Original article

APOE gene is a genetic determinant for alteration of lipid profiles in obesity

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ABSTRACT

Apolipoprotein E (ApoE) is a component of lipoprotein that plays role in lipid metabolism by acting as ligand binding lipoprotein with hepatocyte cell-surface receptors to clearance of chylomicrons and VLDL. Genetic study revealed an association of ApoE gene *(APOE)* with dyslipidemia and CVD. To investigate association between *APOE* polymorphism (rs7412 and rs429358) and serum lipid levels in obesity, 93 control persons and 314 obese persons, aged between 45-65 years were recruited from the Electricity Generating Authority of Thailand (EGAT) Cohort study in 2013. Genotypes of *APOE* polymorphism were analyzed by TaqMan real-time PCR. In control group, genotype frequencies of Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ3/Ɛ3, Ɛ2/Ɛ4, Ɛ3/Ɛ4 and Ɛ4/Ɛ4 were 2.2%, 17.2%, 62.4%, 3.2%, 15.1% and 0%, respectively. Allele frequencies of Ɛ2, Ɛ3 and Ɛ4 were 0.12, 0.79 and 0.09, respectively. For obese group, genotype frequencies of Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ3/Ɛ3, Ɛ2/Ɛ4, Ɛ3/Ɛ4 and Ɛ4/Ɛ4 were 1.3%, 18.8%, 55.4%, 5.4%, 17.8% and 1.3% respectively. Allele frequencies of Ɛ2, Ɛ3 and Ɛ4 were 0.13, 0.74 and 0.13, respectively. The *APOE* polymorphism showed influences on serum lipid levels in obese group. Individuals with Ɛ4/Ɛ4 genotype in obese group had higher TC than Ɛ2/Ɛ4 and higher LDL-C than Ɛ2/Ɛ3, Ɛ3/Ɛ3 and Ɛ2/Ɛ4 genotypes. Additionally, carriers with Ɛ3/Ɛ4 showed significantly higher LDL-C than Ɛ2/Ɛ4 genotype. In conclusion, association between *APOE* polymorphism and serum lipid profiles in obese is existed and this gene may be a useful genetic determinant for obesity and dyslipidemiaas well as cardiovascular disease in Thais.

Key words: *APOE,* Polymorphism, Obesity, Dyslipidemia, TaqMan real-time PCR

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นิพนธ์ต้นฉบับ

อะโปไลโปโปรตีนอีจีนเป็นตัวกำหนดทางพันธุกรรม สำหรับการเปลี่ยนแปลงของระดับไขมันในเลือดในกลุ่มโรคอ้วน

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บทคัดย่อ

อะโปไลโปโปรตีนอี (ApoE) เป็นองค์ประกอบของไลโปโปรตีนในกระบวนการเมแทบอลิซึมของไขมันโดยทำหน้าที่เป็นตัวกลางในการจับกันของไลโปโปรตีนกับตัวรับที่ผิวของเซลล์ตับเพื่อกำจัด chylomicrons และ VLDL การศึกษาทางพันธุกรรมแสดงว่า ApoE จีน (APOE) มีความสัมพันธ์กับภาวะไขมันในเลือดสูงและโรคหลอดเลือดหัวใจเพื่อศึกษาความสัมพันธ์ของความหลากหลายทางพันธุกรรมของ APOE (rs7412 and rs429358) กับระดับไขมันในเลือดในคนไทย คนปกติ 93 คนและคนโรคอ้วน 314 คนอายุระหว่าง 45-65 ปีจากโครงการวิจัยระยะยาวเกี่ยวกับอิทธิพลของปัจจัยเสี่ยงต่อการเกิดโรคหัวใจหลอดเลือดและเมแทบอลิซึมในพนักงานการไฟฟ้าฝ่ายผลิตแห่งประเทศไทย (EGAT Cohort study) ในปี ค.ศ. 2013 ได้รับการวิเคราะห์ APOE polymorphism ด้วยวิธี TaqMan real-time PCR ในกลุ่มควบคุมพบว่ามีความถี่จีโนไทป์ Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ3/Ɛ3, Ɛ2/Ɛ4, Ɛ3/Ɛ4 และ Ɛ4/Ɛ4 เท่ากับ 2.2%, 17.2%, 62.4%, 3.2%, 15.1% และ 0%, ตามลำดับ และมีความถี่อัลลีน Ɛ2, Ɛ3 และ Ɛ4 เท่ากับ 0.12, 0.79 และ 0.09 ตามลำดับ ส่วนในกลุ่มคนโรคอ้วนพบว่ามีความถี่จีโนไทป์ Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ3/Ɛ3, Ɛ2/Ɛ4, Ɛ3/Ɛ4 และ Ɛ4/Ɛ4 เท่ากับ 1.3%, 18.8%, 55.4%, 5.4%, 17.8% และ 1.3% ตามลำดับ และมีความถี่อัลลีน Ɛ2, Ɛ3 และ Ɛ4 เท่ากับ 0.13, 0.74 และ 0.13, ตามลำดับ APOE polymorphism มีผลต่อระดับไขมันในเลือดในกลุ่มคนโรคอ้วนโดยพบว่าผู้ที่มีจีโนไทป์ Ɛ4/Ɛ4 มีระดับคอเลสเตอรอลมากกว่าผู้ที่มีจีโนไทป์ Ɛ2/Ɛ4 และมีระดับ LDL-C มากกว่าผู้ที่มีจีโนไทป์ Ɛ2/Ɛ3, Ɛ3/Ɛ3 และ Ɛ2/Ɛ4 นอกจากนี้ยังพบว่า ผู้ที่มีจีโนไทป์ Ɛ3/Ɛ4 มีระดับ LDL-C มากกว่าผู้ที่มีจีโนไทป์ Ɛ2/Ɛ4 โดยสรุปพบว่ามีความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของ APOE กับระดับของไขมันในเลือดในคนโรคอ้วน และบ่งชี้ว่าจีนนี้น่าจะเป็นหนึ่งในปัจจัยทางพันธุกรรมที่กำหนดความเสี่ยงต่อการเกิดภาวะโรคอ้วนและไขมันในเลือดสูงรวมทั้งโรคหลอดเลือดหัวใจในคนไทย

คำสำคัญ: อะโปไลโปโปรตีนอีจีน ความหลากหลายทางพันธุกรรม ภาวะโรคอ้วน ภาวะไขมันในเลือดผิดปกติ TaqMan real-time PCR

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Introduction

Obesity, an excessive body fat accumulation, is a major cause of public health problem worldwide. Obesity is associated with many metabolic disorders and diseases such as dyslipidemia, hypertension, and diabetes known as metabolic syndrome which contributes to cardiovascular disease (CVD)[1,2]. The prevalence of obesity has been increasing in low- and middle-income countries. The National Health and Nutrition Examination Survey (NHANES) reported that the prevalence of obesity in overall U.S. adults was 39.8% and in adults age 40-59 was 42.8%[3]. Data from WHO demonstrated that the prevalence of Thai adults with BMI more than 25 kg/m^2 continued rising up since 1975 from 8.6% to 32.6% in 2016[4]. In addition, Aekplakorn et al. (2004) reported that the prevalence of obesity was increased to 22.4% and 34.3% in men and women, respectively[5]. Obesity individuals may have lipid abnormalities which were mediated by adipokines and free fatty acids. Moreover, abnormalities of lipid profiles in obesity subjects including elevated triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels as well as decreased high-density lipoprotein (HDL) level were generally reported[2]. In the past decade, there has been a large *increase* in gene-environment interaction *researches*, as one of the mechanisms that determined clinical manifestations of obesity with various phenotypes. Numerous molecular genetic studies have been focused to identify for the genes underlying the variations of obesity with dyslipidemia[6].

Apolipoprotein E (ApoE) is a multifunctional protein component of triglyceride-rich lipoproteins. ApoE plays an important role in lipid metabolism by acts as a ligand binding with the cell-surface receptors at hepatocytes including the low-density lipoprotein receptor (LDLR), LDLR related protein (LRP) and heparin sulfate proteoglycan (HSPG). This can mediates the clearance of VLDL and chylomicrons from blood circulation. Moreover, ApoE stimulates the production of VLDL which can increase TG levels[7].

ApoE gene (*APOE*) is located on the long arm (q) of chromosome 19 at position 13.2. with coding a glycoprotein ApoE that plays a central role in lipid metabolism. This gene is also considered as a candidate gene for studying susceptibility to CVD due to its influence on the obesity related factors and abnormal lipid levels[8]. The *APOE* polymorphism is defined by rs429358 and rs7412 at position 112 and 158, respectively. There are three common alleles of the *APOE* (Ɛ2 Ɛ3 and Ɛ4) which generates six possible genotypes: Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ2/Ɛ4, Ɛ3/Ɛ3, Ɛ3/Ɛ4 and Ɛ4/Ɛ4. *Changes* in the nucleotide base lead to substitutions of amino acid and their functional alterations. Based on three APOE isoforms, the most common isoform is APOE 3 which contains a cysteine at residue 112 and an arginine at residue 158. For APOE 2 and E4, there are an

amino acid substitution of arginine for cysteine at residue 158, and a substitution of arginine for cysteine at residue 112, respectively[9]. Numerous studies reported that genetic variations of *APOE* have interrelation with changes in plasma total and LDL- cholesterol (LDL-C) concentrations. The presence of the ε4 allele was associated with elevations in LDL-C, whereas the presence of ε2 resulted in decreased levels of LDL-C. This association has supported by different isoforms of APOE with various degree of binding affinity to the cell surface receptors and their clearance rates from plasma[10].

The published relevance of *APOE* polymorphism and complex interaction obesity and dyslipidemia was documented. The more BMI the greater increase abnormal lipid levels. Multivariate analysis for evaluation of the interaction between *APOE* polymorphism, and BMI on lipid, and lipoprotein levels was investigated. The significant interactions ($p < 0.04$) between *APOE* alleles and increased BMI were found for total and low-density lipoprotein cholesterol and insulin levels, the increase in those parameters with BMI being stronger among ε4 carriers than among ε3 or ε2 carriers[11]. Villeneuve et al., reported that the significant odds ratio of hyperlipidemia in *APOE* ε2/ε4 carriers were less likely than ε2/ε2 carriers, but more likely than ε3/ε3 carriers, to suffer from hypertriglyceridemia[12]. Previous study in *APOE* polymorphism among Thai individuals with 121 normal lipid profiles and 125 dyslipidemia showed no significant findings with the influence of genetic variations of *APOE* on the risk of dyslipidemia[13]. Clinical evidences indicated that both genetic and environmental factors contributed to the obesity can increase cardiovascular risk through risk factors such as increased fasting plasma TG, high LDL-C, low HDL-cholesterol (HDL-C), elevated blood glucose level and high blood pressure. Therefore, this study was conducted to investigate the relative contributions of genetic background, *APOE* polymorphisms, on serum lipid profiles in obesity subjects.

Materials and Methods

Study population

The 407 subjects from the second survey of the Electricity Generating Authority of Thailand (EGAT) cohort study in 2013 were enrolled in this study. Participants included 93 of control group (68 males, 25 females) and 314 of obese group (246 males, 68 females) with age between 45-65 years. Control group was defined as non-obese (BMI <25 kg/m^2) according to the BMI cut-off point of obesity for Asian adults[14]and normal lipid profiles (TG <150 mg/dL, TC <200 mg/dL, LDL-C <130 mg/dL, and HDL-C >40 mg/dL) according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III)[15]. For obese group, participants had BMI ≥25 kg/m^2, with abnormal lipid levels. Individuals suffering from diseases like cancer, liver disease, renal disease, cardiac disease, respiratory disease or any other acute or

chronic diseases as well as subjects suffering from thyroid disorder, psychiatric illness or on insulin therapy were also excluded. Informed consent was obtained from all subjects. This study was approved by the Ethic Committee on Human Right Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA2017/115).

Data collection

Self-administered questionnaires (gender, age, education, smoking, alcohol consumption and family medical history), and physical examinations (weight, height, waist circumference and blood pressure) were performed. Venous blood was collected in the early morning after the subjects had fasted overnight and before they had begun lipid-lowering treatment as appropriate. Blood samples were centrifuged at 1600 g for 15 min at 4°C, and serum was separated and stored at -30°C until analysis. The lipid profiles (TG, TC, HDL-C, LDL-C and VLDL levels), fasting blood glucose, HbA1C as well as liver and kidney function tests were analysed by the automated analyser cobas® 8000 (Roche).

Genotyping analysis

The genomic DNA was extracted from whole blood in EDTA coated tubes by a standard phenol-chloroform extraction procedure and frozen at -20°C until use. The *APOE* SNPs (rs429358 and rs7412) were genotyped using TaqMan® Real-time polymerase chain reaction (real-time PCR) (Applied Biosystems, Foster City, CA, USA), in 96-well format. There were forward, reverse primers and two reporter probes. The 5′ end of each probe is linked to different fluorescent allele-specific dye: FAM and VIC. The TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA, USA) used contains AmpliTaq Gold® DNA polymerase, dNTP and passive internal reference based on proprietary ROX dye. To prepare the reaction mix to amplify samples, working stock of SNP Genotyping assay were added to universal master mix to be diluted with distilled water. After vortexing, the mixtures were transferred into each well before adding wet genomic DNA. The plate was sealed and inserted into the Step One Applied Biosystems real-time PCR machine. PCR temperature was kept on hold for 10 minutes at 95°C then reduced to 92°C for 15 seconds (denaturation) and further reduced in annealing and extension stages to 60°C for 1 minute each for 40 cycles. The Step One software is linked to the allelic fluorescence detection system which measures and plots different fluorescence signals on partitioning chart (X or Y axis). The discrete clusters with different colours showed wide separation based on their defined genotypes. A cluster which moved horizontally to the bottom side toward X axis represented homozygosity of one allele (XX); signals which moved upward vertically to the Y axis represented homozygous for the other allele (YY) and those located in between X and Y axis represented heterozygous genotype (XY)

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 19.0. Continuous data were expressed as mean ± standard deviation (SD) and analyzed by parametric methods. Continuous variables were tested with the Kolmogorov-Smirnoff test for normality. TG levels were log-transformed and expressed as geometric mean. Differences of mean values between two and three groups were analyzed by T-test and One-way analysis of variance (ANOVA), respectively. Allele frequencies were calculated by the gene counting method. The Fisher's exact test was used to evaluate the concordance of genotype frequencies with Hardy-Weinberg's equilibrium expectations. Both genetic and other clinical variable were evaluated to find out the effect on serum lipid by multiple regression analysis. All tests were two-tailed and differences were considered statistically significant when $p<0.05$.

Results

The characteristics of the study subjects are shown in Table 1. Mean age of both groups were 56 years. No significant differences were found in gender, age, smoking status, alcohol consumption and physical activity. There were some statistical clinical parameters between obese and control groups in BMI (28.42 vs. 21.70kg/m^2, $p<0.05$), systolic blood pressure (SBP) (138.4 vs. 127.5 mmHg), and diastolic blood pressure (DBP) (83.96 vs. 76.83 mmHg). Subjects in obese group had significantly higher levels of lipid profiles and glucose markers than controls; TG (215.5 vs. 80.67 mg/dL), TC (222.5 vs. 178.1 mg/dL), LDL-C (149.3 vs. 106.6 mg/dL), HDL-C (47.08 vs. 64.63 mg/dL), VLDL-C (45.93 vs. 16.92 mg/dL), fasting blood glucose (FBG) (108.2 vs. 91.91 mg/dL) and haemoglobin A1c (HbA1c) (6.12 vs. 5.38%).

The distribution of *APOE* genotypes and alleles in both groups were presented in Table 2. Genotype frequencies of Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ3/Ɛ3, Ɛ2/Ɛ4, Ɛ3/Ɛ4 and Ɛ4/Ɛ4 were 2.2%, 17.2%, 62.4%, 3.2%, 15.1% and 0% for control group and 1.3%, 18.8%, 55.4%, 5.4%, 17.8% and 1.3% for obese group, respectively. Allele frequencies of Ɛ2, Ɛ3 and Ɛ4 in control group were 0.12, 0.79 and 0.09 and in the obese group were 0.13, 0.74 and 0.13, respectively. Comparisons of biochemical data by *APOE* genotypes in control and obese groups were presented in Table 3 and 4, respectively. There were no statistically significant differences in lipid profiles, FBG and HbA1C among the five genotype groups (Ɛ2/Ɛ2, Ɛ2/Ɛ3, Ɛ3/Ɛ3, Ɛ2/Ɛ4, and Ɛ3/Ɛ4,) in controls (Table 3). For the obese group, total cholesterol level in carrier with Ɛ4/Ɛ4 genotype (282.8 mg/dL) was significantly higher than those with Ɛ2/Ɛ4 genotype (202.8 mg/dL, $p<0.05$). Subjects with Ɛ4/Ɛ4 genotype (214.0 mg/dL) had significantly higher in LDL-C than Ɛ2/Ɛ3 (143.4 mg/dL), Ɛ3/Ɛ3 (148.4 mg/dL), and Ɛ2/Ɛ4 genotypes (125.4 mg/dL). Moreover, individuals with Ɛ3/Ɛ4 genotype also showed statistical difference in LDL-C level

(160.9 mg/dL) compared to ε2/ε4 genotype (125.4 mg/dL) (Table 4). Based on significant results related to dyslipidemia, further analysis of LDL-C and different *APOE* genotype was focused. We performed stepwise multiple regression analysis to identify the variables that independently and significantly contributed to the change in LDL-C level. *APOE* genotypes showed a statistical predictor of LDL-C (β=2.846, p=0.007). Other parameters included sex, age, BMI, smoking status, alcohol status and physical activity did not influence on serum LDL-C levels (Table 5).

Table 1: General characteristics and biochemical parameters of the study subjects.

Characteristics	Control group	Obese group
Total (n)	93	314
Male, n (%)	68 (73.1)	246 (78.3)
Female, n (%)	25 (26.9)	68 (21.7)
Age (years) (min-max)	56.34±4.34 (49-64)	56.10±3.77 (49-65)
BMI (kg/m^2)	21.70±2.33	28.42±2.91*
SBP (mmHg)	127.5±14.86	138.4±15.97*
DBP (mmHg)	76.83±9.85	83.96±10.41*
Smokers, n (%)	17 (18.3)	47 (15.0)
Alcohol consumer, n (%)	46 (49.5)	175 (55.7)
Physical activity, n (%)	66 (71.0)	201 (64.0)
TG (mg/dL)	80.67±1.35	215.5±1.39*
TC (mg/dL)	178.1±17.51	222.5±43.27*
LDL-C (mg/dL)	106.6±17.18	149.3±39.56*
HDL-C (mg/dL)	64.63±12.18	47.08±10.31*
VLDL-C (mg/dL)	16.92±5.08	45.93±20.74*
FBG (mg/dL)	91.91±14.51	108.2±34.14*
HbA1c (%)	5.38±0.45	6.12±1.18*

Data are presented as mean±SD and percentage.

* Significant difference from control group ($p<0.05$).

Table 2: Distribution of APOE genotype and allele frequency in study groups.

Genotype/ allele	Control group (n=93)		Obese group (n=314)	
	Observed genotype frequency (%)	Allele frequency	Observed genotype frequency (%)	Allele frequency
Ɛ2/Ɛ2	2 (2.2)	Ɛ2 = 0.12 Ɛ3 = 0.79 Ɛ4 = 0.09	4 (1.3)	Ɛ2 = 0.13 Ɛ3 = 0.74 Ɛ4 = 0.13
Ɛ2/Ɛ3	16 (17.2)		59 (18.8)	
Ɛ3/Ɛ3	58 (62.4)		174 (55.4)	
Ɛ2/Ɛ4	3 (3.2)		17 (5.4)	
Ɛ3/Ɛ4	14 (15.1)		56 (17.8)	
Ɛ4/Ɛ4	0 (0)		4 (1.3)	

Data are presented as number and percentage.

Table 3: Serum lipid profiles, fasting blood glucose and HbA1c in control group according to *APOE* (rs7412 and rs429358) genotypes.

Parameters	Control group (n=93)					
	Ɛ2/Ɛ2 (n=2)	Ɛ2/Ɛ3 (n=16)	Ɛ3/Ɛ3 (n=58)	Ɛ2/Ɛ4 (n=3)	Ɛ3/Ɛ4 (n=14)	Ɛ4/Ɛ4 (n=0)
TG (mg/dL)	94.49±1.25	83.45±1.32	81.21±1.40	70.31±1.28	75.98±1.20	-
TC (mg/dL)	194.5±0.71	168.1±18.92	179.4±16.13	183.7±10.97	180.8±20.23	-
LDL-C (mg/dL)	108.5±13.44	93.88±17.28	109.3±15.70	111.7±14.50	108.7±19.30	-
HDL-C (mg/dL)	78.50±17.68	68.00±12.52	62.90±12.79	67.00±6.08	65.50±7.97	-
VLDL-C (mg/dL)	19.00±0.00	17.44±4.65	17.21±5.74	14.33±3.06	15.43±2.53	-
FBG (mg/dL)	90.50±0.71	99.13±22.17	90.05±9.31	92.00±6.25	91.57±21.36	-
HbA1c (%)	5.55±0.21	5.41±0.50	5.38±0.47	5.33±0.21	5.30±0.42	-

Data are presented as mean±SD.

Table 4: Serum lipid profiles, fasting blood glucose and HbA1c in the obese group according to *APOE* (rs7412 and rs429358) genotypes.

Parameters	Obese group (n=314)					
	Ɛ2/Ɛ2 (n=4)	**Ɛ2/Ɛ3 (n=59)**	**Ɛ3/Ɛ3 (n=174)**	**Ɛ2/Ɛ4 (n=17)**	**Ɛ3/Ɛ4 (n=56)**	**Ɛ4/Ɛ4 (n=4)**
TG (mg/dL)	254.9±1.29	221.4±1.37	216.4±1.39	210.3±1.63	205.2±1.33	205.21±1.28
TC (mg/dL)	228.5±45.44	218.9±40.87	221.0±43.65	202.8±43.19	232.0±41.42	282.8±23.66[c]
LDL-C (mg/dL)	146.8±36.90	143.4±36.21	148.4±39.00	125.4±39.53	160.9±39.07[c]	214.0±20.77[a,b,c]
HDL-C (mg/dL)	46.75±5.97	49.47±12.31	46.59±9.60	45.29±11.61	46.79±10.19	45.25±6.08
VLDL-C (mg/dL)	52.00±13.95	46.88±19.89	46.10±19.59	49.65±44.78	43.09±14.34	42.00±10.49
FBG (mg/dL)	105.5±18.52	110.1±34.00	106.8±31.67	106.2±26.44	111.7±44.51	102.8±23.89
HbA1c (%)	5.98±0.68	6.13±1.22	6.09±1.11	6.15±1.30	6.27±1.40	5.48±0.33

Data are presented as mean±SD.

[a,b,c] Significant difference from Ɛ2/Ɛ3, Ɛ3/Ɛ3 and Ɛ2/Ɛ4 genotype, $p<0.05$, respectively.

Table 5: Effects of sex, age, BMI, lifestyle factors and *APOE* genotype on plasma LDL cholesterol

Variables	Unstandardized Coefficients		Standardized Coefficients	p-value
	β	**Std. Error**	**β**	
Sex	2.207	5.818	0.023	0.705
Age	-1.018	0.594	-0.097	0.088
BMI	-0.558	0.792	-0.041	0.482
Smoking status	-3.347	6.526	-0.030	0.608
Alcohol status	2.358	4.728	0.030	0.618
Physical activity	-0.731	1.189	-0.036	0.539
APOE genotypes	2.846	1.041	0.154	0.007

Discussion

In this study, we investigated *APOE* polymorphisms for their association with dyslipidemia in obese subjects. Our study included 314 subjects with obesity and 93 normal control subjects. The result showed that the *APOE* genotype was an independent risk factor for dyslipidemia in obesity. Obesity can cause various metabolic disorders, including dyslipidemia, hypertension and diabetes. The present findings also support various clinical consequences of obesity. Means of TG, total cholesterol, LDL and VLDL levels in obese group were significantly higher than those in control groups whereas lower of HDL-C (Table 1). It is widely known that obesity is the main cause of dyslipidemia since obesity leads to number of molecular changes in lipid and lipoprotein metabolisms, including impaired peripheral trapping and increased fluxes of free fatty acids from adipocytes to the liver and other tissues as well as hepatic overproduction of very low density lipoprotein, decreased circulating TG lipolysis and the formation of small dense LDL[12]. For HDL-C, its concentration is adversely altered in obesity both the degree and distribution of obesity. The possible mechanisms were obesity promoting reduced HDL_2-subfraction levels and hypertriglyceridemia with enhancing HDL catabolism in obese subjects[16]. A previous study found that the obese state directly enhanced the rate of clearance and might enhance the secretion rate of HDL (measured as apo A-I) into plasma. This hypothesis was supported by the level of intra-abdominal fat, as measured by magnetic resonance imaging, strongly correlated with the clearance rate of HDLapo A-I ($r = 0.98$, $p = 0.003$)[17].

Dyslipidemia with obesity can lead to endothelial damage and the loss of physiological vasomotor activity that may become manifested as increased blood pressure. In addition, angiotensin II released from adipose tissue can increases blood pressure by its vasoconstrictive effects[18]. Similar to our finding with high systolic and diastolic blood pressure in obese subjects compared to controls, Wilson et al., demonstrated that the age-adjusted relative risk (RR) for new hypertension was highly associated with overweight status (male: RR, 1.46; female: RR, 1.75)[19].

The association of obesity with type 2 diabetes has been recognized for decades, and the major basis for this link is the ability of obesity to bring forth insulin resistance. Insulin resistance is a fundamental aspect of the etiology of type 2 diabetes. High FBG and HbA1C levels point to insulin resistance or diabetes. In the present study, we have found statistically higher levels of FBG and HbA1C in obese subjects compared to those with normal BMI. These findings were in agreement with previous study that found a significant positive correlation between BMI and FBG ($r=0.751$, $P <0.0001$)[20]. Generally, obesity is related to insulin resistance byincreased free fatty acid (FFA) which inhibit the glucose uptake by muscle. Excess FFA together with angiotensin II

can lead to impair the function of the pancreas, and finally to insulin resistance. In addition, adipose tissue has been demonstrated to secrete various adipokines such as leptin, adiponectin, resistin and retinol-binding protein 4 (RBP4) implicating their involvement in insulin resistance[2,21]. Obesity is also a state of low-grade inflammation which relates to the development of insulin resistance. Pro-inflammatory cytokines such as TNF-α and IL-6 are secreted from macrophages in adipose tissue also down-regulate the sensitivity of insulin[22].

Apo E is a glycoprotein consisting of 299 amino acids and plays a fundamental role in the lipid metabolism. Important functions of Apo E have been documented. It participates in the clearance of chylomicron remnants and VLDL by serving as a ligand for LDL receptors as well as in intestinal cholesterol absorption and plasma lipid maintenance. The association of A*POE* with lipid levels and hyperlipidemia suggest that *APOE* alleles contribute to the genetic risk of developing atherosclerotic vascular disease and other CVDs. We found that the distribution of allele frequencies was in agreement with earlier studies in Thais[13, 23]. In this study, ε3 allele was the most common allele (0.79 and 0.74 in control and obese group, respectively). None of the subjects in the control group had ε4/ε4 genotype (Table 2). Another study among Han Chinese population reported that the frequency of genotypes ε3/3, ε3/4, and ε2/3 was found to be 75.00, 10.70, and 11.90%, respectively, and 0.60, 1.20, and 0.60% for ε2/2, ε2/4, and ε4/4 in total studied subjects. The derived allele frequencies for ε3, ε4, and ε2 alleles were 86.31, 6.55, and 7.14%, respectively[23].

The influences of *APOE* genotypes on lipid parameters were analyzed. For the obese group, the ε4/ε4 genotype had an effect on LDL-C levels compared to ε2/ε3, ε3/ε3, and ε2/ε4 genotypes. The carrier with ε4/ε4 genotype had an effect on TC levels compared to ε2/ε4 genotype. This consistent with previous studies[20,24] and supported the theory explanation that the ε4 allele had an influence on TC and LDL-C levels due to the enhanced uptake of chylomicron remnants and the down-regulation of LDL receptor[25,26]. Moreover, the ε4 isoform has amino acid substitution differ from the ε2 and ε3 isoforms. The ε4 isoform has arginine residues at position 112 and 158 while the ε2 isoform has cysteine at both sites and the ε3 isoform has cysteine and arginine residues, respectively. The ε4 preferentially bind to high affinity lipoprotein particles like VLDL to cell-surface receptors. Therefore, it delayed the clearance of VLDL which associated with the down-regulation of LDL receptors as described above[27]. In addition, obese subjects with ε3/ε4 genotype had higher LDL-C levels than ε2/ε4 genotype (Table 4). This similar to the previous study that the ε2 carriers had lower levels of LDL-C than the ε3/ε3 genotype. The observed results indicated that the ε2 allele has a protective

effect on atherosclerosis. Because the ε2 allele impaired clearance of chylomicron remnants and the up-regulation of LDL receptor, lead to lower LDL-C levels[25,26]. The effects of *APOE* polymorphism on LDL-cholesterol concentrations was emphasized in multiple linear regression model and suggested that the *APOE* could be a predictive factor of dyslipidemia, especially LDL-C levels (Table 5). Our results were thus consistent with previous observations[28-30].

In the present study, we observed that the polymorphisms of *APOE* were associated with high LDL-cholesterol, with the E2 isoform being protective against this condition and the E4 isoform representing risk factors for this condition. However, in interpreting our findings, we should consider several aspects. First, this is a cross-sectional analysis and we did not determine the temporal sequence of the observed results. Second, several studies have addressed the relationship between the *APOE* and dyslipidemia in obesity, however there are *other candidategenes (i.e. APOA5, APOC3, APOA1,* and *LPL) involving in* prediction of the genetic risk for these conditions.

Conclusion

APOE was involved in the regulation of lipid metabolism. The ε2 allele is associated with lower levels of serum TC, and LDL-Cas compared with the ε3 allele, whereas the ε4 allele was associated with higher levels. Our results confirm that this gene is one of the determinants of LDL-cholesterol in obese subjects and suggesting for its possible effects on obesity and dyslipidemia development. *APOE* is considered as a possible conventional risk factor for lipid metabolic disorders and CVD.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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