EFFECTS OF *PCSK1* GENETIC VARIANTS ON OBESITY AMONG THAI CHILDREN AND THEIR FAMILY MEMBERS: IN RELATION TO HEALTH RISK, AND BIOCHEMICAL AND ANTHROPOMETRIC PARAMETERS

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Abstract. Single nucleotide polymorphisms (SNPs) in *PCSK1*, namely, rs6234, rs6235, and rs271939 have been linked to obesity in European population; and rs3811951 has also been connected to type 2 diabetes and obesity parameters in Chinese population. In this family-based case-control study, we analyzed links between *PCSK1* genetic variants and obesity in Thai children and their families. Eleven obese children with a percent weight for height ≥140 who had family history of obesity and 69 family members were recruited. SNPs rs6234, rs6235, rs3811951, and rs271939 of *PCSK1* were analyzed using PCR and gene sequencing methods. DNA of 200 normal weight subjects was used as control. Participants with variant genotypes in the rs6234-6235 pair are at significantly more risk of being obese [OR = 2.44 (1.35-4.43), $p = 0.003$], and also at increased risk of being severely obese (obese class III) [OR = 3.03 (1.20-7.66), $p = 0.015$]. Variant rs3811951 showed no association with being obese, but is significantly linked to an increased risk of being severely obese [OR = 3.59 (1.42-9.08) $p = 0.005$]. Moreover, high density lipoprotein (HDL)-C levels between normal and variant rs3811951 group differed considerably, with patients with variant genotype having a lower HDL-C level ($p = 0.037$). Thus, Thais carrying SNPs rs6234-5 are at increased risk of being obese, and the risk of severe obesity increases when carrying both rs6234-5 and

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rs3811951, but not with rs271939. Furthermore, patients with genetic variations at rs3811951 are at risk of having low HDL-C levels.

Keywords: *PCSK1* gene, genetic variant, obesity, BMI

INTRODUCTION

In the 21st century, obesity is a leading cause of health problems across the world, including in Thailand, with increasing prevalence in both adults and children (Haslam, 2007). Different researchers have found early-onset obesity involves a single-nucleotide variation of genes which control energy balance, such as proprotein convertase subtilisin/kexin type 1 (*PCSK1*) gene, which encodes prohormone convertase 1/3 (PC1/3) (Choquet and Meyre, 2010; Chung, 2012). In energy homeostasis, PC1/3 cleaves pro-opiomelanocortin (POMC) to become α-melanocortin-stimulating hormone (α-MSH) for binding with its receptor, MC4R, in order to inhibit food intake (List and Habener, 2003).

PCSK1 mutation in mice has been linked to obesity, hyperphagia, and increased metabolic efficiency (Lloyd *et al*, 2006; Scamuffa *et al*, 2006). As with mice, mutations in *PCSK1* in humans lead to loss of PC1/3 function, causing early-onset obesity, as well as impaired glucose tolerance (Jackson *et al*, 1997, 2003). Six independent case-control and family-based cohort studies comprising of 13,659 European individuals found that *PCSK1* missense variants containing N221D (rs6232) and Q665E-S690T pair (rs6234-rs6235) might be linked to risk of obesity (OR = 1.34; 95%CI: 1.20-1.49 and OR = 1.22; 95%CI: 1.15-1.29, respectively) (Benzinou *et al*, 2008).

However, other studies failed to find any association between obesity and *PCSK1* variance. A study in 3,885 non-diabetic Swedes found no links between obesity and rs6235 (Renstrom *et al*, 2009). Another study in 20,249 individuals by EPIC-Norfolk UK also failed to find a clear relationship between obesity and rs6235; links were only found between rs6232, obesity and body-mass index (BMI) specifically in younger individuals (aged < 59 years) (Kilpelainen *et al*, 2009).

In a Chinese Han population-based cohort of 3,210, researchers found no significant relationship between rs6234 and being overweight, obesity, BMI, waist circumference, or body fat percentage (Qi *et al*, 2010). However, after being stratified by sex, the rs6234 G-allele demonstrated strong links to an increased risk of the combined phenotype of being overweight and obesity (OR = 1.21; 95%CI: 1.03-1.43) in men, but not in women. Moreover, significant links were discovered between rs6234 G-allele and an increase of the homeostatic model assessment-beta cell function (HOMA-B) (p = 0.006) and a decrease of the homeostatic model assessment-insulin sensitivity (HOMA-S) (p = 0.035) in all participants (Qi *et al*, 2010). In addition, this study among the Chinese population found links between rs3811951 (A>G; intron 2) and total body fat percentage, high density lipoprotein (HDL)-C, fasting glucose, fasting insulin, and the homeostatic model assessment-insulin resistance (HOMA-IR) (Chang *et al*, 2010).

Most recently, rs6232 and rs6235 were genotyped in a population of 2,382 Mexicans (1,206 children and 1,176 adults), where it was found rs6232 is strongly

Table 1
Obesity classification.

BMI for Asian populations (kg/m^2)	Percent weight for height in children	Obesity classification
18.50-22.99	90 - <110%	Normal weight
23.00-24.99	110 - <120%	Overweight
25.00-29.99	120 - <140%	Obese class I
30.00-34.99	140 - <160%	Obese class II
≥35.00	≥160%	Obese class III (Severe obesity)

Adapted from: [1]National Growth References for children under 20 years of age, Ministry of Public Health, Thailand; [2]The presentation and use of height and weight data for comparing the nutritional status of groups of children under the age of 10 years, WHO (Waterlow *et al*, 1977).

linked to childhood obesity and severe obesity in adults (OR = 3.01; 95%CI: 1.64-5.53); on the other hand, rs6235 shows no significant association with obesity in any group (Villalobos-Comparan *et al*, 2012). Furthermore, rs271939 (G>A), located downstream to the gene, has been shown to be linked to obesity in French Caucasian adults (OR = 0.83; 95%CI: 0.73-0.94) (Benzinou *et al*, 2008).

In Thailand, evidence of early-onset obesity due to genetic variation of *PCSK1* has yet to be revealed. Therefore, this research was aimed at examining whether variations of *PCSK1* are linked with obesity in Thai children and their obese family members.

MATERIALS AND METHODS

Study subjects

In this family-based case-control study, subjects were selected from obese children, both male and female, at the Pediatric Outpatient Departments of Siriraj and Ramathibodi Hospitals. *PCSK1* variations were investigated in each patient's family member to cover at least 2 generations. Subject groups were defined as follows as part of the inclusion criteria. For obese volunteers, they were between 8-20 years of age, with percent weight for height ≥140, and had exogenous obesity and an obese family member in at least 2 generations. For proband's obese family members, adults had BMI ≥25 kg/m^2, and children and adolescents had percent weight for height ≥120. For non-obese family members, adults had BMI between 18.50 and 24.99 kg/m^2, and children and adolescents had percent weight for height between 90 and <120.

In adults, we categorized obesity status by using the BMI for Asian populations (WHO/IASO/IOTF, 2000; Kagawa *et al*, 2006; Thaikruea *et al*, 2006). Moreover, we used percent weight for height to classify overweight and obesity in children by National Growth References for children under 20 years of age from the Ministry of Public Health, Thailand and WHO (Waterlow *et al*, 1977) (Table 1).

We investigated 11 families (80 persons), comprising of 9 participants of normal weight, 11 overweight persons, 22 in obese class I, 16 in obese class II and 23 in obese class III. All subjects were aged between 8-70 years old, 28 men and 52 women. We used DNA of 200 normal

weight subjects as a control group (BMI < 25 kg/m^2) for analyzing the minor allele frequency of single nucleotide polymorphisms (SNPs) in Thai people. DNA of control subjects were collected from the gene bank at the Department of Medicine, Division of Molecular Genetics, Faculty of Medicine Siriraj Hospital, Mahidol University. Exclusion criteria for all subjects were having serious underlying diseases that cause obesity, such as Cushing syndrome, hypothyroidism, pseudohypoparathyroidism, or taking medication or undergoing hormonal treatment which might affect weight, and also having any genetic disorders which cause obesity, such as Prader-Willi or Bardet-Biedl syndrome.

The study protocol, informed consent/assent documents, and questionnaire were approved by the Ethics Committees of the Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University; Faculty of Medicine Ramathibodi Hospital, Mahidol University, and the Faculty of Tropical Medicine, Mahidol University.

Anthropometric measurements

Nutritional status of subjects was assessed by means of anthropometric measurements. The body weight of each individual dressed in light clothing was measured (ZEPPER TCS -150L, China). Height of each individual was measured using a vertical-measuring rod (Microtoise, Poissy, France). BMI was calculated as weight (in kg) divided by squared height (in m^2). Bioelectrical impedance analysis (BIA) (HBF-361 Model; Omron, Kyoto, Japan) was used for the measurement of total body fat percentage. Waist circumference was measured, taken as the midpoint between the lower border of the ribs and the upper border of the pelvis.

Laboratory measurements

Fasting blood glucose, insulin, hemoglobin A1C (HbA1C), cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were sent for examination (Cobas Integra® 800, Roche, Mannheim, Germany) at the laboratory of Siriraj Hospital. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from the formula: glucose (mg/dl) x insulin /405).

PCR and genotyping

Genotyping of the four common DNA sequence variants, namely, rs6234 (C>G), rs6235 (G>C), rs3811951 (A>G), and rs271939 (A>G), of human *PCSK1* was carried out by PCR and DNA sequencing. The 3 pairs of PCR primers designed using the Primer-BLAST program were as follows: rs6234 and rs6235 (496 bp) (F) GTTGAAGCCCAAGTCCATGT and (R) CGGTCGTCTCTGTGCTTGTA; rs3811951 (376 bp) (F) ATCCTGGGTGATGAATGAGC and (R) AGGGCAGTCTTAGTGGATGG; rs271939 (482 bp) (F) TGTGAAATCCTTCCCAGAGG and (R) ATGGATTCTGGGGAAAAACC. Thermocycling consisted of 94ºC/5 minutes, 30 cycles for [94ºC/45 seconds, 59ºC/30 seconds, 72ºC/30 seconds], followed by 72ºC/5 minutes for rs6234, rs6235 and rs3811951 and 94ºC/5 minutes, 30 cycles for [94ºC/45 seconds, 56ºC/30 seconds, 72ºC/30 seconds], followed by 72ºC/5 minutes for rs271939 were performed by Biometra Thermal cycler (Göttingen, Germany). Purified PCR amplicons were sequenced using an automatic DNA sequencer (Macrogen, Seoul, South Korea), and if DNA variants were found, they were confirmed by sequencing the anti-sense strands.

Statistical analysis

SPSS version 16.0 computer program

Table 2
Characteristics of the study population.

	Non-obese (BMI <25 kg/m^2)	Obese (BMI ≥25 kg/m^2)	Total	*p*-value
n (%)	20 (25)	60 (75)	80 (100)	
Age (year)	39.5 (32.3-52.0)	42.0 (19.5-52.5)	41.0 (21.8-52.0)	0.947
BMI (kg/m^2)	23.1 (20.4-23.7)	32.2 (28.3-38.4)	29.4 (24.7-35.8)	**<0.001**
Waist circumference (cm)	77.5 (74.2-83.6)	98.2 (92.0-112.9)	95.3 (82.0-107.8)	**<0.001**
Total body fat percentage	27.5 (17.7-31.7)	36.5 (31.2-39.0)	35.1 (27.6-38.0)	**<0.001**
Total cholesterol (mg/dl)	197.5 (173.5-220.0)	196.0 (174.3-219.8)	196.5 (174.3-219.8)	0.934
Triglyceride (mg/dl)	81.0 (52.0-93.0)	120.5 (93.3-153.0)	112.5 (81.5-148.0)	**<0.001**
LDL-C (mg/dl)	116.9 (96.7-146.8)	119.1 (103.4-147.1)	118.9 (101.6-147.1)	0.773
HDL-C (mg/dl)	62.0 (51.3-75.3)	48.5 (42.0-60.0)	53.0 (43.3-61.0)	**0.001**
Fasting glucose (mg/dl)	87.5 (83.3-92.8)	91.5 (86.3-112.8)	90.5 (85.3-102.0)	**0.014**
HbA1C (%)	5.6 (5.3-5.8)	6.0 (5.6-6.8)	5.9 (5.6-6.3)	**0.001**
Fasting insulin (μU/ml)	5.0 (3.5-7.1)	13.3 (9.9-26.1)	11.5 (6.4-21.2)	**<0.001**
HOMA-IR	1.14 (0.71-1.45)	3.56 (2.35-8.40)	2.66 (1.41-5.45)	**<0.001**

Data are *n* (%) or median (interquartile range). BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *p*-value is compared between non-obese and obese groups.

was used to analyze data. Descriptive statistics, such as median, interquartile range, frequency and percentage, were employed to describe the general characteristics and allele frequency. Hardy-Weinberg equilibrium (HWE) test was also performed for all polymorphisms found in each group. Mann-Whitney *U* test was used to determine differences of anthropometric and biochemical parameters between non-obese and obese groups. Logistic regression test was employed to examine risks and links between polymorphisms of *PCSK1* and obesity, adjusting for age and sex. Results are considered statistically significant for $p < 0.05$.

RESULTS

Minor allele frequency of rs6234, rs6235, rs3811951, and rs271939 of *PCSK1* in Thais

Analysis of 200 normal weight Thai subjects showed that the minor allele frequency (MAF) of rs6234 (C>G), rs6235 (G>C), rs3811951 (A>G), and rs271939 (A>G) was G = 27.5%, C = 27.5%, G = 23.0%, and G = 43.2%, respectively. In obese Thai children and their family members, MAF of rs6234, rs6235, rs3811951, and rs271939 was G = 33.1%, C = 33.1%, G = 26.2% and G = 50.6%, respectively.

PCSK1 variants and obesity parameters in the family-based study

HWE was tested in *PCSK1* variants rs6234, 6235, 3811951, and 271939, showing equilibriums of $p = 0.911$, $p = 0.911$, $p = 0.382$ and $p = 0.824$, respectively. The characteristics of studied populations are shown in Table 2. All parameters show no significant difference between homozygous and heterozygous variants, so we grouped them together as one variant group for analysis. Genotype of rs6234 shows a significant difference of BMI, waist circumference and total body fat

Table 3
Analysis of *PCSK1* rs6234-rs6235 pair, rs3811951, and rs271939 in relation to obesity and related metabolic parameters.

	rs6234 (C >G)			rs3811951 (A > G)			rs271939 (A > G)		
	CC(*n*=36)	CG + GG (*n*=44)	*p*-value	AA(*n*=42)	AG +GG (*n*=38)	*p*-value	AA(*n*=20)	AG +GG(*n*=60)	*p*-value
BMI (kg/m^2)	26.6 (23.0-32.5)	31.9 (27.5-39.2)	**0.001**	27.4 (23.1-32.6)	32.2 (26.0-40.6)	**0.004**	30.2 (27.0-38.5)	29.2 (23.9-35.7)	0.158
Waist circumference (cm)	87.0 (77.5-101.0)	97.4 (90.8-112.8)	**0.003**	91.0 (77.5-98.1)	100.3 (89.3-117.3)	**0.004**	96.5 (88.0-110.2)	93.5 (81.0-107.8)	0.279
Total body fat percentage	30.4 (25.5-35.8)	36.5 (31.7-38.9)	**0.022**	32.8 (26.2-37.7)	36.0 (31.0-38.5)	0.054	36.0 (27.0-38.9)	35.0 (28.9-37.9)	0.328
Cholesterol (mg/dl)	197.0 (188.0-219.8)	194.0 (169.3-223.0)	0.739	201.5 (186.8-221.0)	191.5 (163.5-218.3)	0.332	191.0 (166.3-218.3)	197.0 (175.3-219.8)	0.158
Triglyceride (mg/dl)	93.0 (70.5-144.3)	123.0 (91.8-162.8)	0.186	101.5 (73.5-138.8)	130.0 (85.5-156.3)	0.215	126.0 (95.5-162.8)	104.0 (69.5-147.6)	0.225
LDL-C (mg/dl)	119.4 (106.3-147.4)	118.5 (100.3-144.0)	0.829	118.8 (104.7-147.5)	119.7 (98.6-134.5)	0.610	115.6 (94.2-125.3)	122.9 (103.7-147.6)	0.099
HDL-C'(mg/dl)	53.0 (43.5-64.0)	53.0 (43.3-61.0)	0.245	58.5 (46.0-64.25)	49.0 (42.8-58.5)	**0.037**	49.0 (43.8-60.8)	54.0 (43.3-63.0)	0.688
Fasting glucose (mg/dl)	88.5 (84.0-96.5)	91.5 (86.5-112.3)	0.904	90.0 (84.0-103.8)	91.0 (86.0-100.5)	0.984	91.5 (84.3-104.5)	90.0 (85.3-98.8)	0.761
HbA1C (%)	5.75 (5.40-6.18)	6.00 (5.63-6.80)	0.618	5.85 (5.40-6.33)	5.90 (5.60-6.30)	0.915	5.80 (5.60-6.20)	5.90 (5.53-6.38)	0.961
Fasting insulin (μU/ml)	10.02 (4.96-15.66)	13.12 (7.81-24.59)	0.459	10.29 (5.19-15.19)	14.08 (7.42-25.45)	0.460	11.83 (7.48-22.12)	11.18 (5.46-21.11)	0.704
HOMA-IR	2.31 (1.06-4.47)	3.28 (2.30-6.70)	0.758	2.36 (1.09-4.68)	3.24 (2.16-7.40)	0.671	2.64 (1.92-4.66)	2.69 (1.30-6.31)	0.575

Data presented are medians (interquartile range). The results of rs6235 were similar to rs6234, but the minor allele is C (G>C)*p*-value was adjusted for age and sex. Insulin and HOMA-IR were adjusted for BMI, age and sex using logistic regression. BMI, body mass index. HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 4
Association between *PCSK1* variants and obesity in all case and control groups (cut-off at BMI ≥25 kg/m^2 for adults and %WFH ≥120 for children) (N = 280).

SNP	Genotype	Non-obese (%) (n = 220)	Obese (%) (n = 60)	OR(95%CI)	p-value
rs6234 (C > G)	CC	125 (57)	21 (35)	2.44 (1.35-4.43)	**0.003**
	CG+GG	95 (43)	39 (65)		
rs6235 (G > C)	GG	125 (57)	21 (35)	2.44 (1.35-4.43)	**0.003**
	CG+CC	95 (43)	39 (65)		
rs3811951 (A > G)	AA	134 (61)	29 (48)	1.67 (0.94-2.96)	0.080
	AG+GG	86 (39)	31 (52)		
rs271939 (A > G)	AA	68 (31)	19 (32)	0.97 (0.52-1.76)	0.911
	AG+GG	152 (69)	41 (68)		

OR, odds ratio, representing the effects of risk allele (gene variance). OR and p-value are adjusted for age and sex.

Table 5
Association between *PCSK1* variants and obese class III in all cases and control group (cut-off at BMI ≥35 kg/m^2 for adult or %WFH ≥160 for children) (N = 243).

SNP	Genotype	Non-obese (%) (n = 220)	Obese class III (%) (n = 23)	OR(95%CI)	p-value
rs6234 (C > G)	CC	125 (57)	7 (30)	3.03 (1.20-7.66)	**0.015**
	CG+GG	95 (43)	16 (70)		
rs6235 (G > C)	GG	125 (57)	7 (30)	3.03 (1.20-7.66)	**0.015**
	CG+CC	95 (43)	16 (70)		
rs3811951 (A > G)	AA	134 (61)	7 (45)	3.59 (1.42-9.08)	**0.005**
	AG+GG	86 (39)	16 (70)		
rs271939 (A > G)	AA	68 (31)	7 (30)	1.04 (0.41-2.64)	0.938
	AG+GG	152 (69)	16 (70)		

OR, odds ratio, representing the effects of risk allele (gene variance). OR and p-value are adjusted for age and sex.

percentage between CC (wild type) and CG+GG (variant) after being adjusted for age and sex (p = 0.001, p = 0.003, and p = 0.022, respectively) (Table 3). Results of rs6235 were similar to those of rs6234, but here the wild type was GG and the variant was GC+CC. Furthermore, rs3811951 shows a significant difference of BMI and waist circumference between AA (wild type) and AG+GG (variant) after being adjusted for age and sex (p = 0.004 and p = 0.004, respectively) (Table 3). Patients with gene variants had a BMI and waist circumference greater than those of the wild type genotypes. However, rs271939 demonstrates no significant difference in anthropometric parameters between wild type and variant group after being adjusted for age and sex (Table 3).

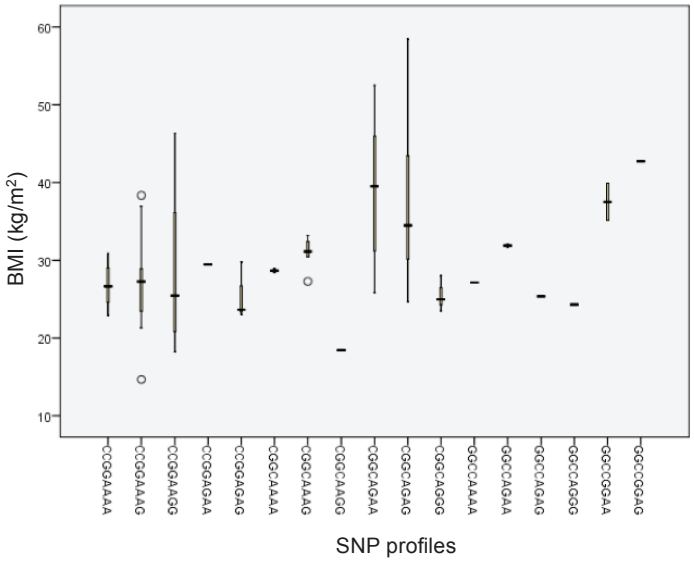
Table 6
SNP profile and BMI of 80 family members.

	SNP profile	*n*	Mean			Median	
			Mean BMI (kg/m^2)	95% confidence interval	SE	BMI (kg/m^2)	Percentile 25^{th}-75^{th} (Q1-Q3)
1	CCGGAAAA	4	26.8	21.6-32.0	1.63	26.6	23.6-29.9
2	CCGGAAAG	13	27.4	23.4-31.4	1.84	27.3	23.3-32.5
3	CCGGAAGG	16	29.3	24.1-34.6	2.49	25.4	20.8-37.0
4	CCGGAGAA	1	29.5	-	-	29.5	-
5	CCGGAGAG	3	25.5	16.2-34.8	2.16	23.6	23.0-29.8
6	CGGCAAAA	2	28.6	25.3-32.0	0.27	28.6	21.3-21.7
7	CGGCAAAG	6	30.9	28.8-33.1	0.84	31.1	29.6-32.6
8	CGGCAAGG	1	18.4	-	-	18.4	-
9	CGGCAGAA	7	38.9	29.5-48.2	3.83	39.5	27.0-47.6
10	CGGCAGAG	16	36.7	31.8-41.7	2.30	34.5	29.7-43.8
11	CGGCAGGG	3	25.5	19.8-31.2	25.49	25.0	23.5-28.0
12	GGCCAAAA	1	27.1	-	-	27.1	-
13	GGCCAGAA	2	31.9	29.5-34.2	0.16	31.9	23.7-24.0
14	GGCCAGAG	1	25.3	-	-	25.3	-
15	GGCCAGGG	1	24.3	-	-	24.3	-
16	GGCCGGAA	2	37.5	7.1-67.9	2.39	37.5	35.1-39.9
17	GGCCGGAG	1	42.7	-	-	42.7	-

There is a statistically significant difference of HDL-C between wild type and variant group after being adjusted for age and sex (p=0.037) in rs3811951 only (Table 3). No SNPs investigated were found to be significantly linked to cholesterol, LDL-C, triglyceride, fasting glucose, HbA1C, fasting insulin, or HOMA-IR (Table 3).

***PCSK1* variants and risk of obesity in family members and controls**

Analysis of data of 80 family members, together with 200 controls revealed that in terms of links between SNPs in *PCSK1* and risk of obesity, patients who had variant genotype, CG+GG of rs6234 and GC+CC genotype of rs6235 had a 2.44-fold greater risk of becoming obese than those who had wild type genotype, CC (rs6234) and GG (rs6235) (95% CI: 1.35-4.43, p=0.003) (Table 4). However, we found no significant association between risk of obesity in either rs3811951 or rs271939 genotype. Furthermore, when comparing patients with obese class III (n=23) with normal weight subjects (n=220), patients with variant genotype of rs6234 and rs6235 had a 3.03-fold greater risk of becoming obese class III than those who had wild type genotype (95%CI: 1.20-7.66, p=0.015) (Table 5). Patients with variant genotype (AG+GG) of rs3811951 were at 3.59-fold greater risk of becoming obese class III than those who had wild type genotype (AA) (95%CI: 1.42-9.08, p=0.005) (Table 5). However, rs271939 genotype



"-", median of BMI of each SNP profile and "o", outlier data.

Fig 1–Box plot of SNP profiles and BMI of 80 family members.

presented no significant association with risk of severe obesity.

SNP profile analysis of rs6234, rs6235, rs3811951, and rs271939 of *PCSK1*

We arranged the genotypes of 4 SNPs together and called them SNP profiles, of which there are rs6234-rs6235-rs3811951-rs271939. The SNP profiles of rs6234, rs6235, rs3811951 and rs271939 in relation to BMI are shown in Fig 1. Four types of SNP profiles were associated with a mean BMI ≥ 35 kg/m^2 (obese class III), 2 profiles with a mean BMI of 30.0-34.9 kg/m^2 (obese class II), and 9 profiles with a mean BMI of 25.0-29.9 kg/m^2 (obese class I) (Table 6). The SNP profile, GGCCGGAG, associated with the highest BMI (42.7 kg/m^2) was found in only one patient.

DISCUSSION

In this study, we found allele frequencies of 4 SNPs in *PCSK1* varied with ethnicity. MAFs of rs6234 (G = 27.5%) and rs6235 (C = 27.5%) in Thais were similar to those found in the European population (G = 26.7% and C = 26.7%, respectively) (HapMap-CEU). However, the Japanese population (HapMap-JPT) demonstrated a lower MAF (4.4%) for rs6234 and rs6235 , whereas the Chinese population (HapMap-HCB) demonstrated a higher MAF (37.8%) in both SNPs. Similarly, MAF of rs3811951 in Thais (G = 23.0%) was similar to that of the European population (G = 26.7%), whereas the Japanese population showed a lower MAF (13.6%) and the Chinese population a higher MAF (33.3%). On the other hand, rs271939 of Thais (G = 43.2%) had a MAF similar to the Japanese population (G = 48.9%), but both the Chinese and European populations showed lower MAF at 36.7% and 33.1%, respectively (data from HapMap). In addition, the result of rs271939 genotyping in normal weight controls was opposite to the study in French Caucasians, which showed the A allele having a MAF (G > A) of 34.8% (Benzinou *et al*, 2008). This could be explained by ethnic difference. Even among European population, the finding from French Caucasian study (Benziou *et al,* 2008) differed from Hapmap data.

We also confirmed the linkage between rs6234 and rs6235 SNPs, which have the same MAF. Our results underlined the existing links between *PCSK1* rs6234-rs6235 pair and risk of obesity, which had previously been reported among European (Benzinou *et al*, 2008) and Chinese populations (Qi *et al*, 2010). However, our results differed from other studies in Europe, namely the two independent genome-wide association studies (GWAS) in Caucasian Europeans

(Scamuffa *et al*, 2006; Renstrom *et al*, 2009) and also the study of European descendants in the EPIC-Norfolk UK project (Kilpelainen *et al*, 2009), which reported no association between the rs6234-6235 pair and risk of obesity. A possible explanation is that our study observed dependent samples, and categorized obese status at BMI ≥25 kg/m^2, which was lower than the cut-off used in European population (BMI ≥30 kg/m^2). Furthermore, we found an interaction between *PCSK1* variants and abdominal obesity, where waist circumference is linked significantly to the rs6234-6235 pair, similar to the study of rs6234 in the Chinese Han population, but that particular research showed an association only in men (Qi *et al*, 2010). In addition to this, our results differed from the study in the Chinese population in rs3811951, which found no association between BMI and waist circumference with rs3811951 variant (Chang *et al*, 2010), whereas our study did. The different findings between ours and that of the Chinese study could be partly explained by the difference in characteristic of studied population. Ethnic difference might influence the result. The Chinese study recruited subjects with hypertension along with their siblings. Their mean BMI was about 25.3 kg/m^2 (Michael *et al*, 2004), while our subjects were mostly obese and had median BMI of 29.4 kg/m^2.

We investigated the interaction between *PCSK1* gene variants and severity of obesity in the Thai population, and found patients who have variant genotypes at rs6234-6235 and rs3811951 are more likely to become severely obese (BMI ≥35). This result is in contrast with the study in childhood and adult class III obesity in the Mexican population, which shows rs6235 has no significant association with class III obesity (Villalobos-Comparan *et al*, 2012). Furthermore, rs271939 demonstrates no real difference in terms of parameters between wild type and variant groups after being adjusted for BMI, age and sex, and shows no significant link to obesity or severe obesity. Our results differed in this respect from the study in French Caucasians, which found a protective effect of this SNP and obesity (Benzinou *et al*, 2008). However, a lack of association between genetic variations at rs271939 and obesity in our study might be from the limited sample size. Furthermore, based on the SNP profile of obese Thai family members, all participants with a BMI ≥35 kg/m^2 showed variations in 3 SNPs (rs6234, rs6235, and rs3811951) and had rs271939 AA or AG genotypes, suggesting that interactions among these 4 SNP variants could actually enhance development of severe obesity.

In relation to lipid profiles, we found that only *PCSK1* rs3811951 shows significant relationship with HDL-C. Our results replicated those of the Chinese population study, which found links between HDL-C and rs3811951 (Chang *et al*, 2010). Nonetheless, other studies have shown a relationship between *PCSK1* polymorphism and an increased risk of insulin-resistance and DM type II (Chang *et al*, 2010; Heni *et al*, 2010). In our study, all SNPs demonstrated no significant association with fasting glucose, fasting insulin and HOMA-IR when adjusted for BMI, age and sex. This result is contrary to the study in the Chinese population, which found an interaction between rs3811951 and fasting glucose, fasting insulin, and HOMA-IR (Chang *et al*, 2010). This could be explained by the difference in ethnicity and characteristic of studied population.

In summary, this family-based Thai study revealed that SNPs of rs6234-rs6235 pair and of rs3811951 were linked to an

increase in BMI and waist circumference, and to risk of having severe obesity. There is a significant link of rs3811951 genotype to decreas levels of HDL-C and there was an additive effect of the 4 SNPs as most participants with severe obesity almost always had a genetic variation in all 4 positions. The major limitation of this research study was the small sample size as we only selected subjects from obese families because we wanted to observe the effects of gene variance on obesity. Further research should look into the interactions between each of the 4 SNPs in a larger sample size, as well as their effects in the normal population.

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