APOA1/C3/A5 haplotype and risk of hypertriglyceridemia in Taiwanese

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Abstract

Background: Apolipoprotein A5 gene (*APOA5*) has been shown to modulate plasma triglyceride concentrations. We investigated 2 distinct *APOA1/C3/A5* haplotypes roles for hypertriglyceridemia.

Methods: We recruited 308 cases of hypertriglyceridemia and 281 normal controls from a hospital. Twelve single nucleotide polymorphisms (SNPs) across the *APOA1/C3/A5* gene region were genotyped.

Results: One haplotype containing the minor alleles of the *APOA5* (−1131T>C, c.553G>T) and *APOA1* (−3013C>T, −75G>A) was more prevalent in cases than in controls (11.3% vs. 1.1%, respectively) and was statistically significantly associated with high triglycerides (adjusted odds ratio: 12.83, 95% confidence interval [CI]: 5.1–32.4, $P<0.001$). Another haplotype that was associated with hypertriglyceridemia (adjusted odds ratio 2.13, 95% CI, 1.37–3.29, $P=0.001$). Participants carrying both minor alleles of *APOA5*-1131CC and c.553TT had a 116% higher triglyceride concentration compared with those carrying common allele.

Conclusions: The *APOA1/C3/A5* haplotype represents an important locus for predicting risk of hypertriglyceridemia among Taiwanese.

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1. Introduction

Hypertriglyceridemia is a common metabolic disease in general population and is associated with the risk of coronary heart disease [1,2]. Increases of serum triglyceride concentrations may be due to either overproduction or accumulation of chylomicrons, very-low-density lipoproteins (VLDL) in circulation and genetic control plays an important role for triglyceride metabolism. Primary hypertriglyceridemia has been associated with lipoprotein lipase (LPL) deficiency, apolipoprotein (APO) CII deficiency or hepatic lipase deficiency [3,4]. Several genes associated with triglyceride metabolism were related to triglyceride concentration in general population. Among the candidate

genes, *APOA1/C3/A5* gene cluster is located on chromosome 11q23 in human and has been well documented as a predictor for triglyceride concentrations. The SstI (3238C>G) single nucleotide polymorphism (SNP) in 3′-flanking region and variation of −482C>T within the insulin-responsive element in the promoter and 1100C>T in exon 3 of *APOC3* has consistently shown an association with a variation in plasma triglycerides [5–9].

A minor haplotype of *APOA5* (−1131C, c.−3G, IVS3+476A and 1259C) and another *APOA5* haplotype (−1131T, c.56G, IVS3+476G and 1259T) associated with high plasma triglyceride concentrations were reported [10–16]. The c.553G>T polymorphism in the coding region of *APOA5* has shown an association with hypertriglyceridemia in Chinese. In addition to this polymorphism in the *APOA5*, the haplotype of *APOA5* (IVS3+476G, c.457G and c.553T) is independently associated with hypertriglyceridemia [17]. Furthermore, minor alleles at the *APOA1* loci have been associated with combined hyperlipidemia, or hypertriglyceridemia in some studies [18–20], but without

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replication [21,22]. We conducted the case-control study on hypertriglyceridemia in Taiwanese and performed the genetic association study on the numerous SNPs on the *APOA1/C3/A5* gene cluster.

2. Materials and methods

2.1. Subjects and design

Three hundred and eight patients with hypertriglyceridemia were selected for study. Hypertriglyceridemia was diagnosed on the basis of the lipid concentration (serum triglyceride >400 mg/dl) through the metabolic clinic of National Taiwan University Hospital. Patients with secondary hyperlipoproteinemia, hypertension, diabetes, taking primary lipid-lowering drugs, or endocrine or metabolic disorders were excluded. The control subjects consisted of 281 individuals who were recruited from the health checkup in the same hospital. All subjects are Taiwanese and gave their informed consent before participation. The Medical Ethics Committee of National Taiwan University Hospital approved the study protocol. Anthropometric measurements including body weight and body height were obtained in each participant.

2.2. DNA analysis

The DNA direct sequence or polymerase chain reaction (PCR) then restriction enzyme digestion were carried out to genotype all markers. Primer sequences for genotyping and the PCR conditions were posted on line (Supplementary Tables 1 and 2). To analyze both *APOC3* -455T>C and -482C>T variants, a touch down PCR was carried out. Restriction enzymes were added to the PCR products and resolved on 3% agarose gels. All PCRs were performed in a GeneAmp® PCR System (Applied Biosystems Foster City, CA).

APOA5 c.1269T>C, c.553 G>T, IVS+476G>A polymorphisms were genotyped as previously described [17]. *APOA5* c.-3A>G and c.56C>G polymorphisms were genotyped by direct sequencing by using a 3100 DNA genetic analyzer (Applied Biosystems). Nucleotides were numbered as suggested by the Human Genome Variation Society [23], with nucleotide number 1 being the A of the ATG initiation codon.

2.3. Lipid analysis

Serum total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, and triglyceride (TG) concentrations were measured enzymatically on a Hitachi 7450 Analyzer (Hitachi, Japan) using Roche reagents. Total percentage coefficients of variation using pooled sera ranged from 3.5 to 7.6% for total cholesterol, 4.7 to 11.4% for triglyceride, 4.2 to 10.8% for LDL cholesterol and 4.1 to 11.3% for HDL cholesterol.

2.4. Statistical analyses

Clinical characteristics of cases and control subjects were compared by the Student's *t* test. We estimated the allele frequencies by gene counting methods and calculated from the EM algorithm. We tested the Hardy–Weinberg equilibrium in cases and control subjects separately. Contingency χ^2 statistics were used to test differences in allele frequencies between cases and control subjects. We calculated pair-wise linkage disequilibrium, *D'* and correlation (r^2) values and the disequilibrium coefficient was divided by maximum disequilibrium and respective significance concentrations [24]. Serum triglyceride concentration difference among every genotype was tested with multiple linear regression models, after adjusting for age, gender and body mass index. The adjusted odds ratios were calculated by using multivariate logistic regression analysis, and we used the co-dominant modes of inheritance to avoid assumptions, with 0 coded for homozygous wild-type and two dummy variables coded for heterozygous and homozygous variants. We designated the inferred haplotype distribution in the study participants accounting for haplotype uncertainty by multiple imputation methods [25]. For haplotype analysis, we estimated the haplotype frequency of 12 genotype markers by PM (permutation and model-free analysis) and EH (estimating haplotypes) programs, which were

implemented in SAS/Genetics software [26,27]. We evaluated the haplotype effects if the haplotype frequency was >1% in the study population. The adjusted odds ratios of various haplotypes were estimated after adjusting for age, gender and body mass index effects. We also estimated the triglyceride concentrations according to various haplotypes among control subjects using general linear models after adjusting for age, gender and body mass index.

3. Results

3.1. Lipid profile between patients and controls and DNA sequencing

The gender distributions between the hypertriglyceridemic and control subjects were similar. Compared with control subjects, the hypertriglyceridemic patients had significantly higher age (55.6 vs. 50.3 y), body mass index (26.1 vs. 23.1 kg/m²), triglyceride (648 vs. 90 mg/dl) and low HDL-C (38.7 vs. 51.8 mg/dl) concentrations than control subjects.

A total of 12 SNPs were analyzed with 4 in each of the 3 genes. The *APOA5* IVS+476G>A, c.553G>T and 1259T>C were published previously [17]. Thus, Table 1 lists the other 9 SNPs. The genotype frequencies of these variable sites were all in Hardy–Weinberg equilibrium. Of these, 5 sites (*APOA1* 2169G>C, *APOC3* 3238C>G, -455T>C, -482C>T and *APOA5* -1131T>C) revealed significant differences in genotype frequencies between the hypertriglyceridemic and control groups ($P<0.05$). The other 4 sites (*APOA1* -3013C>T, -75G>A, IVS3+135C>T and *APOC3* IVS1+122G>C) were insignificant. When allele frequency was analyzed by gene counting *APOA1* IVS3+135C>T became statistically different between the hypertriglyceridemic and control groups ($P<0.02$). The other 3 SNPs at *APOA1* -3013C>T, -75G>A and *APOC3* IVS1+122G>C remained no differences between groups. No polymorphisms were found for both *APOA5* c.-3A>G and c.56C>G (data not shown) in this population. Both sites were not analyzed afterward.

3.2. Linkage disequilibrium and association analysis

There was significant linkage disequilibrium between large numbers of SNPs (data not shown). Seventeen percent of all pair-wise *D'* values were >0.9 in normal subjects. Strong linkage disequilibrium was found in *APOA5* -1131T>C with all the other *APOC3* variants and *APOA1* IVS3+135C>T. While *APOA5* c.553G>T showed strong linkage disequilibrium with *APOA5* -1131T>C and *APOA1* -3013C>T, there was no significant linkage disequilibrium with all the other *APOC3* variants. We found in normal controls a significant association between serum triglyceride concentrations and 3 of the 9 SNPs analyzed in this study (Table 2). The minor allele of each of these three polymorphisms (*APOA1* IVS3+135T, *APOC3* 3238G and *APOA5* -1131C, respectively) was associated with higher serum triglyceride concentrations. Multiple logistic regression for best selection model in above genotypes after adjusting age, gender and body mass index is shown in Table 3. The *APOA5* -1131C minor allele remains the most prominent that is associated to hypertriglyceridemia risk with an odds ratio of 6.37 (95% confidence interval [CI], 4.08–9.95, $P<0.0001$).

In addition, we found that carriers for the *APOA1* 2169C are protective against hypertriglyceridemia (odds ratio of 0.38, 95% CI, 0.18–0.83, $P=0.015$) but *APOA1* IVS3+135C>T became a no significant predictor of risk of hypertriglyceridemia. However, neither *APOA5* c.553G>T nor *APOA5* –1131T>C is linked to *APOA1* 2169G>C (the coefficients of linkage disequilibrium are 0.0182, 0.2567, respectively).

3.3. Haplotype analysis and classification of triglyceride-raising haplotypes

Table 4 summarized the 14 most common haplotypes with their relative frequencies. Of these, 4 (haplotypes 1–4) among

Table 1
Comparison of genotype and allele frequencies of the polymorphisms in the *APOA1/C3/A5* gene between control and hypertriglyceridemia group

		Controls (n)	Hypertriglyceridemia (n)	P
<i>APOA1</i> –3013C>T	C/C	0.529(145)	0.463(130)	NS
	C/T	0.358(98)	0.423(119)	
	T/T	0.113(31)	0.114(32)	
	C allele	0.708(388)	0.674(379)	NS
	T allele	0.292(160)	0.326(183)	
<i>APOA1</i> –75G>A	G/G	0.486(119)	0.470(132)	NS
	G/A	0.396(97)	0.423(119)	
	A/A	0.118(29)	0.107(30)	
	G allele	0.684(335)	0.681(383)	NS
	A allele	0.316(155)	0.319(179)	
<i>APOA1</i> IVS3+135C>T	C/C	0.514(125)	0.430(122)	0.069
	C/T	0.391(95)	0.422(120)	
	T/T	0.095(23)	0.148(42)	
	C allele	0.710(345)	0.641(364)	0.017
	T allele	0.290(141)	0.359(204)	
<i>APOA1</i> 2169G>C	G/G	0.115(28)	0.050(14)	0.006
	G/C	0.885(215)	0.950(267)	
	C/C	0(0)	0(0)	
	G allele	0.942(458)	0.975(548)	0.007
	C allele	0.058(28)	0.025(14)	
<i>APOC3</i> 3238C>G	C/C	0.493(139)	0.391(117)	0.041
	C/G	0.411(116)	0.478(143)	
	G/G	0.096(27)	0.131(39)	
	C allele	0.699(394)	0.630(377)	0.014
	G allele	0.301(170)	0.370(221)	
<i>APOC3</i> IVS1+122G>C	G/G	0.894(219)	0.905(256)	NS
	G/C	0.098(24)	0.095(27)	
	C/C	0.008(2)	0(0)	
	G allele	0.943(462)	0.952(539)	NS
	C allele	0.057(28)	0.048(27)	
<i>APOC3</i> –455T>C	T/T	0.372(105)	0.244(73)	0.003
	T/C	0.426(120)	0.482(144)	
	C/C	0.202(57)	0.274(82)	
	T allele	0.585(330)	0.485(290)	0.001
	C allele	0.415(234)	0.515(308)	
<i>APOC3</i> –482C>T	C/C	0.362(102)	0.247(74)	0.011
	C/T	0.418(118)	0.492(147)	
	T/T	0.220(62)	0.261(78)	
	C allele	0.571(322)	0.493(295)	0.008
	T allele	0.429(242)	0.507(303)	
<i>APOA5</i> –1131T>C	T/T	0.578(163)	0.191(56)	0.000
	T/C	0.376(106)	0.430(126)	
	C/C	0.046(13)	0.379(111)	
	T allele	0.766(432)	0.406(238)	0.000
	C allele	0.234(132)	0.594(348)	

Table 2

Triglyceride levels (mg/dl) according to different SNPs in control group

		Triglyceride (mean±SD)	P
<i>APOA1</i> –3013C>T	C/C	90.9±34.3	NS
	C/T	90.4±38.3	
	T/T	85.9±35.7	
<i>APOA1</i> –75 G>A	G/G	93.5±35.6	NS
	G/A	89.4±39.5	
	A/A	84.0±24.5	
<i>APOA1</i> IVS3+135C>T	C/C	84.0±31.7	0.003
	C/T	100.8±38.4	
	T/T	89.3±40.9	
<i>APOA1</i> 2169G>C	G/G	91.6±36.3	NS
	G/C	87.1±34.9	
	C/C	–	
<i>APOC3</i> 3238C>G	C/C	83.4±31.9	0.008
	C/G	97.1±38.5	
	G/G	92.8±38.6	
<i>APOC3</i> IVS1+122G>C	G/G	90.9±36.4	NS
	G/C	90.2±35.5	
	C/C	80.0±28.3	
<i>APOC3</i> –455T>C	T/T	83.7±32.5	0.079
	T/C	93.9±37.8	
	C/C	93.0±36.6	
<i>APOC3</i> –482C>T	C/C	84.5±32.3	NS
	C/T	94.7±39.7	
	T/T	89.7±32.9	
<i>APOA5</i> –1131T>C	T/T	80.9±29.7	<0.0001
	T/C	100.5±39.1	
	C/C	116.8±44.2	

control subjects and 3 (haplotypes 1, 2, and 5) among the hypertriglyceridemia cases had a frequency >10%. Haplotype 1 denotes the major allele; haplotype 2 is composed of 5 major and 7 minor alleles, haplotype 3, 10 majors and 2 minors of *APOA1* –3013T and –75A, and haplotype 5, 8 majors and 2 minors of *APOA5* –1131C and c.553T, in addition to the two minors in haplotype 3. Haplotype 5 occurred at a significantly higher frequency in hypertriglyceridemia than in control subjects ($P<0.0001$). Significant differences were found between normal controls and hypertriglyceridemia for haplotypes 1 (19.2% vs. 10.7%, $P=0.005$), 2 (10.4% vs. 18.3%, $P=0.006$), 3 (19.1% vs. 7.2%, $P=0.000$) and 5 (1.1% vs. 11.3%, $P=0.000$). The odds ratios and 95% CI of various haplotypes in the multiple logistic regression model in various haplotypes after adjusting age, gender and body mass index are also listed in Table 4. Haplotype 5 was still the most significantly related to hypertriglyceridemia risk with an adjusted odds ratio of 12.83 (95% CI, 5.08–32.4, $P<0.0001$). These analyses suggested that haplotype 5 seems to be specific for hypertriglyceridemic subjects.

Moreover, significant differences in serum triglyceride concentrations were found according to different haplotypes among control subjects. Of the 14 most common haplotypes, 10, 11, and 13 were not found in normal control subjects. Normal control subjects who carried the most common haplotype 1, accounting for 19.2%, had a mean triglyceride concentration of 86.7 mg/dl (Table 4). Subjects who carry 5, 2, 6, and 4 haplotypes had triglycerides higher than those with haplotype 1. Furthermore, subjects with haplotype 5, a predictor

Table 3

Odds ratio and 95% confidence intervals in the multiple logistic regression models after adjusting for age, gender and body mass index

	Genotype	Odds ratio (95% CI)	P
<i>APOA1</i> –3013C>T	(CT and TT)/CC	1.57 (1.06–2.32)	0.026
<i>APOA1</i> –75 G>A	(GA and AA)/GG	1.29 (0.86–1.93)	NS
<i>APOA1</i> IVS3+135C>T	(CT and TT)/CC	1.58 (0.80–3.14)	NS
<i>APOA1</i> 2169G>C	(GC and CC)/GG	0.38 (0.18–0.83)	0.015
<i>APOC3</i> 3238C>G	(CG and GG)/CC	1.59 (1.08–2.33)	0.018
<i>APOC3</i> IVS1+122G>C	(GC and CC)/GG	0.95 (0.50–1.80)	NS
<i>APOC3</i> –455T>C	(TC and CC)/TT	1.72 (1.13–2.61)	0.011
<i>APOC3</i> –482C>T	(CT and TT)/CC	1.69 (1.11–2.56)	0.014
<i>APOA5</i> –1131T>C	(TC and CC)/TT	6.37 (4.08–9.95)	<0.0001

for hypertriglyceridemia, had triglyceride concentration of 125.5 mg/dl, the highest among all groups. Because haplotype 5 is different from haplotype 3, which is found in controls with an averaged triglyceride concentration of 86.3 mg/dl, by two additional minor alleles at *APOA5* –1131C and c.553T, the latter 2 SNPs are better predictors for hypertriglyceridemia than *APOA1* –3013C>T and –75G>A carried in both haplotypes 3 and 5. Two (haplotypes 2 and 6) of the other 3 haplotypes had higher triglyceride concentrations than the subjects carrying the *APOA5* 1259C minor allele. Subjects with haplotype 9 had the

lowest TG concentration (73.8 mg/dl) among all normal control subjects. The haplotype 9 is identical to haplotype 1 in 11 of the 12 SNPs and different in *APOA1* 2169G>C. The results suggested that *APOA1* 2169C was a protective factor for the risk of hypertriglyceridemia.

Because control subjects carrying haplotype 5, harboring both *APOA5* –1131C and c.553T, had the highest triglyceride concentrations, we next examined the effect of minor allele homozygotes on triglyceride concentrations. Compared with the participants with major alleles (*APOA5* –1131TT and c.553GG), those homozygous for both SNPs (*APOA5* –1131CC and c.553TT) had higher triglyceride concentrations (Fig. 1). Subjects homozygous for only *APOA5* c.553TT were not recruited in this study. Subjects homozygous for –1131CC had higher triglycerides than those with major alleles (–1131TT). Furthermore, participants carrying both minor allele of *APOA5* –1131CC and c.553TT showed a significant higher triglyceride concentration. These results indicated that *APOA5* –1131C and c.553T were significantly predictive for hypertriglyceridemia risk.

4. Discussion

In this case-control association study, two distinct haplotypes, haplotypes 2 and 5, from the 12 SNPs of the *APOA1/C3/*

Table 4

Common haplotype derived from all polymorphic sites using all the genotype data

Haplo type	Frequency, %				P	<i>APOA1</i>				<i>APOC3</i>				<i>APOA5</i>				OR ^c	95% CI	P
	All	Normal (TG ^a)	HTG ^b			–3013 C>T	–75 G>A	IVS3+135 C>T	2169 G>C	3238 C>G	IVS1+122 G>C	–455 T>C	–482 C>T	–1131 T>C	IVS3+476 G>A	c.553 G>T	c.1259 T>C			
1	14.5	19.2 (86.7)	10.7	0.005	C	G	C	G	C	G	T	C	T	G	G	T		0.4	0.25, 0.62	<0.0001
2	14.2	10.4 (108.7)	18.3	0.006	C	G	T	G	G	G	C	T	C	A	G	C		2.13	1.37, 3.29	0.001
3	13.3	19.1 (86.3)	7.2	0.000	T	A	C	G	C	G	T	C	T	G	G	T		0.35	0.22, 0.55	<0.0001
4	8.9	10.3 (89.1)	7.5	NS	C	G	T	G	G	G	C	T	T	G	G	T		0.59	0.35, 1.00	0.052
5	6.1	1.1 (125.5)	11.3	0.000	T	A	C	G	C	G	T	C	C	G	T	T		12.83	5.08, 32.41	<0.0001
6	4.7	3.6 (97.6)	5.8	NS	T	A	C	G	C	G	T	C	C	A	G	C		2.73	1.32, 5.64	0.007
7	4.1	4.9 (81.7)	3.0	NS	C	G	C	G	C	C	C	T	T	G	G	T		0.65	0.32, 1.34	NS
8	3.6	4.5 (78.1)	3.1	NS	C	G	C	G	C	G	C	T	T	G	G	T		0.27	0.12, 0.64	0.003
9	3.5	4.9 (73.8)	2.1	NS	C	G	C	C	C	G	T	C	T	G	G	T		0.43	0.17, 1.04	0.06
10	2.0	0.0	3.4	0.002	C	G	T	G	G	G	C	T	C	G	T	T				
11	1.9	0.0	3.4	0.002	T	A	C	G	C	G	C	T	C	G	T	T				
12	1.6	0.9	2.2	NS	C	G	C	G	C	G	C	T	C	G	T	T				
13	1.5	0.0	2.1	0.031	C	G	C	G	C	G	T	C	C	A	G	C				
14	1.0	1.8 (76.5)	0.0	0.025	C	G	T	G	G	G	T	C	T	G	G	T				

The 14 depicted haplotypes account for 80.9% of all haplotypes, none of the other predicted haplotypes had a frequency of greater than 1%. The minor alleles that define the haplotypes are highlighted in bold.

^a Indicates average triglyceride concentration in mg/dl.

^b Indicates hypertriglyceridemia.

^c Odds ratio.

Fig. 1. Triglyceride concentrations in normal subjects after adjusting age, gender and BMI, according to combined *APOA5* -1131T>C and *APOA5* c.553G>T genotypes. Subjects carrying *APOA5* -1131CC and c.553TT had higher mean serum triglyceride concentrations than those with wild-type.

A5 gene cluster, were significantly associated with the risk of hypertriglyceridemia in Taiwanese. These associations were independent of age, gender and body mass index and were not explained by other confounding factors. Our findings provide a new, relevant evidence of risk in Taiwanese and comprehensive information on the *APOA1/C3/A5* gene.

Our data suggested that haplotype 5 was a strongly predictive factor for hypertriglyceridemia, which were supported from the data on control subjects. Haplotype 5 contains 8 major and 4 minor alleles (*APOA1* -3013T, -75A, and *APOA5* -1131C, c.553T) in the 12 SNPs, compared with other haplotypes that varied in *APOA1* -3013T and -75A. Pennacchio et al. reported that a minor haplotype of *APOA5* (-1131C, IVS3+476A and c.1259C) was associated with a 20–30% elevation in plasma triglyceride concentrations in Caucasian men and women [11], but we cannot replicate the results among participants carrying this haplotype (i.e. haplotype 6). Our findings confirmed that the genetic predisposition to hypertriglyceridemia varied between different ethnicity.

We are convinced that the SNPs in *APOA5* were important for controlling hypertriglyceridemia risk. Talmud et al. [12] reported that *APOA5* c.56G and -1131C had 52% and 40%, respectively, higher triglyceride concentrations compared with the common allele homozygotes (c.56CC and -1131TT), and the two SNPs' effects were independent and additive. Pennacchio and colleagues showed that in Caucasian the predictive genotypes for the disease are *APOA5* c.-3A>G and c.56C>G [13], and that the minor allele of the c.56C>G associated with familial combined hyperlipidemia was also reported in Dutch family [28]. However, neither *APOA5* c.-3A>G nor c.56C>G

polymorphism was found in this ethnicity study. While *APOA5* -1131T>C had a significant independent effect on the serum triglyceride concentration in Japanese [14], this association was not significant in a population-based Spanish control group [15]. Our data identified that the allele frequencies of *APOA5* -1131T>C polymorphism were significantly higher in hypertriglyceridemic patients, which was consistent with the Japanese data. Furthermore, although there is strong linkage disequilibrium between *APOA5* -1131T>C and *APOA5* c.553G>T, subject carrying both minor allele of *APOA5* -1131CC and c.553TT still predicted additive high triglyceride concentrations, indicating that *APOA5* c.553G>T enhanced the risk of hypertriglyceridemia by *APOA5* -1131T>C. These synergistically additive effects were biologically plausible and supported by the pathway of triglyceride metabolism: Fruchart-Najib et al. reported an increased hydrolysis of VLDL in vitro by free LPL in the presence of very high amounts of recombinant *APOA5* [29], and Schaap et al. showed an influence of *APOA5* on LPL-mediated lipolysis of triglyceride-rich, apolipoprotein-free emulsions [30]. Merkel et al. also demonstrated that in the presence of proteoglycans, *APOA5* led to a significant and dose-dependent increase in LPL-mediated hydrolysis of VLDL triglycerides [31]. Qi and colleagues demonstrated that *APOA1/C3/A4/A5* haplotypes affected triglyceride and HDL cholesterol among women with diabetes [16]. All these data suggested that the importance of *APOA5* in the regulation of plasma triglyceride concentrations. Because the c.553G>T polymorphic site is located in the translated region of the *APOA5*, affecting the amino acid residue 185 causing a substitution of a cysteine which contains sulfur atom, and easily forming disulfide bond

for a glycine residue, this amino acid change may enhance the conformation or function of the APOA5 related to hypertriglyceridemia.

The frequency of haplotype 2 was significantly higher in hypertriglyceridemia cases than in control subjects. Due to carrying the minor alleles of *APOA5* –1131T>C, IVS3+476G>A and c.1259T>C, haplotype 2 is similar to the *APOA5**2 haplotype that was previously reported to associate with increased plasma triglyceride concentrations [13]. Haplotype 2 is distinguished from haplotype 6 with its comparably higher frequency among hypertriglyceridemia cases and its variations in 3 of the 4 SNPs in both *APOC3* and *APOA1* loci. Our data suggested that carriers of the *APOA1* 2169C minor allele had a marginally significant lowering effect on triglycerides.

The limitations of this study include that fact our participants were recruited from hospital; therefore, the data are not applicable to general population. Second, because we selected the SNPs from literature and did not cover the extensive *APOA1/C3/A4/A5* gene locus, we might miss some information from other SNPs. We did not include *APOA4* SNPs in this study, because there is no *APOA4* SNP is associated with hypertriglyceridemia, except *APOA4* T347S associated with a TG-lowering effect [32]. Nevertheless, we constructed haplotypes with tagged SNPs and the results are valid for the association study on the risk of hypertriglyceridemia. Finally, we did not correct the significant values induced by multiple testing and the type I error rates might be inflated. We considered the one-locus SNP study as descriptive results, and we used the global test of haplotypes.

In this case-control study, we established that 2 distinct haplotypes contribute to the hypertriglyceridemia in Taiwanese. Both haplotypes comprise neither *APOA5* c.–3A>G nor c.56C>G polymorphism which were reported among Caucasians and other ethnicity. The results of our study extend and add new information to the existing data regarding the association between *APOA1/C3/A5* gene cluster and hypertriglyceridemia. Further studies are warranted to determine the molecular mechanism of these haplotypes on the regulation of triglyceride concentrations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cca.2007.12.014.

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