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

1. Introduction

Hypertriglyceridemia is a common metabolic disease in general population and is associated with the risk of coronary heart disease [1,2]. Elevation of serum triglyceride levels 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 LPL deficiency, apolipoprotein (APO) CII deficiency or HL deficiency [3,4]. Several genes associated with triglyceride metabolism were related to triglyceride level 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 levels. The *Sst*I (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 levels were reported [10,11,12-15]. 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 [16]. Furthermore, minor alleles at the *APOA1* loci have

been associated with combined hyperlipidemia, or hypertriglyceridemia in some studies [17-19], but without replication [20, 21].

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. We focused on the haplotype patterns and estimated the relative risks after adjusting gender, age, and body mass index. Of all haplotypes, we established 2 distinct haplotypes that contribute remarkably to the hypertriglyceridemia in Taiwanese.

2. Materials and methods

2.1 Subjects

Three hundred and eight patients with hypertriglyceridemia were selected for study. Hypertriglyceridemia was diagnosed on the basis of the lipid level (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 and detailed medical history 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. 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 Division of Perkin-Elmer Corp.).

APOA5 c.1269T>C, c.553 G>T, IVS +476G>A polymorphisms were genotyped as previously described [16]. *APOA5* c.-3A>G and c.56C>G polymorphisms were genotyped by direct sequencing by using a 3100 DNA genetic analyzer (Applied Biosystems Division of Perkin-Elmer Corp.). Nucleotides were numbered as suggested by the Human Genome Variation Society [22], 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 levels 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. 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 levels [23]. Serum triglyceride level 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. We designated the inferred haplotype distribution in the study participants accounting for haplotype uncertainty by multiple imputation methods [24]. 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 [25,26]. We evaluated the haplotype effects if the haplotype frequency was more than 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 levels 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

No statistically significant differences were found between genders. The hypertriglyceridemic subjects were likely to be older, and have a higher body mass index and a higher total cholesterol concentration, compared with the control subjects.

A total of 12 SNPs were analyzed with 4 in each of the three genes. The *APOA5* IVS+476G>A, c.553G>T and 1259T>C were published previously [16]. 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

The linkage disequilibrium amongst the variants in normal subjects is shown in Table 2. There was significant linkage disequilibrium between large numbers of SNPs. Seventeen percent of all pair wise *D'* values were greater than 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 *APOA*5 -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 levels and 3 of the 9 SNPs analyzed in this study (Table 3). The minor allele of each of these three polymorphisms (*APOA1* IVS3+135T, *APOC3* 3238G and *APOA5* -1131C, respectively) was associated with higher serum triglyceride levels. Multiple logistic regression for best selection model in above genotypes after adjusting age, gender and body mass index is shown in Table 4. 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.

3.3 Haplotype analysis and classification of triglyceride-raising haplotypes

Table 5 summarized the 14 most common haplotypes with their relative frequencies. Of these, 4 (haplotypes 1, 2, 3, and 4) among control subjects and 3 (haplotypes 1, 2, and 5) among the hypertriglyceridemia g 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 5. 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 level of 86.7 mg/dl (Table 5). Subjects who carry 5, 2, 6, and 4 haplotypes had triglycerides higher than those with haplotype 1. Furthermore, subjects with haplotype 5, a predictor for hypertriglyceridemia, had triglyceride level 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 level 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 levels than the subjects carrying the *APOA5* 1259C minor allele. Subjects with haplotype 9 had the lowest TG level (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 hat *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 levels, we next examined the effect of minor allele homozygotes on triglyceride levels. 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 levels (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 level. These results indicated that *APOA5* -1131C and c.553T were significantly predictive for hypertriglyceridemia risk.

4. Discussion

In this well-designed case-control association study, two distinct haplotypes, haplotypes 2 and 5, from the 12 SNPs of the *APOA1/C3/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 levels in Caucasian men and women [11], but we can not

replicate the results among participants carrying this haplotype (i.c. 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 levels 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 [27]. 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 level 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 levels, 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 and colleagues reported an increased hydrolysis of VLDL in vitro by free LPL in the presence of very high amounts of recombinant APOA5 [28], and Schaap et al. showed an influence of APOA5 on LPL-mediated lipolysis of triglyceride-rich,

apolipoprotein-free emulsions [29]. 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 [30]. All these data suggested that the importance of APOA5 in the regulation of plasma triglyceride levels. 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 levels [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/A5* gene locus, we might miss some information from other SNPs. Nevertheless, we constructed haplotypes with tagged SNPs and the results are valid for the association study on the risk of hypertriglyceridemia.

In this well-designed 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 levels.

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Table 1

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

		Controls (*n*)	Hypertriglyceridemia (*n*)	*P*
APOA1 -3013C>T	C/C	0.529(145)	0.463(130)	0.249
	C/T	0.358(98)	0.423(119)	
	T/T	0.113(31)	0.114(32)	
	C allele	0.708(388)	0.674(379)	0.225
	T allele	0.292(160)	0.326(183)	
APOA1 -75G>A	G/G	0.486(119)	0.470(132)	0.791
	G/A	0.396(97)	0.423(119)	
	A/A	0.118(29)	0.107(30)	
	G allele	0.684(335)	0.681(383)	0.940
	A allele	0.316(155)	0.319(179)	
APOA1 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)	
APOA1 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)	
APOC3 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)	
APOC3 IVS1 +122G>C	G/G	0.894(219)	0.905(256)	0.311
	G/C	0.098(24)	0.095(27)	
	C/C	0.008(2)	0(0)	
	G allele	0.943(462)	0.952(539)	0.491
	C allele	0.057(28)	0.048(27)	
APOC3 -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)	
APOC3 -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)	
APOA5 -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

Linkage disequilibrium between all the variants in normal subjects. Left lower triangle part as *D'*, and right upper part as *P* values for pair-wise linkage disequilibrium.

	APOA1 -3013 C>T	*APOA1* -75 G>A	*APOA1* IVS3 +135 C>T	*APOA1* 2169 G>C	*APOC3* 3238 C>G	*APOC3* IVS1 +122 G>C	*APOC3* -455 T>C	*APOC3* -482 C>T	*APOA5* -1131 T>C	*APOA5* IVS3 +476 G>A	*APOA5* c.553 G>T	*APOA5* c.1259 T>C
APOA1 -3013C>T		0	0	0.1658	0	0.0091	0	0	0.3294	0.1238	0.0314	0.1106
APOA1 -75G>A	0.9129		0	0.0087	0	0.0088	0	0	0.2500	0.3370	0.2895	0.3901
APOA1 IVS3 +135C>T	0.9679	0.8330		0.0137	0	0.0137	0	0	0.0001	0	0.0786	0
APOA1 2169G>C	0.5279	1	1		0.0124	0.3406	0.0111	0.0063	0.5861	0.4820	0.7983	0.4549
APOC3 3238C>G	0.9685	0.8335	0.9796	1		0.0125	0	0	0	0	0.1126	0
APOC3 IVS1 +122G>C	1	1	1	1	1		0	0.0000	0.0349	0.4666	0.6441	0.2402
APOC3 -455T>C	0.8810	0.7918	0.8206	0.7982	0.8269	1		0	0	0	0.4669	0.0000

APOC3 -482C>T	0.8651	0.7771	0.8144	0.8336	0.8329	1	0.9541		0.0001	0	0.6141	0.0005
APOA5 -1131T>C	0.1671	0.1973	0.2998	0.2567	0.3554	1	0.4227	0.3653		0	0	0
APOA5 IVS3 +476G>A	0.3010	0.1901	0.5775	0.3826	0.6194	0.3983	0.5691	0.5051	0.8948		0.0600	0
APOA5 c.553G>T	0.3668	0.2074	0.7919	0.0182	0.6178	0.5402	0.2210	0.1488	0.7145	1		0.6713
APOA5 c.1259T>C	0.285	0.1536	0.4069	0.3671	0.4621	0.5802	0.4119	0.3409	0.7008	0.9123	0.2066	

Table 3

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

		Triglyceride (mean±SD)	*P*
APOA1 -3013C>T	C/C	90.9±34.3	0.782
	C/T	90.4±38.3	
	T/T	85.9±35.7	
APOA1 -75 G>A	G/G	93.5±35.6	0.408
	G/A	89.4±39.5	
	A/A	84.0±24.5	
APOA1 IVS3 +135C>T	C/C	84.0±31.7	0.003
	C/T	100.8±38.4	
	T/T	89.3±40.9	
APOA1 2169G>C	G/G	91.6±36.3	0.537
	G/C	87.1±34.9	
	C/C	-	
APOC3 3238C>G	C/C	83.4±31.9	0.008
	C/G	97.1±38.5	
	G/G	92.8±38.6	
APOC3 IVS1 +122G>C	G/G	90.9±36.4	0.911
	G/C	90.2±35.5	
	C/C	80.0±28.3	
APOC3 -455T>C	T/T	83.7±32.5	0.079
	T/C	93.9±37.8	
	C/C	93.0±36.6	

APOC3 -482C>T	C/C	84.5±32.3	0.112
	C/T	94.7±39.7	
	T/T	89.7±32.9	
APOA5 -1131T>C	T/T	80.9±29.7	<0.0001
	T/C	100.5±39.1	
	C/C	116.8±44.2	

Table 4

Multiple logistic regression after adjusting age, gender and BMI

	Variable	Odds ratio (95% CI)	*P*
APOA1 -3013C>T	(CT and TT)/CC	1.57 (1.06-2.32)	0.026
APOA1 -75 G>A	(GA and AA)/GG	1.29 (0.86-1.93)	0.212
APOA1 IVS3 +135C>T	(CT and TT)/CC	1.58 (0.80-3.14)	0.190
APOA1 2169G>C	(GC and CC)/GG	0.38 (0.18-0.83)	0.015
APOC3 3238C>G	(CG and GG)/CC	1.59 (1.08-2.33)	0.018
APOC3 IVS1 +122G>C	(GC and CC)/GG	0.95 (0.50-1.80)	0.864
APOC3 -455T>C	(TC and CC)/TT	1.72 (1.13-2.61)	0.011
APOC3 -482C>T	(CT and TT)/CC	1.69 (1.11-2.56)	0.014
APOA5 -1131T>C	(TC and CC)/TT	6.37 (4.08-9.95)	<0.0001

Table 5

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

Haplotype	Frequency, %			P	*APOA1*				*APOC3*				*APOA5*				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	0.306	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	0.323	**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	0.384	C	G	C	G	C	**C**	**C**	**T**	T	G	G	T	0.65	0.32, 1.34	0.24
8	3.6	4.5 (78.1)	3.1	0.505	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	0.100	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	0.288	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			

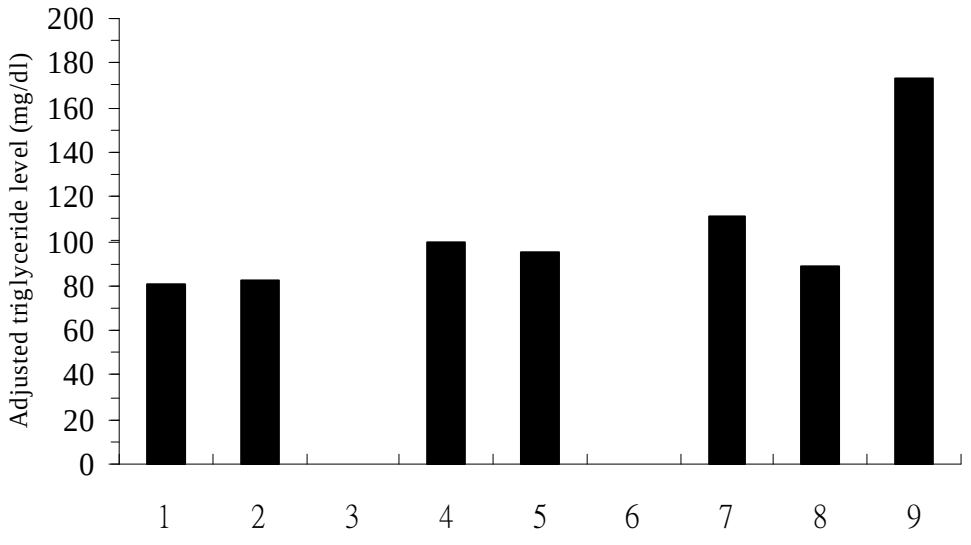
[a] indicates average triglyceride concentration in mg/dl.

[b] indicates hypertriglyceridemia.

[c] Odds ratio.

Fig. 1. Triglyceride levels 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 had higher mean serum triglyceride levels than those with wild type (111.2 mg/dl for *APOA5* c.553GG, 88.8 mg/dl for *APOA5* c.553GT versus 80.4 mg/dl for those wild type).

Fig. 1.



	1	2	3	4	5	6	7	8	9
Observed number	158	5	0	85	20	0	10	2	2
APOA5 -1131T>C	TT	TT	TT	TC	TC	TC	CC	CC	CC
APOA5 c.553G>T	GG	GT	TT	GG	GT	TT	GG	GT	TT

Evaluation:

We have finished our project and have submitted the manuscript for publication. The results reached our goal.