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

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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.

Abbreviations: 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.

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(8). Previously it has been shown that gene polymorphisms in the APOAI locus directly affected the plasma lipid levels (9). Apolipoproteins A1 (APOAI), CII (APOC3) and AIV (APOAI) genes were found to be located on the same segment in the long arm of chromosome 11 (11q23-24) APOAI. The APOAI 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 correlated with obesity (11).

A common polymorphism described in the human APOAI 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 APOAI gene, a cytosine (C) to thymine (T) (+83 bp) transition (7). The frequency of the +83 bp substitution was found to be present at the +83 bp site in the first intron of the APOAI 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 substitutions 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, we examined the relationship between lipid levels in obese children with the APOAI -75 G/A MspI polymorphism. The related gene segments were amplified by PCR and variable patterns determined by denaturing 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 APOAI gene MspI polymorphisms were amplified using the primer pairs: forward 5’-GC*-AGG GAC AGC ACG 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’GCCGCCGCGCGCCGCGCCGCGCCGCGCCGCGCGCCGCGGCG CGCGCGCGCGCGCGCGCGCGCGC-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 10x PCR buffer (containing no MgCl2), 4 μl (25 mM) MgCl2, 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 distilled water, 4μl 5x 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 using Template Suppression Reagent solution and sequenced on an ABI PRISM 310 Genetic Analyzer. Samples were analyzed using the Prosseq 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 mean±SD.
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.
The allele frequencies for the -75(G/A) transition in the \textit{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 \((p=0.035, \text{Chi-square}=4.425, \text{OR}=3.019, \text{95\% CI}=1.05-8.63)\). There were no significant differences between the obese, Control I and healthy Control II groups for any alleles (Table II).

The VLDL-cholesterol \((p=0.010, 95\% \text{ CI}=5.54\pm27.18)\), LDL \((p=0.021, 95\% \text{ CI}=4.05\pm39.41)\) and TG \((p=0.002, 95\% \text{ CI}=50.67\pm143.72)\) levels were statistically higher in the patients carrying the GA genotype than in Control I group and BMI \((p=0.002, 95\% \text{ CI}=2.93-8.03)\), VLDL-cholesterol \((p=0.005, 95\% \text{ CI}=8.15\pm230.01)\) and TG \((p=0.001, 95\% \text{ CI}=61.99\pm2159.84)\) 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 \((p<0.010, 95\% \text{ CI}=3.89\pm226.48)\) (Table III).

### 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 \textit{MspI} -75(G/A) promoter polymorphism was first recorded by Pagani \textit{et al.} (20). It has been postulated that the A allele causes an increase in \textit{APOAI} gene expression (21). Previous studies have reported the effect of genetic and environmental factors on Apo AI and HDL-cholesterol levels, however, the gene–environment interactions are still incompletely known. The underlying mechanism by which the -75(G/A) polymorphism affects
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 APOAI -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, Carmen et al. reported that familial hypercholesterolemia subjects carrying the A allele for the APOAI -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.

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