in vivo *25*: 425-430 (2011)

Comparison of Lipid Profiles with *APOA1 MspI* Polymorphism in Obese Children with Hyperlipidemia

BAHAR TOPTAS[1], UZAY GÖRMÜŞ[1], ARZU ERGEN[1], HAKAN GÜRKAN[2], FATİH KELEŞOĞLU[3], FEYZA DARENDELİLER[3], FİRDEVS BAS[3], ALTAY BURAK DALAN[1], GÜLDAL İZBIRAK[4] and TURGAY ISBIR[5]

[1]*Department of Molecular Medicine, Institute of Experimental Medicine, and*
[2]*Department of Medical Biology, Institute of Health Sciences, Istanbul University, Capa-Istanbul, Turkey;*
[3]*Department of Pediatric Endocrinology, Faculty of Medicine, Istanbul University, Istanbul, Turkey;*
[4]*Department of Family Medicine, and* [5]*Department of Medical Biology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey*

Abstract. *Background: Obesity is a multifactorial, chronic disorder leading to adverse metabolic effects on plasma lipid levels. Apolipoprotein AI (Apo AI) is the major structural component of high-density lipoprotein (HDL) and is involved in the esterification of cholesterol as a cofactor of lecithin-cholesterol acyltransferase (LCAT) and thus plays a major role in cholesterol efflux from peripheral cells. The APOA1 gene is associated with changes in lipid metabolism. A common gene polymorphism described in the APOA1 promoter region consists of the exchange of guanine (G) for adenine (A) at a position -75 bp upstream of the transcription origin. The relationship between lipid levels in obese children and the APOA1 MspI polymorphisms, was examined. Materials and Methods: Three separate groups were included, the patient group of obese children with hyperlipidemia; the obese control group (control group I) consisted of obese children without hyperlipidemia; and the healthy control group (control group II) contained healthy children with neither hyperlipidemia nor obesity. The related gene segments were amplified by polymerase chain reaction and determined different patterns were determined using denaturating gradient gel electrophoresis and positive results were confirmed automatic sequence analysis. All the results were analyzed by Proseq and BioEdit computer programmes. Results: The A allele was found to be more frequent in control group I compared to the patient group ($p=0.035$). Very low-density lipoprotein (VLDL), LDL and triglyceride (TG), levels were statistically higher in the patients carrying the GA genotype than in control group I, and body mass index (BMI), VLDL and TG levels were statistically higher than in control group II ($p<0.05$). There was no relationship between -75(G/A) polymorphism and serum lipid HDL-cholesterol levels when patient values were compared to those of the controls ($p>0.05$). Additionally, according to the -75 GA genotypes, those in control group I with the GA genotype had elevated total cholesterol levels compared to those with the GG genotype ($p<0.010$). In conclusion, carrying the A allele could confer a higher risk of hyperlipidemia in obese children.*

Obesity is defined as excessive accumulation of body fat (1). The prevalance of obesity has been increasing worldwide (2). Childhood obesity also seems to increase cardiovascular risk, involving altered lipid levels and impaired glucose tolerance which could contribute to the development of atheroma plaque and coronary heart disease in adult life (3, 4). Recent studies have revealed that obesity is associated with higher morbidity than smoking and alcoholism (5).

High-density lipoprotein (HDL) serves many functions in reverse cholesterol transport, while decremental oxidation of lipid content occurs in low density lipoprotein (LDL) particles. Apolipoprotein AI (Apo AI) is the major structural component of HDL and is involved in the esterification of cholesterol as a cofactor of lecithin-cholesterol acyltransferase (LCAT) and thus plays a crucial role in lipid transport and metabolism (6,7). in particular in cholesterol efflux from peripheral cells. Apo AI has been shown to increase HDL function to inhibit atherosclerosis and restore antiinflammatory and antioxidant properties to HDL

Abbrevations: Apo AI, Apolipoprotein AI; BMI, body mass index; DGGE, denaturating gradient gel electrophoresis; EDTA, ethylenediaminetetra-acetic acid; HDL-C, high-density lipoprotein-cholesterol; LCAT, lecithin-cholesterol acyltransferase; LDL-C, low-density lipoprotein-cholesterol; PCR, polymerase chain reaction; SDS, standard deviation score; TG, triglycerides.

Correspondence to: Professor Dr. Turgay Isbir, Department of Medical Biology, Medical Faculty, Yeditepe University, Istanbul, Turkey, İnönü Mah. Kayışdağı Cad. August 26 Campus, 34755 Kadıköy - İstanbul. Tel/Fax: +90 2165780000, e-mail: tisbir@superonline.com

Key Words: Apo AI, DGGE, hyperlipidemia, obesity, polymorphism.

(8). Previously it has been shown that gene polymorphisms in the *APOA1* locus directly affected the plasma lipid levels (9). Apolipoproteins AI *(APOA1),* CIII *(APOC3)* and AIV *(APOA4)* genes were found to be located on the same segment in the long arm of chromosome 11 (11q23-24) *APOA1*. The *APOA1* gene is associated with changes in lipid metabolism (6), implying that this gene region influences plasma lipid levels in hyperlipidemic individuals. Hyperlipidemia is characterized by high plasma levels of total cholesterol, LDL-cholesterol, triglycerides (TG) and lower levels of HDL-cholesterol (10). Hyperlipidemia is highly corelated with obesity (11).

A common polymorphism described in the human *APOA1* promoter region consists of the exchange of guanine (G) for adenine (A) at a position -75 bp upstream of the transcriptional start site. Another polymorphism is known to be present at the +83 bp site in the first intron of the *APOA1* gene, a cytosine (C) to thymine (T) (+83 bp) transition (7). The frequency of the +83 bp substitution was found to be less common than -75 bp in a healthy population. The -75A and +83T alleles both have base substitions resulting in the loss of *MspI* restriction sites. Both polymorphisms can be detected by using a unique procedure of polymerase chain reaction (PCR) and *MspI* digestion (12).

In this study, were examined the relationship between lipid levels in obese children with the *APOA1* -75 G/A *MspI* polymorphism. The related gene segments were amplified by PCR and variable patterns determined by denaturating gradient gel electrophoresis (DGGE) were confirmed by automatic sequence analysis.

Materials and Methods

Patient selection and clinical investigation. Three separate groups were included: the patient group was obese children with hyperlipidemia; the obese control group (Control I) consisted of obese children without hyperlipidemia and the healthy control group (Control II) contained healthy children with neither hyperlipidemia nor obesity.

The follow-up cases of the Endocrinology Department in Istanbul University were used and the study was approved by the Ethical Committee of the Istanbul University Faculty of Medicine. The patient group consisted of 38 individuals (mean age:11.50±3.61; 26 female and 12 male); Control I consisted of 37 individuals (mean age: 11.51±3.30; 24 female and 13 male); Control II consisted of 13 individuals (mean age: 8.15±2.15; 5 female and 8 male). Standard deviation score (SDS) for childhood and body mass index (BMI) were used and the calculations of SDS and BMI were as described in previous studies (13).

Blood was drawn from individuals fasted for 12 hours. LDL-cholesterol, TG and HDL-cholesterol were analyzed by a biochemical autoanalyser at the Department of Clinical Laboratory of Pediatric Endocrinology, Faculty of Medicine, Istanbul University within 4 hours of collection

Childhood cholesterol and TG values change depending on age and gender. For this reason, the 95th percentile data was used as the limit value in this study (Figures 1 and 2) (14).

Method of genotyping. Blood specimens were collected in tubes containing ethylenediaminetetra-acetic acid (EDTA). DNA samples were extracted from the whole blood using a Roche Blood Kit (Roche MagNa Pure Compact Nucleic Acid Isolation Kit-I). The *APOA1* gene *MspI* polymorphisms were amplified using the primer pairs: forward 5'- GC*AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG - 3'and reverse 5'-TTA GGG GAC ACC TAC CCG TCA GGA AGA GCA- 3' (15).

Each PCR primer in each pair included a 40-base GC-rich segment 'GC-clamp' 5'GCGCGCGCGCGCGCGCGCGCGCGCGC GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC CGCGCGCGCGCGCGC-3' attached to its 5' end to facilitate the detection of mutations by DGGE. The PCR reaction was performed under standard conditions in a volume of 50 μl containing 36 μl sterile distilled water, 3 μl 10× PCR buffer (containing no $MgCl_2$), 4 μl (25 mM) $MgCl_2$, 3 μl (10 nmols) of each deoxyribonucleoside triphosphate, 1 μl (10 pmols) of each primer, 1μl (1.5 units) of DNA Taq polymerase and 1 μl (25-175 ng) of human genomic DNA. The reaction consisted of denaturation at 96˚C for 1 minute, followed by 35 cycles of 96˚C for 30 seconds, 62˚C for 30 seconds and 72˚C for 60 seconds, with a final extension at 72˚C for 8 minutes. The final PCR products were electrophoresized using 2% agarose gel to test the amplification reaction.

Mutation detection. DGGE is an electrophoretic method capable of detecting differences between DNA fragments of the same size but with different sequences. These fragments can be separated in a denaturing gradient gel based on their differential denaturation (melting) profile (16). DGGE was performed according to the literature (17).

The technique was based on the electrophoretic separation of PCR-generated double stranded DNA in an acrylamide gel containing a gradient of a denaturant. For DGGE, 15 μl PCR products were analyzed using a 6% polyacrylamide gel with a 40-60% denaturing gradient of urea formamide (UF); 100% UF was 7M urea and 40% deionized formamide in 1×TAE (40 mM Tris–acetate, 1 mM EDTA, pH 8.0) buffer. DGGE was performed in 1×TAE at 60˚C for 16 hours with 75 V. Staining of the gel was performed with ethidium bromide was photographed with a UV source.

Sequencing. In DGGE analysis, the PCR products with different band patterns were purified using a Roche High Pure PCR Product Purification kit for DNA sequencing. Fifty nanograms of the purified PCR product was used for cycle sequencing with a Dynamic ET Terminator Cycle Sequencing Kit. The purified PCR products without the GC clamp were amplified again by using 2.4 μl sterile distiled water, 4 μl 5× sequencing buffer, 0.6 μl forward or reverse primer, 3 μl purified PCR product total volume 10 μl. After the amplification using the conditions of 95˚C for 20 seconds, 50˚C for 25 seconds and 60˚C for 2 minutes for 35 cycles. The amplified products were purified with Template Suppression Reagent solution and sequenced on an ABI PRISM 310 Genetic Analyzer. Samples were analyzed using the Proseq and BioEdit programmes. The base substitution of the -75(G/A) gene polymorphism of *MspI* region is shown in Figure 3.

Statistical analysis. Statistical analyses were performed using the SPSS software package (revision 11.5; SPSS Inc., Chicago, IL, USA). The clinical laboratory data are expressed as means±SD. The

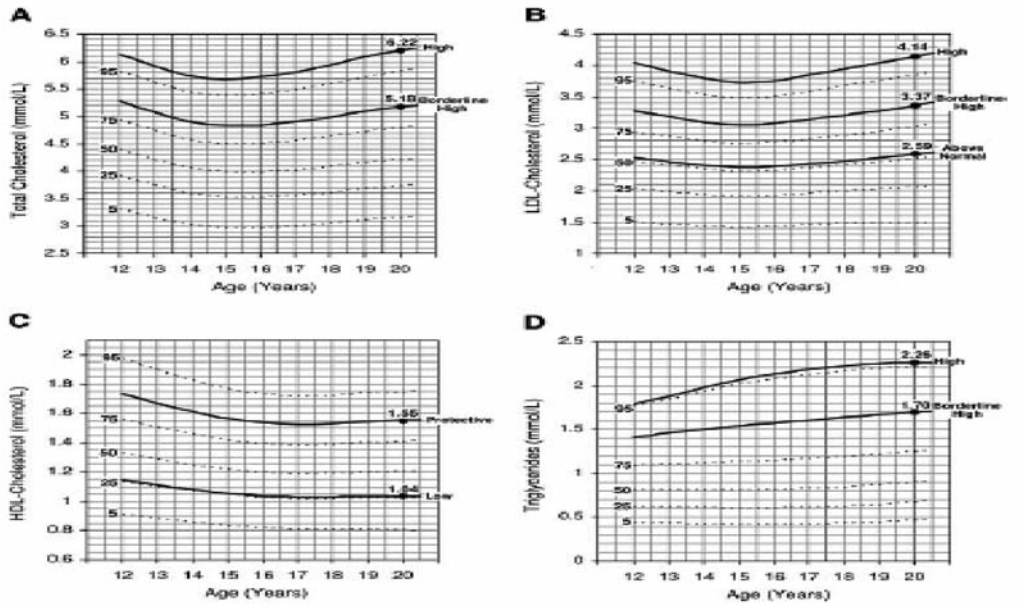

Figure 1. *Age-specific cut-off points for (A) total cholesterol, (B) LDL-cholesterol, (C) HDL-cholesterol, and (D) TG for males 12 to 20 years of age.*

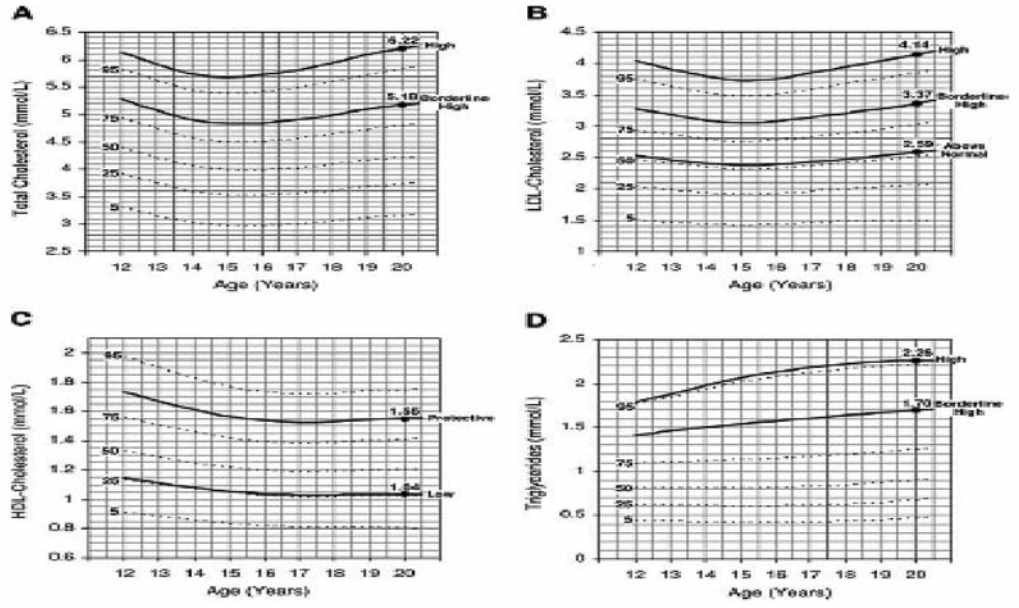

Figure 2. *Age-specific cut-off points for (A) total cholesterol, (B) LDL-cholesterol, (C) HDL-cholesterol, and (D) TG for females 12 to 20 years of age.*

mean values were compared between patients and controls by unpaired Student's *t*-test. Differences in the distribution of genotypes or alleles between cases and controls were tested using the Chi-square statistic, respectively. Fisher's exact test was used if the number in any cell of the 2×2 contingency table was <5. Relative risk at 95% confidence intervals (CI) was calculated as the odds ratio (OR). Values of $p<0.05$ were considered statistically significant.

Results

The demographic characteristics of the study population are shown in Table I. The patient group had increased total cholesterol, TG, LDL-cholesterol ($p<0.001$), VLDL cholesterol ($p=0.036$), VLDL-cholesterol and BMI levels ($p<0.001$) compared to Control I and Control II groups, respectively.

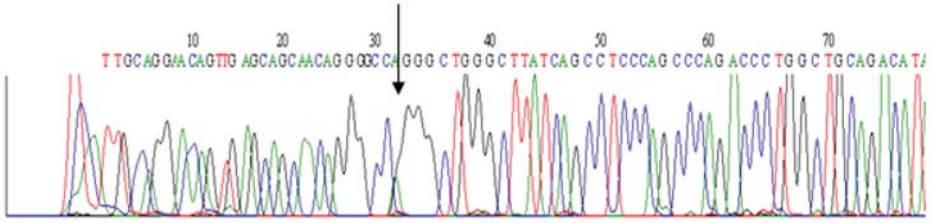

Figure 3. *The sequence of the -75 (G/A) polymorphism.*

Table I. *Demographic characteristics of the study population.*

	Patients n=38	Control I n=37	Control II n=13	Total n=88
Gender (female/male)	26/12	24/13	5/8	55/33
Age (years)	11.50±3.61	11.51±3.30	8.15±2.15	11.01±3.48
BMI (kg/m^2)-SDS	6.27±2.79	6.18±3.07	0.14±1.06*	5.29±3.49
Triglyceride (mg/dl)	149.92±64.54	77.92±22.87*	80±32.67*	110±58.63
Total cholesterol (mg/dl)	181.79±35.6	145.97±18.19*	164±33.49	164.10±33.29
HDL-cholesterol (mg/dl)	49.58±13.24	49.62±10.04	56.18±10	50.44±11.65
LDL-cholesterol (mg/dl)	104.10±26.25	81.86±15.07*	89.64±28.37	92.69±24.52
VLDL-cholesterol (mg/dl)	30.50±13.36	19.81±27.24**	18.36±6.65*	24.35±20.68
Total cholesterol/HDL-cholesterol (mg/dl)	3.89±1.23	3.03±0.57	2.94±0.51	3.40±1.01

n: Number of individuals; Patient group: obese children with hyperlipidemia; Control I: obese children without hyperlipidemia; Control II: healthy children with neither hyperlipidemia nor obesity. The results are shown as means±SD, *p<0.001 and **p=0.036 *versus* patient group.

The allele frequencies for the -75(G/A) transition in the *APOA1* gene are given in Table II. The A allele was found to be more frequent in Control I group compared to the patient group (p=0.035, Chi-square=4.425, OR=3.019, 95% C=1.05-8.63). There were no significant differences between the obese, Control I and healthy Control II groups for any alleles (Table II).

The VLDL-cholesterol (p=0.010, 95% C=5.54±27.18), LDL (p=0.021, 95% C=4.05±39.41) and TG (p=0.002, 95% C=50.67±143.72) levels were statistically higher in the patients carrying the GA genotype than in Control I group and BMI (p=0.002, 95% Cl=2.93-8.03), VLDL-cholesterol (p=0.005, 95% C=8.15±230.01) and TG (p=0.001, 95% Cl=61.99±2159.84) levels were statistically higher than in Control II group. There was no relationship between the -75(G/A) polymorphism and serum lipid HDL-cholesterol levels when the patients' values were compared to those of the controls (p>0.05). Additionally, in Control I group individuals with the GA genotype had elevated total cholesterol levels compared to those with the GG genotype (p<0.010, 95% C=3.89±226.48) (Table III).

Table II. *Prevalance of the APOA1 (-75 (G/A) alleles in the study groups.*

APOA1 (-75 (G/A)) Allele	Patients (n=38)	Control I (n=37)	Control II (n=13)
G (GG+GA)	68 (89.5%)	59 (79.7%)	18 (69.2%)
A (AA+GA)	8 (10.5%)	15 (20.3%)*	8 (30.8%)

n: Number of individuals; Patient group: obese children with hyperlipidemia; Control I obese children without hyperlipidemia; Control II: healthy children with neither hyperlipidemia nor obesity. Chi-square test *p=0.035 compared to the patient group.

Discussion

Obesity is a multifactorial, chronic disorder leading to adverse metabolic effects on plasma lipid levels (18, 19).

The determination of structural variants of Apo AI has allowed the characterization of the structure–function relationship in the protein. Mutations in the promoter region might result in an altered rate of gene expression, and thus in an altered rate of synthesis and secretion of Apo AI from the liver or intestine. The *MspI* -75(G/A) promoter polymorphism was first recorded by Pagani *et al.* (20). It has been postulated that the A allele causes an increase in *APOA1* gene expression (21). Previous studies have reported the effect of genetic and environmental factors on Apo AI and HDL-cholesterol levels, however, the gene–enviroment interactions are still incompletely known. The underlying mechanism by which the -75(G/A) polymorphism affects

Table III. *Comparison of APOA1 -75 (G/A) genotype and lipid levels and BMI.*

Group	Patients			Control I		Control II	
-75(G/A) Genotype	GG (n=31)	GA (n=6)	AA (n=1)	GG (n=22)	GA (n=15)	GG (n=9)	AA (n=4)
Triglyceride (mg/dl)	145.23±68.27	174.67±44.47[ab]	147.00	78.23±24.93	77.47±20.31	89.29±35.80	63.75±63.75
Total cholesterol (mg/dl)	184.81±31.04	164.17±56.24	194.00	139.82±17.25	155.00±16.06[c]	164.29±38.06	163.50±29.01
HDL-cholesterol (mg/dl)	50.13±14.03	51.40±10.78	55.00	48.41±9.56	51.40±10.79	57.71±11.05	53.50±8.58
VLDL- cholesterol (mg/dl)	29.58±13.90	32.83±10.34[ab]	45.00	22.09±35.29	16.47±4.41	21±6.78	13.75±3.30
LDL-cholesterol (mg/dl)	103.03±28.28	108.67±16.23[a]	11.00	78.41±13.42	86.93±16.37	85.86±32.10	96.25±22.99
BMI	6.33±2.91	5.36±2.06[b]	8.81	6.25±3.49	6.08±2.48	0.26±1.13	0.12±0.95

n: Number of individuals. The results are shown as mean±SD: ab, b, a $p<0.05$, $p=:001$; [a]*versus* control I, [b]*versus* control II, [c]*versus* control I.

plasma lipid levels, especially those of LDL-cholesterol and total cholesterol levels, is uncertain (22).

In vivo studies have shown that the presence of the A allele was associated with elevated HDL levels (20, 22-24), however, some studies were not able to confirm this (25, 26). Similarly, no relationship was found between this polymorphism and HDL-cholesterol levels in the present patients compared to the controls, nor was there a relationship between the groups.

Gonzalez-Amieva *et al*. reported that the presence of the GA genotype was associated with total cholesterol, LDL and TG levels more than the GG genotype in heart transplant patients with hyperlipidemia (10). VLDL-cholesterol and LDL-cholesterol levels were also statistically higher in the present patients carrying the GA genotype than in the Control I group.

Xu *et al*. investigated an association between the *APOA1* -75(G/A) polymorphism and plasma lipid levels in Italian boys. They observed that plasma LDL-cholesterol levels were significantly elevated in subjects carriers of the A allele but no effect was found on plasma HDL-cholesterol levels (27). Similarly, in the present study, VLDL-cholesterol, LDL and TG levels were statistically higher in the patients carrying the GA genotype than control I group and BMI, VLDL and TG levels were statistically higher than control II group.

In contrast to the present study, Carmena *et al*. reported that familial hypercholesterolemia subjects carrying the A allele for the *APOA1* -75(G/A) polymorphism had significantly lower total and LDL-cholesterol levels (28).

In conclusion, carrying the A allele could increase the risk of hyperlipidemia in obese children. This is the first study to determine the relationship between hyperlipidemia, *MspI* polymorphism and childhood obesity in Turkish people.

Acknowledgements

This study was supported by the Istanbul University Research Fund Project No: 1704.

References

1 Noller DT and Paulk D: Childhood obesity curbing an American epidemic. JAAPA *18*: 12, 2005.
2 Grundy SM: Obesity, metabolic syndrome and cardiovascular disease. J Clin Endocrinol Metab *89(6)*: 2595-2600, 2004.
3 Choi YJ, Jo YE, Ahn SM, Jung SH, Kim HJ, Chung YS, Lee KW and Kim DJ: High plasma concentration of remnant lipoprotein cholesterol in obese children and adolescents. Diabetes Care 29(10), 2006.
4 Strauss R: Childhood obesity. Curr Probl Pediatr *29*: 5-29, 1999.
5 Lavie CJ, Milani RV and Ventura HO: Obesity and cardiovascular disease. J Am Coll Cardiol *53*: 1925-1932, 2009.
6 Groenendijk M, Cantor RM, Bruin TW and Dallinga-Thie GM. The apoAI-CIII-AIV gene cluster. Atherosclerosis *157*: 1-11, 2001.
7 Reguero JR, Cubero GI, Batalla A, Alvarez V, Hevia S, Cortina A and Coto E: Apolipoprotein A1 gene poymorphisms and risk of early coronary disease. Cardiology *90*: 231-235, 1998.
8 Kontush A and Chapman MJ: Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, ınflammation, and atherosclerosis. Pharmacol Rev *58*: 342-374, 2006.
9 Chen ES, Mazzotti DR, Furuya TK, Cendorogio MS, Ramos R, Araujo LQ, Burbano RR and Smith CM: Apolipoprotein A1 gene polymorphisms as risk factors for hypertension and obesity. Clin Exp Med 0051-3, 2009.
10 Gonzalez-Ameiva A, Lopez-Miranda J, Marin C, Prez- Marinez P, Gomez P, Paz-Rojas E, Arizon JM, Jimenez-Pereperz JA, Concha M and Perez-Jimenez F: The Apo A-I gene promoter region polymorphism determines the severity of hyperlipidemia after heart transplation. Clin Transplant *17*: 56-62, 2003.
11 Freedmam DS, Dietz WH, Srinivasan SR and Berenson GS: The relation of overweight to cardiovascular risk factors among children and adolescents: The Bogalusa Heart Study. Pediatrics *103*: 1175-1182, 1999.
12 Wang XL, Badenhop R, Humphrey K and Wilcken DEL: New *MspI* polymorphism at +83 bp of the human apolipoprotein AI gene: association with increased circulating high density lipoprotein cholesterol levels. Genet Epidemiol *13*: 1-10, 1996.

13 Cole TJ, Bellizzi MC, Flegal KM and Dietz WH: Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ *320*: 1240-1243, 2000.
14 Jolliffem CJ and Janssen I: Distribution of lipoproteins by age and gender in adolescents. Circulation *114*: 1056, 2006.
15 Meng Q, Pajukanta P, Valsta L, Aro A, Pıetınen P and Tıkkanen M, Influence of apolipoprotein polymorphism on lipid levels and responses to dietary change in Finnish adults. J Inter Med *241*: 373-378, 1997.
16 Ercolini D: PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. J Microbiol Met *56*: 297-314, 2004.
17 Myers RM, Maniatis T and Lerman LS: Detection and localization of single base changes by denaturing gradient gel electrophoresis. Methods Enzymol *155*: 501-527, 1987.
18 Nisoli E and Carruba M: Emerging aspects of pharmacotherapy for obesity and metabolic syndrome. Pharmacological Res *50*: 453-469, 2004.
19 Steinberger J and Daniels SR: Obesity, insulin resisitance diabetes and cardiovascular risk in children: an American Heart Association scientific statement from the Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Commitee (Council on Nutrition, Physcal Activity, and Metabolism). Circulation *107*: 1448-1453, 2003.
20 Pagani F, Sidoli A, Giudici GA, Barenghi L, Vergani C and Baralle FE: Human apolipoprotein A-I gene promoter polymorphism: association with hyperalphalipoproteinemia. J Lipid Res *31*: 1371-1377, 1990.
21 Angotti E, Mele E, Costanzo F and Avvedimento EV: A polymorphism (G to A transition) in the −78 position of the apolipoprotein A-I promoter increases transcription efficiency. J Biol Chem *269*: 17371-17374, 1994.
22 Talmud PJ, Shu Y and Humphries SE: Polymorphism in the promoter region of the apolipoprotein A-I gene associated with differences in apolipoprotein A-I levels: the European Atherosclerosis Research Study. Genet Epidemiol *11*: 265-280, 1994.
23 Saha N, Tay JSH, Low PS and Humphries SE: Guanidine to adenine (G/A) substitution in the promoter region of the apolipoprotein A-I gene is associated with elevated serum apolipoprotein A-I levels in Chinese non-smokers. Genet Epidemiol *11*: 255-264, 1994.
24 Peacock RE, Hamsten A, Johansson J, Nilsson-Ehle P and Humphries S: Association of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis and high density lipoprotein subclasses. Clin Genet *46*: 273-282, 1994.
25 Peacock RE, Temple A, Gudnason V, Rosseneu M and Humphries SE: Variation at the lipoprotein lipase and apolipoprotein AI-CIII gene loci are associated with fasting lipid and lipoprotein traits in a population sample from Iceland: interaction between genotype, gender, and smoking status. Genet Epidemiol *14*: 265-282, 1997.
26 Kamboh MI, Aston CE, Nestlerode CM, McAllister AE and Hamman RF: Haplotype analysis of two *APOA1/MspI* polymorphisms in relation to plasma levels of apo A-I and HDL-cholesterol. Atherosclerosis *127*: 255-262, 1996.
27 Xu CF, Angelico F, Del Ben M and Humphries S: Role of genetic variation at the apo AI-CIII-AIV gene cluster in determining plasma apo AI levels in boys and girls. Genet Epidemiol *10*: 113-122, 1993.
28 Carmena R, Ordovas J, Ascaso J, Real J and Priego M: Influence of genetic variation at the apo A-I gene locus on lipid levels and response to diet in familia hypercholesterolemia. Atherosclerosis *139*: 107-113, 1998.

Received October 27, 2010
Revised January 12, 2011
Accepted January 13, 2011