Apolipoprotein E receptor 2 Gene Polymorphisms Associated with Dyslipidemia among Thai Population

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Background: *Dyslipidemia is an abnormal amount of lipids and/or lipoproteins in the blood. It is a major risk factor of coronary heart disease and atherosclerosis.*
Objective: *This study investigated two single nucleotide polymorphisms (SNPs) in the apolipoprotein E receptor 2 (ApoER2) gene in association with risk of dyslipidemia in the Thai patients.*
Material and Method: *Four hundred blood samples including dyslipidemia patient (200) and unrelated normal control (200) were included in this study. Serum lipids were examined. DNAs were extracted and genotyped by using polymerase chain reaction (PCR) followed by high-resolution melting (HRM) analysis. The differences in genotype distribution between patient and normal control were assessed by Chi-square test of the SPSS software version 11.5.*
Results: *The data analysis revealed that two SNPs (rs3737984 and rs2297660) in ApoER2 gene had significant association with dyslipidemia. The rs3737984 showed significant association at p-value = 0.001, in which A alleles informed the decreased risk of dyslipidemia [odds ratio and 95% CI of A allele, 0.42 (0.28-0.65)]. In contrast, the rs2297660 exhibited strongest association with an increase risk of dyslipidemia [p-value = 0.001, odds ratio and 95% CI for the A allele was 2.38 (1.49-3.80)].*
Conclusion: *The rs2297660 may be used as biomarker for the risk of dyslipidemia in Thai ethnic.*

Keywords: *Dyslipidemia, Single nucleotide polymorphisms, Apolipoprotein E receptor 2, High-resolution melting analysis*

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Dyslipidemia is an abnormal amount of lipids (e.g. cholesterol and triglyceride) and/or lipoprotein in the blood. It may be exhibited by an increase of the total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein cholesterol (HDL-C) concentration in the blood[1]. Normally, dyslipidemia causes no symptoms but it is a major risk factor for atherosclerosis, cardiovascular, coronary heart, cerebrovascular and peripheral arterial diseases. Primary and secondary causes lead to dyslipidemia in varying degrees[2,3]. The primary causes are single or multiple gene mutations that result in either overproduction or defective clearance of triglyceride and LDL cholesterol, or in underproduction or excessive clearance of HDL[4]. The secondary causes contribute to most cases of dyslipidemia in adults such as lifestyle with excessive intake of fats, alcohol overuse, diabetes mellitus, chronic kidney disease, hypothyroidism, primary biliary cirrhosis and other cholestatic liver diseases[4].

The genetic variability of several genes were observed to be associated with dyslipidemia including the Thr56Met missense mutation of the autosomal recessive hypercholesterolemia *(ARH)* gene[5]. The P143L polymorphism of the *LCAT* gene was assumed to play a role in decreased HDL-C levels, and increase risk of dyslipidemia[6]. A genome-wide association study (GWAS) examined the concentrations of HDL-C and triglycerides in European ethnic[7-11] and identified the SNPs at 15 loci which associated with HDL-C levels (such as, APOA1/C3/A4/A5 gene cluster) and SNPs at 12 loci which associated with triglycerides (such as *APOB, APOE* genes). A meta-analysis of GWAS of blood lipoprotein and lipid phenotypes in European population showed SNPs at 30 loci were associated with LDL-C, HDL-C and triglyceride[7].

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Polymorphisms on the *apolipoprotein E (ApoE)* locus have consistently showed a significant association with total and LDL-cholesterol.

ApoE is a class of apolipoprotein found in the chylomicron and intermediate-density lipoprotein (IDL) that binds to a specific receptor on liver cells and peripheral cells[8]. *ApoE* gene polymorphism positions among the major factors involved in determining inter-individual differences in the initiation and progression of atherosclerosis[9,10]. It plays an important role in the particle lipoprotein and bind to LDL receptor (LDLR) on the liver cell[11].

Apolipoprotein E receptor, a members of low density lipoprotein receptor (LDLR) family, is involved in cellular recognition and internalization of ApoE lipoproteins. VLDLR modulates the binding and uptake of ApoE containing lipoprotein, including chylomicron and VLDL, and regulates TG level in plasma[12]. The specific receptor of three isoforms of ApoE *(E2, E3, E4)* is apolipoprotein E receptor 2 (ApoER2), that they have similar affinity to this receptor. The ApoER2 is a member of the LDLR gene family that plays a role in endocytosis and signal transduction. Human *ApoER2* related gene spans approximately 60-kb and contains 19 exons[13]. It located on chromosome 1p32.3 that encodes 963 amino acids protein.

This study two SNPs in *ApoER2* gene were investigated for their association with risk of dyslipidemia in the Thai ethnic.

Material and Method

Participants

Two hundred dyslipidemia patients (78 males and 122 females) and 200 unrelated normal controls (82 males and 118 females) participated in this study based on their serum lipid levels. Dyslipidemia was defined as either triglyceride levels >150 mg/dL, total cholesterol >200 mg/dL, LDL-C >100 mg/dL and HDL-C <60 mg/dL, whereas normal control was defined as triglyceride <150 mg/dL, total cholesterol <200 mg/dL, LDL-C <100 mg/dL, and HDL-C >60 mg/dL[14]. The characteristics of dyslipidemia subject were classified as the high range of total cholesterol and triglyceride levels whereas LDL was in borderline high while HDL of both patient and healthy control were within normal ranges[14]. This study was approved by the ethical committees of the Faculty of Medicine, Srinakharinwirot University, Thailand. Written informed consent was obtained prior to participation.

Determination of serum lipids level

Level of serum triglycerides and total cholesterol were determined by the enzymatic colorimetric method[15,16] whereas HDL-C and LDL-C were analyzed by using homogeneous method[17]. The analysis was performed at laboratory of HRH Princess Mahachakri Sirindhorn Medical Center, Faculty of Medicine, Srinakharinwirot University. All lipid profile tests were run on Dimension RXL chemistry analyzer (Dade Behring, USA).

DNA extraction and genotyping

Genomic DNAs of patient and control were extracted from whole blood (5 ml) using Flexigene DNA kit (Qiagen, Germany). Two SNPs (rs3737984 and rs2297660) in *ApoER2* gene were selected from JSNP, NCBI database with a minor allele frequency (MAF) exceeding 0.2 in populations of Japanese descent[18]. PCR primers were designed from website http://snp.ims.u-tokyo.ac.jp/search.html. The nucleotide sequence of PCR primers of each SNPs were shown in Table 1.

Genotyping was accomplished by using real-time PCR and HRM. The PCR reaction was performed in a total volume of 10 µl composed of 1 µl of DNA (25 ng), 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 5 µl of quantiprobe, 1 µl of Syto 9 green fluorescent, and 2 µl of sterile-distilled water. PCR reaction was carried out for 40 cycles as follows pre-denaturing at 95°C for 10 min, denaturation at 95°C for 10 sec, annealing at 66.8°C for rs2297660 or 57.5°C for rs3737984 for 15 sec, and extension at 72°C for 20 sec. HRM analysis was performed at temperature ranging from 70°C to 85°C using a rotor gene 6000 (Corbett,

Table 1. SNP primers of *ApoE receptor 2* (*ApoER2)* gene used in PCR amplification

Gene	db SNPs ID	Location	Primer sequence 5' to 3'	PCR product (bp)
ApoER2	rs2297660	CDS*3	F1: TAGTTCCGCTTCACCAGGTC R1: AAGCCAGTGGCAGAGTCTTG	153
ApoER2	rs3737984	intron	F3: GCATTTCGGGAAAAGGAATTA R3: TTGAGTCTGGAGAGGGGATA	185

Australia). Genotype was validated by sequencing using ABI 3730xl (Applied Biosystems).

Statistical analysis

Clinical characteristics of patient and normal controls were compared using the t-test. The goodness-of-fit test for Hardy-Weinberg equilibrium (HWE) was performed using the equation p2 + q2 + 2pq, where p and q represented the wild-type and variant allele of a gene. Association analysis was accomplished using Pearson's Chi-square test implemented in SPSS program version 11.5 for Windows. Statistical significant level was set at $p<0.05$.

Results

The clinical characteristic of dyslipidemia patients and normal controls are summarized in Table 2. Number of normal control and patient were equal. Age and sex were not significantly difference. Levels of total cholesterol, triglyceride, LDL-C of dyslipidemia patient were higher than control (*p*-value = 0.001) whereas HDL-D was lower with *p*-value = 0.001.

The goodness-of-fit test was equal to 1 indicated that both of SNPs tested were in Hardy-Weinberg equilibrium. The genotype and allele frequencies of rs2297660 and rs3737984 of *ApoER*2 gene in patients and controls and association analysis are summarized in Table 3. The SNPs in *ApoER2* gene showed significant differences in the genotype distribution between patients and controls in the Thai population ($p<0.05$). The rs2297660 and rs3737984 in *ApoER2* gene were significantly associated with dyslipidemia (*p*-value = 0.001, OR and 95% CI were 2.38 (1.49-3.80) and (*p*-value = 0.001, OR and 95% CI were 0.42 (0.28-0.65), respectively.

Discussion

Dyslipidemia is mainly characterized by high levels of total cholesterol, triglyceride and low-density lipoprotein cholesterol, and low levels of high-density lipoprotein cholesterol. It is an important etiological factor for the development of cardiovascular disease (CVD)[19]. GWAS of an ethnic Indian demonstrated a strong association of the cholesterol ester transfer

Table 2. Clinical characteristic of dyslipidemia patients and unrelated normal controls

Variables of subjects	Normal control	Dyslipidemia	*p*-value
Sample number (n)	200	200	
Male	82 (42%)	78 (39%)	
Female	118 (58%)	122 (61%)	
Age (years)	47.91±0.5	47.01±0.5	0.220
Total cholesterol (TC)	194.04±2.7*	243.09±4.2*	0.001
Triglyceride (TG)	117.81±5.7*	288.04±53.5*	0.001
Low-density lipoprotein (LDL)	114.49±3.2**	149.31±7.3**	0.001
High-density lipoprotein (HDL)	58.81±1.3**	51.04±0.8**	0.001

* Number of control = 200, and patients = 200; ** Number of control = 164, and patients = 167

Table 3. Distributions of genotype and allele frequencies of *ApoER*2 SNPs in dyslipidemia and controls

SNPs ID		Genotype		Allele		*p*-value*	OR (95%CI)**
rs2297660	A/A	A/C	C/C	A	C		
Patients	109 (54.5%)	56 (28.0%)	35 (17.5%)	0.27	0.13	0.001	2.38 (1.49-3.80)
Controls	54 (27.0%)	79 (39.5%)	67 (33.5%)	0.19	0.21		
rs3737984	A/A	A/C	C/C	A	C		
Patients	28 (14.0%)	84 (42.0%)	88 (44.0%)	0.14	0.26	0.001	0.42 (0.28-0.65)
Controls	56 (28.0%)	94 (47.0%)	50 (25.0%)	0.21	0.19		

* The *p*-vaules of Chi-square test of genotype frequencies; ** Odds ratio and the 95% CI confidence intervals of allele frequencies

protein (CETP) rs3764261 with HDL-C, and SNPs (rs964184 and rs12286037) from BUD13-ZNF259 near the *APOA5-A4-C3-A1* genes with TG levels[20]. A variety of genetic studies reported several genes associated with dyslipidemia such as *ApoA1*, *ApoC3*, *ApoA4*, *ApoA5*, *ApoB*, *ApoE*, *LPL*, *LCAT*, *LDLR, and CETP*, etc.

Apolipoprotein E receptor 2 are members of the LDL receptor family, a group associated with cellular cholesterol homeostasis[21]. In human, ApoE ligand has a key role in lipid transport both in the plasma and in the central nervous system by binding specifically to apolipoprotein E receptor 2. Polymorphisms of *ApoER2* gene may affect the affinity of binding and normal function of *ApoE* in transportation of lipid leading to the development of dyslipidemia.

The polymorphisms in *ApoER*2 gene in association with lipid level in dyslipidemia were investigated in Thai ethnic. The first SNP, rs3737983, which is in coding region of the *ApoER2* gene was shown to associate with dyslipidemia[22]. The other two SNPs in the exon and intron regions of *ApoER2* gene-rs2297660 and rs3737984, respectively, appeared to be significantly associated with a risk of dyslipidemia in a Thai population ($p<0.05$). Among the three SNPs, the rs2297660 tend to be the strongest SNP that associated with risk of dyslipidemia because of its low *p*-value ($p = 0.001$) and higher odd ratio value and 95% CI [2.38 (1.49-3.80)], in which the A allele imparted the increase risk of dyslipidemia. The rs3737984 was significantly associated with dyslipidemia ($p = 0.001$), whereas the A allele informed the decreased risk of dyslipidemia [odds ratio and 95% confidence interval of the A allele, 0.42 (0.28-0.65)] Because the strong association of rs2297660, it may be used as biomarker for indicating the risk of dyslipidemia in Thai ethnic.

Conclusion

Two SNPs in exon and intron regions of *ApoE receptor 2* gene, rs2297660 and rs3737984, were significantly associated with dyslipidemia. The rs2297660 showed the strongest association, therefore, it may be used as biomarker for a risk of dyslipidemia in the Thai population.

What is already known on this topic ?

The polymorphisms in *ApoER*2 gene in association with lipid level in dyslipidemia were first investigated in Thai ethnic by our group.

The rs3737983, which is in the coding region of the *ApoER2* gene, showed association with dyslipidemia.

What this study adds ?

This study analyzed two more SNPs, rs2297660 and rs3737984, in *ApoER2* gene and found that both SNPs were significantly associated with a risk of dyslipidemia.

Among the three SNPs, the rs2297660 was the strongest SNP that associated with risk of dyslipidemia in the Thai ethnic and can be used as biomarker for this disease.

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Potential conflicts of interest

None.

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การแปรผันของเบสหนึ่งเบสในจีนตัวรับอะโปไลโปโปรตีนอี 2 มีความสัมพันธ์กับภาวะไขมันในเลือดผิดปกติ ในประชากรไทย

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ภูมิหลัง: ไขมันผิดปกติเป็นภาวะที่มีระดับไขมันหรือไลโปโปรตีนในเลือดผิดปกติ ทำให้มีความเสี่ยงต่อการเกิดโรคหลอดเลือดต่าง ๆ ได้แก่ โรคหลอดเลือดหัวใจ โรคหลอดเลือดแดงแข็ง เป็นต้น

จุดประสงค์: เพื่อวิเคราะห์หาความสัมพันธ์ระหว่างการแปรผันของเบสหนึ่งเบส (สนิปส์) ในจีนตัวรับอะโปไลโปโปรตีนอี 2 (ApoER2) กับการเกิดภาวะไขมันในเลือดผิดปกติในประชากรไทย

วัสดุและวิธีการ: ตัวอย่างเลือดทั้งหมด 400 ตัวอย่าง ประกอบด้วยเลือดจากคนไข้ที่มีภาวะไขมันในเลือดผิดปกติจำนวน 200 คน และเลือดจากคนที่มีไขมันปกติจำนวน 200 คน ทำการหาระดับไขมันในซีรัม สกัดดีเอ็นเอและหาสนิปส์โดยเทคนิคพีซีอาร์ และตามด้วยไฮเรสโซลูชั่นเมลติ้ง (เอชอาร์เอ็ม) จากนั้นวิเคราะห์หาความถี่ของจีโนไทป์ในคนไข้และคนปกติโดยใช้ Chi-square test โปรแกรม SPSS รุ่น 11.5

ผลการศึกษา: พบความสัมพันธ์อย่างมีนัยสำคัญของสนิปส์ในจีนตัวรับอะโปไลโปโปรตีนอี 2 กับการเกิดโรคไขมันผิดปกติ 2 ตำแหน่ง คือ rs3737984 และ rs2297660 โดยที่ rs3737984 มีความสัมพันธ์ในด้านลดความเสี่ยงต่อการเกิดโรคไขมันผิดปกติ [ค่า p เท่ากับ 0.001, odds ratio และ 95% CI ของอัลลีล A เท่ากับ 0.42 (0.28-0.65) ส่วน rs2297660 มีความสัมพันธ์ในด้านการเพิ่มความเสี่ยงต่อการโรค [ค่า p เท่ากับ 0.001 ค่า odds ratio และ 95% CI ของอัลลีล A เท่ากับ 2.38 (1.49-3.80)]

สรุป: สนิปส์ rs2297660 ในจีนตัวรับอะโปไลโปโปรตีนอี 2 อาจใช้เป็นตัวบ่งชี้ทางชีวภาพสำหรับทำนายความเสี่ยงของการเกิดโรคไขมันในเลือดผิดปกติในคนไทย