Analysis of Gene Variation in the Middle Region of Melanocortin 4 Receptor Gene in Normal-Weight Thai Individuals

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ABSTRACT

Nowadays, obesity is a major global health issue, with the number of obese patients rapidly increasing in many countries, including Thailand, where obesity rates have been continuously rising. This study investigates the mid-region variants of the melanocortin 4 receptor (*MC4R*) gene in Thai volunteers with normal body weight, to be used as a database for the prognosis of obesity. *MC4R* gene plays a crucial role in regulating body weight and energy balance through the leptin-melanocortin signaling pathway. Nucleotide variations (Single Nucleotide Polymorphisms; SNPs) in this gene affect the response to appetite regulation. Firstly, DNA was extracted from hair roots of 48 Thai volunteers, aged 18-60 years old, with a Body Mass Index (BMI) between 18.50 and 22.99. The extracted DNA was then used to amplify the mid-region of the *MC4R* gene (position5,555-6,166) using Polymerase Chain Reaction (PCR). The PCR products were analyzed by agarose gel electrophoresis, which revealed a single, specific PCR product of 612 base pairs. The concentration of the PCR product was sufficient for nucleotide sequencing using the Big-Dye Terminator technique. All PCR products were sent to APICAL SCIENTIFIC SDN. BHD. (Malaysia) for nucleotide sequence analysis with the MC4R-F1 primer. In the final step, the nucleotide sequence of the *MC4R* gene mid-region in the volunteers were aligned and compared with the reference sequence from the National Center for Biotechnology Information (NCBI) to identify SNPs. The middle region of the *MC4R* gene from 48 DNA samples were analyzed and no SNP was observed. Although nucleotide variation was absent in the analyzed region, this result provided a valuable reference for establishing a control group for comparison with individuals with obesity. Moreover, it could help improving the ability to distinguish obesity-related SNPs from nucleotide variations found in normal weight individuals, thereby contributing to more accurate genetic screening. These findings support the development of more effective obesity screening models, paticularly within the Thai population, ultimately improving the quality of life for individuals with obesity and reducing the overall public health burden in Thailand.

OBJECTIVES

- To investigate the mid-region variants of the *MC4R* gene in Thai volunteers with normal body weight.
- To apply this data as a database for the prognosis of obesity in Thai population.

INTRODUCTION

Obesity is a significant global health challenge, with its prevalence steadily increasing in Thailand. The prevalence of obesity in Thailand has shown a significant upward trend over the past decade. Starting at 27.9% in 2010, the obesity rate has steadily risen to 48.35% in 2023, indicating a sharp increase of nearly 20% over just 13 years.

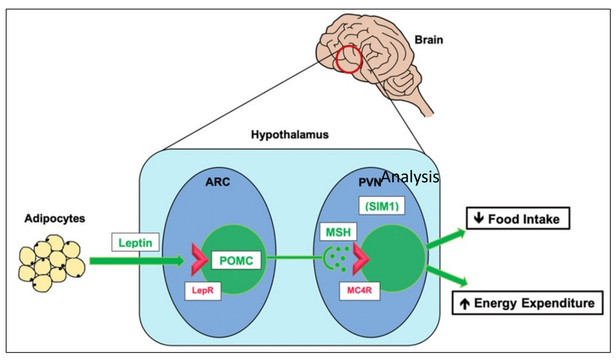

Figure 1. The leptin-melanocortin pathway (modified from Wallis and Raffan, 2020)

While lifestyle and environmental factors play major roles, genetic variations also contribute to an individual's susceptibility to obesity. One of the key genes involved in appetite regulation and energy balance is the Melanocortin 4 Receptor (*MC4R*) , which functions within the leptin-melanocortin pathway to control food intake and energy expenditure (Figure 1).

Single Nucleotide Polymorphisms (SNPs) of *MC4R* gene have been linked to obesity in various populations, but their distribution and impact in the Thai population remain uncertain. This study focuses on analyzing the nucleotide variations within the mid-region of *MC4R* in normal-weight Thai individuals, aiming to establish a control dataset that can serve as a genetic reference for future obesity-related research.

METHODOLOGY

Volunteer Recruitment

Hair root samples were collected from Thai individuals (18-60 years old) with normal body weight (BMI: 18.50-22.99 kg/m²).

Genomic DNA Extraction

DNA was extracted with PrimeWay Genomic DNA Extraction Kit (1st Base, Malaysia).

Agarose Gel Electrophoresis

Quality of genomic DNA was analyzed by 1.2% agarose gel electrophoresis.

Gradient PCR

Gradient PCR was performed to optimize the annealing temperature for PCR amplification of the *MC4R* gene.

Table 1. Annealing temperatures examined by gradient PCR

Annealing Temperature (°C)												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
	57.0	57.6	58.9	61.0	63.4	65.8	68.2	70.6	73.0	75.1	76.4	77.0

Table 2. Nucleotide sequences of primers used for *MC4R* gene amplification

Primer	Name	Nucleotide sequence from 5' -> 3'	Tm (°C)	G+C (%)
Forward primer	MC4R-F1	GTCTCTCCTGAGGTGTTTGTG	65.0	52
Reverse primer	MC4R-R1	GATGGTCAAGGTAATCGCTCC	65.0	52

Data Analysis

Electropherograms were analyzed using Chromas. Next, nucleotide sequences were aligned and compared with the NCBI reference sequence using BioEdit version 7.7.1.

Nucleotide Sequence Analysis

A total of 48 PCR products were submitted to APICAL SCIENTIFIC SDN. BHD. (Malaysia) for nucleotide sequence analysis by Big Dye Terminator using MC4R-F1 primer.

PCR Amplification of *MC4R* Gene

Table. 3 Preparation of PCR reaction to amplify the *MC4R* gene

PCR Components	Stock Concentration	Final Concentration	Volume (µL)
DNA	10 ng/µL	20 ng/reaction	3.0
KOD One™ PCR Master Mix	2X	1X	25.0
Forward primer	10 µM	0.6 µM	0.3
Reverse primer	10 µM	0.6 µM	0.3
Sterile DI	-	-	16
Total volume	-	-	50.0

Table 4. PCR cycles for *MC4R* gene amplification using the optimum annealing temperature

PCR Reaction steps	Temp (°C)	Time	Cycle
Initial denaturation	95.0	10 min	1
Denaturation	94.0	30 sec	30
Annealing	65.8	30 sec	
Extension	68.0	1 min	
Final extension	68.0	10 min	1
Hold	10.0	∞	-

RESULTS & DISCUSSION

Optimization of *MC4R* gene amplification by gradient PCR

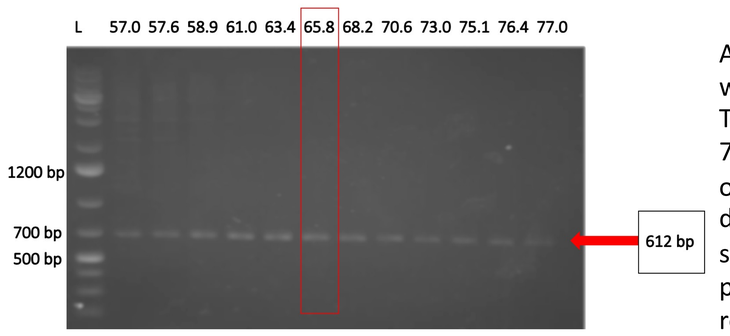

Figure 2. Examination of gradient PCR products by 1.2% agarose gel electrophoresis Lane L : GeneRuler 1 kb Plus DNA ladder Lane 1-12 : Gradient PCR products obtained from the annealing temperatures of 57.0-77.0, respectively

Annealing temperature optimization was performed by gradient PCR. Temperatures ranging from 57.0°C – 77.0°C were analyzed and the optimum annealing temperature was determined to be **65.8°C**, of which a single band of the expected PCR product (612 bp) was obtained with relatively high intensity (Figure 2).

All 48 DNA samples gave single specific PCR product of 612 bp with relatively high intensity. The absence of PCR product in Lane N (negative control) confirmed that no DNA contamination from unrelated sources occurred during the PCR process (Figure 3). The obtained PCR products were suitable for nucleotide sequence analysis by big-dye terminator.

PCR AMPLIFICATION OF *MC4R* GENE

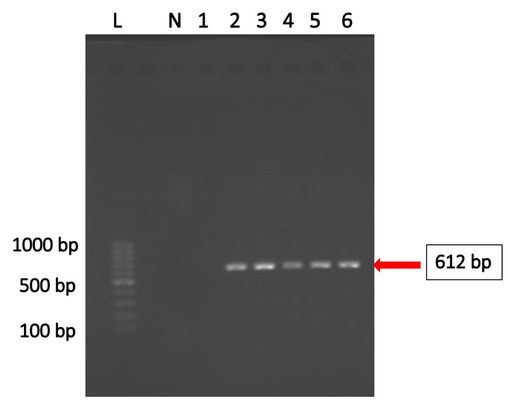

Figure 3. Examination of PCR product by 1.2% agarose gel electrophoresis Lane L : GeneRuler 100 bp DNA ladder Lane N : negative control Lane 1 : sample 007 ; Lane 2 : sample 051 ; Lane 3 : sample 073 ; Lane4:sample077;Lane5:sample:078 Lane6:sample 081 ; Lane 7 : sample 0084

Nucleotide Sequence Analysis

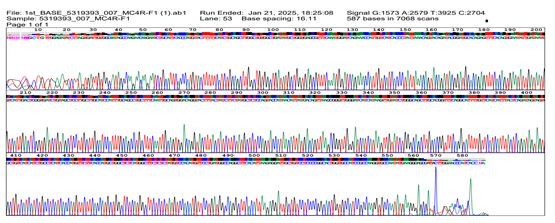

Figure 4. Electropherogram of Sample 007

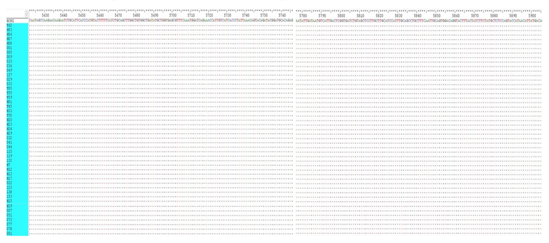

Figure 5. Nucleotide sequence alignment of 48 DNA samples with NCBI reference sequence

Electropherograms, displaying single peak, were subsequently exported as nucleotide sequence and compared with the reference sequence from NCBI.

Nucleotide sequences from all samples were aligned and compared with the reference sequence from NCBI, however, no SNP was observed in the analyzed region. These findings suggested a high degree of conservation in the region under investigation within the studied population. The lack of observed nucleotide variation may also reflect the limited variability within the population sample.

CONCLUSIONS

- The optimum annealing temperature for *MC4R* gene amplification was 65.8°C of which the expected PCR product of 612 bp was obtained with relatively high intensity.
- No SNP was identified in the analyzed region, suggesting high conservation in the studied population.
- The findings established a genetic reference dataset for future obesity-related studies.
- This study contributed to the development of more precise obesity risk screening tools for the Thai population.

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REFERENCES

Farooqi, I. S., Keogh, J. M., Yeo, G. S. H., Lank, E. J., Cheetham, T., & O'Rahilly, S. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *New England Journal of Medicine*, 348(12), 1085-1095.