ASSOCIATION OF MELANOCORTIN 4 RECEPTOR (*MC4R*) GENE MUTATION, PHYSICAL ACTIVITY, AND FOOD INTAKE BETWEEN NORMAL AND OVERWEIGHT MALAY CHILDREN

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TAN YEE LIN

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Tan Yee Lin

MSc (Sports Science)

Sports Science Unit

School of Medical Sciences, Universiti Sains Malaysia

Health Campus, 16150 Kelantan, Malaysia

Introduction: Overweight and obesity has become an alarming issue worldwide and the problem is on the rise. Obesity arises when there is energy imbalance where energy intake exceeding energy expenditure. It can be resulted from the interaction between genetic, behavioral and environmental factors. Melanocortin 4 receptor (*MC4R*) mutation is the most common known monogenic cause of human obesity that impairs energy homeostasis by increasing food intake and decreasing energy expenditure. *MC4R* mutation N62S which was found in children of Pakistani origin was found to partially impair the receptor function which might cause obesity. On the other hand, behavioral factors leading to obesity include increased energy intake and reduced physical activity.

Objectives: The aims of the study were to determine the presence and frequency of *MC4R* mutation N62S among Malay children, and to determine the

influence of mutation N62S, physical activity level and total caloric intake on overweight.

Subjects and Methods: One hundred and twenty children of the age of 9-11 years were recruited and analyzed for the presence of mutation N62S through high resolution melt (HRM) analysis; activity counts by using accelerometer GT3X+ (Actigraph, UK); physical activity level based on activity count; and total caloric intake and percentage of fat intake goal achieved per day through the subjects' food diary analysis by DAPlus software (Esha Research, USA).

Results: N62S mutant was not found in all Malay children from both of the normal and overweight groups. No significant difference was found between the two groups in terms of activity count ($P = 0.282$) and total caloric intake ($P = 0.179$). No association was found between BMI status and physical activity level ($P = 0.660$). Normal weight children achieved significantly higher percentage of fat intake goal than overweight children with the mean(standard deviation) of 91.17(40.91) and 63.60(27.98) %, respectively ($P < 0.001$).

Conclusions: The absence of N62S mutant in the Malay children in the present study might be due to its low prevalence. The belief that overweight children take in more energy and fat, and have lesser energy output than non-overweight children was not proven in the present study.

Limitations and Recommendations: These findings suggested that overweight and obesity might be resulted from other environmental factors, or even an underreport of fat intake, which worth future study taking all these factors into consideration. Besides, future study with larger sample size is suggested to determine if the N62S mutant is really absent in Malay children, and inactivity and high energy intake among children would be the important factors for childhood obesity development in Malaysia.

Dr. Mohd Nidzam Bin Jawis: Supervisor

Dr. Surini binti Yusoff: Co-Supervisor

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

HUBUNGKAIT MUTASI GEN '*MELANOCORTIN 4 RECEPTOR*' (*MC4R*), AKTIVITI FIZIKAL DAN PENGAMBILAN MAKANAN ANTARA KANAK-KANAK MELAYU YANG MEMPUNYAI BERAT BADAN NORMAL DAN BERLEBIHAN

ABSTRAK

Masalah berat badan berlebihan dan obesiti telah menjadi isu yang membimbangkan di seluruh dunia dan masalah ini terus meningkat. Ketidakseimbangan penggunaan tenaga optima di mana pengambilan tenaga melebihi penggunaan tenaga menyebabkan obesiti. Masalah obesiti boleh disebabkan oleh interaksi antara faktor genetik, tingkahlaku dan persekitaran. Mutasi *MC4R* merupakan mutasi yang paling lazim dikenali sebagai punca '*monogenic obesity*' dalam manusia. Ia menjejaskan homeostasis tenaga dengan meningkatkan pengambilan makanan dan mengurangkan penggunaan tenaga. Adalah didapati bahawa mutasi '*melanocortin 4 receptor*' (*MC4R*) N62S yang ditemui dalam kalangan kanak-kanak yang berasal dari Pakistan telah menjejaskan sebahagian fungsi reseptor dan hal tersebut mungkin menyebabkan obesiti. Faktor-faktor tingkahlaku pula yang meyebabkan obesiti adalah termasuk peningkatan dalam pengambilan tenaga dan kekurangan aktiviti fizikal. Oleh itu, kajian ini bertujuan untuk mengenalpasti kehadiran dan kekerapan mutasi *MC4R* N62S dalam kalangan kanak-kanak Melayu, dan untuk mengenalpasti pengaruh mutasi N62S, tahap aktiviti fizikal dan jumlah pengambilan kalori

terhadap masalah berat badan berlebihan. Seratus dua puluh kanak-kanak yang berusia antara 9-11 tahun telah diambil dan dianalisis untuk mengenalpasti kehadiran mutasi N62S melalui analisis '*high resolution melt*' (HRM); aktiviti fizikal dengan menggunakan '*accelerometer*' GT3X+ (Actigraph, UK); tahap aktiviti fizikal berdasarkan *'activity count*' mengikuti Yildirim *et al.* (2011); dan jumlah pengambilan kalori dan peratusan matlamat pengambilan lemak yang dicapai setiap hari melalui analisis diari makanan dengan menggunakan perisian DAPlus (Esha Research, USA). Mutan N62S tidak ditemui dalam semua kanak-kanak Melayu dari kedua-dua kumpulan iaitu yang normal dan berlebihan berat badan. Tiada perbezaan yang signifikan didapati di antara kedua-dua kumpulan dari segi jumlah aktiviti (P = 0.282) dan jumlah pengambilan kalori (P = 0.179). Tiada kaitan ('*association*') didapati antara status BMI dan tahap aktiviti fizikal (P = 0.660). Kanak-kanak yang mempunyai berat badan normal mencapai peratusan matlamat pengambilan lemak yang jauh lebih tinggi daripada kanak-kanak berlebihan berat badan, iaitu masing-masing dengan min(sisihan piawai) bernilai 91.17 (40.91) dan 63.60 (27.98) %. Perbezaan tersebut adalah signifkan, $P < 0.001$. Salah satu faktor yang mungkin akan menyebabkan mutan N62S tidak dapat ditemui dalam kalangan anak-anak Melayu dalam kajian ini ialah kekerapan atau prevalen mutasi *MC4R* yang rendah. Kepercayaan bahawa kanak-kanak belebihan berat badan mengambil kalori/tenaga dan lemak yang banyak, serta menggunakan tenaga yang kurang berbanding dengan kanak-kanak dengan berat badan normal tidak dapat dibuktikan dalam kajian ini. Penemuan ini mencadangkan bahawa masalah berlebihan berat badan dan obesiti mungkin dipengaruhi oleh faktor

persekitaran yang lain, ataupun kegagalan subjek untuk mencatatkan pengambilan lemak yang tepat. Kajian pada masa hadapan boleh mengambil kira faktor-faktor tersebut. Selain itu, kajian pada masa hadapan boleh mengambil saiz sample yang lebih besar untuk mengenalpasti samada mutan N62S wujud atau tidak dalam kalangan kanak-kanak Melayu, dan samada ketidakaftifan dan pengambilan tenaga yang tinggi dalam kalangan kanak-kanak Melayu merupakan faktor-faktor meyebabkan obesiti kanak-kanak.

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percentage of fat intake goal achieved per day through the subjects' food diary analysis by DAPlus software (Esha Research, USA). N62S mutant was not found in all Malay children from both of the normal and overweight groups. No significant difference was found between the two groups in terms of activity count (P = 0.282) and total caloric intake (P = 0.179). No association was found between BMI status and physical activity level (P = 0.660). Normal weight children achieved significantly higher percentage of fat intake goal than overweight children with the mean(standard deviation) of 91.17(40.91) and 63.60(27.98) %, respectively (P < 0.001). The absence of N62S mutant in the Malay children in the present study might be due to its low prevalence. The belief that overweight children take in more energy and fat, and have lesser energy output than non-overweight children was not proven in the present study. These findings suggested that overweight and obesity might be resulted from other environmental factors, or even an underreport of fat intake, which worth future study taking all these factors into consideration. Besides, future study with larger sample size is suggested to determine if the N62S mutant is really absent in Malay children, and inactivity and high energy intake among children would be the important factors for childhood obesity development in Malaysia.

CHAPTER 1

INTRODUCTION

1.1 OVERWEIGHT AND OBESITY

1.1.1 GLOBAL PREVALENCE OF OVERWEIGHT AND OBESITY

Overweight and obesity has become an alarming issue worldwide and the problem is on the rise. The prevalence of adults being overweight and obese can go up to >70 and >35 %, respectively (Low *et al.*, 2009) while some countries have childhood overweight levels of >25 % (de Onis *et al.*, 2010).

The prevalence of adult obesity, and childhood overweight (including obesity) are found to be increasing in some reviews (Low *et al.*, 2009; de Onis *et al.*, 2010). Globally, there was a relative increase of 60 % of prevalence of childhood overweight and obesity, namely from 4.2 (1990) to 6.7 % (2010). This trend is expected to continue and there will be relative increase of prevalence of 36 % from 2010 by reaching a prevalence of 9.1 % in 2020 (de Onis *et al.*, 2010).

As for adults more than 20, 35 % were overweight (body mass index; BMI ≥ 25 kg/m^2) with 34 % men and 35 % of women in 2008. In ten years time from 1980 to 2008, the worldwide prevalence of obesity has more than doubled. The

prevalence of obesity of 5 % for men and 8 % for women (BMI ≥ 30 kg/m^2) in 1980 has increased to 10 and 14 %, respectively. It was estimated that 205 million men and 297 million women over the age of 20 were obese which is a total of more than half a billion adults worldwide (WHO, n.d.).

1.1.2 DEFINITION OF OVERWEIGHT AND OBESITY

Body mass index (BMI) is a simple index of body weight-for-height. It is calculated by dividing the body weight in kilograms by the square of the body height in meters (kg/m^2) and it is commonly used to classify underweight, overweight and obesity.

Overweight is defined as a condition when a person is weighing more than a standard level for height and age with a BMI of ≥ 25 kg/m^2; while obesity is a condition when someone is having excessive fat with a BMI of ≥ 30 kg/m^2 (Field *et al.*, 2002). On the other hand, prevalence of childhood overweight (including obesity) is usually determined by using the International Obesity Task Force (IOTF) cut-off points which are based on BMI centile curves that passed through the adult cut-off points of BMI 25 and 30 kg/m^2 for being overweight and obese, respectively (Cole *et al.*, 2000; Low *et al.*, 2009). Table 1.1 shows the international cut off points for BMI for overweight and obesity by sex between 2 to 18 years of age which are defined to pass through BMI of 25 and 30 kg/m^2 at age 18 where below the cut-off points is considered normal while above the cut of points is considered overweight. The data were obtained by averaging data from 6 countries, namely, Brazil, Great Britain, Hong Kong, Netherlands,

Singapore, and the United States (Cole *et al.*, 2000). Nevertheless, both overweight and obesity may impair health (WHO, 2011).

Table 1.1 International cut-off points for BMI for overweight and obesity by sex between 2-18 years, defined to pass through body mass index of 25 and 30 kg/m^2 at age 18, obtained by averaging data from Brazil, Great Britain, Hong Kong, Netherlands, Singapore, the United States (Cole *et al.*, 2000)

	Body mass index 25 kg/m^2		Body mass index 30 kg/m^2	
Age (years)	Males	Females	Males	Females
2	18.4	18.0	20.1	20.1
2.5	18.1	17.8	19.8	19.5
3	17.9	17.6	19.6	19.4
3.5	17.7	17.4	19.4	19.2
4	17.6	17.3	19.3	19.1
4.5	17.5	17.2	19.3	19.1
5	17.4	17.1	19.3	19.2
5.5	17.5	17.2	19.5	19.3
6	17.6	17.3	19.8	19.7
6.5	17.7	17.5	20.2	20.1
7	17.9	17.8	20.6	20.5
7.5	18.2	18.0	21.1	21.0
8	18.4	18.3	21.6	21.6
8.5	18.8	18.7	22.2	22.2
9	19.1	19.1	22.8	22.8
9.5	19.5	19.5	23.4	23.5
10	19.8	19.9	24.0	24.1
10.5	20.2	20.3	24.6	24.8
11	20.6	20.7	25.1	25.4
11.5	20.9	21.2	25.6	26.1
12	21.2	21.7	26.0	26.7
12.5	21.6	22.1	26.4	27.2
13	21.9	22.6	26.8	27.8
13.5	22.3	23.0	27.2	28.2
14	22.6	23.3	27.6	28.6
14.5	23.0	23.7	28.0	28.9
15	23.3	23.9	28.3	29.1
15.5	23.6	24.2	28.6	29.3
16	23.9	24.4	28.9	29.4
16.5	24.2	24.5	29.1	29.6
17	24.5	24.7	29.4	29.7
17.5	24.7	24.8	29.7	29.8
18	25	25	30	30

1.1.3 COMMON HEALTH CONSEQUENCES OF OVERWEIGHT AND OBESITY

Raised body mass index (BMI) is a major risk factor for quite a number of diseases, including cardiovascular disease (mainly heart disease and stroke), which was the leading cause of death in 2008; diabetes; musculoskeletal disorders (especially osteoarthritis); some cancers (e.g. endometrial, breast and colon); and/or even mortality. The risk of getting the diseases increases with the increase in BMI (Field *et al.*, 2002; Russell and Hamill, 2010; WHO, 2011).

Overweight and obesity are not only affecting adults but is also greatly affecting children. It is basically a vicious cycle where childhood obesity increases the chances of the children to be obese in adulthood besides disability and premature death. In addition to the future risks, obese children experience breathing difficulties, increased fracture risk, hypertension, early markers of cardiovascular disease, insulin resistance and also psychological effect (WHO, 2011). A study reported that the risk of adult obesity was at least twice as high for obese children as for non-obese children and the risk of adult obesity was greater for children who were at higher levels of obesity and for children who were obese at older ages (Serdula *et al.*, 1993).

1.1.4 LIFESTYLE OF OVERWEIGHT AND OBESE CHILDREN

Overweight and obese children are less active than their healthy counterparts (Janssen *et al.*, 2005; Lioret *et al.*, 2007). This may be a result of

vicious cycle of overweight and obese in childhood. Healthy children who practiced a sedentary lifestyle and in addition, taking in a lot of unhealthy food such as food high in fat and sugar for a prolonged period will lead them into overweight or mild obese. Heavier body weights lead the children to be lazy to move and this will result in even more serious obesity. Conditions such as musculoskeletal disorders, asthma, diabetes, psychological problems (depression and low esteem) and others will be developed in severe obesity cases and activities of the severely obese children will be restricted to a greater extent (Figure 1.1) (Jung, n.d.).

Overweight and obese children are assumed to be taking in extra calories and fat. Excessive sugar intake by soft drink, increased portion size, and steady decline in physical activity are some supporting evidences that play major roles in the rising rates of obesity all around the world. As a consequence, lifestyle of over-consumption of calories and reduced physical activity leads to childhood obesity (Dehghan *et al.*, 2005).

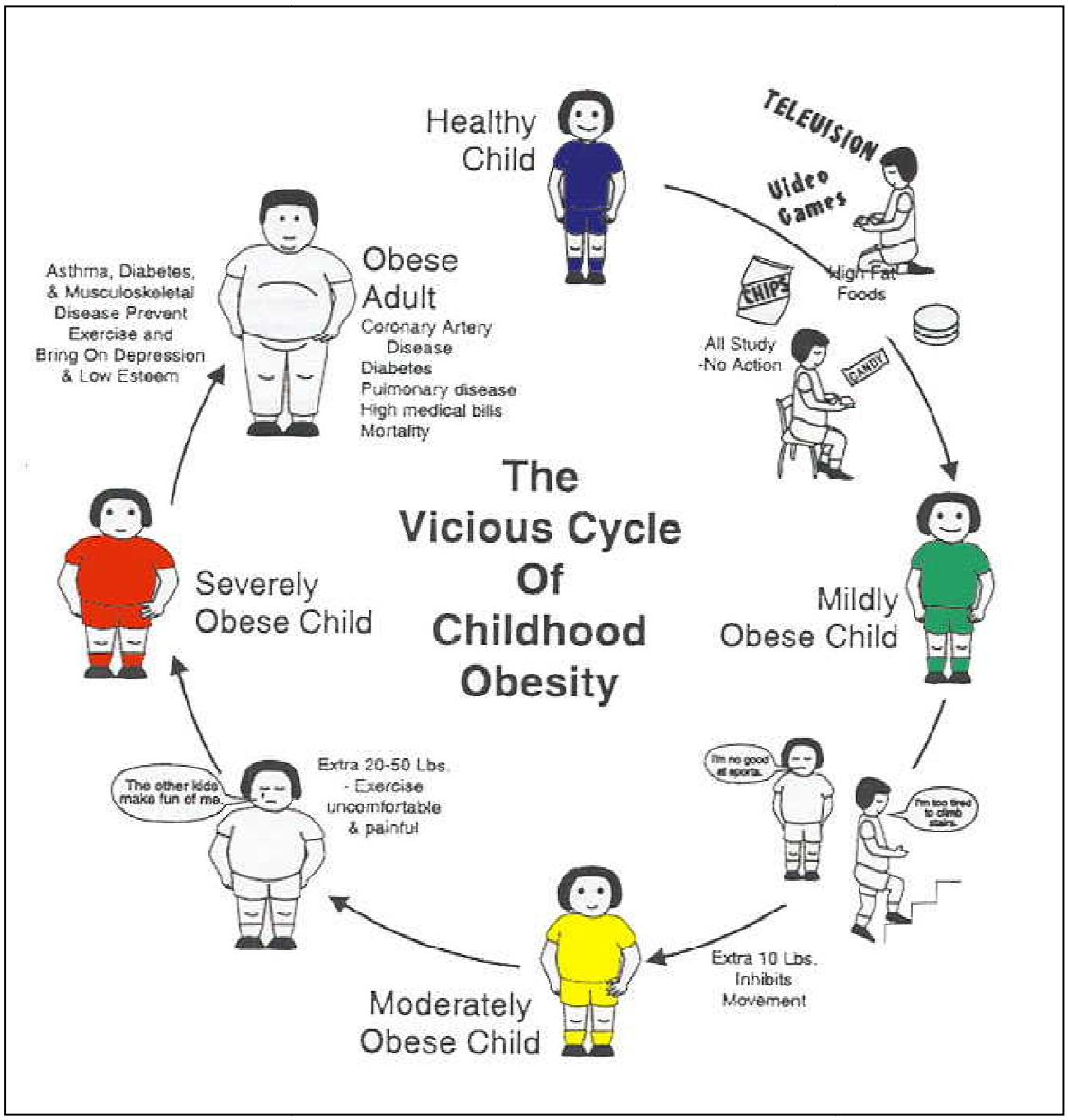


Figure 1.1 The vicious cycle of childhood obesity (Jung, n.d.)

1.2 GENETICS AND OBESITY

Obesity is a complex disease which can be resulted from the interaction between lifestyle and genetic factors. Unhealthy or unbalanced dietary patterns and sedentary habits are lifestyle factors leading to obesity. Currently, genetic

factors are estimated to account for 40 to 70 % of the variance in human adiposity (Razquin *et al.*, 2011).

1.2.1 MELANOCORTIN 4 RECEPTOR (*MC4R*) GENE

Cytogenetic location of melanocortin 4 receptor (*MC4R*) gene is 18q22 i.e. *MC4R* gene is located at the long (q) arm of chromosome 18 at position 22 (Figure 1.2). Its molecular location is from base pairs (bp) 58,038,563 to 58,040,000 (Genetic Home Reference, 2012). Thus, it has a total of 1438 bp with 332 amino acids (NCBI, 2011). The nucleotide sequence of *MC4R* gene is shown in Figure 1.3.

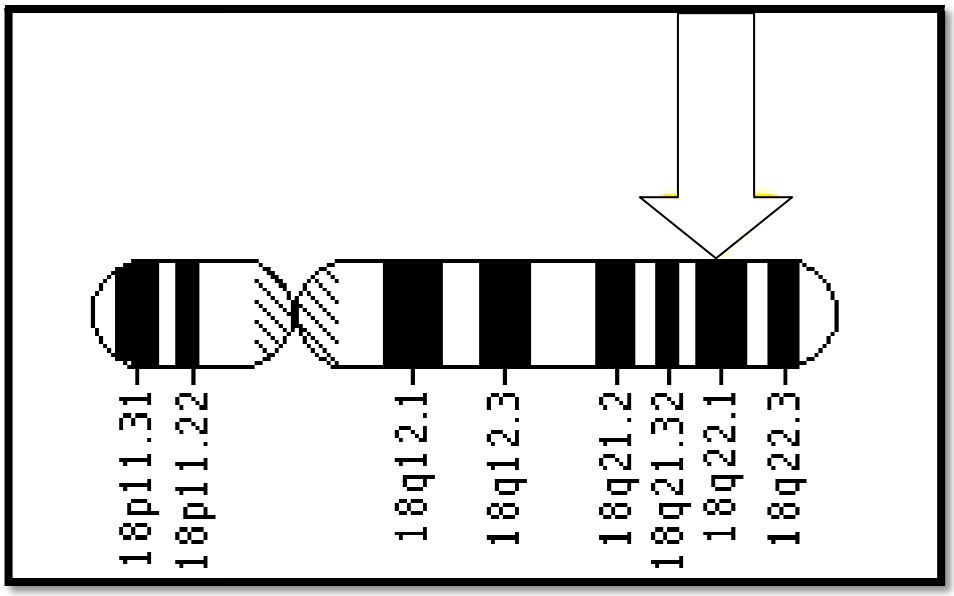
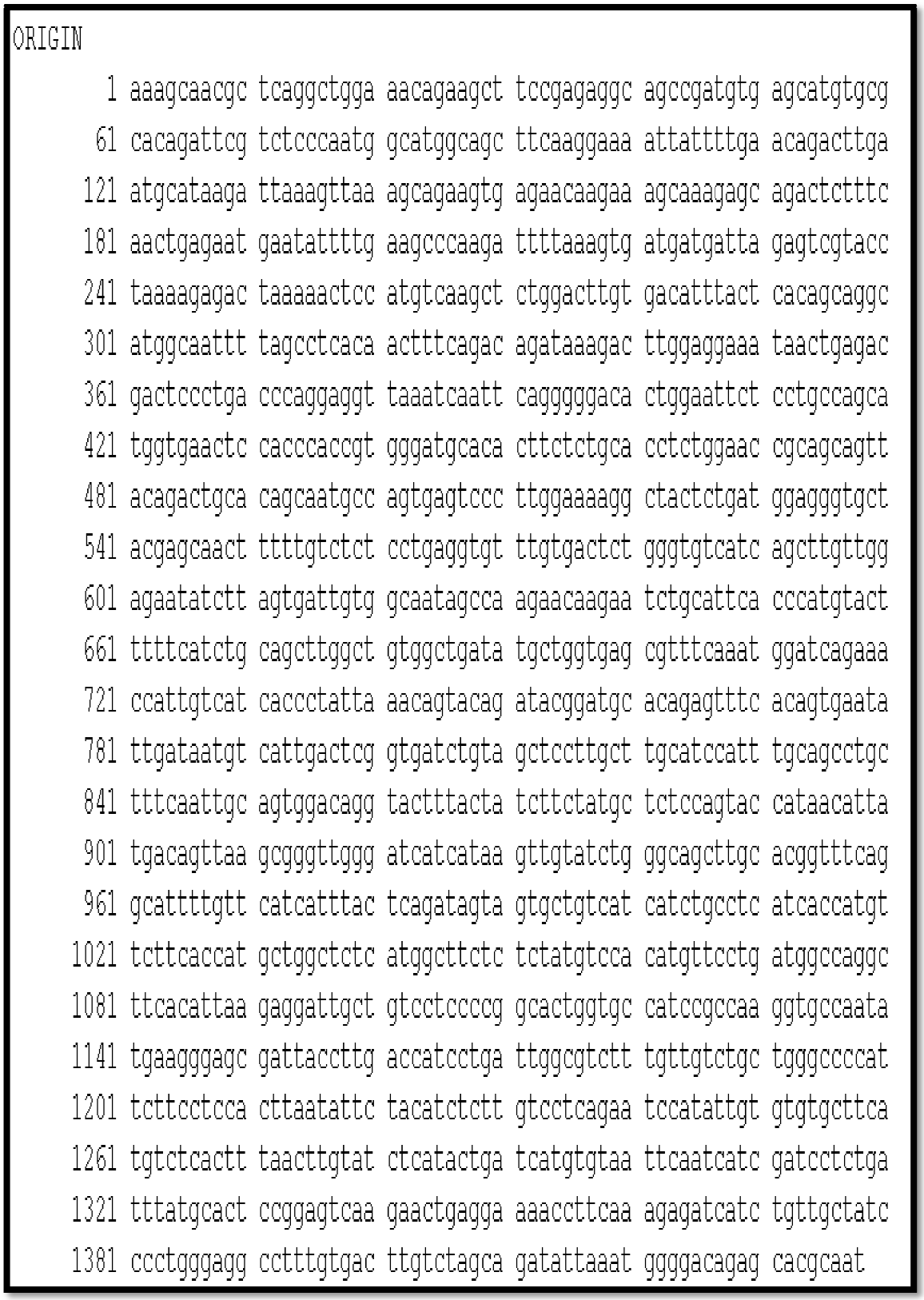


Figure 1.2 Location of melanocortin 4 receptor (*MC4R*) gene (Genetic Home Reference, 2012)



```
ORIGIN
       1 aaagcaacgc tcaggctgga aacagaagct tccgagaggc agccgatgtg agcatgtgcg
      61 cacagattcg tctcccaatg gcatggcagc ttcaaggaaa attattttga acagacttga
     121 atgcataaga ttaaagttaa agcagaagtg agaacaagaa agcaaagagc agactctttc
     181 aactgagaat gaatattttg aagcccaaga ttttaaagtg atgatgatta gagtcgtacc
     241 taaaagagac taaaaactcc atgtcaagct ctggacttgt gacatttact cacagcaggc
     301 atggcaattt tagcctcaca actttcagac agataaagac ttggaggaaa taactgagac
     361 gactccctga cccaggaggt taaatcaatt caggggggaca ctggaattct cctgccagca
     421 tggtgaactc cacccaccgt gggatgcaca cttctctgca cctctggaac cgcagcagtt
     481 acagactgca cagcaatgcc agtgagtccc ttggaaaagg ctactctgat ggagggtgct
     541 acgagcaact ttttgtctct cctgaggtgt ttgtgactct gggtgtcatc agcttgttgg
     601 agaatatctt agtgattgtg gcaatagcca agaacaagaa tctgcattca cccatgtact
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     721 ccattgtcat caccctatta aacagtacag atacggatgc acagagtttc acagtgaata
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    1321 tttatgcact ccggagtcaa gaactgagga aaaccttcaa agagatcatc tgttgctatc
    1381 ccctgggagg cctttgtgac ttgtctagca gatattaaat ggggacagag cacgcaat
```

Figure 1.3 Sequence of melanocortin 4 receptor (*MC4R*) gene (NCBI, 2011)

MC4R gene is an intronless gene where it is encoded by a single exon gene (Lubrano-Berthelier *et al.*, 2003). It is expressed primarily in the brain, including hypothalamus (OMIM, 2011). Protein encoded by this gene namely, *MC4R* belongs to the family of seven transmembrane G-protein coupled receptors (GPCR) and transduces signal by coupling to the heterotrimeric Gs protein and activating adenylate cyclase (Lubrano-Berthelier *et al.*, 2003; Tao, 2010). It is one of the members of the melanocortin receptor family which can be activated by members of the melanocortin family which are α, β and γ-melanocyte stimulating hormone (MSH), and adrenocorticotropic hormone (ACTH) (Tao, 2010).

Melanocortins involve in a wide range of physiological functions which include pigmentation, energy homeostasis, inflammation, immunomodulation (i.e. change in the body's immune system which can be caused by agents that activate or suppress its function), steroidogenesis (i.e. production of steroids by adrenal glands) and temperature control (Tao, 2010).

MC4R can influence food intake and energy expenditure (Yildirim *et al.*, 2011). This can be explained when the binding of α-MSH to *MC4R* activates anorexigenic signals. Through a series of further steps, the anorexigenic signals are believed to reduce food intake by creating the perception of satiety (Tao, 2010).

1.2.2 MELANOCORTIN 4 RECEPTOR (*MC4R*) GENE MUTATION

Obesity is caused by defects on the melanocortin 4 receptor (*MC4R*) gene in an autosomal dominant type (Genetic Home Reference, 2012). The receptor function is disrupted due to the obesity-linked *MC4R* mutations which in turn, prevents the activation of anorexigenic signals in response to α-MSH binding. Therefore, individuals with obesity-linked *MC4R* gene mutations may not experience the feeling of satiety which may cause them to take in too much calories (Lubrano-Berthelier *et al.*, 2003; Nijenhuis *et al.*, 2003; Yeo *et al.*, 2003). It is the most common monogenic form of obesity (Nijenhuis *et al.*, 2003).

Nevertheless, there was a study showing that deletions in the chromosomal region that contains the *MC4R* were not associated with obesity. Thus, although mutations in *MC4R* predispose ones to the development of obesity, not for every individual mutation's role on pathogenesis of obesity is clear, and susceptibility to *MC4R* gene mutations may vary between individuals (Nijenhuis *et al.*, 2003).

Although there are many amino acid changes in human *MC4R*, not all changes are associated with obesity. For instance, amino acid changes of V103I, I251L, and T112M are found in both obese and control subjects and they have no effect on *MC4R* signaling (Farooqi *et al.*, 2003; Yeo *et al.*, 2003).

On the other hand, N62S mutant appears to retain partial signaling capabilities (Yeo *et al.*, 2003; Tao and Segaloff, 2005). It was found in

homozygous form in 5 children with severe obesity from a highly consanguineous extended family of Pakistani origin which is also an Asian country as Malaysia (Farooqi *et al.*, 2000).

1.3 METHOD OF ANALYZING GENE MUTATION: HIGH RESOLUTION MELT (HRM) ANALYSIS

High resolution melt (HRM) analysis is a type of fluorescent deoxyribonucleic acid (DNA) melting analysis. It is a homogeneous, highly powerful method for fast, high-throughput post-PCR analysis of genetic mutations or variance in nucleic acid sequences.

1.4 ACTIVITY MONITOR: ACCELEROMETER GT3X+

Accelerometer GT3X+ (Figure 1.4) is an activity monitor. It is used to record the physical activity done by the participants wearing it. The recorded data will then be analyzed using ActiLife software (ActiGraph, n.d.) . It allows the researcher to collect data in raw format. It provides the following physical activity measurements, namely:

i. Activity counts and vector magnitude
ii. Energy expenditure
iii. Steps taken
iv. Activity intensity levels
v. MET’s etc.

Figure 1.4 Activity monitor: Accelerometer GT3X+ (ActiGraph, n.d.)

1.5 OBJECTIVES OF THE STUDY

1. To identify the presence and frequency of melanocortin 4 receptor (*MC4R*) gene mutation (N62S) among Malay children
2. To determine the association between *MC4R* gene mutation (N62S) and body mass index (BMI) status (normal and overweight groups) among Malay children
3. To determine the difference of physical activity level between normal and overweight Malay children
4. To determine the difference of dietary intake between normal and overweight Malay children

1.6 HYPOTHESIS OF THE STUDY

1. H_O1: Melanocortin 4 receptor (*MC4R*) gene variant (N62S mutant) is not present among Malay children

   H_A1: *MC4R* gene variant (N62S mutant) is present among Malay children

2. H_O2: There is no association between *MC4R* gene variant (N62S mutant) with body mass index (BMI) status (normal and overweight groups) of Malay children

   H_A2: There is an association between *MC4R* gene variant (N62S mutant) with BMI status (normal and overweight groups) of Malay children

3. H_O3: There is no significant difference between physical activity count between normal and overweight Malay children

   H_A3: There is a significant difference between physical activity count between normal and overweight Malay children

4. H_O4: There is no significant difference between dietary intake between normal and overweight Malay children

   H_A4: There is a significant difference between dietary intake between normal and overweight Malay children

1.7 SIGNIFICANCE OF THE STUDY

1. Presence and frequency of melanocortin 4 receptor (*MC4R*) gene variant N62S among overweight Malay children could be identified
2. Specific education on body weight control (through physical activity and food intake) could be suggested to the overweight Malay children if they have the gene variation
3. Upon identification, further research on the mechanism of *MC4R* gene variant N62S in affecting food intake and energy homeostasis could be done

CHAPTER 2

LITERATURE REVIEW

2.1 OVERWEIGHT

2.1.1 CLASSIFICATION OF OVERWEIGHT AND OBESITY IN CHILDREN AND ADOLESCENTS

The prevalence of obesity and overweight reported in different studies differ considerably as different criteria or cut-off points were used for their definition (Lobstein *et al.*, 2004).

Body mass index (BMI) is currently accepted as a valid indirect measure of adipose tissue in children. World Health Organization (WHO) recommends the use of BMI cut-off points of 25 and 30 kg/m^2 to define adult overweight and obesity, respectively. Since the children's BMI will normally change with age and vary by gender, age- and gender-specific BMI cut-offs are necessary so that overweight and obesity in children and adolescents can be classified. American National Centre for Health Statistics (NCHS) reference, the UK reference, and the French reference are some popular BMI-for-age reference charts that have been developed. Nonetheless, WHO/NCHS and the International Obesity Task Force (IOTF) are the two most widely used international references (Lobstein *et al.*, 2004).

2.1.1 (a) WORLD HEALTH ORGANIZATION/NATIONAL CENTRE FOR HEALTH STATISTICS (WHO/NCHS) REFERENCE

Based on the US National Health and Nutrition Examination Survey (NHANES) I data collected by the US NCHS, Centres for Disease Control and Prevention (CDC) in 1971 to 1974, the age-sex-specific body mass index (BMI) 85th and 95th percentiles were developed, and have been widely used to classify overweight and obesity, respectively, in child and adolescent populations (Lobstein *et al.*, 2004). Moreover, BMI cut-offs for international use to define 'at risk of overweight' for adolescents 10 to 19 years old was recommended by a WHO expert committee (Lobstein *et al.*, 2004). For children under 10 years of age, WHO expert committee suggested to use the body weight-for-height Z-score where a value of WHZ > 2 is used to classify 'overweight' based on the US NCHS reference (Lobstein *et al.*, 2004).

2.1.1 (b) INTERNATIONAL OBESITY TASK FORCE (IOTF) REFERENCE

With the support from International Obesity Task Force (IOTF), Cole and colleagues have developed a series of age- and sex-specific body mass index (BMI) cut-off points based on data collected from Brazil, UK, Hong Kong, Singapore, the Netherlands, and the USA, in order to classify overweight and obesity in young people of 2 to 18 years of age (Cole *et al.*, 2000; Lobstein *et al.*, 2004). These BMI cut-offs were derived from sex- specific BMI age curves that pass through a BMI of 25 and 30 kg/m^2 which are the cut-off points used in

adults to define overweight and obesity, respectively) at 18 years of age, respectively (Cole *et al.*, 2000).

As compared to World Health Organization (WHO) reference cut-off points that were based on a US reference population, the cut-off points proposed by Cole and colleagues are less arbitrary and more internationally based than current alternatives which should help to provide internationally comparable prevalence rates of overweight and obesity in children (Cole *et al.*, 2000; Wang and Lobstein, 2006).

2.1.2 PREVALENCE OF OVERWEIGHT AND OBESITY IN MALAYSIA

Second National Health and Morbidity Survey (NHMS II) that was carried out in 1996 showed that the prevalence of overweight and obesity in Malaysian adult population were 16.6 and 4.4 %, respectively. As an overall, there were 21.0 % of Malaysian adults being overweight/obese in 1996 (Malaysian Diabetes Association, August 16, 2010).

Next, third National Health and Morbidity Survey (NHMS III) was conducted in 2006. The prevalence of overweight (body mass index; BMI 25.0 to 29.9 kg/m^2) and obesity (BMI > 30 kg/m^2) in Malaysian adult population were 29.1 and 14.0 %, respectively. The overall prevalence of Malaysian adults being overweight/obese in 2006 was 41 % (Malaysian Diabetes Association, August 16, 2010).

In the 10 years from 1996 to 2006, the prevalence of overweight in Malaysia increased for at least 1.8 times. Besides, the rate of obesity in 2006 was 3.2 times higher than in 1996. The combined problem of overweight and obesity in 2006 was 2 times higher as compared to 10 years ago (Malaysian Diabetes Association, August 16, 2010).

Prevalence of overweight children in Malaysia (below 18 years old) was established only in NHMS III. 5.4 % of them were overweight. The prevalence of overweight boys was higher than that of girls, i.e. 6.0 vs. 4.7 %, respectively. There were more overweight children in urban as compared to rural areas, i.e. 6.3 vs. 4.0 %, respectively (Malaysian Diabetes Association, August 16, 2010).

2.2 FACTORS INFLUENCING CHILDHOOD OVERWEIGHT AND OBESITY

Obesity arises when the situation of energy intake exceeding energy expenditure remains undisputed (Corander *et al.*, 2009). It can be resulted from the interaction between genetic, behavioral and environmental factors (Razquin *et al.*, 2011; CDC, 2012).

Genetic factors are estimated to account for 40 to 70 % of the variance in human adiposity and twin studies suggested that approximately 50 % of the tendency towards obesity is inherited (Kiess *et al.*, 2004; Razquin *et al.*, 2011).

Behavioral factors include food intake pattern and activity lifestyle. The development of a high degree of body fatness can be largely contributed by the

combination of rapid changes in the availability, composition, and consumption of energy-dense food which lead to over fat intake, and a downturn in physical activity levels in all aspects of daily life, e.g. the excessive use of modern media particularly television viewing and lack of physical activity i.e. sedentary lifestyle (Kiess *et al.*, 2004).

Nonetheless, environments like home, child care, school, and community can influence children's behaviors related to food intake and physical activity (CDC, 2012).

2.2.1 GENETIC FACTORS OF OVERWEIGHT CHILDREN

The complexes of multiple genetic and non-genetic influences lead to most cases of "typical obesity". Nevertheless, researchers recently have identified some very rare cases of "simple" obesity due to mutations in single genes which is called monogenic forms of obesity (Beamer, 2003).

2.2.1 (a) MELANOCORTIN 4 RECEPTOR (*MC4R*)

Mutations in the gene encoding for leptin (*LEP*) (Montague *et al.*, 1997), leptin receptor (*LEPR*) (Clement *et al.*, 1998), prohormone convertase 1 (*PC1*) (Jackson *et al.*, 1997), pro-opiomelanocortin (*POMC*) (Krude *et al.*, 1998), and melanocortin 4 receptor (*MC4R*) (Böttcher *et al.*, 2012) are some monogenic causes of obesity in humans. Nevertheless, mutation of *MC4R* is one of the most common single-gene disorders resulting in obesity in humans as

suggested by a study of the general population in the United Kingdom with a finding of *MC4R* mutational frequency of 1 in 1000 (Alharbi *et al.*, 2007).

The *MC4R* is a seven, transmembrane G-protein-coupled receptor with the α-melanocyte-stimulating hormone (α-MSH) i.e. a posttranslational derivative of POMC as its ligand (Cody *et al.*, 1999). The human *MC4R* is an intronless gene i.e. it is consisting of only a single exon (Tao, 2010). It is located at the long (q) arm of chromosome 18 at position 22 which is 18q22 in short (NCBI, 2011). It has a total of 1438 base pairs (bp) with an open reading frame of 999 bp that encodes a protein of 332 amino acids (Tao, 2010; NCBI, 2011).

The *MC4R* are expressed primarily in the central nervous system. The regulatory energy homeostasis pathway which includes the regulation of food intake and energy expenditure, of which *MC4R* is a part, has become an area of intense interest because of its potential role in obesity (Cody *et al.*, 1999; Tao, 2010).

The central melanocortin system involves arcuate neurons i.e. POMC and agouti-related protein (AgRP) neurons as well as their downstream second-order neurons expressing *MC4R* as shown in Figure 2.1 (Corander *et al.*, 2009). POMC neurons project from the arcuate nucleus to other regions of the brain, including other hypothalamic regions such as the paraventricular nucleus (PVN); thalamus and medial amygdala; brainstem and spinal cord (Corander *et al.*, 2009). AgRP is a potent melanocortin antagonist at the *MC4R* (Corander *et al.*, 2009). The *MC4R* expressed in the PVN and amygdala influence the regulation

of food intake, whereas *MC4R* expressed in other neurons influence the control of energy expenditure (Balthasar *et al.*, 2005; Corander *et al.*, 2009). Change in food intake only accounts for 60 % of the effect of the *MC4R* on energy balance while the other 40 % is accounted for by changes in energy expenditure (Balthasar *et al.*, 2005).

The central melanocortin system is said to be related to leptin which is an adipose-derived cytokine that is produced in proportion to fat cell mass as it can modulate central melanocortin tone to cause changes in food intake and energy expenditure (Cone, 2006; Corander *et al.*, 2009). The production of leptin increases with overfeeding and decreases with starvation (Corander *et al.*, 2009). It acts through receptors expressed on POMC and AgRP neurons within the hypothalamus (Corander *et al.*, 2009).

Besides leptin, gut peptides, especially cholecystokinin (CCK), stimulated by meal intake that mediates satiety through centres in the brainstem can also influence the melanocortin activity which, in turn, is capable of affecting energy homeostasis (Cone, 2006; Tao, 2010). Through the neural connections to the hypothalamus, the satiety signals are thought to interact primarily with long-term weight regulation centres to regulate total daily energy intake by adjusting meal size, number, or both (Tao, 2010).

During fed state, there is increased POMC neuronal activity with inhibition of AgRP neurons (Corander *et al.*, 2009). Melanocortins binds to *MC4R* to activate anorexigenic signals by increasing melanocortin tone in regions of the

brain expressing *MC4R* which will, in turn, decrease food intake and increase energy expenditure (Corander *et al.*, 2009). On the other hand, there will be inactivation of POMC neurons (causing the reduction of melanocortin levels) but stimulation of AgRP activity during the states of negative energy balance e.g. fasting. Stimulation of feeding and reduction of energy expenditure occurred due to the decreased *MC4R* signaling (Corander *et al.*, 2009).

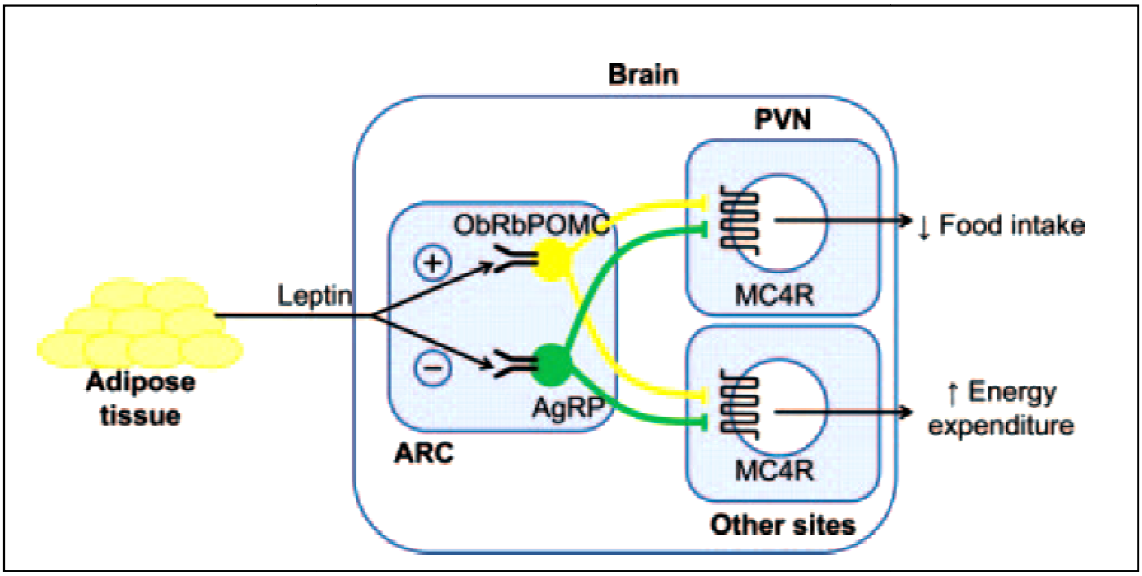

Figure 2.1 The leptin-melanocortin system and energy homeostasis (Corander *et al.*, 2009)

The arcuate nucleus within the hypothalamus has a critical role in integrating circulating nutrition-dependent signals from the periphery and thereby communicating energy balance status to the central nervous system. The adipose-derived cytokine leptin is produced in proportion to fat cell mass, increasing with overfeeding and decreasing with starvation. Acting via receptors expressed on POMC and AgRP neurons within the hypothalamus, leptin can modulate central melanocortin tone to effect changes in food intake and energy expenditure. ObRb indicates leptin receptor; ARC, arcuate nucleus; and PVN, paraventricular nucleus (Corander *et al.*, 2009)

2.2.1 (b) EFFECT OF MELANOCORTIN 4 RECEPTOR (*MC4R*) GENE MUTATIONS

An intact central melanocortin signaling pathway is crucial for normal energy homoeostasis (Corander *et al.*, 2009). Any defects in synthesis, processing, and action of pro-opiomelanocortin (POMC) peptides may result in obesity (Coll *et al.*, 2004). This can be seen when deletion or inactivation of melanocortin 4 receptor (*MC4R*) gene leads to the development of obesity associated with hyperphagia, hyperinsulinemia, and hyperglycemia in mice and human (Huszar *et al.*, 1997; Farooqi *et al.*, 2003). For instance, when there is an increase in the fat content of the diet, wild-type mice responded by rapidly increasing diet-induced thermogenesis and by increasing physical activity (Butler *et al.*, 2001). However, these could not be seen in *MC4R* knockout mice (Butler *et al.*, 2001). The inability of *MC4R* knockout mice in eliciting these responses leads to a decrease in their insulin sensitivity (Sutton *et al.*, 2006).

2.2.1 (c) PREVALENCE OF MELANOCORTIN 4 RECEPTOR (*MC4R*) GENE MUTATIONS

The prevalence of melanocortin 4 receptor (*MC4R*) gene mutations is different across different populations. A study taking place in France found a high frequency (4 %) of rare heterozygous *MC4R* gene mutations in a large population of morbidly obese patients (Vaisse *et al.*, 2000). On the other hand, in another study in France, the identified global prevalence of carriers of functionally relevant *MC4R* gene mutations of severely obese adults was 2.6 %

(Lubrano-Berthelier *et al.*, 2006). In Germany, 3.3 % of *MC4R* mutations and 5.5 % of *MC4R* polymorphisms were found in the analysed obese children and adolescents (Zakel *et al.*, 2005). A study in Denmark indicated a carrier frequency of 2.5 % of pathogenic mutations in the *MC4R* gene in obese men (Larsen *et al.*, 2005). In North American, the total prevalence of rare *MC4R* mutations in severely obese adults was 2.25 % compared with 0.64 % in lean controls (Calton *et al.*, 2009). There was approximately 2 % of children with obesity have a mutation in the *MC4R* gene in Dutch (van den Berg *et al.*, 2012). 1.6 % in Norwegian paediatric and 0.8 % adult patients were having *MC4R* gene mutations (Wangensteen *et al.*, 2009). There was a study indicating that *MC4R* mutations in the obese population from southern Italy was as low as 0.5 % of the patients (Miraglia Del Giudice *et al.*, 2002). A study in Pakistan indicated that homozygous *MC4R* gene mutations were present in 3.2 % of the subjects (Saeed *et al.*, 2012).

2.2.1 (d) TYPES OF MELANOCORTIN 4 RECEPTOR (*MC4R*) GENE POLYMORPHISMS AND MUTATIONS

Mutation in melanocortin 4 receptor (*MC4R*) gene can be missense, nonsense or frameshift (which includes insertion or deletion of nucleotide base) and it can be in the form of heterozygous or homozygous.

However, not all amino acid changes reported in the human *MC4R* are associated with obesity. Numerous studies have been done to assess the function of *MC4R* in relation to the different types of mutation (Vaisse *et al.*,