Single nucleotide polymorphism in exon of *ApoE* receptor 2 gene associated with dyslipidemia in a Thai population

Polphet Thongket*, Siriporn Chatsuriyawong, Weeranuch Seesom, and Wasana Sukhumsirichart

***Abstract*—** Dyslipidemia is an abnormal amount of lipid in the blood. It is a major risk factor of atherosclerosis, cardiovascular disease, and coronary heart disease. It cause by environment and genetic factors. A genome-wide association study of blood lipoprotein and lipid revealed that the SNPs in apolipoprotein E (*ApoE*) gene associated with the high level of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride. In the present study, the SNPs in exon of the ApoE receptor 2 (*ApoER*2) gene was analyzed for association with dyslipidemia in a Thai population. A total of 400 subjects including 200 dyslipidemia patients and 200 unrelated normal controls with mean age of 47.01±0.5 and 47.91±0.5 years, respectively, were participated in the study using lipid level such as triglyceride, total cholesterol, LDL-C, and HDL-C as criteria to allocate the subjects into control and patient groups. The DNAs were extracted from whole blood using Flexigene DNA kit (QIAGEN, German). Genotyping was performed using real-time PCR and high-resolution melting (HRM) analysis. The difference in genotype distribution between patient and control was assessed by Chi-square test of the SPSS software version 11.5. The result revealed that the SNP in the *ApoER*2, rs3737983, showed significant association with the risk of dyslipidemia in the Thai population (*P*-value = 0.011). Further study is required for clarification the role of genetic variation in expression pattern of the *ApoER*2 which may be involved in dyslipidemia.

***Keywords*—** Dyslipidemia, Single nucleotide polymorphism, Apolipoprotein E receptor 2, High-resolution melting analysis

I. Introduction

DYSLIPIDEMIA is an abnormal amount of lipids (e.g. cholesterol and/or triglyceride) in the blood**.** The prolonged elevation of insulin levels can lead to dyslipidemia. It may be manifested by elevation 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] Dyslipidemia itself usually causes no symptoms but can lead to symptomatic vascular disease because it is a major risk factor for coronary heart disease. In previous studies, the genetic variability of several genes were observed to be associated with dyslipidemia such as the Thr56Met missense mutation of the autosomal recessive hypercholesterolemia (*ARH*) gene.[2] The P143L polymorphism may play a role in causing decreased HDL-C levels, leading to increased risk of dyslipidemia.[3] A genome-wide association study (GWAS) examined the concentrations of HDL-C and triglycerides in Europeans [4]-[6] 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 convincingly associated with LDL cholesterol, HDL cholesterol and triglyceride.[4] Polymorphisms at the Apolipoprotein E (ApoE) locus have consistently showed a significant association with total and LDL-cholesterol.

In addition, there are also genes associated with dyslipidemia including apolipoprotein E , there is no reported association polymorphism of apolipoprotein E with dyslipidemia in Thailand. Therefore, this study aims to investigate the single nucleotide polymorphisms (SNPs) of *ApoE* receptor 2 genes in healthy controls and dyslipidemia patients and determine for the association with the risk of dyslipidemia in Thai population.

II. Materials and Methods

Subjects

A total of 400 subjects including 200 dyslipidemia patients (78 males and 122 females) with a mean age of 47.01± 0.5 years and 200 unrelated normal controls (82 males and 118 females) with a mean age of 47.91± 0.5 years were participated in this study after signed informed consent which was approved by the Human Ethics Committees of Faculty of Medicine, Srinakharinwirot University, Thailand. Blood lipid levels were used as criteria for discrimination of healthy control and dyslipidemia. Dyslipidemia is defined as either triglyceride levels >150 mg/dL, total cholesterol >200 mg/dL, LDL- C >100 mg/dL and HDL- C <60 mg/dL.

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Analytical methods

Serum triglycerides and total cholesterol were determined by the enzymatic colorimetric method [7]-[8]. HDL-C and LDL-C were directly measured by homogeneous method [9]. All lipid profile tests were run on Dimension RXL chemistry analyzer (Dade Behring, USA).

Genotyping

Genomic DNAs of patients and control were extracted from whole blood (5 ml) using Flexigene DNA kit (Qiagen, German). Genotyping was performed by using polymerase chain reaction and high-resolution melting analysis (HRM). The SNP primers were designed from the website http://snp.ims.u-tokyo.ac.jp/search.html and http://www.ncbi.nlm.nih.gov/projects/SNP/. The sequences of forward (F) and reversed (R) primers of rs3737983 were as follows; F: GCCAATCTGAGCAGTTCTCC; R: GTGATAGCCCTCCTGTGCAT. The Real-time PCR reaction was performed in total volume of 10 µl containing 5 µl of quantiprobe, 1 µl of DNA (25 ng), 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 1 µl of Syto9 green fluorescent and 2 µl of sterile-distilled water. PCR reaction was carried out for 40 cycles: pre-denaturing at 95ºC for 10 min, denaturation at 95ºC for 10 s, annealing at 64ºC (depending on primer to used) for 15 s and extension at 72ºC for 20 s. HRM analysis was performed at temperature ramping from 70-85 ºC using a rotor gene. The difference in genotype distribution between patient and control was used for association analysis by using Pearson's Chi-square test implemented in SPSS program version 11.5 for Windows. Significant level was set at $P<0.05$.

III. RESULTS

The clinical characteristic of the 200 dyslipidemia patients and the 200 unrelated normal controls are summarized in Table 1. The level of triglyceride, total cholesterol, and LDL-C of dyslipidemia patient were significantly higher than normal control whereas the level in Thai population.

TABLE I
Clinical characteristic of dyslipidemia patients and unrelated normal controls

Variables of subjects	Control group	Dyslipidemia group	*P*-vaule
Number of sample (n)	200	200	
Male	82 (42%)	78 (39%)	
Female	118 (58%)	122 (61%)	
Age (years)	47.91±0.5	47.01±0.5	>0.22
Total cholesterol (mg/dL)	194.04±2.7	243.09±4.2	<0.001
Triglyceride (mg/dL)	117.81±5.7	288.04±53.5	<0.001
HDL-C (mg/dL)	58.81±1.3	51.40±0.8	<0.001
LDL-C (mg/dL)	114.49±3.2	149.31±7.3	<0.001

We tested the SNP that located in the exon of ApoE receptor 2 (*ApoER*2) gene with dyslipidemia in Thai population. The result of the association analysis is summarized in Table 2 (Genotype crosstabulation). The SNP, rs3737983, showed significant differences in the genotype distribution between patients and controls in Thai population (*P*-value = 0.011)

TABLE II
Result of the association analyses (Genotype crosstabulation)

Sample		Genotype				*P*-vaule[a]
		AA	GG	AG	Total	
Control	Count	86	29	85	200	
	Expected Count	78.5	41.0	80.5	200.0	
	% within sample	43.0%	14.5%	42.5%	100.0%	
	% within genotype	54.8%	35.4%	52.8%	50.0%	
	% of total	21.5%	7.3%	21.3%	50.0%	
Patient	Count	71	53	76	200	
	Expected Count	78.5	41.0	80.5	200.0	
	% within sample	35.5%	26.5%	38.0%	100.0%	0.011
	% within genotype	45.2%	64.6%	47.2%	50.0%	
	% of total	17.8%	13.3%	19.0%	50.0%	
Total	Count	157	82	161	400	
	Expected Count	157.0	82.0	161.0	400.0	
	% within sample	39.3%	20.5%	40.3%	100.0%	
	% within genotype	100.0%	100.0%	100.0%	100.0%	
	% of total	39.3%	20.5%	40.3%	100.0%	

[a]*P*-vaule of chi-square test of genotype frequencies

IV. DISCUSSION

In the present study, SNP in exon of ApoE receptor 2 gene, rs3737983 were shown to be significantly associated with risk of dyslipidemia in a Thai population. ApoE receptor 2 is a specific receptor for apolipoprotein E (ApoE) has been demonstrated in the liver [7]-[8], apoE plays a central role in lipid metabolism. As a consequence of this important role, there is much interest in determining the region of the apoE molecule that interacts with lipoprotein receptors [9].

Apolipoprotein E receptor2 are members of the LDL receptor family, a group associated with cellular cholesterol homeostasis [10]. In human apoE has a key role in lipid transport both in the plasma and in the central nervous system. Its three common structural isoforms differentially affect the risk of developing atherosclerosis and neurodegenerative disorder, including Alzheimer's disease. Because the function of apoE is dictated by its structure, understanding the structural properties of apoE and its isoforms is required both to determine its role in disease and for the development of the therapeutic strategies [11]. The failure of the apoE receptor 2 to affect the functions of apoE, it isn't transport lipid because it is a major risk factor for this disease . In the future may be use to biomarker for this disease.

V. CONCLUSION

This study demonstrated that the SNP in exon of ApoE receptor 2 gene, rs3737983, associated with risk of dyslipidemia in Thai population. Further study is required for clarification the role of genetic variation in expression pattern of the ApoE receptor 2 gene which may be involved in dyslipidemia.

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