulin resistance, diabetes and cardiovascular disease lead to increased risk of these disorders

(Matsuzawa, Funahashi, Kihara, & Shimomura, 2004; Yamamoto, Hirose, Saito, Nishikai, & Saruta, 2004). Adiponectin plays crucial role in the pathogenesis of metabolic syndrome as it is correlated with components of metabolic syndrome such as triglycerides, insulin resistance, hyperinsulinaemia and lipoprotein cholesterol levels (Matsuzawa, et al., 2004).

Adiponectin concentration is under genetic control (Gu, 2009; Jee et al., 2010; Ling et al., 2009). For example, Pima Indians in Arizona who has a high prevalence of diabetes associated with obesity, exhibit significantly lower level of plasma adiponectin (Lindsay, et al., 2003). Association between adiponectin level and obesity, serum lipid, triglycerides and cholesterol parameters with genetic variants have been proven in many different populations (Matsuzawa, et al., 2004; Meilleur et al., 2010).

Previous studies have shown that compared to other biomarkers, adiponectin is a potential target for therapeutic intervention in obesity, diabetes and other cardiovascular disorders (Ahima, 2006; Galic, Oakhill, & Steinberg, 2010; Koerner, et al., 2005). Adiponectin exert protective effects on the development of the cardiovascular diseases and diabetes (Berg & Scherer, 2005). Adiponectin has mild effect on reducing body weight and it significantly improves insulin resistance (Matsuzawa, et al., 2004). In addition, adiponectin cause suppression of proliferation and activation of immune cells. It also suppresses secretion of inflammatory markers such as TNF-Alpha in the atherogenic process (Fantuzzi, 2005). Adiponectin appears to inhibit the development of atherosclerotic plaques on the vascular wall (Goldstein & Scalia, 2004). Moreover, adiponectin indirectly exerts its effect to lower severity of dyslipidemia and other cardiovascular risk factors (Hug & Lodish, 2005).

2.11.3 Resistin as a biomarker

Resistin was discovered independently by three groups as a novel factor secreted by the adipocytes (Holcomb, et al., 2000; Kim, et al., 2001; Steppan, Bailey et al., 2001). Resistin levels do not correlate with total energy (Yannakoulia et al., 2003). There is a positive correlation between resistin and body fat content (Steppan & Lazar, 2002). Recent studies reported that resistin level is higher in obese subjects compared to lean controls (Degawa-Yamauchi et al., 2003; Schaffler et al., 2004; Vendrell et al., 2004). There is a positive correlation between resistin with alterations in BMI and visceral fat area (Azuma et al., 2003; Vozarova de Courten, Degawa-Yamauchi, Considine, & Tataranni, 2004).

Plasma resistin levels have been shown to be stable throughout the day (Reilly et al., 2005). This is due to the fact that resistin does not appear to be regulated by eating and is unaffected by fasting. A few studies reported gender-based differences in the plasma resistin levels (Lee et al., 2007). Women tend to have higher levels of plasma resistin compared to men. This may be contributed by the female sex hormones in which plasma resistin is elevated during pubertal maturation (Gerber et al., 2005). Latest data showed that serum resistin levels are under genetic control in different populations (Asano, et al., 2010; Cho, et al., 2004; Hivert et al., 2009; Ukkola, et al., 2008).

Associations of resistin levels with anthropometric and metabolic parameters are inconsistent. Resistin concentration has been shown to be positively associated with obesity and also with metabolic parameters (Azuma, et al., 2003; Degawa-Yamauchi, et al., 2003). In contrast, a few studies have reported lack of association between resistin and obesity and anthropometric parameters (Lee et al., 2003; Sonestedt, et al., 2009). The mechanism underlying expression, regulation, secretion and circulating levels of

resistin remain unclear. The effect of resistin on the central nervous system and β-cell function is still yet to be explored.

2.12 Summary of the genes involved in the development of obesity

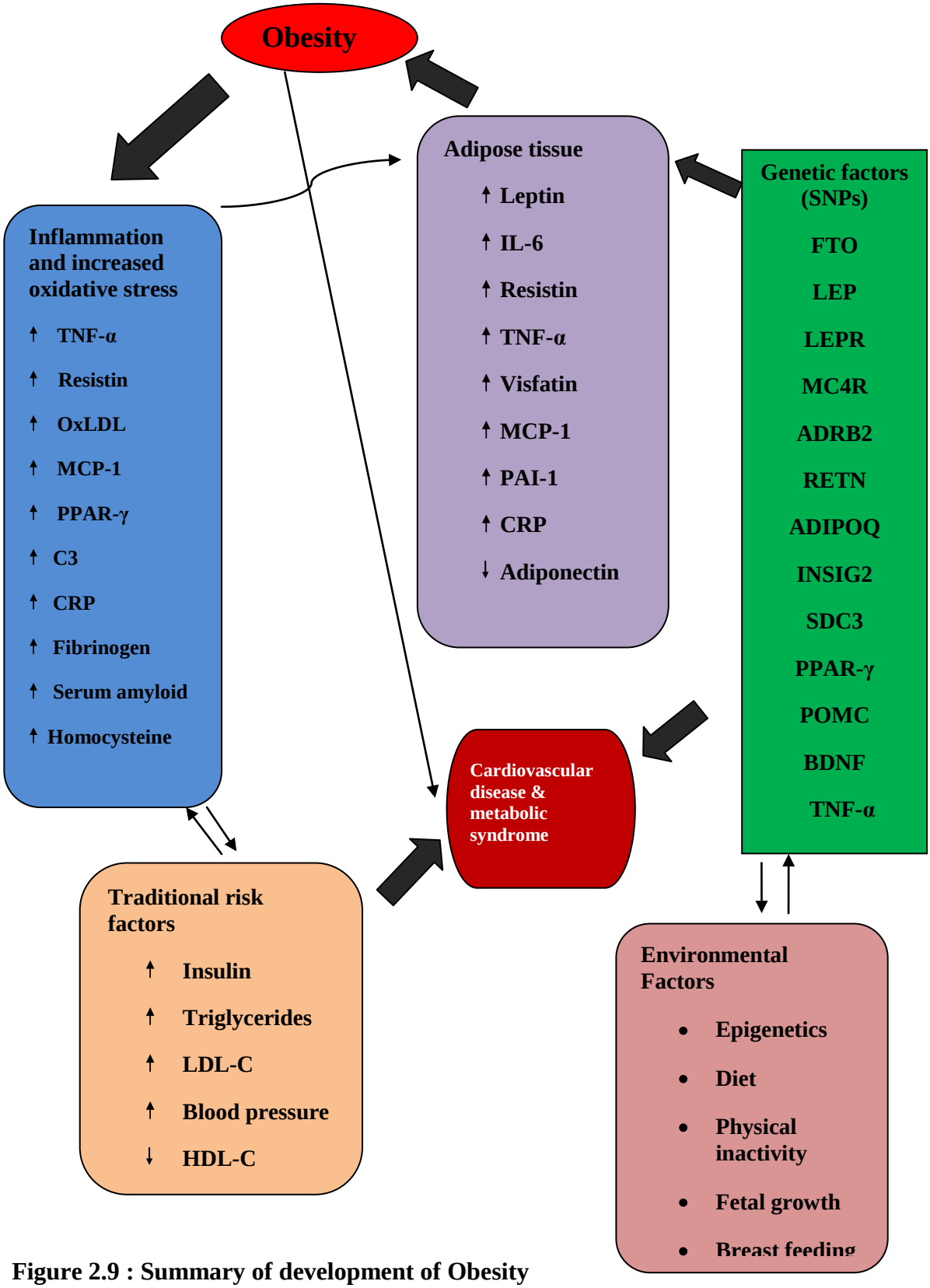


Figure 2.9 : Summary of development of Obesity

Obesity occurs due to many factors. Figure 2.9 shows a summary of the development of mulfactorial obesity and the associated genes. Development of obesity is crucially contributed by biomarkers secreted by adipose tissue. Adiponectin, resistin and visfatin have effects on insulin resistance and inflammation, leptin acts on food intake and fat mass, while Interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) act on inflammatory process, Macrophage chemo attractant protein-1 (MCP-1) and Plasminogen activator inhibitor-1 (PAI-1) act collectively on macrophage attraction roles in the course of obesity.

Genetic and environmental factors interact to cause alterations in the expression of biomarkers from adipose tissue. Genetic factors such as SNPs, CNVs, haplotypes and other variants play pivotal role in the predisposition of obesity. There are numerous genes involved in the pathogenesis of obesity including FTO, MC4R, INSIG2, LEP, LEPR, ADRB2, ADRB3, RETN, ADIPOQ, PPAR-γ, TNF-α, BDNF, POMC, SDC3 and many others. In addition, traditional risk factors such as high lipid, insulin and blood pressure interact with inflammatory and oxidative stress mechanism that involves pro-inflammatory and anti-inflammatory mediators to contribute to obesity. This results in cardiovascular events and metabolic syndrome.

Against this background, this study was carried out to investigate the candidate genes and biomarkers study of obesity in the Malaysian Malays. As we know, obesity is a complex disorder; hence we aimed to investigate the contribution of genetic variants and biomarkers in Malaysian Malays. Identifying a handful of genetic variants which is convincingly associated with obesity-related traits in this population may change the pace of discoveries in the genetics of obesity. Therefore, this study is aimed at providing new data on genetic profiling of obesity which may provide valuable new insights into the pathophysiology and pathways that underlie development of obesity. The advances in the research of identifying causal variants of obesity using recent technologies have

raised hopes for therapeutic interventions as well as management and prevention of obesity. Many challenges have yet to be discovered as modes of actions and functional implication of the genetic variants in different ethnicities. Results from this study will add data on genetic profiling of candidate genes that may aid in understanding the pathogenesis of obesity. This in future will aid in translating genome based profiling into healthcare and clinical practice in Malaysia.