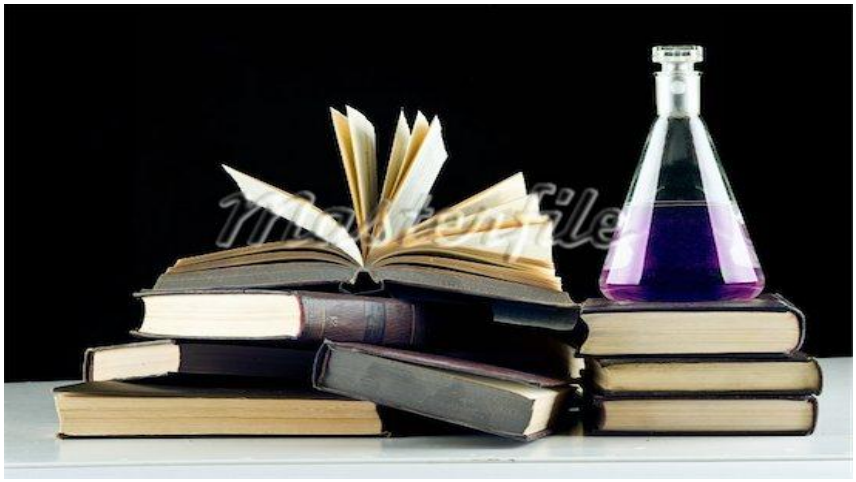
# CHAPTER 5

# DISCUSSION



# 5.0 DISCUSSION

## 5.1 Distribution of anthropometric and obesity-related parameters in the study subjects

A false-negative or a false-positive result may be obtained by chance in any genotype-phenotype study including this study. This issue has taken into consideration by having good-quality genotyping data combined with close attention to Hardy-Weinberg Equilibrium (HWE) testing. When the Hardy-Weinberg is assumed for the genotype distribution, the sample size is assumed to be enough, thereby ruling out sample error and therefore the results are considered to be reliable. Hardy- Weinberg Equilibrium (HWE) test enables the comparison between the actual genetic structure of the population over time and the expected genetic structure. If SNPs satisfy the HWE, they can be used as genetic markers in the population, where their genotype frequencies do not change over generation. A population is said to be in Hardy- Weinberg Equilibrium if the population fulfilled the following criteria:

I. Population size is large enough to eliminate the role of 'chance' in determining which allele gets passed to the next generation.
II. No selection bias exists and that all genotypes are likely to contribute to the genes of the next generation.
III. No mutation are present or that if the mutations do occur, they occur backwards and forwards at the same rate.
IV. No immigration of other people occurs from where the allele frequencies are different. Emigration does not affect this unless one type of genotype is more likely to emigrate than another.
V. Random mating occurs, whereby each genotype is equally likely to mate every other genotype.

In the present study, obesity parameters such as height, body weight, waist circumference, hip circumference, and waist hip ratio were higher in obese compared to non-obese when all the subjects were grouped according to BMI.

Systolic and diastolic blood pressure was significantly higher in obese subjects compared to non-obese subjects in the current study. The prevalence of high blood pressure with increasing BMI was also similar for white, black, Mexican American as observed in many other populations (Brown et al., 2000; Poirier et al., 2006). Elevated blood pressure was strongly associated with obesity (Grundy, Brewer, Cleeman, Smith, & Lenfant, 2004). Previous studies reported that obese subjects are prone to hypertension and that hypertensive subjects seems to be prone to have increased weight (Julius, Valentini, & Palatini, 2000). The exact mechanism by which obesity correlates with hypertension is still unclear. Nevertheless, studies have suggested that the factors secreted by adipose tissue, neuroendocrine mechanism and activation of renin-angiotensin-aldosterone system due to increased sympathetic activity, insulin and leptin resistance, endothelial dysfunction and procoagulatory activity may play major roles in obesity-related hypertension (Engeli & Sharma, 2000; Narkiewicz, 2006; Wiecek, Kokot, Chudek, & Adamczak, 2002). Therefore, reduction in weight can greatly improve the blood pressure. A study has clearly shown that diet-induced weight loss can prevent hypertension (Klein et al., 2004).

HDL cholesterol level was significantly lower and triglycerides were significantly higher in obese subjects compared to non-obese subjects in the Malaysian Malay population. Triglycerides levels are usually elevated in obese subjects or type 2 diabetes (Ford, Bozian, & Knowles, 1968; Howard et al., 1984; Krentz, 2003; Thelle et al., 1983). This indicates that a reduction in BMI is very useful to lower the levels of

these metabolic traits and hence to prevent the metabolic factors such as hypertension, hypercholesterolemia and abdominal obesity.

There was no significant difference in the leptin, adiponectin and resistin levels between the obese and non-obese participants. However, a trend towards higher leptin and lower adiponectin levels were observed in obese participants compared to non-obese participants. Previous studies provided evidence that leptin levels are increased in obesity. Studies reported that obesity is characterized by hyperleptinemia (Caro, Sinha, Kolaczynski, Zhang, & Considine, 1996; Spiegelman & Flier, 1996). Increased in fat storage will result in elevated leptin, which inhibits the satiety centre in hypothalamus and also influences other neuroendocrine systems (Lee, 2009). Studies reported that adiponectin levels are reduced in obese subjects and adiponectin appears to have cardioprotective effects (Diez & Iglesias, 2003; Hotta et al., 2000). In contrast to adiponectin, resistin shows low circulating levels (Galic, et al., 2010). This was also observed in the Malaysian Malays. Numerous studies reported that role of resistin in obesity is conflicting and it is unclear whether production of resistin is decreased or increased obesity (Steppan, Bailey, et al., 2001; Steppan & Lazar, 2002; Way et al., 2001) . Recent studies provided evidence that was not associated with obesity (Iqbal et al., 2005). There was no significant difference in resistin levels between the obese and non-obese subjects in this study.

## 5.2 Genetic profiling of candidate genes of obesity

A total of 8 obesity candidate genes were analyzed. These candidate genes were fat mass and obesity associated (FTO) gene, melanocortin-4 receptor gene (MC4R), β2-adrenoceptor gene (ADRB2), leptin gene (LEP), resistin gene (RETN), insulin-induced gene 2 (INSIG2), adiponectin gene (ADIPOQ) and syndecan 3 gene (SDC3). Single locus analysis, LD and haplotype analysis were performed in this study.

### 5.2.1 Genetic profiling of fat mass and obesity associated (FTO) gene

This is the first study that has reported on FTO polymorphisms in Malaysian Malays. A total of 31 FTO SNPs were included in this study. One of the SNPs, namely FTO SNP rs1861869, deviated from the Hardy-Weinberg equilibrium thus this SNP was removed from further analysis. There were no significant differences between the allelic and genotype frequencies for 30 FTO SNPs in obese and non-obese groups after 5000 permutation tests and Bonferroni correction for multiple markers. The allelic and genotype frequency appears to vary among different ethnicities across the globe. Generally it was observed that the 30 FTO SNPS have high MAF (>5%) in the Malaysian Malays. The 1000 Genomes project was released in 2011 included genotype data from individuals from worldwide. The MAF for each SNP reported by this project in dbSNP is referred to as the global MAF.

The MAF frequency varies in different ethnic group. For example MAF of FTO rs9939609 SNP is 0.39 in Caucasians, while the MAF is only 0.19 in Asians, (Frayling, et al., 2007). The 1000 Genomes project included subjects from different ethnic groups worldwide: four ethnic groups from European ancestry, four ethnic groups from Americans, four ethnic groups from East Asian ancestry, two ethnic groups from West African ancestry and one ethnic group from South Asian ancestry. Table 5.1 shows that as predicted, there were differences observed between the MAF frequencies of FTO SNPs in Malaysian Malays and global MAF reported by the 1000 Genomes Project. For example, the MAF of FTO rs9939609 SNP in the Malaysian Malays is 0.31 while the global MAF is 0.36; MAF of FTO rs16952522 SNP in the Malaysian Malays is 0.16 while the global MAF is 0.04. Similarly, the MAF in the Malaysian Malays were different compared to the global MAF reported by the 1000 Genomes Project for all

other SNPs studied. Variation in genetic inheritance and background of study populations might explain these discrepancies.

**Table 5.1 : MAF of FTO SNPs from 1000 Genomes Project and Malaysian Malays**

| SNP ID | Current study | 1000 Genomes Project |
|---|---|---|
| **rs1077128** | 0.34 | 0.31 |
| **rs11643744** | 0.36 | 0.31 |
| **rs7186521** | 0.32 | 0.39 |
| **rs13334933** | 0.34 | 0.32 |
| **rs16952517** | 0.27 | 0.17 |
| **rs6499643** | 0.23 | 0.18 |
| **rs4784323** | 0.13 | 0.25 |
| **rs7206790** | 0.30 | 0.41 |
| **rs9939973** | 0.34 | 0.35 |
| **rs1421085** | 0.31 | 0.25 |
| **rs1558902** | 0.31 | 0.25 |
| **rs10852521** | 0.27 | 0.40 |
| **rs16952522** | 0.16 | 0.04 |
| **rs17817288** | 0.30 | 0.49 |
| **rs1121980** | 0.34 | 0.37 |
| **rs16945088** | 0.08 | 0.13 |
| **rs17817449** | 0.31 | 0.32 |
| **rs8050136** | 0.31 | 0.34 |
| **rs9935401** | 0.31 | 0.34 |
| **rs3751812** | 0.31 | 0.24 |
| **rs9939609** | 0.31 | 0.36 |
| **rs7190492** | 0.13 | 0.31 |
| **rs7204609** | 0.34 | 0.23 |
| **rs17218700** | 0.14 | 0.08 |
| **rs11642841** | 0.15 | 0.22 |
| **rs1861867** | 0.13 | 0.31 |
| **rs11075994** | 0.13 | 0.23 |
| **rs1421090** | 0.34 | 0.31 |
| **rs17818902** | 0.25 | 0.26 |
| **rs7191513** | 0.32 | 0.43 |

The FTO rs9939609 SNP was chosen as one of the key representatives of the FTO SNPs in this study as this locus was highlighted in many studies for its strongest effect on obesity as well as the key signal for obesity as identified through GWAS (Frayling, et al., 2007). A meta-analysis reported that 21 out of 29 studies have shown significant association between obesity and FTO rs9939609 SNP (Andreasen, Stender-Petersen, et al., 2008; Chang et al., 2008; Frayling, et al., 2007; Gonzalez-Sanchez et al., 2009; Hinney, et al., 2007; Hotta, Nakata et al., 2008; Jacobsson, et al., 2008; Jacobsson et al., 2009; Karasawa, et al., 2010; Li et al., 2008; Liu et al., 2010; Muller, et al., 2008; Peeters, et al., 2008; Peng et al., 2011; Price, Li, & Zhao, 2008; Song, et al., 2008; Tabara et al., 2009; Villalobos-Comparan, et al., 2008; Willer, et al., 2009; Zabena, et al., 2009).

A meta-analysis reported that MAF for FTO rs9939609 SNP is different across the global population. Minor allele frequency (MAF) of FTO rs9939609 polymorphism is lower (0.31) in the Malaysian Malay population compared to previously reported range 0.38-0.46 in European populations (Hinney, et al., 2007; Karasawa, et al., 2010; Peeters, et al., 2008). Generally, non-Europeans and Chinese Han population have much smaller MAF of FTO variants compared to the European populations (Liu, et al., 2010). Table 5.2 shows MAF of rs9939609 in various populations around the world. MAF of rs9939609 is 0.31 to 0.37 in Hispanics, 0.34 to 0.44 in Caucasians, 0.17 in South Americans, 0.36 in Africans and 0.11 to 0.20 in Asians (Dina, et al., 2007; Hennig, et al., 2009; Peng, et al., 2011; Teo, Sim et al., 2009). A meta-analysis reported that in MAF of FTO rs9939609 SNP is 12-14% in Chinese Han and Koreans (Cha, Koo, Park et al., 2009; Cha et al., 2008; Chang, et al., 2008; Hu et al., 2009; Li, et al., 2008; Li et al., 2010; Liu, et al., 2010; Ng, et al., 2008; Ng et al., 2010; Sha et al., 2009), 30-33% in Singapore Malays and Singapore Indians (Chambers et al., 2008; Tan et al., 2008), 30-

33% in Indians from India (Chauhan et al., 2011; Sanghera et al., 2008; Yajnik et al., 2009), 18-20% Japanese and Filipinos (Hotta, Nakata, et al., 2008; Karasawa, et al., 2010; Marvelle, Lange, Qin, Adair, & Mohlke, 2008; Takeuchi et al., 2009). Therefore, it is obvious that Southeast Asians including Malaysian Malays and Singaporeans and South Asian Indians have similar MAF values.

**Table 5.2 : MAF of FTO rs9939609 SNP in various populations**

| Populations | MAF |
|---|---|
| Hispanics | 0.31-0.37 |
| Caucasians | 0.34-0.44 |
| South Americans | 0.17 |
| Africans | 0.36 |
| Asians | 0.11-0.20 |
| Singaporeans Chinese | 0.13 |
| Chinese Han and Koreans | 0.12-0.14 |
| Singaporean Malays and Indians | 0.30-0.33 |
| Indians from India | 0.30-0.33 |
| Japanese and Filipinos | 0.18-0.20 |
| Malaysian Malays | 0.31 |

South Asian and South East Asians have higher MAF of FTO rs9939609 compared to East Asians. The differences in MAF FTO rs9939609 SNP exists in South and Southeast Asian populations compared to East Asians. This may be due to genetic differences in these populations. Phylogenetic analysis from a previous study showed that significant differences exist between Southeast and Northeast Asian populations (Kim, Shin, Harihara, & Kim, 2000). The overall distribution of few alleles is strongly determined by historical relationship among populations (Coop et al., 2009). Closely related populations would commonly experience similar environmental pressures. Natural selection acts to alter the allele frequencies in populations. Consequently geographically localized selection will result in differences in allelic frequency between populations (Cavalli-Sforza, 1966). Many populations do face sharp differences in

selective pressure due to differences in diet, climate, pathogens and other factors (Hancock et al., 2008; Perry et al., 2007; Tishkoff et al., 2007). The geographical and climate differences between Malaysian Malays and other Southeast Asians and Indian who live in tropical climate, when compared to East Asian who live in mild and continental climates (Figure 5.1), may lead to divergences in MAF of FTO SNP. Further studies in other parts of Southeast Asia will be needed in order to fully elucidate the reason to support this. Results from this study in Malaysian Malays also support the genetic heterogeneity that occurs in this continent. A previous report indicates that Southeast Asia was the major geographical source of East Asians and North Asians populations and suggested that genetic stratification is needed when conducting genetic and pharmacogenomics studies in this continent (Abdulla et al., 2009).

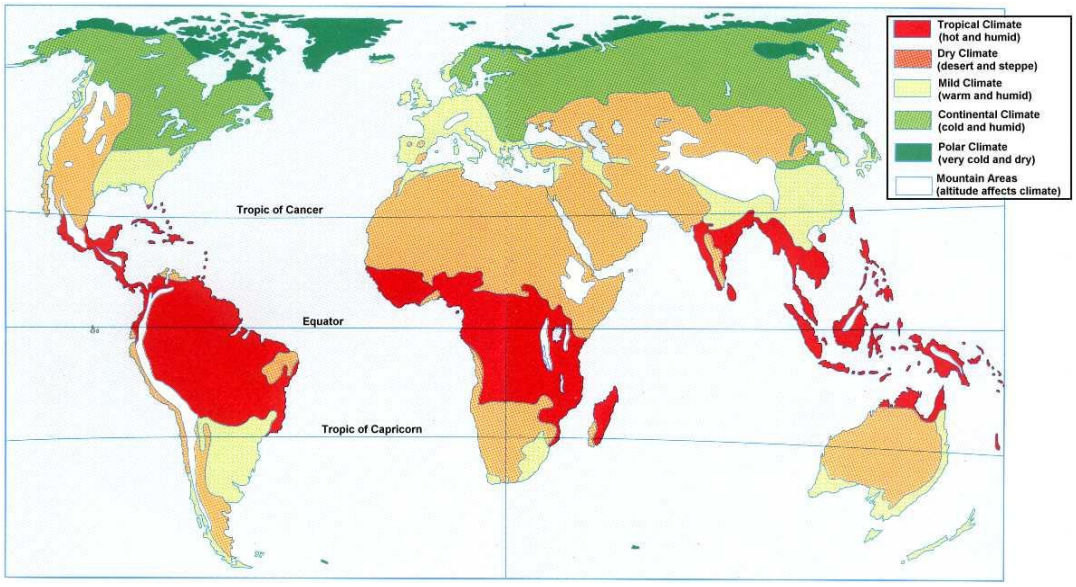



**Figure 5.1 : Climate Zones of the World**

Table 5.3 shows differences in MAF of FTO SNPs between Malaysian Malays and Caucasians. The minor allele frequency (MAF) for FTO rs1421085 SNP is 0.31 in Malaysian Malays. In contrast, for the FTO rs1421085 SNP Caucasian appears to have higher MAF ≥0.40 (Meyre et al., 2009; Peeters, et al., 2008; Peng, et al., 2011; Tonjes,

et al., 2010). For the FTO rs17817449 SNP, the MAF ranges from 0.36-0.60 in Caucasians (Dina, et al., 2007). However, MAF of FTO rs17817449 SNP in Malaysian Malays in this study was found to be 0.31. For the FTO rs8050136 SNPs the MAF is 0.39 in Caucasians, 0.11-0.18 in Asians and 0.42-0.44 in Africans (Cheung et al., 2010; Grant, et al., 2008; Hinney, et al., 2007; Li, et al., 2008; Song, et al., 2008). The MAF of this SNP is 0.31 in Malaysian Malays. The MAF of FTO rs1121980 SNP is 0.34 in Malaysian Malays. Previous studies reported that MAF of FTO rs1121980 is 0.41 in Caucasians and 0.21 in Asians (Hinney, et al., 2007; Hotta, Nakata, et al., 2008). Interestingly, the minor allele frequency (MAF) for FTO rs1421085, rs1558902, rs17817449, rs3751812, rs9939609 and rs8050136 SNPs was similar across these 6 SNPs in this study. MAFs for the SNPs are 0.31. This finding was similar to a reported meta-analysis that FTO rs1421085, rs17817449, rs9939609 and rs8050136 SNPs have very similar MAF values (Peng, et al., 2011). The allelic frequencies in FTO rs1421090 SNP showed no significant difference between obese and non-obese subjects after 5000 permutation correction. Similarly, previous findings reported that rs1421090 was not a risk variant for obesity in Sorbians and Croatians (Tonjes, et al., 2010; Zhang et al., 2010).

**Table 5.3 : Difference in MAF of FTO SNPs between Malaysian Malays and Caucasians**

| SNP ID | Caucasians | Malaysian Malays |
|---|---|---|
| rs1421085 | ≥0.40 | 0.31 |
| rs17817449 | 0.36-0.60 | 0.31 |
| rs8050136 | 0.39 | 0.31 |
| rs1121980 | 0.41 | 0.34 |

Genetic association between 30 FTO SNPs with obesity was investigated in this study. The variants were selected based on significant findings of previous GWAS and association studies (Frayling, et al., 2007; Karasawa, et al., 2010; Peeters, et al., 2008; Scuteri, et al., 2007). In this study, it was found that none of the 30 FTO SNPs was associated with obesity after correction for 5000 permutation test. Among all the genetic factors involved in obesity, FTO variants are known to have maximal effect in the predisposition of obesity in the Europeans (Loos & Bouchard, 2008). A number of studies in Europeans and non-European ethnicity have shown the association between FTO variant and obesity (Adeyemo et al., 2010; Chauhan, et al., 2011; Legry et al., 2009; Scuteri, et al., 2007; Tonjes, et al., 2010; Zhang, et al., 2010). However, the few studies in the Asian populations reported conflicting results (Horikoshi et al., 2007; Hotta, Nakata, et al., 2008; Omori et al., 2008). Nevertheless, two meta-analyses reported that two of the FTO variants (rs9939609 and rs8050136) were found to have significant association with obesity in Asians (Li et al., 2011; Liu, et al., 2010). The contradictory results of the studies in various ethnic groups from different geographical regions might indicate ethnic specific effect of FTO variants on obesity in Malaysian Malays.

The effects of FTO SNPs on obesity-related traits and lipid levels were tested. Quantitative trait analysis after adjustment with age and gender and Bonforreni correction for 30 SNPs revealed that the FTO rs17817288 SNP was significantly associated with LDL-C levels in Malaysian Malays. Recent studies highlighted the SNPs in the FTO gene which contribute to obesity and obesity-related traits (Al-Attar, et al., 2008; Hassanein et al., 2010; Hunt, et al., 2008; Karasawa, et al., 2010; Maes, et al., 1997; Zhang, et al., 2010). A previous study showed that as a transcriptional coactivator, FTO may play an important role in transcription regulation of adipogenesis.

The previous study suggested that FTO might be involved in the regulation of fat development and maintenance (Xi & Mi, 2009). Therefore, the rs17817288 SNP may effect on adipogenesis in Malaysian Malays which is in agreement with findings by Wu et al.(2010) on functional effects of FTO gene.

None of 30 FTO SNPs were associated with logBMI levels in the Malaysians Malays. In contrast, FTO rs9939609 SNP was significantly associated with logBMI in the African- and Europeans Americans (Liu et al., 2011). In the Japanese, FTO rs9939609 SNP was significantly associated with BMI in which AA homozygotes had significantly higher BMI, blood pressure and triglycerides and lower HDL-C levels compared to TT and TA carriers after adjustment for age and gender (Tabara, et al., 2009). A similar trend towards higher logBMI, SBP, logTG and lower HDL-C was observed in the Malaysian Malays. Continuous traits analysis showed FTO rs9939609 was associated with BMI but not with WC, WHR in Hispanic American (Wing et al., 2009), with BMI, weight, WC and HC in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010), with WC in the Indians (Chauhan, et al., 2011) and BMI, TG and WC but not with LDL-C, HDL-C, WHR, SBP and DBP in the Singaporean Chinese, and BMI and WC but not with LDL-C, HDL-C, WHR in Singaporean Malays and not associated with obesity-traits in the Singaporean Indians (Tan, et al., 2008).

Single marker analysis showed that FTO rs1421085 and FTO rs1121980 SNPs were not associated with obesity traits in the Malaysian Malays. However, FTO rs1421085 SNP was associated with BMI but not with WC and WHR in the Hispanic Americans (Wing, et al., 2009). FTO rs1421085 SNP was associated with BMI and WC in the Indians (Chauhan, et al., 2011), with weight, WC, HC and BMI but not with height and WHR in the Island population from the Eastern Adriatic Coast of Croatia

(Zhang, et al., 2010), with weight and BMI but not with height, WC, HC and WHR in the Belgian population and with BMI in the Chinese and Singaporean Malays (Tan, et al., 2008). Previous studies showed that FTO rs1121980 SNP was associated with BMI and WC but not with WHR in the Hispanic Americans (Wing, et al., 2009), with weight, WC, HC and BMI but not with height and WHR in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010) and with BMI in the Chinese and Singaporean Malays (Tan, et al., 2008).

Single marker analysis showed that FTO rs17817449 and rs8050136 SNPs were not associated with obesity traits in the Malaysian Malays. Recent studies showed that FTO rs17817449 SNP was associated with BMI but not with height, HC, WC and WHR in the Hispanic Americans (Wing, et al., 2009), with weight, WC, HC and BMI but not with height and WHR in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010) and with BMI in the Chinese and Singaporean Malays (Tan, et al., 2008). Recent studies showed that FTO rs8050136 was associated with BMI but not with height, HC, WC and WHR in the Hispanic Americans (Wing, et al., 2009), with weight, WC, HC and BMI but not with height and WHR in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010) and with BMI in the Chinese and Singaporean Malays (Tan, et al., 2008).

The FTO rs3751812, rs7204609, rs1861869 and rs7206790 SNPs were not associated with obesity traits in the Malaysian Malays. Recent studies showed that FTO rs3751812 SNP was associated with BMI and WC but not with height, HC and WHR in the Hispanic Americans (Wing, et al., 2009) and with weight, WC, HC and BMI but not with height and WHR in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous reports showed that FTO rs7204609 SNP was associated with BMI and WC but not with height, HC and WHR in the Hispanic

Americans (Wing, et al., 2009). FTO rs1861860 SNP was associated with weight, WC but not with height, WHR, HC and BMI in Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous report showed that FTO rs7206790 SNP was associated with WHR, weight, WC and BMI but not with height and HC in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010).

Single marker analysis of showed that FTO rs9939973, rs10852521, rs17817288 and rs9935401 SNPs were not associated with obesity traits in the Malaysian Malays. However, FTO rs9939973 SNP was associated with BMI, weight, WHR, HC and WC but not with height in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010), with BMI in the Singaporean Malays and Chinese but not with BMI in the Singaporean Indians (Tan, et al., 2008). Previous report showed that FTO rs10852521 SNP was associated BMI, WC, HC, weight and WHR but not with height and HC in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010) and not associated with obesity-traits in the Hispanic Americans (Wing, et al., 2009). Previous report showed that FTO rs17817288 SNP was associated with WHR, weight, HC, WC and BMI but not with height in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous report showed that FTO rs9935401 SNP was associated weight, HC, WC and BMI but not with height and WHR in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010).

Single marker analysis showed that FTO rs7190492, rs11642841, rs1861867, rs11075994 and rs16952522 SNPs were not associated with obesity traits in the Malaysian Malay. Previous report showed that FTO rs7190492 SNP was associated with HC, weight, WC and BMI but not with height and WHR in Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous report showed that FTO rs11642841 SNP was associated with WHR, weight and WC but not with height,

HC and BMI in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous report showed that FTO rs1861867 SNP and weight, WC, HC and BMI but not with height and WHR in Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous report showed that FTO rs11075994 SNP was associated with WHR but not with height, HC, WC, weight and BMI in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Previous report showed that FTO rs16952522 SNP was associated with BMI but not with height, HC, WC, weight and WHR in Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010).

Quantitative analysis of FTO rs1077128, rs11643744, rs6499643, rs4784323, rs16945088, rs17218700 and rs1421090 SNPs showed that these polymorphisms were not associated with any obesity traits in the Island population from the Eastern Adriatic Coast of Croatia (Zhang, et al., 2010). Quantitative analysis of FTO rs11643744, rs13334933, rs16952517, rs4784323 and rs10852521 SNPs was not associated with any obesity traits in the Hispanic Americans (Wing, et al., 2009). These findings were consistent with results in the Malaysian Malays from this study. This may due to the reason that there is absence of genetic effects of these FTO SNPs on obesity-traits in the Malaysian Malays. Although the present study is composed of moderate sample size compared with previous studies which included from 240 to 5380 subjects in different studies across the global population (Peng, et al., 2011), this study was sufficiently powered with a homogenous genetic population of the Malaysian Malays.

LD structure of the 30 SNPs was investigated in the Malaysian Malays. The 30 SNPs of FTO are generally in high LD. Linkage analysis showed 57 regions with complete LD for the FTO gene. In the samples of the Malaysian Malays three FTO SNPs rs9935401, rs16945088 and rs10852521 SNPs were in complete LD (D'=1.0) with

rs9939609. The result from current study showed that fifteen SNPs out of thirty FTO SNPs (50%) are in high LD (D' ≥ 0.88) with FTO rs9939609 SNP. Recent studies reported that the genetic variability in the FTO gene are in high LD to cause risk of obesity in the Spanish population (Gonzalez et al., 2011) and in African Americans (Hassanein, et al., 2010).

In the HapMap sample of Utah residents from northern and western Europe ancestry (CEU), the three FTO SNPs (rs10852521, rs16945088 and rs9935401) are in complete LD (D'=1.0) with FTO rs9939609 SNP as observed in our sample in the Malaysian Malays. Similarly, HapMap sample of the Japanese from Tokyo, Japan (JPT) showed that the FTO rs9939609 SNP was in complete LD with FTO rs10852521 SNP and with high LD for FTO rs16945088 SNP (D'=0.96). In the HapMap sample of the Han Chinese in Beijing, China (CHB), FTO rs9939609 and rs10852521 SNPs was in complete LD (D'=1.0) as is so in our study, but the strength of LD of rs9939609 with FTO rs16945088 SNP (D'=0.44) was reduced. Similarly, in the HapMap samples of the Chinese in Metropolitan Denver, Colorado (CHD), FTO rs9939609 and rs10852521 SNPs were in complete LD (D=1.0) and was with strong LD rs16945088 (D'=0.95). In contrast, in the HapMap samples in the Yoruba in Ibadan, Nigeria (YRI), the FTO rs9939609 and rs10852521 SNPs was not in strong LD (D'=0.48).

HapMap samples of African ancestry in Southwest USA (ASW), Utah residents with Northern and Western European ancestry (CEU), Han Chinese in Beijing, China (CHB), Chinese in Metropolitan Denver, Colorado (CHD), Gujarati Indians in Houston, Texas (GIH), Japanese in Tokyo, Japan (JPT), Luhya in Webuye, Kenya (LWK), Mexican ancestry in Los Angeles, California (MEX), Toscans in Italy (TSI) and Yoruba in Ibadan, Nigeria (YRI) showed complete LD (D=1.0) with FTO rs9939609 SNP at FTO rs16945088 and rs17817449 SNPs. A similar strength of LD was observed in the

samples of the Malaysian Malays. Interestingly, results from HapMap samples showed that FTO rs9939609 SNP was in complete LD with FTO rs10852521 SNP with samples from Asia JPT, CHD and CHB which was also replicated in the samples of the Malaysian Malays. In contrast, the strength of LD of FTO rs9939609 and rs10852521 SNPs was reduced in samples of the African ancestry such as YRI, ASW, LWK and MKK (D'>0.35) (Frazer et al., 2007).

The Singaporean Genome Variation Project (SGVP) analyzed the linkage disequilibrium in the 98 Singaporean Malays (MAS) using the Affymetric Genome-Wide Human SNP array and Illumina Human1M single Beadchip genotyping platforms (Teo, Sim, et al., 2009). In the present study, it was found that Linkage Disequilibrium pattern of all the regions with complete LD in the Malaysian Malays was found to be similar to the MAS samples except for FTO rs17218700 and FTO rs7190492 SNPs. The LD of FTO rs17218700 SNP with FTO rs7190492 SNP was less in MAS (D'=0.74) compared to this study. By using different genotyping platform Sequenom MassARRAY®iPLEX platform with much larger sample size (n=587) in this study, it was found that the LD pattern based on data from this study in the Malaysian Malays is very similar to that of MAS. Therefore, we could predict the similar pattern of LD in FTO gene ancestry of Malays in Southeast Asia due to the genetic homogeneity. However, further studies will be needed to address this in the Malays in other parts of Southeast Asia. Differences exist in the LD structure of FTO in diverse ethnic populations (Peng, et al., 2011). For example, previous studies have shown that the degree of LD in the African-ancestry population is less than that of the European populations (Reich, et al., 2001). The major difference in the extent of LD between populations explains differences due to population history, most likely bottleneck or

founder effect that occurred among the ancestors of north Europeans after the divergence from the ancestors of the Nigerians (Reich, et al., 2001).

The haplotypes in block 1 and block 2 of FTO were not associated with obesity in Malaysian Malays. However, the majority of haplotypes frequencies of FTO were found to be more than 5% in the Malaysian Malays. Major haplotypes in the ancestry of Malaysian Malays may also be present in the Malays in other parts of Southeast Asia. However, studies in different population will be needed to verify this finding.

### 5.2.2 Genetic profiling of MC4R gene

This is the first study that reports on association between MC4R polymorphisms and obesity in the Malaysian Malays. A total of 6 MC4R SNPs were included in this study: MC4R rs571312, rs2229616, rs7227255, rs1016862, rs1295734 and rs17700144 SNPs. However, MC4R rs1295734 and rs17700144 SNPs deviated from the Hardy-Weinberg equilibrium. The MC4R rs1016862 SNP was monomorphic. Therefore these three SNPs were excluded from further analysis. There were no significant differences between the allelic and genotype frequencies for MC4R rs571312, rs2229616 and rs7227255 SNPs in obese and non-obese groups after 5000 permutation test and Bonferroni correction for multiple markers. Generally it was observed that MC4R SNPS (rs2229616 and rs7227255) have low MAF (<5%) while MC4R rs571312 SNP have high MAF (>5%) in the Malaysian Malays.

The 1000 Genomes project included subjects from different ethnic groups worldwide. Table 5.4 shows that there are differences between MAF frequencies of MC4R SNPs (rs571312 and rs7227255) in the Malaysian Malays with global MAF reported by the 1000 Genomes Project. The MAF appears to vary among different ethnicities across the globe. The MAF of MC4R rs2229616 SNP was almost similar in

the Malaysian Malays with that of the global MAF reported by the 1000 Genomes Project. The MAF for rs2229616 was in the range of 0.01-0.02 in Europeans (Stutzmann, et al., 2007). This is in agreement with results in Malaysian Malays. Frequency of the A allele was 2.5% for MC4R rs2229616 SNP in the Malaysian Malay population which is higher compared to the European population in which the frequency of the A allele ranges from 1.49-2.24% (Stutzmann, et al., 2007).

**Table 5.4 : MAF of MC4R SNPs from 1000 Genome Project and Malaysian Malays**

| SNP ID | Current Study | 1000 Genomes Project |
|---|---|---|
| **rs571312** | 0.13 | 0.24 |
| **rs2229616** | 0.03 | 0.02 |
| **rs7227255** | 0.01 | 0.07 |

According to the International HapMap project, a SNP is considered as monomorphic in a particular population if not a single heterozygous individual could be detected in the population. The MC4R rs1016862 SNP was monomorphic among Malaysian Malays because there is no heterozygous genotype detected in the study sample. The MC4R rs1016862 SNP was also monomorphic in the Japanese population (Okuda et al., 2002). Similar monomorphic pattern of MC4R rs1016862 SNP was observed in the Singaporean Malays, Chinese and Indians (Teo, Sim, et al., 2009). The wild homozygotes AA was not detected for the MC4R rs2229616 and rs7227255 SNPs, in the Malaysian Malays. The AA genotype for MC4R rs2229616 SNP was also undetected in the Finnish population (Rutanen et al., 2004).

Quantitative traits analysis was performed to investigate the association between MC4R SNPs and the obesity-traits. There were significant differences between rs571312 with logBMI and SBP after Bonferroni correction for multiple markers and

adjustment for age and gender (p=0.008 and p=0.005) respectively in the Malaysian Malays. The MC4R rs571312 SNP was significantly associated with BMI in the Singaporeans (Dorajoo et al., 2012). This finding is consistent with results in the current study that this polymorphism has an effect on logBMI in the Malaysian Malays. There were no significant differences between MC4R rs7227255 SNP with obesity traits in the Malaysian Malays after Bonforreni correction and adjustment with age and gender. However, recently, a GWAS reported that MC4R rs7227255 SNP was significantly associated with BMI in the Europeans (Speliotes, et al., 2010). Previous studies have shown that MC4R rs2229616 SNP (V103I) protects against human obesity, by negative association with obesity in the Europeans (Stutzmann, et al., 2007), UK populations (Young, et al., 2007) and East Asians (Wang, et al., 2010).

There is a significant association between MC4R rs2229616 polymorphism and serum total cholesterol level after Bonferroni correction for multiple markers and adjustment for age and gender (p=0.016). This showed the possible involvement of MC4R rs2229616 SNP on cholesterol metabolism in the Malaysian Malay population but further verification in functional studies is needed. There is no association between MC4R rs2229616 polymorphism and BMI in the Malaysian Malay population as was observed in the European populations. There is a trend towards higher logBMI in the GA heterozygotes compared to GG homozygotes in the Malays. In the Finnish population, there was significant increase in BMI in GA heterozygotes compared to GG homozygotes (Rutanen, et al., 2004). The GA heterozygous showed a significant decrease in BMI compared to GG homozygotes in the white and Swedish population (Heid et al., 2005; Rosmond, et al., 2001). Although, the polymorphism was not significantly associated with serum triglycerides levels, triglycerides level was lower in the GA carriers in the Malaysian Malay population as was observed in previous findings

in the Swedish and European populations (Bronner et al., 2006; Green, Turki, Innis, & Liggett, 1994; Rosmond, et al., 2001). Obesity is closely linked to increased serum triglycerides level (Carr & Brunzell, 2004). Changes in triglyceride might be due to the influence of MC4R on body weight. A recent finding which reported negative association of this polymorphism with obesity recommended that rigid body weight control should be recommended for subjects carrying the A allele, which can subsequently cause reduction in triglyceride levels (Stutzmann, et al., 2007). There were no significant differences in height, weight, logBMI, WC, HC, WHR, SBP, DBP, HDL-C and LDL-C levels in the Malaysian Malays. Findings from this study were consistent with results reported in the Finnish population (Rutanen, et al., 2004).

The LD analysis showed that strength of LD between MC4R SNPs in the Malaysian Malays is low. The MC4R rs7227255 SNP is in low LD (D'=0.67) with the relatively rare MC4R missense variant (rs2229616). In contrast, the MC4R rs7227255 SNP was in perfect LD with MC4R rs2229616 SNP in a European ancestry reported by the Genetic Investigation of Anthropometric Traits (GIANT) Consortium genome-wide association meta-analysis (Speliotes, et al., 2010). The difference in genetic pattern may explain the differences in strength of LD in these two different populations. Haplotype analysis showed the haplotypes of MC4R in the Malaysian Malays are not risk factors for the obesity. This may be due to the low frequency of these rare MC4R variants in this study population.

### 5.2.3 Genetic profiling of ADRB2 gene

This is the first study that reports on the association between ADRB2 polymorphisms and obesity in the Malaysian Malays. A total of 5 ADRB2 SNPs were included in this study: ADRB2 rs1042713, rs1042714, rs1042717, rs1042718 and rs1042719 SNPs, all of which did not deviate from the Hardy-Weinberg equilibrium.

After 5000 permutation test, there was no significant difference in allelic frequency of each of the ADRB2 SNPs between the obese and non-obese group. Generally, it was observed that ADRB2 SNPS have high MAF (>5%) in the Malaysian Malays.

The 1000 Genomes project included subjects from different ethnic groups worldwide. The MAF frequency of ADRB2 varies in different ethnic group. Table 5.5 shows that there are differences in MAF frequencies of ADRB2 SNPs in the Malaysian Malays with global MAF reported by the 1000 Genomes Project. The MAF of rs1042713 was almost similar in the Malaysian Malays with the global MAF reported by the 1000 Genomes Project. MAF of ADRB2 rs1042714 SNP in the Malaysian Malays is lower compared to the global MAF. MAF of ADRB2 rs1042717, rs1042718 and rs1042719 SNPs were higher in the Malaysian Malays compared to global MAF reported by the 1000 Genomes Project.

**Table 5.5 : MAF of ADRB2 gene from 1000 Genomes Project and Malaysian Malays**

| SNP ID | Current Study | 1000 Genomes Project |
|---|---|---|
| **rs1042713** | 0.45 | 0.47 |
| **rs1042714** | 0.08 | 0.23 |
| **rs1042717** | 0.44 | 0.28 |
| **rs1042718** | 0.44 | 0.27 |
| **rs1042719** | 0.45 | 0.35 |

The frequency of the G (Glu27) allele was reported to be 30.3% across the global populations. Glu is the abbreviation for Glutamic Acid. Interestingly, previous studies showed that there was prominent difference in the G allele frequency across the different ethnic groups. The range was reported to be from 6.71% to 78.29% across the global population. The highest frequency was observed in the Caucasians (40.7%) while the smallest percentage (7%) was observed among the populations living around the Pacific Ocean and the surrounding islands, such as the Japanese and the Taiwanese

Chinese. The frequency of G allele was 8% in the Malaysian Malays, which is in line with a meta-analysis that reported an average of 7% for G allele frequency in the Asians (Jalba, Rhoads, & Demissie, 2008; Litonjua, et al., 2010).

The current study showed that the ADRB2 rs1042714 SNP is associated with DBP in the Malaysian Malays (Apalasamy, Ming, Rampal, Bulgiba, & Mohamed, 2011). These results therefore suggest that the beta-adrenoceptor polymorphism has a role in the regulation of blood pressure in the Malaysian Malay population. ADRB2 is a catecholamine which regulates blood pressure in human (Insel, 1996). Association of ADRB2 rs1042714 polymorphism with blood pressure was also observed in the North American population (Bray et al., 2000). ADRB2 rs1042714 polymorphism was not associated with BMI in the Malaysian Malays in agreement with a meta-analysis carried out in the Koreans, the Olivetti subjects, Japanese, African Americans, Danish men and Swedish subjects (Echwald, et al., 1998; Galletti, et al., 2004; Hayakawa, et al., 2000; Hedenbro JL, 1999; Jalba, et al., 2008; Kao WHL, 1999; Kim, et al., 2002). The ADRB2 rs1042714 SNP was significantly associated with obesity in the Japanese (Ishiyama-Shigemoto, Yamada, Yuan, Ichikawa, & Nonaka, 1999; Mori, et al., 1999). Association between ADRB2 rs1042714 SNP and obesity was found in the Pacific Islanders but not in the Europeans (Jalba, et al., 2008), Austrian women, (Oberkofler, Esterbauer, Hell, Krempler, & Patsch, 2000) Koreans (Kim, et al., 2002) and Japanese men (Hayakawa, et al., 2000). However, a trend towards increased logBMI level was observed in the CC carriers in our study as was similarly seen in the Swedish and Australian subjects (Ehrenborg, et al., 2000; Large, et al., 1997; Lin, et al., 2001). A trend towards increased triglycerides levels was observed in the GG homozygotes in the Malaysian Malays. However, it is the CC homozygotes which showed increased trigycerides levels in the Taiwanese and Swedish subjects (Kao, Yen, & Lung, 2008). A

western blot analysis suggesting that a delay in degradation of β2-adrenoceptor in ADRB2 rs1042714 polymorphism might result in increase in triglyceride level (Green, et al., 1994). In the Koreans, ADRB2 rs1042714 SNP was not associated with weight, BMI, WC, SBP, SBP, TG and HDL-C as observed in the Malaysian Malays (Park, Shin, & Lee, 2008).

There was no significant difference between ADRB2 rs1042713 SNP with obesity-traits in the Malaysian Malays after adjustment with age and gender. The rs1042713 was not associated with obesity in the Japanese man (Hayakawa, et al., 2000) while this polymorphism was associated with obesity in the French-Canadian men. A meta-analysis reported that ADRB2 rs1042713 and rs1042714 SNPs were not associated with obesity (Jalba, et al., 2008). The AA homozygotes showed trend towards higher weight, DBP, logTG and HDL-C and lower logBMI and weight compared to AG and GG carriers. Similarly, in subjects who participated in the Olivetti Heart Study, there was no significant association between ADRB2 rs1042713 SNP with BMI, WC and TG (Galletti, et al., 2004). In the Koreans and Japanese men, ADRB2 rs1042713 SNP was not associated with weight, BMI, WC, SBP, SBP, TG and HDL-C as observed in the Malaysian Malays (Hayakawa, et al., 2000; Park, et al., 2008).

There were no significant differences between ADRB2 rs1042717, rs1042718 and rs1042719 SNPs with obesity-traits in the Malaysian Malays after adjustment with age and gender. However, the AA homozygotes showed trend towards lower WC, DBP, TC and LDL-C compared to GG and AG carriers. In the Koreans, ADRB2 rs1042717 SNP was significantly associated with weight in which the AA homozygotes had higher weight (Park, et al., 2008). The GG homozygotes showed trend towards lower weight, logBMI, WC, HC, SBP and DBP compared to CG and CC carriers. In the Koreans, ADRB2 rs1042717, rs1042718 and rs1042719 SNPs were not significantly associated

with weight, BMI, WC, SBP, SBP, TG and HDL-C as observed in the Malaysian Malays (Park, et al., 2008).

In the Malaysian Malays, ADRB2 rs1042713 SNP was in high LD with ADRB2 rs1042717 SNP (D'=0.90) and ADRB2 rs1042718 SNP (D'=0.94). However, ADRB2 rs1042713 SNP was in complete LD (D'=1.0) with ADRB2 rs1042717 SNP and ADRB2 rs1042718 SNP in the HapMap sample of the Utah residents with ancestry from the northern and western Europe (CEU), Yoruba in Ibadan, Nigeria (YRI), African ancestry in Southwest USA (ASW), Han Chinese in Beijing, China (CHB), Chinese in Metropolitan Denver, Colorado (CHD), Gujarati Indians in Houston, Texas (GIH), Mexican ancestry in Los Angeles, California (MEX), Toscans in Italy (TSI), Maasai in Kinyawa, Kenya (MKK), Luhya in Webuye, Kenya (LWK) and Japanese in Tokyo Japan (JPT). In the present study, LD pattern between ADRB2 rs1042713 SNP with ADRB2 rs1042717 SNP in the Malaysian Malays was found to be similar with the samples in the Singaporean Indians, Malays and Chinese (Teo, Sim, et al., 2009).

In the Malaysian Malays, ADRB2 rs1042717 SNP was in high LD with ADRB2 rs1042718 SNP (D'=0.87). However, ADRB2 rs1042717 SNP was in complete LD with ADRB2 rs1042718 SNP in the HapMap samples of YRI, CEU, ASW, CHB, CHD, GIH, JPT, LWK, MEX and Singaporean Chinese, Indian and Malays (Teo, Sim, et al., 2009). In the Hapmap samples of MKK and TSI, the ADRB2 rs1042717 SNP was in high LD (D'=0.96 and D'=0.98) with ADRB2 rs1042718 SNP respectively. In the Malaysian Malays, ADRB2 rs1042713 SNP was in low LD with ADRB2 rs1042714 SNP (D'= 0.67). Similarly, ADRB2 rs1042713 SNP was in low LD with ADRB2 rs1042714 SNP in the France men (Dallongeville, Helbecque, Cottel, Amouyel, & Meirhaeghe, 2003). ADRB2 rs1042713 SNP was also in low LD with ADRB2 rs1042714 SNP (D'= 0.79) in the Swedish men (Ehrenborg, et al., 2000). A total of 5

haplotypes were found in a 9kb haplotype block. Haplotype analysis of the ADRB2 gene revealed that the haplotypes are not associated with obesity in the Malaysian Malays.

### 5.2.4 Genetic profiling of LEPTIN gene

A total of 8 LEP SNPs were included in this study; LEP SNPs rs11770725, rs1349419, rs12535708, rs12535747, rs7799039, rs2167270, rs2278815 and rs12706832. LEP SNPs rs1349419, rs12535708, rs12535747, rs7799039, rs2167270, rs2278815 and rs12706832 did not deviate from the Hardy-Weinberg equilibrium (HWE). However, the LEP rs11770725 SNP deviated from HWE, and was thus removed from further analysis. After 5000 permutation test, there was no significant difference in allelic frequency of each of the LEP SNPs between the obese and non-obese group. Generally, it was observed that LEP SNPS have high MAF (>5%) in the Malaysian Malays.

The 1000 Genomes project included subjects from different ethnic groups worldwide. Table 5.6 shows that there is a difference between MAF frequencies of LEP SNPs in Malaysian Malays with that of the global MAF reported by the 1000 Genomes Project. The MAF of LEP rs2167270 SNP (0.17 versus 0.35), LEP rs2278815 SNP (0.25 versus 0.49) and LEP rs12706832 SNP (0.28 versus 0.50) were almost twice in global MAF reported by the 1000 Genomes Project compared to the Malaysian Malays. MAF for LEP rs1349419, rs12535708, rs12535747 and rs7799039 SNPs were higher in the Malaysian Malays compared to global MAF reported by the 1000 Genomes Project.

**Table 5.6 : MAF of LEP gene from 1000 Genome Project and Malaysian Malays**

| SNP ID | Current Study | 1000 Genomes Project |
|---|---|---|
| **rs1349419** | 0.25 | 0.48 |
| **rs12535708** | 0.16 | 0.29 |
| **rs12535747** | 0.16 | 0.28 |
| **rs7799039** | 0.27 | 0.43 |
| **rs2167270** | 0.16 | 0.35 |
| **rs2278815** | 0.25 | 0.49 |
| **rs12706832** | 0.28 | 0.50 |

The reported MAF of LEP SNPs varies in different ethnic populations. The MAF of LEP rs1349419 SNP was 0.41, LEP rs12535708 SNP was 0.35, LEP rs2167270 SNP was 0.34, LEP rs12535747 SNP was 0.35 and LEP rs2278815 SNP was 0.41 in the Americans (Jiang, et al., 2004). The results from the current study indicate that MAF of LEP SNPs is generally lower in the Malaysians compared to other ethnic groups such as the Americans. The G allele frequency of LEP rs7799039 SNP was higher in obese compared to controls in the North American women (0.65 versus 0.49), Europeans (0.64 versus 0.54) that showed a positive association with obesity (Yiannakouris, et al., 2003).

There was no significant difference between LEP SNPs with obesity-traits in the Malaysian Malays after Bonferroni correction and adjustment with age and gender. The LEP rs1349419, rs12535708, rs12535747, rs2167270 and rs2278815 SNPs were not associated with BMI in the Americans (Jiang, et al., 2004). The LEP rs7799039 SNP was associated with extreme obesity in the Taiwanese indigenous population (Wang, et al., 2006). The LEP rs7799039 SNP was not associated with BMI, WC and WHR but with leptin levels in the Brazilian women (Hinuy et al., 2008). The LEP rs7799039 SNP was not associated with BMI, WC, WHR, TC, LDL-C, HDL-C, SBP and DBP but with leptin levels in the Romanian subjects (Constantin et al., 2010). The LEP rs7799039

SNP was not associated with BMI, WC, WHR, TC, LDL-C and HDL-C, but with leptin levels in the Tunisian subjects (Ben Ali, et al., 2009). The present study showed that there is no significant association between the LEP rs7799039 gene polymorphism with BMI but the GG homozygotes exhibited higher BMI compared to the GA and AA carriers. This was similarly found in the USCI (Malaysia) students cohort (Koh Yuan Ni, 2009). The LEP rs12706832, rs2278815, rs2167270, rs12535747, rs12535708 and rs1349419 SNPs were not associated with log-Leptin levels after Bonferroni correction for the multiple markers.

The LD analysis between the LEP SNPs indicates strong LD in the Malaysian Malays. Complete LD was observed at LEP rs1349419 with rs12535708, rs2167270 and rs12535747 SNPs. Complete LD was observed at LEP rs12535708 SNP with LEP rs12535747 SNP and with LEP rs2167270 SNP. Complete LD was observed at LEP rs12535747 SNP with LEP rs2167270 SNP. Similarly, complete LD was observed between LEP rs1349419 SNP with LEP rs12535708 SNP, LEP rs1349419 SNP with LEP rs2167270 SNP and LEP rs12535708 SNP with LEP rs2167270 SNP in the Americans (Jiang, et al., 2004). Complete LD was observed in LEP rs1349419 SNP with LEP rs2278815 SNP, LEP rs2278815 SNP with LEP rs12535708 SNP and LEP rs2167270 SNP in the Americans (Jiang, et al., 2004) and in the Malaysian Malays, it was in high LD between these regions (D'=0.99). The LEP rs2167270 SNP was in high LD (D'=0.95) with LEP rs12706832 SNP in the Malaysian Malays while the LEP rs2167270 and rs12706832 SNPs were in complete LD (D'=1.0) in HapMap samples of the Japanese (JPT), GIH, CHB and Singaporean Malays (Teo, Sim, et al., 2009). The LEP rs2167270 and rs12706832 SNPs were in high LD in the HapMap samples of CEU (D'=0.97), CHD (D'=0.97), MEX (D'=0.89), Singaporean Indians (INS) (D'=0.93) and

Singaporean Chinese (CHS) (D'= 0.97). The rs2167270 and rs12706832 were in low LD in the ASW (D'=0.65), MKK (D'=0.73), LWK (D'=0.40) and YRI (D'=0.46).

A haplotype block was identified in the of LEP gene. All the seven SNPs in the risk haplotype are in a 9kb region. Five haplotypes were found in this region. The GCCGGAA haplotype was significantly associated with obesity. This showed that the LEP variants are involved in predisposition to obesity in the Malaysian Malays.

### 5.2.5 Genetic profiling of RETN gene

A total of 3 RETN SNPs were included in this study, namely, RETN SNPs rs34861192, rs1862513 (-420C>G) and rs3219175, and all of these SNPs did not deviate from the Hardy-Weinberg equilibrium. After 5000 permutation test, there was no significant difference in allelic frequency of each of the RETN SNPs between the obese and non-obese groups. All the RETN SNPs were in high frequencies (>5%) in this population. This shows that resistin gene does not play a major role in predisposition of obesity in the Malaysian Malays. The RETN rs1862513 SNP was not associated with obesity in the Koreans (Cho, et al., 2004).

The 1000 Genomes project included subjects from different ethnic groups worldwide. Table 5.7 shows that there is a difference between MAF frequencies of RETN SNPs in the Malaysian Malays with that of the global MAF as reported by the 1000 Genomes Project. The MAF of RETN rs34861192, rs1862513 and rs3219175 SNPs were higher in the Malaysian Malays compared to the global MAF reported by the 1000 Genomes Project. The MAF of RETN rs34861192 and RETN rs3219175 SNPs in the Malaysian Malays were two times higher than the global MAF as reported by the 1000 Genomes Project. The RETN rs34861192 SNP was found to be monomorphic in the Hispanic and Europeans in the EGP_HISP-PANEL (Hispanic) and EGP_CEPH-PANEL (Europeans) Panel of dbSNP.

**Table 5.7 : MAF of RETN gene from 1000 Genomes Project and Malaysian Malays**

| SNP ID | Current Study | 1000 Genomes Project |
|---|---|---|
| **rs34861192** | 0.15 | 0.06 |
| **rs1862513** | 0.46 | 0.31 |
| **rs3219175** | 0.14 | 0.08 |

The MAF of RETN rs34861192 SNP was 0.22 in the Japanese which was higher than in the Malaysian Malays. The MAF of RETN rs1862513 SNP was 0.37 in the Japanese which was lower than in the Malaysian Malays (0.46). The MAF of RETN rs3219175 SNP was 0.22 in the Japanese which was higher than in the Malaysian Malays (0.14) (Asano, et al., 2010). Other study in the Japanese men reported that MAF of RETN rs1862513 and rs3219175 SNPs were 0.34 and 0.21 respectively (Yoshihiro Miyamoto, 2009). The ethnic differences may contribute to the differences in MAF.

Number of the AA homozygotes was smaller compared to AG and GG carriers. Therefore, to confirm the findings, the association analysis between the obesity traits with the SNPs analyzed using two different genetic models known as additive and dominant models. There were significant differences between RETN rs34861192 and rs3219175 SNPs with plasma log-resistin levels and weight in the Malaysian Malays after Bonferroni correction and adjustment with age, gender and log-resistin. Similarly, the RETN rs34861192 SNP in the 5' flanking regions is associated with plasma resistin levels in the Japanese and Finnish populations (Asano, et al., 2010; Ukkola, et al., 2008). The RETN rs1862513 SNP was not associated with obesity-traits in the Malaysian Malays. RETN SNPs rs34861192, rs1862513 and rs3219175 SNPs were not associated with metabolic traits and lipid parameters in the Japanese population (Asano, et al., 2010). In the Korean populations, RETN rs1862513 SNP was associated with TG but not with BMI and other metabolic traits (Ukkola, et al., 2008). The RETN

rs1862513 and RETN rs3219175 SNPs were not associated with plasma resistin levels in the Japanese population (Asano, et al., 2010). On the other hand, the RETN rs1862513 SNP was associated with plasma resistin concentrations in the Koreans (Cho, et al., 2004). A meta-analysis in the Europeans reported that RETN rs1862513 SNP was not associated with resistin levels in two European populations, the Framingham Offspring study and a cohort from Italy (Hivert, et al., 2009; Menzaghi et al., 2006).

In the Malaysian Malays, RETN rs1862513 was in complete LD (D'=1.0) with RETN rs3219175 SNP. The strength of LD between RETN rs1862513 and RETN rs3219175 SNPs was reduced in the Japanese population and Japanese men (Asano, et al., 2010; Yoshihiro Miyamoto, 2009). The RETN rs34861192 and RETN rs3219175 SNPs was in perfect LD in the Japanese but the strength of LD between these two SNPs was slightly reduced in Malaysian Malays (D'=0.99). The RETN rs34861192 and RETN rs1862513 SNPs was in high LD (D'=0.90) in the Malaysian Malays but the strength was reduced in the Japanese (Asano, et al., 2010).

A haplotype block was identified in the RETN gene. There are three SNPs in the haplotype block. Three haplotypes were found in this region. The haplotypes were in high frequencies (>5%). None of the RETN haplotypes were significantly associated with obesity. This showed that the RETN variants do not play a major role in obesity in the Malaysian Malays.

### 5.2.6 Genetic profiling of INSIG2 gene

The INSIG2 rs7566605 SNP was included in this study. This SNP did not deviate from the Hardy-Weinberg equilibrium. There were no significant differences in allelic and genotype frequencies of INSIG2 rs7566605 SNP between the obese and non-obese groups. The genotype frequency of the CC homozygotes of INSIG2 rs7566605 SNP was higher in obese subjects compared to non-obese subjects in the Malaysian

Malay population as observed in other studies (Goodman, Dolan, Morrison, & Daniels, 2005; Hotta, Nakamura, et al., 2008; Wang, et al., 2008).

The global MAF reported by the 1000 Genomes Project for INSIG2 rs7566605 SNP was 0.30. The MAF of INSIG2 rs7566605 SNP in the Malaysian Malays was 0.43. This shows that MAF in the Malaysian Malays is higher compared to the global MAF. From the data from the International HapMap project for INSIG2 rs7566605 polymorphism, different frequency of C allele can be observed in different ethnic groups. From this study, the frequency of C allele is 44% in the Malaysian Malay population. The frequency of C allele of INSIG2 rs7566605 polymorphism were 23% in the Uyghur population, 28% in the American white, 36% in the Chinese Han and Japanese, 41% in the African, 37% and 31% in those with the Western European ancestry (Herbert, et al., 2006).

This study showed no association between INSIG2 rs7566605 SNP with obesity. The lack of association found in the present study is in agreement with that of the Nurses' Health Study Cohort in which 2726 subjects participated and with the Caucasian population in which 1428 subjects participated (Hall, Rahman, Avery, & Keavney, 2006; Herbert, et al., 2006). The lack of association may be due to the small sample size compared to the previous report from GWAS. A GWAS by Herbert et al in 2006 reported association of INSIG2 rs7566605 polymorphism with BMI with OR for obesity 1.22 [95% CI 1.05-1.42]; p=0.008. Following this initial finding, replication of this findings was seen in the Western European ancestry, African Americans and a few other studies (Herbert, et al., 2006; Hotta, Nakamura, et al., 2008; Liu, Li et al., 2008; Lyon, et al., 2007; Orkunoglu-Suer et al., 2008; Yang, et al., 2008; Zhang, et al., 2008). However, a few other studies failed to show the association between INSIG2 rs7566605 SNP with obesity in the French, Europeans, German, Danish, British Caucasian,

American-Samoans and Mexican-American populations (Andreasen, Mogensen et al., 2008; Bressler, et al., 2009; Deka, et al., 2009; Dina, et al., 2007; Hall, et al., 2006; Loos, Barroso, O'Rahilly, & Wareham, 2007; Rosskopf, et al., 2007). In Asians, the association of INSIG2 rs7566605 SNP with obesity is inconsistent. Significant association between INSIG2 rs7566605 SNP with obesity was found in the Japanese population (Hotta, Nakamura, et al., 2008) while lack of association was found in the Chinese and Indian populations (Kumar, Sunkishala, Karthikeyan, & Sengupta, 2007; Wang, et al., 2008)

The INSIG2 rs7566605 SNP had no effect on obesity-traits in the Malaysian Malays. INSIG2 rs7566605 SNP was associated with BMI but not with WHR, SBP, DBP, TG and cholesterol levels in the Chinese minority group in Uyghurs (Zhang, et al., 2008). INSIG2 rs7566605 SNP was not associated with BMI, lipoprotein parameters and free fatty acid levels in the Utah and Austria populations (Boes, et al., 2008). Similarly, INSIG2 rs7566605 had no effect on TC, TG, HDL-C, LDL-C and blood pressure parameters in the Chinese population (Feng et al., 2007). In addition, INSIG2 rs7566605 SNP had no effect on triglyceride levels in two UK-based cohorts (Smith, Cooper, Li, & Humphries, 2007). Lack of association was observed between this polymorphism with obesity-related traits except WHR in the white, Hispanic and African-American subjects (Bressler, et al., 2009). In the Indian subjects, there was no association between INSIG2 rs7566605 SNP with BMI and obesity-related traits (Kumar, et al., 2007).

INSIG2 has been found to play a role in cholesterol metabolism (Xi & Mi, 2009). In the present study, INSIG2 rs7566605 SNP was not found to be significantly associated with levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides in the Malaysian Malays. This was similarly observed in the Korean and

Japanese population (Cha, Koo, Choi, et al., 2009; Hotta, Nakamura, et al., 2008; Oki, et al., 2009). The INSIG2 rs7566605 SNP was not associated with BMI, WHR, plasma levels of cholesterols and triglycerides in the Slavonic Caucasian population (Hubacek, et al., 2010).

### 5.2.7 Genetic profiling of ADIPOQ gene

The ADIPOQ rs3774261 and rs17366568 SNPs were included in this study. This SNP did not deviate from the Hardy-Weinberg equilibrium. There were no significant differences in allelic and genotype frequencies of ADIPOQ rs3774261 between the obese and non-obese groups. However, significant association was found in allelic frequencies of ADIPOQ rs17366568 SNPs between the obese and non-obese groups. Table 5.8 shows that there is a difference between MAF frequencies of ADIPOQ SNPs in the Malaysian Malays with that of the global MAF as reported by the 1000 Genomes Project. The global MAF reported by the 1000 Genomes Project for ADIPOQ rs3774261 SNP was 0.49. The MAF of ADIPOQ rs3774261 SNP in the Malaysian Malays was 0.46. The global MAF reported by the 1000 Genomes Project for ADIPOQ rs17366568 SNP was 0.05. The MAF of ADIPOQ rs17366568 SNP was 0.04 in the Malaysian Malays. This shows that MAF in this population is lower compared to the global MAF.

**Table 5.8 : ADIPOQ gene from 1000 Genomes Project and Malaysian Malays**

| SNP ID | Current Study | 1000 Genomes Project |
|---|---|---|
| **rs3774261** | 0.46 | 0.49 |
| **rs17366568** | 0.04 | 0.05 |

Current study showed no association between ADIPOQ rs3774261 SNP with obesity in the Malaysian Malays. The ADIPOQ rs3774261 SNP had no effect on log-adiponectin levels, BMI, WC, WHR, SBP, DBP, LDL-C, HDL-C, TG and TC levels in the Malaysians. The ADIPOQ rs3774261 SNP was associated with adiponectin levels but not with HDL-C, triglycerides and BMI in the Turkish and Europeans (Ling, et al., 2009). In future, linkage studies involving more rare variants will be needed in order to elucidate whether the DNA variations at ADIPOQ influences plasma adiponectin levels in the Malaysian Malays.

The ADIPOQ rs17366568 SNP was significantly associated with obesity in the Malaysian Malays. There was a significant difference between the obese and non-obese groups for the allelic frequencies of ADIPOQ rs17366568 SNP. Number of the AA homozygotes was smaller compared to AG and GG carriers. Therefore, to confirm the findings, the association analysis between the obesity traits with the ADIPOQ rs17366568 SNP was analyzed using two different genetic models known as additive and dominant models. Quantitative traits analysis revealed that the ADIPOQ rs17366568 SNP was not significantly associated with obesity parameters in Malaysian Malays. Similarly, this SNP was not associated with metabolic parameters in the European population (Ling, et al., 2009). The ADIPOQ rs17366568 SNP showed no effect on the adiponectin levels. However, this SNP was found to have effect on the adiponectin levels in the European subjects (Heid et al., 2010). Previous studies have shown that genetic polymorphisms play important role in the determination of the adiponectin levels (Breitfeld, Stumvoll, & Kovacs, 2012). However, the exact mechanism for the association between the ADIPOQ SNPs with adiponectin levels and metabolic traits is still unclear and yet to be investigated.

The strength of LD between ADIPOQ rs17366568 and rs3774261 was low in the Malaysian Malays. Similar pattern of low LD was observed between these two SNPs in the GEMS study that was conducted in the European subjects (Heid, et al., 2010; Ling, et al., 2009). A haplotype block was identified in the ADIPOQ gene region. The AG haplotypes was significantly associated with obesity in the Malaysian Malays. The other haplotypes of ADIPOQ, GG and GA was not associated with obesity in this population.

### 5.2.8 Genetic profiling of SDC3 gene

The SDC3 rs2491132 SNP was included in this study. This SNP deviated from the Hardy-Weinberg equilibrium. The genotype frequency of the TT homozygotes in SDC3 rs2491132 polymorphism of obese individuals was higher than in control which is different from the results found in the Korean population (Masuo, et al., 2006). The frequency of the CT heterozygotes was two times higher than the obese subjects as observed in the Korean population. The frequency of the C and T in the Malaysian Malays (0.89 and 0.11) is different from that of the Korean population which is 0.75 and 0.25 respectively (Masuo, et al., 2006). Interestingly, the C and T allele frequencies of the obese Malaysian Malays in the present study (0.88 and 0.12) is similar to that of the Korean population and closer to the European population (0.76 and 0.24) (Schuring, et al., 2009).

## 5.3 Gene-gene interaction between the FTO and MC4R

FTO and MC4R are reported to have effect in increased risk of obesity (Frayling, et al., 2007; Loos, et al., 2007). Since their respective influence was found to be modest, a study in European population investigated combined effects of MC4R and FTO on obesity. The study showed the combined effects of MC4R and FTO on obesity

was modest and this may be influenced by other environmental factors (Cauchi, et al., 2009). The potent genetic variants of FTO and MC4R variants in Malaysian Malays were tested for gene-gene interaction. The result showed that there were no combined effects of these variants on obesity parameters in this population.

## 5.4 Analysis of obesity biomarkers

Plasma log-adiponectin and log-leptin were found to be significantly correlated with obesity-traits in the Malaysian Malays. However, log-resistin was not correlated with obesity-traits in this study. Plasma log-adiponectin was negatively correlated with WC, HC, logTG and logBMI in this study. Previous studies have shown that adiponectin exert its effect on lipid metabolism and energy homeostasis (Ahima, 2006; Hardie, 2003). Adiponectin levels have been found to be linked to metabolic syndrome and dyslipidemia (Hara et al., 2002). Plasma adiponectin was strongly correlated with HDL-C in GEM study in the Turkish and Europeans (Ling, et al., 2009).

Plasma adiponectin levels are negatively correlated with BMI (Ling, et al., 2009). Plasma log-adiponectin level was positively correlated with HDL levels and SBP in the Chinese population (Rong LUO, 2007). Plasma adiponectin level was positively correlated with HDL levels and negatively correlated with BMI, WC, WHR, TG and blood glucose parameters in the Malaysians (Lau & Muniandy, 2011). In this current study, similar correlation was replicated in the Malaysian Malays as observed in the previous study (Lau & Muniandy, 2011). The strongest correlation of plasma log-adiponectin levels was with logTG and HDL-C levels. This was also observed in the previous findings in the Malaysian subjects (Lau & Muniandy, 2011). There is no significant difference in adiponectin level between the men and women. However, there was a trend towards lower adiponectin level in men compared to women. Previous studies reported that adiponectin is lower in men than in women probably due to

suppression of the levels by androgens (Combs, et al., 2003). In addition, a study reported that women have proportions of high MW adiponectin then men (Nishizawa, et al., 2002).

The role of resistin in obesity and metabolic disorders is controversial. The correlation between resistin with HDL-C and LDL-C is inconsistent as few studies show negative relation while others show positive relation (Al-Daghri et al., 2005; Janowska, Zahorska-Markiewicz, & Olszanecka-Glinianowicz, 2006; Norata et al., 2007; Piestrzeniewicz, et al., 2008). Plasma resistin level was inversely correlated with serum HDL-C and positively with BMI in the Japanese population (Asano, et al., 2010). Plasma resistin was positively correlated with HDL-C and SBP in the Chinese population (Rong LUO, 2007). Plasma resistin levels have shown to be positively correlated with BMI, WC, WHR, and LDL-C while negatively correlated to HDL-C but not with blood pressure (Piestrzeniewicz, et al., 2008). Previous studies have shown no relation between resistin and lipid metabolism (Burnett et al., 2005; Lee, et al., 2003). Resistin have shown not to be not correlated to WC, BMI and total cholesterol is hence unlikely to be involved in energy homeostasis in human (Yang et al., 2003). Previous studies reported that there was no correlation between human plasma or serum resistin levels with any adiposity markers, blood pressure, fasting plasma glucose and lipid profile (Chen et al., 2005; Lee, et al., 2003). Plasma log-resistin levels were not correlated with obesity parameters in this study. There is no significant difference in resistin level between the men and women. Therefore, resistin is most unlikely to be involved in the pathogenesis of obesity in the Malaysian Malays.

Plasma leptin level is positively correlated with logBMI, WC and HC in the Malaysian Malays. Leptin is involved in body weight regulation, energy expenditure and food intake (Zhang, et al., 1994). Previous studies indicated that circulating leptin

levels is positively correlated with BMI and other obesity-traits (Rosenbaum et al., 1996). Studies have indicated that there was a strong relationship between leptin levels and BMI (Considine, et al., 1996). Leptin concentration was positively correlated with BMI, WC, HC, WHR, cholesterol, triglycerides and HDL-C in the South Indian population (Malathi, 2007). There is a significant difference in leptin level between the genders in the Malaysian Malays. Leptin level is significantly higher in women compared to men. The relationship between gender and leptin levels is inconsistent (Malathi, 2007; Wauters & Van Gaal, 1999). A recent report indicated that serum leptin level in women was three times more when compared with that of men in a South Indian population (Malathi, 2007). Interestingly, data from this study shows that there is a fourfold elevation in plasma leptin level in women compared with men in the Malaysian Malays. In a study conducted in the South Indians, sex steroids such as testosterone and β-estradiol were found to have an influence on leptin production. This interaction could be a part of the hypothalamic-pituitary-gonadal-adipose tissue axis that play a role in the regulation of body weight and reproductive function (Malathi, 2007). In this study, we lack the data on levels of sex hormone to elucidate the reason behind the gender effect on leptin levels. It is possible that gender contributes a significant independent role in the prediction of plasma leptin level in the Malaysian Malays.

## 5.5 Limitations and strength of the study

The limitations and strengths of this study as discussed in the section below.

### 5.5.1 Limitations

Since the participants of this study are middle-aged and elderly individuals, these findings cannot be generally extrapolated to children and adolescents. In addition, this study was conducted in Malaysian Malays. Therefore the findings cannot be

generalized to other ethnic groups in Malaysia or Malays in other parts of Southeast Asia.

Participants involved in this study were those from the University of Malaya Wellness Program, from whom both blood and buccal swabs were collected in order to extract DNA and routine blood chemistries. For the participants from the Bera district of Pahang we did not manage to collect their blood samples for blood assays due to problems of storage.

Although heritability of obesity is high, varying between 30% and 70%, (Bell, Walley, & Froguel, 2005) life style and environmental factor such as diet and physical inactivity also modifies the risk of obesity. Differences in genetic susceptibility of obesity observed in different populations might be caused by population specific environmental and lifestyle factors. However, lack of data on diet and physical activity in this study does not allow us to make any deductions in this regard.

### 5.5.2 Strengths

Obesity traits are influenced by many different genes. Various genes are contributing to a small amount to the overall risk of obesity. Therefore, studying a broad range of genes is crucial and this study attempts to do so by investigating few genes that linked to obesity. In addition, this study also uses the full range of BMI in both quantitative and qualitative analysis, whereas participants with intermediate BMI were not excluded.

False-negative or false-positive factors has taken into consideration by having good-quality genotyping data combined with close attention Hardy-Weinberg (HWE) testing, which demonstrated equilibrium for all the SNPs which were tested for

association with obesity traits. Since the Hardy-Weinberg is assumed for the genotype distribution, the sample size is assumed to be enough, thereby ruling out genotyping error and therefore the results are reliable.

Regarding limitations, it need to be noted that this study belong to a homogenous single ethnic, the Malays in Malaysia. Careful attention has been paid to population stratification as population structure is a big concern in interpreting results on any candidate-gene association studies. Studies have shown that differences in allelic frequency due to population structure can result in variations in the patterns of LD between populations (Hanchard et al., 2007). Differences in LD between populations, particularly in the presence of opposing LD, can also manifest in increased rates of false associations in the absence of functional variant for case control designs with biased sampling across populations. It has been proven that in the presence of population structure as defined as LD differences, combining data across populations can reduce power in association studies (Teo, Small et al., 2009).

Furthermore, it is not easy to get an exact representative sample of the general population. Nevertheless, since this study involved subjects from both rural area (Bera district of Pahang) and urban area (University of Malaya Wellness Program in Kuala Lumpur, Malaysia), it should be a close representation of the general population of Malays in Malaysia.

# CHAPTER 6

# CONCLUSION

# &

# FUTURE STUDIES

## 6.1 Conclusion

In summary, the present study showed significant associations between FTO rs17817288 with LDL-Cholesterol; MC4R rs571312 with logBMI and systolic blood pressure; MC4R rs2229616 SNP with total cholesterol; ADRB2 rs1042714 SNP with diastolic blood pressure; RETN rs3219175 and rs34861192 SNPs with weight and log-resistin levels. There was no significant gene-gene interactions between the FTO and MC4R SNPs. Strong linkage disequilibrium (LD) pattern was observed in the SNPs of the resistin, FTO, ADRB2 and leptin genes in Malaysian Malays. Strength of LD was low in MC4R and ADIPOQ gene regions in this population. The haplotypes of leptin gene, GCCGGAA was associated with obesity in the Malaysian Malays. There was a significant difference between the obese and non-obese groups for the allelic frequencies of ADIPOQ rs17366568 SNP. Association between BMI and SNPs are inconsistent in different populations. What could be the potential reasons for these contradicting results? The difference in genetic background might explain the discrepancies. It may also be because BMI represents a different adiposity phenotype in Asians than in Europeans because of variation in body composition. It is also possible that differences in genetic susceptibility of obesity observed in different population might be due to modulation by population-specific environmental factors and lifestyle. However, absence of data on physical activity and diet does not allow confirmatory conclusion in this regard.

In addition, this study includes haplotype analysis which examines multimarker of genes rather than single locus analysis. Novel haplotypic associations of obesity genes in the Malaysian Malays that were detected in this study will offer additional knowledge of causative variants specific for Malaysians Malays. This study suggests that the genomic region of FTO, RETN, ADRB2 and LEP might have multiple variants

that influence predisposition to obesity in the Malaysians Malays. Therefore, this indicates the importance of studying a broader range of SNPs compared to single locus analysis.

Biomarkers may be important indicators in obesity. This study showed that levels of adiponectin and leptin are probably more important obesity indicators compared to levels of resistin in Malaysian Malays. There is a gender effect on leptin concentration in the Malaysian Malays. No significant difference was found between the obese and non-obese participants for the biomarkers levels.

From the clinical point of view, the observed association between the genetic variants and obesity-traits can lead to identification of those requiring preventive intervention in a population. It is common knowledge that BMI, specifically body weight, is a trait that is modifiable by exercise and dietary interventions. Therefore, early detection of at-risk populations using genetic information may be useful in prevention and management of obesity, the incidence of which is rising, and of obesity-related disorders in Malaysia.

This is the first study that is carried out in the Malaysian Malays, which provides data on the association between genetic variants such as FTO, MC4R, INSIG2, RETN, ADIPOQ and ADRB2 SNPs with obesity related traits. The data are important for understanding the genetic basis of obesity in the Malaysian Malay population. This information may contribute to epidemiological and functional studies on obesity and obesity-related risk factor such as hypertension, hypercholesteremia and hyperlipidemia.

## 6.2 Future studies and recommendations

Future studies on genetic factors affecting obesity in the Malaysian population should include studies on copy number variants. Since CNV have a role in causing high penetrant obesity, understanding the CNVs will add knowledge in to variants that are associated with obesity in this population. Many disease-causing genetic variants are found within the exomes. Exomes are gene coding regions. It is also useful to focus on key genomic regions to detect the rare and common variants in the exome. The variants that can be focused are SNPs, insertions, CNVs and deletions that are linked to obesity. By looking closer at the regions of interest, we may discover causal variants for a variety of complex diseases including obesity in the Malaysian population. Finding the novel causal variants with the use of latest high-throughput Next Generation Sequencing (NGS) technologies will aid in knowing the detailed molecular and physiological pathways linked to obesity.

Nutrients can alter the mechanisms involved in epigenetic process. However, the knowledge concerning nutritional epigenetics is largely unknown. Future studies to investigate this are crucial in order to find measures to curb obesity through nutritional interventions. Genes interact with nutrients and nutrients alter genetic expression. Therefore, the analysis of this whole system is important to understand complex biological systems which may lead to useful outcomes. Future studies will be needed to assess whether diet intake modulates the association between gene polymorphisms and obesity parameters. This in future will benefit those individuals affected by chronic metabolic disorders such as obesity from individually adjusted dietary recommendation. Obesity is becoming increasingly a major problem in Malaysia, and this approach may aid in the prevention of obesity through genome-tailored diet counseling and thus open new era of nutrigenomics in Malaysia.

Due to its cardioprotective effect, adiponectin is growingly becoming a promising biomarker for therapeutic intervention instead of leptin or resistin. The effects of SNPs on adiponectin levels are strongly modulated by ethnic variation. Therefore, the human genetic studies on different population on this area will provide valuable data. Due to the anti-inflammatory, anti-diabetic and anti-atherogenic nature of the adiponectin, study on more polymorphisms of ADIPOQ gene may aid in the identification of specific role of this biomarker linked to the pathophysiology of various metabolic diseases in Malaysia.

Endocrine disruptors are environmental chemical compounds that are formed by human action. These agents may exert effect either by mimicking or blocking hormonal actions. These chemicals are known to alter lipid metabolism and adipogenesis that may lead to development or exacerbation of obesity. These agents may interact with other obsogenic environment for predisposition of obesity. Therefore, the effect of endocrine disruptors should be taken into consideration with regard to obesity in the Malaysians.

The current study identified LD pattern of certain genes of obesity in the Malaysian Malays. Hence future studies will be needed to verify the LD patterns in Malays in other parts of Southeast Asia. Large-scale genetic association studies should be carried out in future in the Malaysian Malays and in other ethnic groups within the Malaysian population with more data on various genetic mutations, diet, sedentary lifestyle, physical activity, behavioural aspects and epigenetics factors. This is crucial in order to elucidate role of various factors on predisposition to obesity in the Malaysians. Understanding of the function and structure of genes is essential to develop genetic diagnosis, preventive medicine and develop new therapies based on evidence of molecular genetics and biomarker studies in Malaysia.

Now we are in revolution era of genomic medicine in which diagnosis and treatment is based on the information of an individual's entire sequence or genome. Increased knowledge on genomics and utilization of genetic data will lead to prediction of disease and identification of more effective therapies. Advances in sequencing technology which are rapid and accurate have resulted in global genomic knowledge base for development and expansion of genetic research. This will eventually offer an important reference point for future testing and research. For this national DNA database might be needed in the future. Perhaps outcome of gene research in Malaysian population may lead to new medication for obesity if some of these genes turn out to be important drug-able targets. Nevertheless this will take a lot more years to accomplish as it needs detailed physiological and pharmacological research on obesity.