Impact of Genetic Polymorphism on LDL-C Response to Plant Stanol Ester Intake

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Background: *The high blood cholesterol level could be prevented by plant stanol ester (Staest) consumption. In addition, the genetic polymorphism of cholesterol transporters might be related with lipid profile and subsequently response to Staest intake.*

Objective: *To investigate the effect of single nucleotide polymorphism in ATP-binding cassette hetero-dimeric G5/G8 (ABCG5/G8) and Niemann-Pick C1 Like1 protein (NPC1L1) gene on LDL-C response subsequent to plant Staest intake in Thai Subjects.*

Material and Method: *The blood samples were collected from 113 subjects for genotyping. The single nucleotide polymorphisms (SNPs) of ABCG5/G8 positions; rs6720173 (Q604E), rs4148211 (C54Y), rs4148217 (T400K), rs3806471 (5'UTR-145), and NPC1L1; positon; rs2072183 (L272L) were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.*

Results: *After Staest intake, the subjects with QE genotype (Q604E of ABCG5) showed a 4-fold significant decrease in LDL-C level (14.17±10.67%) compared to subjects with QQ genotype (3.50±10.65%) ($p = 0.003$). Moreover, the pronounced effect of Q604E polymorphism was observed in subjects after intake of Staest with meal. However, no significant difference in these markers was observed in subjects carrying other mutations.*

Conclusion: *Thus, it could be suggested that non-synonymous gene polymorphism resulted substitution of uncharged glutamine (Q) with negatively charged glutamic acid (E) at position 604, thereby possibly alter the function of transporter proteins. Besides, the genetic variation in these genes might be related with serum lipid profiles. Moreover, Q604E mutation of ABCG5 in each individual with meal effect could lead to particular response towards LDL-C level after Staest intervention.*

Keywords: *ABCG5/G8, Single nucleotide polymorphism, Plant stanol ester, Nutrigenetic, Thai subject*

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Cardiovascular disease (CVD) is the most common cause of death and public health concern worldwide[1]. An important risk factor in CVD pathogenesis is hypercholesteroemia or dyslipidemia status, especially low-density lipoprotein cholesterol (LDL-C) condition. The major causes of hypercholesterolemia, a conspicuous pre symptom of CVD are hypothesized to be genetic and lifestyle factors. The former involves genetic polymorphism, while the later includes dietary pattern or physical activity[2].

Nowadays, medicinal treatments using synthetic drugs such as statins with lowering serum cholesterol effect are commonly used by most CVD patients. Nevertheless, these drugs produce adverse effects such as nausea, diarrhea, and constipation in CVD patients. The usage dose of these drugs varies from person to person, thereby making CVD treatment more specific to each individual. Thus, green medicines (natural products) that could decrease serum cholesterol level have become a target strategy for CVD prevention. Moreover, it was previously reported that cholesterol absorption in human, especially LDL-C level, could be decreased via competition of cholesterol influx mechanism after consumption of some functional foods or medicinal plants[3]. The difference in effective response against cholesterol level after treatment or consumption of food such as dietary plant stanol ester (Staest) was observed[4,5]. This inter-individual response towards cholesterol absorption had been hypothesized as the effect of genetic variation in intestinal cholesterol transportation[6]. Several studies had reported the relationship between genetic variation (resulting in mutation in transporter protein) and regulation of sterol transportation via small intestine and other organs in vivo[7,8].

Two major transporter proteins including hetero-dimeric ATP-binding cassette (G5 and G8 or

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ABCG5/G8) and Niemann-Pick C1 Like1 protein (NPC1L1) play significant roles in sterol excretion and absorption, respectively. The genes coding for ABCG5/G8 are located on chromosome 2p21. However, a rare genetic mutation on these genes could cause sitosterolemia, resulting in high serum cholesterol and increased risk of cardiovascular disease[9]. The ABCG5/G8 transporters are mostly located at apical membrane of small intestine and liver in order to efflux sterol from cell to lumen, control sterol level that passes to intestinal cell, and stimulate sterol excretion into bile. In addition, the studies on single nucleotide polymorphism (SNPs) of ABCG5/G8 had been reported that non-cholesterol sterol level could be decreased in response to treatment of cholesterol-lowering drug or functional food consumption[6,10]. On the other hand, NPC1L1, a key cholesterol transporter protein, is required for sterol influx from micells to intestinal mucosa cells, the function that is opposite to ABCG5/G8 protein[11]. The NPC1L1 gene is located in 7p13, in which some genetic variants have been reported to inter-individual response towards decreasing of LDL-C level in lipid lowering drug treated patients (such as ezetimibe) in vivo[12].

Despite this information, the detailed mechanism of intestinal cholesterol transportation and gene variation regarding decreased lipid level after Staest intervention in Thai subjects is still unavailable. Therefore, the aim of the present study was to investigate the effect of cholesterol transporter gene polymorphisms, including ABCG5/G8 and NPC1L1, on serum lipid level in mild hypercholesterolemia Thai subjects undergoing Staest intervention. The SNPs; rs6720173 (Q604E, G>C) of ABCG5 replaces glutamine (Q) with glutamic acid (E) at position 604 in peptide sequence. The rs4148211 (cysteine change to tyrosine at 54; C54Y, G>A), rs4148217 (threonine change to lysine at 400; T400K, C>A), rs3806471 (5'untranslated region at 145 nucleotides; 5'UTR-145, A>C) are located in ABCG8 gene and the rs2072183 (synonymous SNP with leucine at 272; L272L, C>G) are in NPC1L1 gene. This present study might provide useful information for physician regarding the inter-individual response after cholesterol lowering treatment as well as promote CVD prevention by natural product (i.e., Staest) consumption.

Material and Method

Subjects and study design

The present genetic study was a cross sectional design and collected data about the clinical parameter from previous clinical trial study[4]. One hundred thirteen volunteer (58 placebo controls and 55 Staest subjects) were included in the research of randomized, double-blind, placebo controlled study with 4-week intervention phases in 2013[4]. A daily intake of 2-gram plant stanols as a plant Staest biscuit was carried out in the intervention group. The protocol was approved by the Ethics Committee of the Mahidol University Institutional Review Board (Registration number 2013/004.1001), which was registered as part of Clinical Trials (NCT02331043). The informed written consent was obtained from all the study participants. Further details were reported elsewhere[4].

Blood collection

Twelve hour-fasting blood samples were taken before the study began (run-in), baseline (at the end of week 2), and at the end of the intervention study (week 6). All samples (weeks 0 and 6) were measured for serum total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG). The concentrations of lipid parameters were analyzed by enzymatic colorimetric methods using an Olympus/AU 400 auto analyzer. Interassay variability was 3.24% for TC, 1.11% for LDL-C, 0.75% for HDL-C, and 1.09% for TGs. The detailed were described in previous report[4]. The EDTA coated tube (3 mL) was used for DNA analysis in the present study.

Analysis of genetic variation in ABCG5/G8 and NPC1L1

In the present study, SNP with allele frequency higher than 10% in individuals (reportedly using lipid profile, both cross sectional and Staest intervention) was chosen. Genomic DNA was extracted from peripheral leukocytes of the EDTA-treated blood samples using a Flexi gene DNA kit (Qiagen, Hilden, Germany). The genetic variants in the cholesterol transporters were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using a T100 thermocycler (BioRad, California, USA). The reaction consisted of 100 ng of genomic DNA in 25 µL of PCR reagent (10 mM ris-HCl (pH 8.3) containing 50 mM KCl, 1.5 mM $MgCl_2$, 0.24 mM deoxynucleotide triphosphates, 0.24 µM of forward and reverse primer[13,14], and 0.2 unit Taq DNA polymerase. The PCR performed in vitro amplification of nucleic acids. Initial denaturation of sample was carried out at 94°C for 5 minutes, followed by 32 cycles of further denaturation at 94°C. The annealing step was performed at 59-63°C (Q604E, C54Y, T400K,

Table 1. The parameters on baseline data of the study subjects (n = 113), mean ± SD

Parameter	Total subjects	Placebo (n = 58)	Plant stanol (n = 55)
Age (years)	42.5±9.3	42.9±9.5	42.3±9.2
Sex (female), n (%)	85 (75)	44 (75)	41 (75)
BMI (kg/m^2)	24.56±4.17	24.39±3.57	24.59±4.74
Total cholesterol (mg/dl)	232.66±27.40	232.23±23.93	232.83±30.19
Triglyceride (mg/dl)	122.90±56.65	124.15±61.21	121.73±49.69
HDL-C (mg/dl)	61.18±15.56	61.45±17.71	61.24±13.10
LDL-C (mg/dl)	159.68±26.18	159.71±23.33	159.59±29.02

BMI = body mass index; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol

5'UTR-145, and L272L) and then extension step at 72°C. Each step lasted within 30 seconds, with a final extension for 5 minutes at 72°C. The PCR products undergone enzymatic digestion as previously described[15] and visualized on 14% polyacrylamide or 3% agarose gel electrophoresis.

Statistical analysis

The statistical software program SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used to analyze the individual parameters. Mean comparison of each genotypes was performed and the data was presented as mean ± standard deviation. The statistical differences between groups in genotypic distribution (Hardy-Weinberg equilibrium) were assessed by Chi-square test. The difference between genotype and serum lipid level was tested by the independent t-test (two-tailed). A $p<0.05$ was considered statistically significant.

Results

The genetic variations in ABCG5/G8 and NPC1L1 were investigated using 113 mild hypercholesterolemic subjects. Among the subjects, there were 75% female and 25% male with mean age of about 43 year old and BMI about 25 kg/m^2 (Table 1). Mild cholesterolemia were found in these subjects with the values of 232.66±27.40 mg/dL TC, 122.9±56.65 mg/dL TG, and 159.68±26.18 mg/dL LDL-C. However, HDL-C level was found with the value of 61.18±15.56 mg/dL.

Based on the DNA analysis, SNP at Q604E, T400K, C54Y, 145UTR, and L272L on ABCG5/G8 and NPC1L1 did not showed any significant deviation from Hardy-Weinberg equilibrium (HWE) (Table 2). Besides, minor allele frequency (MAF) was depended on ethnicity. It was noted that the MAF of ABCG5/G8 and NPC1L1 gene polymorphism was similar to the previous report in Asian population such as Japanese and Chinese[8,15], but it was not found in case of mutant EE genotype.

Table 2. Genotype distribution and allele frequencies on the gene polymorphisms of cholesterol transporters, ATP-binding cassette hetero-dimeric ABCG5/G8 and NPC1L1

Gene	Position	Mutation		MAF	*p*-value for HWE
ABCG5					
rs6720173	Exon 13	Q604E	G>C	C = 0.10	0.228
ABCG8					
rs4148211	Exon 2	C54Y	G>A	A = 0.18	0.073
rs4148217	Exon 8	T400K	C>A	A = 0.12	0.149
rs3806471	5'UTR	145	C>A	A = 0.18	0.088
NPC1L1					
rs2072183	Exon 2	L272L	C>G	G = 0.3	0.468

MAF = minor allele frequency; HWE = Hardy-Weinberg equilibrium

The effect of gene polymorphism on serum lipid of all subjects at baseline (pre-PS consumption) indicated that the subjects with CC genotype encoded for QQ phenotype (ABCG5,Q604E) had significantly higher cholesterol level (237.89±23.51 mg/dL) and HDL-C level (60.43±15.47 mg/dL) as compared to QE phenotype carriers (cholesterol level of 226.09±21.95 mg/dL and HDL-C level of 52.91±12.07 mg/dL) (Table 3). On the other hand, similar LDL-C levels with no statistical significant difference were observed in subjects with ABCG5/G8 gene polymorphism. Likewise, the investigation in NPC1L1 gene suggested that individuals carrying silent mutation L272L with CC genotype (44% of subjects) had higher LDL-C level (165.90±25.96 mg/dL) as compared to CG + GG genotype carriers (56% of subjects) who had significantly lower LDL-C level (156.41±22.40 mg/dL) ($p = 0.036$). Nevertheless, other SNPs did not showed

Table 3. Baseline serum levels of TC, TG, HDL-C, and LDL-C based on classification by gene polymorphisms

Gene	Number	Serum lipid concentration (mg/dl), mean ± SD							
		TC	*p*-value	HDL-C	*p*-value	LDL-C	*p*-value	TG	*p*-value
ABCG5									
Q604E	QQ = 90	237.89±23.51	0.031*	60.43±15.47	0.032*	161.80±24.54	0.293	117.32±54.55	0.477
	QE = 23	226.09±21.95		52.91±12.07		155.78±23.91		126.52±58.02	
ABCG8									
C54Y	CC = 73	235.42±22.97	0.961	59.67±15.62	0.437	159.25±23.96	0.482	120.04±58.16	0.824
	CY+YY = 40	235.65±4.96		57.59±14.24		162.98±25.53		117.63±49.95	
T400K	TT = 86	235.82±24.31	0.804	58.91±15.35	0.987	160.56±25.24	0.985	120.55±55.33	0.634
	TK = 27	234.52±21.54		58.96±14.55		160.67±22.07		114.74±55.28	
145UTR	CC = 73	235.66±24.17	0.925	53.39±15.79	0.653	160.47±25.22	0.946	119.79±55.27	0.871
	CA+AA = 40	235.22±22.81		58.05±13.90		160.80±23.19		118.02±55.55	
NPC1L1									
L272L	CC = 50	238.06±25.60	0.310	57.02±13.74	0.228	165.90±25.96	0.036*	115.35±53.55	0.508
	CG+GG = 63	233.44±21.83		60.46±16.07		156.41±22.40		122.27±56.62	

TC = total cholesterol; TG = triglyceride
Asterisk values are significantly different ($p<0.05$)

any significant correlation between genotype and plasma lipid concentration.

Interestingly, the subjects that undergone Staest intervention had significantly lower TC and LDL-C levels than placebo group, whilst TG and HDL-C levels remained unchanged (Table 4). The association between gene polymorphisms in ABCG5/G8 and NPC1L1 with percentage change in lipid level after the consumption of plant sterol was presented.

Only subjects with Q604E genotype of ABCG5 (QE) had a significantly decrease the mean serum LDL-C level compared to QQ genotype by -23.54±13.81 mg/dL and -6.79±16.40 mg/dL, respectively (Table 4). QE genotype showed a 4-fold greater decreased LDL-C level (14.17%) than that of QQ genotype (80% of Staest group) (3.50%) (Fig. 1).

When concerned about the meal effect to Staest intervention, it was also found that QE genotype of ABCG5 showed a significant effect to the responsiveness of decreasing LDL-C level in subject who consumed Staest alone (p-value = 0.019) but QE genotype showed less association in subject who consumed Staest with meal (p-value = 0.062) (Table 5). In addition, no association between other SNPs and serum lipid changes was observed.

Discussion

The lowering LDL-C level is a strategy to control or prevent cardiovascular diseases, and plant stanol consumption might be an optional food for the lipid regulation in human. Individual variation in the response after intervention with drug or Staest is the major problem for clinical trial studies[16,17], and it could be associated with genetic factors[10]. The present study is the first report that showed the association of sterol transporter genes, ABCG5/G8 (Q604E, C54Y, T400K, and 145UTR) and NPC1L1 (L272L) polymorphisms, which possibly affect the lipid profiles and the responsiveness of Staest intervention in Thai subjects. The selected polymorphisms in the present study have demonstrated the association of cholesterol and plant sterol metabolism in both Caucasian[18,19] and Asian populations[8,13].

Baseline data suggested that subjects with ABCG5 gene polymorphism (Q604E) with QQ genotype

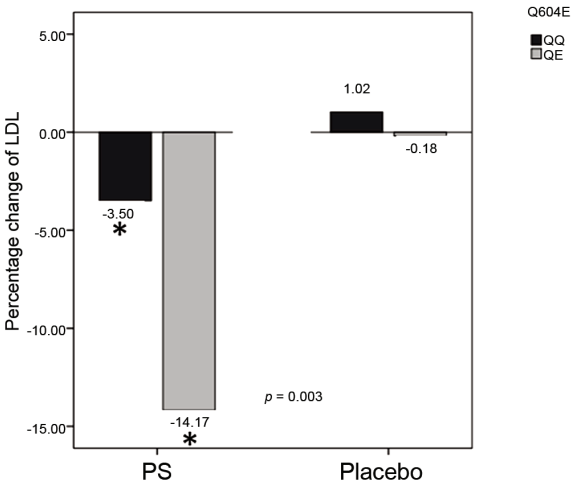

Fig. 1 The greater fold changes in plasma LDL-C in response to plant stanol ester intervention among subjects with Q604E polymorphism.

Table 4. The association between gene polymorphism in ABCG5/G8 and NPC1L1 with change in lipid level (mg/dL, mean ± SD) after the consumption of plant stanol ester and placebo control

Subjects (n)	TC	LDL-C	HDL-C	TG
Plant stanol group (n = 55)				
ABCG5				
- Q604E				
QQ (44)	-6.59±24.84	-6.79±16.40	1.90±6.82	-10.81±29.63
QE (11)	-17.00±17.75	-23.54±13.81	1.27±4.96	17.55±82.45
p-value	0.198	0.003*	0.773	0.065
ABCG8				
- C54Y				
CC (40)	-9.32±23.79	-10.50±16.75	1.75±6.85	-3.20±51.97
CY + YY (15)	-6.93±24.65	-9.20±18.87	1.87±5.50	-10.33±22.27
p-value	0.744	0.805	0.953	0.611
- T400K				
TT (44)	-10.52±22.80	-11.84±16.06	1.86±6.41	-3.41±49.63
TK (11)	-1.27±27.44	-3.36±20.57	1.45±6.95	-12.09±24.96
p-value	0.253	0.145	0.853	0.578
- 145UTR				
CC (36)	-10.92±23.56	-9.77±14.84	1.86±6.83	-9.75±34.05
CA + AA (18)	-4.89±24.98	-11.67±21.67	1.67±6.01	3.55±64.18
p-value	0.389	0.708	0.919	0.322
NPC1L1				
- L272L				
CC (26)	-8.76±24.10	-11.31±17.91	1.46±5.78	-1.50±23.30
CG + GG (29)	-8.58±23.99	-9.10±16.75	2.07±7.09	-8.41±59.33
p-value	0.978	0.639	0.731	0.580
Placebo group (n = 58)				
ABCG5				
- Q604E				
QQ (46)	4.24±20.07	0.70±20.55	1.83±6.70	-7.28±51.34
QE (12)	3.17±11.63	0.08±12.45	4.00±4.13	-4.50±39.99
p-value	0.860	0.922	0.309	0.862
ABCG8				
- C54Y				
CC (33)	3.39±19.94	-2.45±20.34	2.45±6.94	-6.39±39.11
CY + YY (25)	4.84±16.91	4.56±16.80	2.04±6.11	-7.12±60.32
p-value	0.772	0.167	0.813	0.956
- T400K				
TT (42)	4.90±19.61	-0.21±19.56	2.86±6.59	-9.80±46.28
TK (16)	1.69±15.75	2.63±18.54	0.75±6.35	1.44±55.98
p-value	0.560	0.617	0.277	0.439
- 145UTR				
CC (37)	6.51±16.98	1.30±18.60	2.97±6.63	-8.97±49.18
CA + AA (21)	-0.38±20.74	-0.71±20.25	1.05±6.34	-2.71±49.34
p-value	0.176	0.703	0.285	0.644
NPC1L1				
- L272L				
CC (24)	3.08±19.96	-1.83±20.84	3.79±6.51	-2.68±50.29
CG + GG (34)	4.68±17.76	2.26±17.82	1.21±6.44	7.40±43.20
p-value	0.751	0.425	0.139	0.100

Asterisk values are significantly different ($p<0.05$)

(79% of the subjects) contained higher TC and HDL-C level than those of QE phenotype carriers. This result suggested that genetics could have strong influence on serum lipid concentration. It was previously reported that the ABCG5/G8 play a significant role in cholesterol metabolism by pumping cholesterol back to intestinal lumen and inducing biliary secretion from liver[9]. Besides, the relation between serum lipid level and ABCG5/G8 gene polymorphisms have been reported[20]. It was found that ABCG5, Q604E was related to lipid levels, in which the subjects carrying 604Q allele had higher serum TC than 604E carriers. It was noticed

Table 5. The association between gene polymorphism in ABCG5/G8 and NPC1L1 with percentage change in lipid level after the consumption of plant sterol and concern with meal effect

Without meal	Percentage change of serum lipids, mean ± SD			
	TC	LDL	HDL	TG
ABCG5				
- Q604E				
QQ (18)	1.39±11.36	0.47±12.03	4.33±12.67	-0.61±31.16
QE (4)	-5.50±9.26	-13.83±10.44	-1.50±11.50	12.75±54.28
p-value	0.252	0.062	0.411	0.663
ABCG8				
- C54Y				
CC (14)	-0.71±8.91	-3.33±10.69	2.64±13.83	7.36±41.89
CY + YY (8)	1.63±14.85	-0.05±16.55	4.38±10.27	-7.88±16.63
p-value	0.694	0.625	0.742	0.244
- T400K				
TT (15)	-0.47±8.90	-3.19±10.34	4.53±12.88	6.27±40.56
TK (7)	1.43±15.69	0.14±17.83	0.57±11.83	-7.71±18.13
p-value	0.773	0.658	0.490	0.277
- 145UTR				
CC (9)	0.11±14.68	-4.72±17.71	-1.44±11.92	6.00±33.44
CA + AA (13)	0.15±8.56	-0.34±8.39	6.54±12.12	-1.08±37.33
p-value	0.994	0.505	0.143	0.647
NPC1L1				
- L272L				
CC (10)	-3.30±14.24	-3.03±15.09	-1.00±11.26	7.00±32.26
CG + GG (12)	3.00±7.14	-1.38±11.26	6.83±12.66	-2.50±38.23
p-value	0.226	0.778	0.140	0.534
With meal or snack				
ABCG5				
- Q604E				
QQ (26)	-4.50±9.76	-6.24±8.80	2.31±9.69	-9.00±22.48
QE (7)	-8.0±6.78	-14.37±6.60	3.57±7.61	5.57±23.81
p-value	0.294	0.019*	0.720	0.180
ABCG8				
- C54Y				
CC (26)	-4.88±9.98	-7.41±9.79	2.77±9.59	-5.53±23.90
CY + YY (7)	-6.57±6.29	-10.00±4.62	1.86±8.13	-7.29±22.06
p-value	0.592	0.330	0.805	0.859
- T400K				
TT (29)	-5.66±8.99	-8.54±8.75	1.83±8.49	-5.44±23.26
TK (4)	-2.25±12.09	-3.80±10.65	8.00±13.56	9.25±25.94
p-value	0.620	0.448	0.435	0.278
- 145UTR				
CC (10)	-3.10±9.26	-7.95±11.77	5.90±6.11	-9.00±20.97
CA + AA (23)	-6.17±7.73	-7.97±7.73	1.13±10.02	-4.57±24.42
p-value	0.393	0.996	0.105	0.602
NPC1L1				
- L272L				
CC (16)	-2.93±9.02	-8.07±8.69	4.63±7.80	-2.81±23.29
CG + GG (17)	-7.4±9.21	-7.87±9.45	0.65±10.19	-8.82±23.42
p-value	0.169	0.949	0.216	0.466

that HDL-C level was significantly lowered in 604Q as compared to 604E allele, while higher LDL-C level was found in subjects with 604Q genotype than 604E carriers. It was hypothesized that these differences might be the result of a small sample size effect. On the other hand, the study of ABCG8 gene polymorphism, including C54Y, T400K, and 5'UTR-145, were not associated with serum lipid level in the subjects as being reported in the previous studies[6].

Furthermore, the significant response to LDL-C level after plant sterol intervention was found in QE genotype of Q604E polymorphism. A similar study of gene-diet interaction in which subjects changed the low to a high-dietary-cholesterol diet

with ABCG5; Q604E polymorphism possessed EE genotype, and were found to be associated with a greater increase in serum LDL-C compared to QQ + QE genotype[21].The previous report also suggested that common variation in ABCG5/G8 could interact with serum lipid and non-cholesterol sterol concentration in subjects that consumed plant Staest[10].

In terms of structural protein analysis's perspective, the association of gene polymorphism in Q604E of ABCG5 and lower LDL-C level could explain single amino acid change affected the function of whole protein. The mutation of Q604E led to the change of uncharged side chain-glutamine (Q) to negatively charged glutamic acid (E) at position 604 in transporter protein. The change in polarity might alter intra- and inter-interaction of protein structure, leading to structural change and resulting in higher cholesterol and Staest absorption metabolism. Therefore, it could reduce cholesterol and LDL-C level[22]. The ABCG8 single nucleotide polymorphism including, C54Y, T400K and 5'UTR-145 were not found to be related with the change in lipid level; however, T400K of ABCG8 carriers were reported to be related with serum plant sterol level[6,10].

The present study investigated the L272L in NP1C1 gene due to its association with lipid profile and cholesterol lowering drug (ezetimibe) response[12]. It was also previously reported that primary hypercholesterolemia subjects with wild-type genotype; CC in L272L had higher LDL-C level compared to mutant type (CG + GG)[23]. This mutant might affect the competition between cholesterol and Staest absorption through enterocyte resulting in lower LDL-C. Despite this information, the association of this gene polymorphism and response of cholesterol level in this Staest intervention was not found.

It might be due to nutrigenetic influence of interaction between dietary consumption and gene polymorphism. While comparing the association between meal and ABCG5 gene polymorphism in Staest intervention group, it was found that QE genotype was less related to decrease of serum LDL-C. This genotype still had a strong effect on the decrease of LDL-C in subjects after consuming Staest with meal or snack. The association between diet and plant stanol transporter gene polymorphism could be explained that fat content in meal could promote the sterol absorption mechanism via bile acid secretion[4] as well as the structural change from Q604E polymorphism. Therefore, it might be more affected to LDL-C level after Staest consumption with meal than without any food.

The limitation of the present study included lack of the serum non-cholesterol sterol analysis to confirm the effect of ABCG5/G8 and NPC1L1 gene polymorphisms after plant sterol intervention. Therefore, further study should be conducted in larger population with complete serum sterol analysis.

Conclusion

ABCG5/G8 and NPC1L1 play an important role in cholesterol metabolism. The genetic variation in these genes might be related to serum lipid profiles. In addition, Q604E of ABCG5 had a strong association with the lowering LDL-C after plant sterol intervention but the consumption of Staest without meal could reduce the effect of this gene polymorphism than with meal. It must be noted that food intake and gene are important in Staest intervention. These results might be useful evidence in term of the genetic variations that affected the response of cholesterol treatment or intervention in Thai population.

What is already known on this topic?

Nutrigenetics is an important tool for personalized nutrition.

What this study adds?

Q604E polymorphism of ABCG5 gene in each individual with meal effect might lead to particular response towards LDL-C level in Thai subjects who consumed plant stanol ester.

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Potential conflicts of interest

None.

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ผลกระทบของความแปรผันทางพันธุกรรมต่อการตอบสนองของ LDL-C เมื่อรับประทานแพลนท์สตานอลอีสเทอร์

เชาวนี ชูพีรัชน์, อุทัยวรรณ สุทธิศันสนีย์, ณัฐิรา อ่อนน้อม, วันทนีย์ เกรียงสินยศ

ภูมิหลัง: *การรับประทานแพลนท์สตอนอลเอสเทอร์สามารถลดภาวะคอเลสเตอรอลในเลือดสูงได้ เป็นที่น่าสนใจว่าความแปรผันทางพันธุกรรมของจีนผลิตตัวรับส่งคอเลสเตอรอล มีความสัมพันธ์กับปริมาณไขมันในเลือดเมื่อรับประทานแพลนท์สตานอลเอสเทอร์*

วัตถุประสงค์: *เพื่อศึกษาผลกระทบของจีน ATP-binding cassette hetero-dimeric G5/G8 (ABCG5/G8) และ Niemann-Pick C1 Like1 protein (NPC1L1) ต่อ การตอบสนองของ LDL-C เมื่อรับประทานแพลนท์สตานอลเอสเทอร์ ในอาสาสมัครคนไทย*

วัสดุและวิธีการ: *เก็บตัวอย่างเลือดจากอาสาสมัครจำนวน 113 คน สำหรับศึกษาพันธุกรรมแบบความแปรผันที่เบสเดียว โดยศึกษาตำแหน่ง rs6720173 (Q604E) ที่จีน ABCG5 ตำแหน่ง rs4148211 (C54Y), rs4148217 (T400K), rs3806471 (5'UTR-145) ที่จีน ABCG8 และตำแหน่ง rs2072183 (L272L) ที่จีน NPC1L1 ด้วยวิธี polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)*

ผลการศึกษา: *ในผู้รับประทานแพลนท์สตานอลเอสเทอร์พบว่า อาสาสามัครผู้มีจีโนไทป์แบบ QE ของจีน ABCG5 ตำแหน่ง Q604E มีปริมาณ LDL-C (14.17±10.67 mg/dL) ลดลง 4 เท่า หลังได้รับประทานแพลนท์สตานอลเอสเทอร์เมื่อเปรียบเทียบกับผู้ซึ่งมีจีโนไทป์แบบ QQ (3.50±10.65 mg/dL) (p = 0.003) เมื่อศึกษาในกลุ่มผู้ที่รับประทานแพลนท์สตานอลเอสเทอร์กับอาหารอื่น ๆ เปรียบเทียบกับการรับประทานแพลนท์สตานอลเอสเทอร์เพียงอย่างเดียวพบว่า QE จีโนไทป์ ยังแสดงความสัมพันธ์กับปริมาณ LDL-C ที่ลดลงอีกด้วย อย่างไรก็ตามความแปรผันทางพันธุกรรมตำแหน่งอื่น ๆ นั้น ไม่มีความสัมพันธ์กับการลดลงของ LDL-C*

สรุป: *ความแปรผันทางพันธุกรรมของ Q604E ที่จีน ABCG5 นั้นทำให้ชนิดของกรดอะมิโนในตัวรับส่งคอเลสเตอรอล ABCG5 มีความเปลี่ยนแปลงที่ตำแหน่ง 604 จากกรดอะมิโนกลูตามีนซึ่งไม่มีประจุเป็นกรดกลูตามิคซึ่งมีประจุลบ จึงเป็นไปได้ว่าอาจจะเปลี่ยนโครงสร้างโปรตีนและส่งผลต่อการทำงานของตัวรับส่งคอเลสเตอรอลชนิดนี้ได้ ความแปรผันทางพันธุกรรมในจีนนี้จึงอาจจะส่งผลเกี่ยวข้องกับภาวะไขมันในเลือด โดยเฉพาะ ณ ตำแหน่ง Q604E ของจีน ABCG5 นี้ เมื่อรวมผลจากการรับประทานอาหารอื่นร่วมด้วย จะกระทบต่อประสิทธิผลในการลด LDL-C เมื่อรับประทานแพลนท์สตานอลเอสเทอร์*