Effect of SstI Polymorphism of the Apolipoprotein CIII Gene and Environmental Factors on Risks of Hypertriglyceridemia in Taiwan Aborigines

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Background Hypertriglyceridemia (HTG) is a heterogeneous metabolic disorder. The aim of this study was to examine associations among genetic polymorphisms, SstI polymorphism of apolipoprotein CIII (ApoCIII) and Hind III polymorphism of lipoprotein lipase (LPL), environmental factors and risks of HTG.

Methods and Results Two hundred and forty-nine southern Taiwanese aborigines were recruited for a cross-sectional study, which included 90 subjects with triglyceride (TG) >150 mg/dl (HTG) and 159 with TG ≤150 mg/dl (NTG). The frequencies of SstI major allele (S1) and minor allele (S2) of ApoCIII were 66.1% and 33.9% in HTG and 73.6% and 26.4% in NTG ($p<0.1$). In female subjects, the frequencies of the S2 allele was significantly higher in HTG (0.38) than NTG (0.27) ($p<0.04$). The frequencies of the LPL HindIII major allele (H+) and minor allele (H-) were similar between HTG (H+ 84.3%; H- 15.7%) and NTG (H+ 78.9%; H- 21.1%). In a multivariate adjusted logistic model, education ≤6 year (odds ratio (OR)=3.71, 95% confidence interval (CI): 1.24–8.13), Amis tribe (OR=3.08, 95% CI: 1.41–6.77), body mass index (BMI) ≥25 (OR=2.22, 95% CI: 1.18–4.16), starchy food consumption ≥3 times/week (OR=1.89, 95% CI: 1.00–3.59) and ApoCIII S2S2 genotype (OR=3.35, 95% CI: 1.10–10.19) were independently ($p<0.05$) associated with HTG risks. Among ApoCIII S1S1, S1S2 and S2S2 genotypes, ApoCIII and TG concentrations increased ($p<0.01$) in a dose-responsive manner.

Conclusions The ApoCIII S2 variant and environmental factors, including education, tribal background, BMI and starchy food intake, modulate the risks of HTG in aboriginal Taiwanese. Interaction between genetic and environmental factors warrants further investigation. (Circ J 2006; 70: 1030–1036)

Key Words: Apolipoprotein CIII SstI; HindIII; Hypertriglyceridemia; Lipoprotein lipase polymorphism; Taiwan aborigines

Mounting evidence supports an independent association between hypertriglyceridemia (HTG) and coronary artery disease (CAD);^{1–3} a relationship that likely stems from the atherogenicity of some species of triglyceride (TG)-rich lipoproteins, particularly small very (V)-low-density lipoprotein (LDL) and intermediate-density lipoprotein particles.⁴ Based on the 1993–1996 National Nutrition Survey in Taiwan,⁵ the prevalence of HTG in aboriginal adults (age >19 years) was the highest compared with other non-aboriginal regions investigated. In 2004, the 5th and 6th leading causes of death in aborigines were cerebrovascular and heart diseases, the respective death rates being higher than those of non-aborigines.⁶ During the

period of 1981–1990, it was reported that standardized mortality rates for heart diseases and stroke in aborigines were significantly higher than those for non-aborigines in Taiwan.⁷ A similar trend was also found during 1991–2000. Significantly higher standardized mortality rates for ischemic heart disease (1.13, 95% confidence interval (CI): 1.02–1.26) in male aborigines and cerebrovascular accident in male (1.98, 95% CI: 1.88–2.08) and female (1.64, 95% CI: 1.54–1.76) aborigines were observed compared with non-aborigines in Taiwan.⁸

The ApoAI/CIII/AIV gene complex on chromosome 11q⁹ is a strong candidate for the susceptibility region for common HTG. Apolipoprotein CIII (ApoCIII) is a major apolipoprotein of VLDL and chylomicrons, inhibiting lipoprotein lipase (LPL) of the TG carried by these particles.¹⁰ Overexpression of human ApoCIII causes severe HTG in transgenic mice. A transversion from C to G in the 3' untranslated region (3'UTR) of exon 4 in ApoCIII results in SstI polymorphism.¹¹ Associations between the less common S2 allele of SstI polymorphism and elevated TG concentrations have been reported in different ethnic groups, including Japanese,¹² Asian Indians,¹³ Arabs,¹⁴ Caucasians,¹⁵ and Chinese.¹⁶ However, some other studies have shown contradictory results.^{17,18}

LPL-catalyzed hydrolysis of TG constitutes the rate-limiting step in the removal from the circulation of TG-rich lipoproteins, such as VLDL and chylomicrons, for uptake

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of free fatty acids and glycerol by peripheral tissues for oxidation or storage. The gene for human LPL has recently been cloned, localized to chromosome 8, and found to code for a protein of 65,000 kDa.¹⁹ A common sequence variant, the intron 8 HindIII polymorphism, is reportedly associated with subtle alterations in plasma lipids or atherosclerosis risk. Studies of HindIII polymorphisms have reported associations with HTG,^{16,20} levels of cholesterol (C), and high-density lipoprotein (HDL)²¹ and coronary heart diseases.²²

Approximately 86% of Taiwanese are descended from Han Chinese whose ancestors migrated 400 years ago from Fukkien and Canton provinces.²³ Taiwanese aborigines constitute approximately 2% of the population, rarely mix with Han Chinese and reside in isolated mountainous areas with their own language, customs and social organizations. Genetically, they are markedly different from Han Chinese.²⁴ HTG is a complicated metabolic disease, and both environmental and genetic factors exercise profound influence on determination of blood lipid levels. Considering the high prevalence of HTG, as one of the underlining risk factors in the progression of CAD, in the aboriginal population of Taiwan, the current study aimed to investigate (1) the association of ApoCIII SstI and LPL HindIII polymorphisms with a phenotype of elevated TG in a cross-sectional study, and (2) the effect of these genetic variations with the simultaneous presence of environmental factors on risks of HTG.

Methods

Subjects

From July 2002 to June 2003, community health examinations of a total of 475 subjects were performed in aboriginal villages, Taimali (Tai-Tung) and Machia (Ping-Tung), of southern Taiwan. Parental origin determined a participant's aboriginal tribal classification and only subjects whose parents were from the same tribe were analyzed. After excluding non-aborigines and mixed aborigines, there was a total of 249 unrelated subjects. Of these, 90 had plasma TG >150 mg/dl (HTG) and 159 had TG levels ≤150 mg/dl (NTG), based on the definition of elevated TG set by the National Cholesterol Education Program.³ All subjects gave informed consent for blood samples, anthropometric measurements and questionnaire interviews, and the protocol was approved by the Human Ethical Committee of Kaohsiung Medical University.

Data Collection

Weight and height were measured. Information regarding demographic characters, age and parents' tribal background, use of alcohol, betel nut and cigarettes, and food frequency was obtained by a structured questionnaire completed by each subject.

Blood Collection

After fasting, blood was drawn and plasma/white blood cells were separated within 3 h. An aliquot of plasma was stored at -80°C until ApoCIII analysis using TBA-40FR (Toshiba, Tokyo, Japan) auto-analyzer, and another aliquot of fresh blood was stored at 4°C for routine blood tests, which were performed within 2 days of collecting blood. These measurements included fasting plasma glucose, TG, C, and uric acid using an auto-analyzer (Biotechnica, Hitachi, Tokyo), and HDL-C and LDL-C using a TBA-40FR analyzer (Toshiba, Tokyo, Japan). DNA was extracted using a GFXTM Genomic Blood DNA purification

Table 1 Demographic and Clinical Profile of Taiwan Aborigines With HTG

	HTG (n=90)	NTG (n=159)	p value
Age (years)	57.5±11.7	55.7±15.3	0.32
Body mass index (kg/m ²)	27.8±5.5	26.5±5.7	0.09
Fasting plasma glucose (mg/dl)	134.3±97.3	106.6±42.7	<0.001
Triglyceride (mg/dl)	263.8±167.9	87.7±32.0	<0.001
Log-triglyceride (mg/dl)	2.4±0.2	1.9±0.2	<0.001
Total cholesterol (mg/dl)	194.2±43.3	165.5±35.9	<0.001
LDL-cholesterol (mg/dl)	103.9±41.5	97.3±35.4	0.19
HDL-cholesterol (mg/dl)	42.3±8.9	48.9±12.3	<0.001
Uric acid (mg/dl)	7.0±1.9	6.5±1.7	0.02
Apolipoprotein CIII (mg/dl)	11.6±4.9	7.2±2.9	<0.001

Data are mean±SD; p<0.05 is considered significantly different.

HTG, hypertriglyceridemia, subjects with serum triglycerides >150 mg/dl; NTG, subjects with serum triglycerides ≤150 mg/dl; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SD, standard deviation.

kit (Amersham Pharmacia Biotech, NJ, USA) and stored at -20°C until genotyping.

Genotyping

Polymerase Chain Reaction (PCR) Amplification DNA samples were subjected to amplification by PCR in an automatic thermal cycler. One set of primers was from the DNA sequences of ApoCIII with sense primer of 5'-GGCTGGG TGACCGATGGCTTC-3' and anti-sense primer of 5'-GGG AGGCCAGCATGCCTGGAG-3'. The other set of primers was derived from sequences between exons 8 and 9 in LPL to amplify the sequence around a HindIII restriction site in intron 8 (the forward primer was 5'-TTTAGGCCTGAAG TTTCCAC-3' and the reverse primer was 5'-CTCCCTAG AACAGAAGATC-3'). The 50 µl reaction mixture contained 1×PCR buffer (2.0 mmol MgCl₂), dNTPs at 2.5 mmol/L, 2.0 µg/µl each primer, 2 µg genomic DNA and 0.6 units of Taq DNA polymerase. Amplification of the SstI site of ApoCIII was subject to 35 temperature cycles, which consisted of denaturing at 95°C for 5 min, annealing at 58°C for 1 min and extension at 72°C for 5 min. Amplification of the HindIII site of LPL was performed for 33 thermal cycles consisting of denaturing at 94°C for 5 min, annealing at 60°C for 2 min and extension at 72°C for 2 min.

For detection of restriction fragment length polymorphisms in PCR-amplified DNA, amplified products were digested with SstI or HindIII, and the resulting fragments were separated on 2% agarose gels. After electrophoresis, the gel was treated with ethidium bromide for 20 min and DNA fragments were visualized by UV illumination. Upon digestion with SstI, the presence of the restriction site (S2 allele) resulted in fragments of 322 and 230 bp and 552 bp for the uncut S1 allele. For LPL, the presence of the HindIII site yielded fragments of 600 bp and 700 bp (H+ allele) and the uncut H- allele had 1,300 bp.

Statistical Analysis

T test was used to analyze differences in continuous variables, and the chi-square test was used to analyze distribution of categorical variables (if n<5, Fisher exact test was used). Odds ratio (OR) and 95% CI were used to estimate risks of HTG associated with environmental factors obtained from the questionnaire or serum blood chemistry. Those variables with significant OR in the univariate analysis were further examined by multivariate logistic regres-

Table 2 Genotype Distribution and Allele Frequencies for the ApoCIII-SstI Polymorphism in HTG and NTG Subjects

Subjects	Genotype			Allele frequency	
	S1S1	S1S2	S2S2	S1	S2
Total					
HTG (n=90)	42 (46.7)	35 (38.9)	13 (14.4)	119 (66.1)	61 (33.9)
NTG (n=159)	85 (53.5)	64 (40.2)	10 (6.3)	234 (73.6)	84 (26.4)
p value		0.096		0.078	
Males					
HTG (n=33)	17 (51.5)	15 (44.5)	1 (3.0)	49 (74.2)	17 (25.8)
NTG (n=44)	26 (59.1)	15 (34.1)	3 (6.8)	67 (76.1)	21 (23.9)
p value		0.51		0.85	
Females					
HTG (n=57)	25 (43.9)	20 (35.1)	12 (21.0)	70 (61.4)	44 (38.6)
NTG (n=115)	59 (51.3)	49 (42.6)	7 (6.1)	167 (72.6)	63 (27.4)
p value		0.013		0.036	

Data are number (%), chi-square was used to test the differences. If $n < 5$, Fisher exact test was used. $p < 0.05$ is considered statistically significant.

ApoCIII, apolipoprotein III. Other abbreviations see in Table 1.

Table 3 Genotype Distribution and Allele Frequencies for the LPL-HindIII Polymorphism in HTG and NTG Subjects

	Genotype distribution			Allele frequency	
	H-H-	H-H+	H+H+	H-	H+
Total					
HTG (n=89)	3 (3.4)	22 (24.7)	64 (71.9)	28 (15.7)	150 (84.3)
NTG (n=159)*	3 (1.9)	61 (38.4)	95 (59.7)	67 (21.1)	251 (78.9)
p value		0.082		0.147	
Males					
HTG (n=33)	1 (3.0)	6 (18.2)	26 (78.8)	8 (12.1)	58 (87.9)
NTG (n=44)	0 (0.0)	21 (47.7)	23 (52.3)	21 (23.9)	67 (76.1)
p value		0.017		0.065	
Females					
HTG (n=57)	2 (3.6)	16 (28.6)	38 (67.9)	20 (17.9)	92 (82.1)
NTG (n=115)	3 (2.6)	40 (34.8)	72 (62.6)	46 (20.0)	184 (80.0)
p value		0.696		0.664	

Data are number (%), chi-square was used to test the differences, if $n < 5$, Fisher exact test was used. $p < 0.05$ is considered statistically significant.

LPL, lipoprotein lipase. Other abbreviations see in Table 1.

*One sample missing because of failure of polymerase chain reaction.

sion model to obtain adjusted OR and adjusted 95% CI. Differences were considered statistically significant when $p < 0.05$ or when the 95% CI range was not included in the unity. SAS 8.0 Version (Cary, NC, USA) was used to complete the statistical analyses.

Results

The clinical and biochemical features of the study subjects are listed in Table 1. There were no differences in sex distribution and mean age between the HTG and NTG groups. Concentrations of fasting plasma glucose, C, TG, log-TG, ApoCIII and uric acid levels were significantly ($p < 0.05$) higher in the HTG group than the NTG group and HDL-C was significantly lower in HTG subjects ($p < 0.05$). No differences in body mass index (BMI) and LDL-C were observed between the 2 groups.

The genotype and allelic distributions of the ApoCIII-SstI and LPL-HindIII polymorphisms in the study population and their distribution in the 2 genders are shown in Tables 2 and 3. Overall, the allelic frequencies of the S2 allele of the ApoCIII-SstI polymorphisms were 0.34 ($n=61$) in the HTG group and 0.26 ($n=84$) in the NTG group ($p=0.078$), reaching marginal significance between the 2 groups. As separately analyzed in the female group, significant differences in

S2 distribution was observed between HTG (S2 frequency: 0.39, $n=44$) and the NTG (S2 frequency: 0.27, $n=63$). There were also significant differences between the HTG and NTG groups in the distribution of S1S1, S1S2 and S2S2 genotypes in the female subjects ($p < 0.05$). The frequencies of S1S1, S1S2 and S2S2 were 43.9% ($n=25$), 35.1% ($n=20$) and 21.0% ($n=12$) in the HTG group and 51.3% ($n=59$), 42.6% ($n=49$) and 6.1% ($n=7$), respectively, in the NTG ($p=0.013$). The genotyping of LPL-HindIII of 1 case could not be determined and thus was excluded from the analysis. The allelic frequencies of the H+ allele of the LPL-HindIII polymorphisms were similar between the HTG and NTG groups. Overall, the frequency of the H+ allele was 0.84 ($n=150$) and 0.79 ($n=251$) in the HTG and NTG groups respectively ($p=0.147$). The distribution of the H-/H-, H-/H+ and H+/H+ genotypes was 3.4% ($n=3$), 24.7% ($n=22$) and 71.9% ($n=64$) in the HTG group and 1.9% ($n=3$), 38.4% ($n=61$) and 59.7% ($n=95$), respectively, in the NTG group ($p=0.082$). No differences in the frequencies of the H+ allele and H- allele were found between the 2 groups in either male or female subjects.

Univariate analysis was performed to illustrate associations between environmental factors and HTG risks. The results indicated that educational level (≤ 6 years of education, OR=2.6, 95% CI: 1.3–5.1), tribal background

(Amis, OR=2.6, 95% CI: 1.4–5.2), BMI (≥ 25 , OR=1.9, 95% CI: 1.1–3.4), frequency of starchy food (except for rice) consumption (≥ 3 times/week, OR=2.0, 95% CI: 1.1–3.4), cigarette use (OR=2.1, 95% CI: 1.1–3.8), betel nut or quid chewing (OR=2.4, 95% CI: 1.3–4.3) were positively associated with HTG risk. Age, gender, hypertension and alcohol consumption were not related to HTG risk in the univariate analysis. Upon adjustment for covariance (Table 4), education ≤ 6 year (OR=3.71, 95% CI: 1.24–8.13), Amis tribe (OR=3.08, 95% CI: 1.41–6.77), BMI ≥ 25 (OR=2.22, 95% CI: 1.18–4.16), starchy food consumption ≥ 3 times/week (OR=1.89, 95% CI: 1.00–3.59) and ApoCIII S2S2 (OR=3.35, 95% CI: 1.10–10.19) were independently associated with HTG risk (Table 4).

The TG and ApoCIII concentrations among ApoCIII S1S1, S1S2, S2S2 genotypes are shown in Table 5. TG (p for trend=0.017) and ApoCIII (p for trend=0.007) concentrations were increased in subjects with S1S1, S1S2 and S2S2 genotypes in a dose-responsive manner, although only individuals with S2S2 genotype had significantly higher TG and ApoCIII concentrations compared with the other 2 genotypes (p<0.05). This relation was also examined separately in the male and female groups. In the female group, concentrations of TG (p for trend=0.044) and ApoCIII (p for trend<0.001) remained significantly associated with S1S1, S1S2 and S2S2 genotypes, but not in the male subjects (p>0.05).

Discussion

We report a significant association between ApoCIII SstI polymorphism and risks of HTG in female Taiwan aborigines. A marginal significance (p=0.078) between HTG and NTG for S2 distribution was found in all subjects. As further analyzed in the female participants, S2 allele frequency was significantly (p<0.04) higher in the HTG group than in NTG subjects. Taiwan aborigines are genetically different from Han Chinese, and one theory^{24,25} suggests that they are genetically related to Austronesians who migrated from South-East Asia and arrived 6,000–9,000 years ago in Taiwan; later, Austronesians reached central Polynesia in 200 BC and spread further out across the Pacific Ocean island groups, finally reaching Tahiti, then Hawaii and then New Zealand between 600–800 AD (though there was likely a later migration wave around 1,200 AD from the Society Islands to New Zealand), by that time having evolved to become proto-Polynesians in cultural, language and artifact specializations. Furthermore, recent mitochondrial DNA analysis supports the link between Taiwan aborigines and

Table 4 Multivariate Logistic Regression Analysis of the Effects of ApoCIII (Sst-I) Polymorphism and Environmental Factors on the Risks of HTG

Variable	Classification		Odds ratio (95% CI)
	HTG (n=90)	NTG (n=159)	
	n	n	
Education			
>6 years	13	49	1.0
≤ 6 years	75	110	3.71 (1.24–8.13)
Tribe			
Paiwan	65	139	1.0
Amis	25	20	3.08 (1.41–6.77)
Body mass index			
<25	26	70	1.0
≥ 25	63	86	2.22 (1.18–4.16)
Starchy food intake			
<3 times/week	27	75	1.0
≥ 3 times/week	60	84	1.89 (1.00–3.59)
Cigarette use			
No	61	130	1.0
Yes	28	29	1.41 (0.67–2.98)
Betel nut use			
No	19	62	1.0
Yes	70	97	1.98 (0.99–4.00)
ApoCIII genotype			
S1S1	42	85	1.0
S1S2	35	64	0.8 (0.47–1.69)
S2S2	13	10	3.35 (1.10–10.19)

Model was adjusted for age and sex: CI, confidence interval. Other abbreviations see in Tables 1,2.

If total number of subjects in each variable was not equal to 249, there are missing values (n=1–4).

Polynesians²⁶ Associations between the rare S2 allele of ApoCIII and elevated TG have been reported in various different ethnic groups, including Japanese;² Asian Indians;¹³ Arabs;¹⁴ Caucasians;¹⁵ and Chinese.¹⁶ The present study found frequencies of the S2 allele in NTG and HTG subjects of 0.26 and 0.34, respectively. The frequency of the S2 allele is in line with ranges reported for Chinese (0.30–0.43);¹⁶ Japanese (0.25–0.48);^{12,27} and Indians (0.31);¹³ but is higher than those reported for Caucasians in whom the frequencies of the S2 allele have been 5–10% in most reports (0.0–0.11).^{14,15,28,29} The clinical significance of possessing this allele has been demonstrated in some case-control studies that showed a 2- to 5-fold increase in frequency in patient groups with premature coronary heart and peripheral vascular disease compared with control groups.^{30–33}

Table 5 Relationship Between Concentrations of Plasma Triglycerides or Apolipoprotein CIII and Apolipoprotein CIII SstI Polymorphism

	Mean $\pm$ SD			p for trend
	S1S1	S1S2	S2S2	
Total (n=249)				
Triglycerides (mg/dl)	137.8 $\pm$ 97.5	153.2 $\pm$ 132.6	219.2 $\pm$ 252.5	0.017
Apolipoprotein CIII (mg/dl)	8.2 $\pm$ 3.6	8.6 $\pm$ 4.7	11.5 $\pm$ 4.7	0.007
Males (n=77)				
Triglycerides (mg/dl)	165.9 $\pm$ 112.9	182.9 $\pm$ 167.0	356.0 $\pm$ 553.0	0.122
Apolipoprotein CIII (mg/dl)	9.4 $\pm$ 4.4	10.3 $\pm$ 6.3	15 $\pm$ 8.5	0.323
Females (n=172)				
Triglycerides (mg/dl)	123.4 $\pm$ 86.0	140.3 $\pm$ 113.5	190.4 $\pm$ 148.2	0.044
Apolipoprotein CIII (mg/dl)	7.72 $\pm$ 3.1	7.87 $\pm$ 3.6	11.2 $\pm$ 4.5	<0.001

Data are mean $\pm$ SD and p<0.05 considered statistically significant. Abbreviation see in Table 1.

The biochemical basis for the association of the S2 allele with HTG has yet to be established. Similar to the finding of other studies,^{34–37} we also found a positive association between plasma levels of ApoCIII and genotypes carrying the S2 variant. The gene coding for ApoCIII is situated 2.6 kbp from the 3' end of the ApoAII gene and 6.5 kbp from the 5' end of the ApoAIV gene.³⁸ The SstI polymorphism, arising from a cytosine to guanine substitution, is located in the 3' UTR of ApoCIII. Therefore, it is more likely that the S2 allele is not etiological, but is in linkage disequilibrium with other unknown causative mutations in ApoCIII or nearby genes involved in the metabolism of TG molecules. It has been suggested that certain haplotypes generated from SstI polymorphism of ApoCIII may predispose to HTG;³⁹ however, few of the studies carried out in Caucasians,^{40,41} Taiwanese,¹⁸ Japanese⁴² and Arabs¹⁷ have not found any significant association between SstI polymorphism and HTG. It is speculated that the linkage disequilibrium between the polymorphic site and the causative mutation is weakened or absent in some populations.³⁷ It should be noted that some of the discrepancies reported in the literature could be related to gene/environmental interactions, such as those described for dietary variations.⁴³

In this study, HTG subjects (134.4 mg/dl) also had significantly ($p < 0.01$) higher fasting glucose concentrations than NTG subjects (106.6 mg/dl). Recently, Salas et al⁴⁴ reported that subjects carrying the S2 variant of ApoCIII SstI polymorphism had an elevated insulin response to an oral glucose tolerance test when they consumed diets rich in saturated fat. The mechanism by which this polymorphism modulates insulin sensitivity is unknown. A possible explanation is that the S2 allele is in disequilibrium with 2 other polymorphisms, –455 T>C and –482 C>T, which occur within the insulin responsive element in the ApoCIII promoter region.¹⁴ ApoCIII expression is normally down-regulated 40–50% by insulin. The presence of a rare allele of either variant (–455 T>C or –482 C>T) was proved sufficient to remove the ability of insulin to inhibit ApoCIII expression⁴⁵ which subsequently leads to increased levels of ApoCIII as well as TGs in the blood. Further, it was previously reported that variation at the C–482T determined the response to the oral glucose tolerance test (variant in the ApoCIII promoter), with carriers of the rare T–482 having significantly elevated glucose and insulin concentrations.⁴⁶ That finding demonstrates some of the interactions occurring between lipid and glucose metabolism and supports the possible involvement of ApoCIII in the development of impaired glucose metabolism.

In the multivariate-adjusted model, ApoCIII S2S2 (OR = 3.35, 95% CI: 1.10–10.19) was positively associated with HTG risk, and a significant linear trend ($p < 0.05$) of ApoCIII and TG concentrations for the S1S1, S1S2, S2S2 genotypes was also detected, further confirming the relationship between the rare S2 allele and risk of HTG (Table 5). As we further analyzed gender, the serum TG and ApoCIII concentrations remained significantly associated with S1S1, S1S2 and S2S2 genotypes in a dose-responsive manner ($p < 0.05$) in the female subjects ($n = 172$), but not in the males. This finding agrees with the conclusions of other reports^{47,48} that have shown an influence of gender on relationships between ApoCIII polymorphisms and plasma lipid variables. Espino-Montoro et al⁴⁷ reported an interaction between gender and ApoCIII SstI polymorphism in essential hypertensive patients. Significantly greater concentrations of plasma TG, C, and fasting plasma glucose were observed

predominantly in female subjects carrying the rare S2 allele. Dallongeville et al⁴⁸ demonstrated that serum TG and APOB concentrations were higher in women bearing the rare allele of SstI than in those with the most common allele, but not in men. These observations, as well as the current results, suggest a gender-related impact of ApoCIII SstI polymorphism on lipid-related variables. We postulate that sexual hormones may influence the production or catabolic rates of apolipoproteins and lipoproteins, which may in turn influence the potential role of ApoCIII on lipid metabolism. Alternatively, differences in lifestyle or diet between men and women may also exercise some influence.

Formation of HTG is determined by a complex interaction between genetic and environmental factors. In the current study, besides ApoCIII SstI polymorphism, several environmental factors, such as socioeconomic background and diet, were also investigated. Our multivariate-adjusted model demonstrated that education level (≤ 6 years of education, OR = 3.71, 95% CI: 1.24–8.13), tribal background (Amis tribe, OR = 3.08, 95% CI: 1.41–6.77), BMI (≥ 25 , OR = 2.22, 95% CI: 1.18–4.16), starchy food consumption (≥ 3 times/week, OR = 1.89, 95% CI: 1.00–3.59) were independently related to risk of HTG. Subjects receiving education ≤ 6 years had a greater risk of HTG (OR = 3.71, 95% CI: 1.24–8.13) than those with more than 6 years of education. A similar influence of education level on blood lipid status has been documented previously by Dennis et al⁴⁹ who reported that less educated men had higher blood pressure, higher HDL-C and, furthermore, in multivariate-adjusted logistic modeling, the relative risk for coronary heart diseases of the least-educated men compared with the most-educated men was 1.9. In the current study, subjects with BMI ≥ 25 were related to HTG risk ($p < 0.05$), which is consistent with other reports.⁵⁰ Further, HTG is one of the clinical parameters of metabolic syndrome. Zhang et al⁵¹ suggested that clustering of metabolic syndrome not only occurs more prevalently in persons with BMI ≥ 25 , but also in normal-weight persons with large weight fluctuation. In addition, hypoadiponectinemia is closely associated with the clinical phenotype for metabolic syndrome, so measuring the plasma concentration of adiponectin may be a useful tool for managing metabolic syndrome.⁵² Among dietary factors, diets high in fat and carbohydrates have been shown to elevate blood TG.⁵³ We found that consumption of starchy food ≥ 3 times/week had a greater risk of HTG compared with < 3 times/week. Excessive consumption of sugar and sweets is likely to contribute to a high frequency of HTG.⁵⁴ However, it has been suggested that the classification of carbohydrates into simple and complex carbohydrates may not be the most helpful predictor of their metabolic properties.⁵³ Recently, it has been suggested that impaired dietary antioxidant status is related to presence of various coronary risk factors, including HTG and hypo-HDL.⁵⁵ Thus, further study is needed to investigate the relationships among various antioxidant-related dietary factors and the risk of HTG.

In the current study, we did not find significant associations between LPL-HindIII polymorphisms and HTG. The frequency of the H+ allele of the LPL-HindIII polymorphism was 0.16 and 0.21 in the HTG and NTG subjects, respectively; there were no differences between the 2 groups. The frequency for H– is lower than that reported in Caucasians^{56,57} and Hispanics,⁵⁸ and is in the range of those found for Chinese.¹⁶ Larger sample sizes may be needed to further confirm the relationship between the rare H– allele

and the risk of HTG.

In conclusion, we found a higher prevalence of the rare S2 allele of molecular variants of ApoCIII SstI polymorphism in subjects with HTG among Taiwan aboriginal females. Distribution of the S2 allele in the study population was in line with that for Asians, such as Chinese, Japanese and Indians. In a multivariate-adjusted logistic regression model, the ApoCIII S2S2 genotype was independently (OR=3.35) associated with the risk of HTG, and the plasma ApoCIII and TG concentrations were increased ($p<0.01$) accordingly among the ApoCIII S1S1, S1S2 and S2S2 genotypes. Besides the ApoCIII S2S2 genotype, environmental factors (ie, education ≤ 6 year (OR=3.71), Amis tribe (OR=3.08), BMI ≥ 25 (OR=2.22), starchy food consumption ≥ 3 times/week and (OR=1.89)) were also found independently ($p<0.05$) related to HTG risk. It appears that both genetic and environmental factors are associated with the phenotype of HTG. Further studies are warranted to examine interactions of ApoCIII polymorphism with other genes related to HTG, as well as with environmental factors.

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