Menoufia Medical Journal

PRINT ISSN: 1110-2098 - ONLINE ISSN: 2314-6788

journal hompage: www.menoufia-med-j.com

Volume 29 | Issue 2 Article 24

6-1-2016

# Effect of apolipoprotein B-100 EcoRI polymorphism on serum lipids and insulin resistance in obese children

Belal A. El Mohsen Montaser
*El Menoufia University*, drbelalmontaser@yahoo.com

Emad F. Haleim
*El Menoufia University*

Gehan K. Sead
*El Menoufia University*

Hanzada I. Fattah
*Ain Shams University*

Waleid F. Azeim
*El Menoufia University*



## Recommended Citation

El Mohsen Montaser, Belal A.; Haleim, Emad F.; Sead, Gehan K.; Fattah, Hanzada I.; and Azeim, Waleid F. (2016) "Effect of apolipoprotein B-100 EcoRI polymorphism on serum lipids and insulin resistance in obese children," *Menoufia Medical Journal*: Vol. 29: Iss. 2, Article 24.
DOI: https://doi.org/10.4103/1110-2098.192436

# Effect of apolipoprotein B-100 *Eco*RI polymorphism on serum lipids and insulin resistance in obese children

Belal A. El Mohsen Montaser[a], Gehan K. Sead[a], Emad F. Haleim[a], Hanzada I. Fattah[b], Waleid F. Azeim[a]

[a]Department of Clinical Pathology, Faculty of Medicine, El Menoufia University, El Menoufia, [b]Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Correspondence to Belal A. El Mohsen Montaser, MSc, Clinical Pathology Department, Faculty of Medicine, El Menoufia University, Yassin Abd El Ghaffar Street, Shebin El Kom, El Menoufia, 32511, Egypt
Tel: +20 109 918 9080; Fax: +20 482 228 304; e-mail: drbelalmontaser@yahoo.com

**Received** 11 March 2014
**Accepted** 09 May 2014

**Menoufia Medical Journal** 2016, 29:330–336

**Objective**
The aim of this work was to evaluate the role of apolipoprotein B-100 (*ApoB*-100) *Eco*RI gene polymorphism on serum lipid parameters and BMI in obese children and its possible relationship with insulin resistance. This is the first study in Egypt to determine possible relationships of these parameters.

**Background**
*ApoB*-100 is a large, amphipathic glycoprotein playing a central role in human lipoprotein metabolism and is required for very low-density lipoprotein production in the liver. *ApoB*-100 is also the ligand for low-density lipoprotein-receptor-mediated endocytosis of low-density lipoprotein particles. There are several known *ApoB*-100 polymorphisms that were proven to cause obesity, hyperlipidemia, and cardiovascular diseases. Three common polymorphisms were found, MspI, XbaI, and *Eco*RI (exon 26), which have been associated with variation in lipid levels.

**Materials and methods**
BMI was calculated, fasting serum lipids were assayed by routine techniques on Synchron systems, serum fasting insulin was assayed by ELISA technique, HOMA-IR was calculated, DNA was extracted from whole blood, amplified, and then *ApoB*-100 *Eco*RI polymorphism was assayed using PCR-RFLP.

**Results**
The results of this study revealed that there was a significant statistical difference in BMI, lipid profile, insulin, and insulin resistance between positive *Eco*RI and negative *Eco*RI polymorphisms, and hence there was a direct positive relationship between *ApoB*-100 *Eco*RI polymorphism and all studied parameters. Results were collected, tabulated, and statistically analyzed using the statistical package for the social sciences (SPSS, version 11). Two types of statistics were performed: the Mann–Whitney *U*-test and Pearson's correlation.

**Conclusion**
This study revealed a great association between *ApoB*-100 *Eco*RI gene polymorphism and atherogenic hyperlipidemia and insulin resistance in obese pediatric patients.

**Keywords:**
*ApoB*-100 *Eco*RI polymorphism, hyperlipidemia, insulin resistance, metabolic syndrome, obesity

Menoufia Med J 29:330–336

1110-2098

## Introduction

Obesity is a global epidemic health problem affecting both adults and children. It is a common disease affecting not only developed countries but also developing countries. Approximately, 22 million children under 5 years of age are overweight across the world [1]. In Egypt, the number of overweight children and adolescents has doubled in the last two to three decades, and similar doubling rates are being observed worldwide, including the developing countries [2].

It has been long known that obesity is associated with premature death, as obesity increases the risk for a number of diseases including the two major killers, cardiovascular disease and cancer. It is estimated that, on average, obesity reduces life expectancy between 3 and 13 years [3,4].

Apolipoprotein B (*ApoB*) is a large, amphipathic glycoprotein playing a central role in human lipoprotein metabolism and is coded by the *ApoB* gene located on chromosome 2. One of the two *ApoB* forms is *ApoB*-100, which is required for very low-density lipoprotein production in the liver. In addition to being an essential structural component of very low-density lipoprotein, *ApoB*-100 is also the ligand for low-density lipoprotein (LDL)-receptor-mediated endocytosis of LDL particles [5].

There are several known *ApoB* polymorphisms that were proven to cause hyperlipidemia and cardiovascular disease. One of such polymorphisms is the *Eco*RI polymorphism that results in Glu4154Lys amino acid substitution in the 26th exon. In previous studies, a significant direct relationship between *Eco*RI polymorphism and the serum

levels of both cholesterol and triglyceride was found. It was thought that these polymorphisms reduce the binding capacity of *ApoB* to LDL receptors, and hence cause a decrement in LDL clearance [6].

## Patients and methods

### Patients

The patients of the present study were divided into two groups:

(1) Group I: this involved 40 obese children (20 boys and 20 girl), their ages ranging between 6 and 12 years. They were randomly selected from primary schools in Menoufia governorate between May 2010 and November 2013.

The group I patients were further divided into three subgroups:

(a) Subgroup Ia: this included 10 class 1 obese children (5 boys and 5 girls), their ages ranging between 6 and 12 years.
(b) Subgroup Ib: this included 10 class 2 obese children (5 boys and 5 girls), their ages ranging between 6 and 12 years.
(c) Subgroup Ic: this included 20 class 3 obese children (10 boys and 10 girls), their ages ranging between 6 and 12 years.

(1) Group II: this included 20 apparently healthy age-matched and sex-matched children as a control group (10 boys and 10 girls). The study was approved by the ethics committee of our medical faculty, and written informed consent was obtained from the parents of all children before study entry.

### Methods and techniques

For all participants, the following were performed:

(1) Anthropometric measurements (weight, height, and BMI) to diagnose obesity:

$$\text{BHI} = \frac{\text{Weight in kg}}{\text{Height in m}^2}.$$

(2) A volume of 2 ml of blood sample was collected after 8 h of fasting, under aseptic condition by clean venipuncture without venous stasis for determination of fasting serum glucose and fasting insulin.
(3) A volume of 8 ml of blood sample was collected after 12 h of fasting, under aseptic condition by clean venipuncture without venous stasis. It was divided into two parts:
   (a) A volume of 4 ml was added to an EDTA-containing sterile tube for the determination of *ApoB*-100 polymorphisms. The sample was stored in the refrigerator for not more than a week until extraction of DNA or stored as a cell pellet on lysate for longer period at -80°C until extraction.
   (b) A volume of 4 ml was added to a sterile plain tube for immediate assessment of lipid profile [total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-c), and high-density lipoprotein-cholesterol (HDL-c)] and lipoprotein electrophoresis. The blood was left to clot at 37°C and rapidly centrifuged at 4000 rpm for 10 min.

Biochemical tests for detection of blood glucose and lipid profile were performed on Synchron CX9 Autoanalyzer using kit supplied by Beckman (4862 Innovation Dr, Fort Collins, CO, United States) [7].

(4) Fasting serum insulin was assayed by ELISA technique (product code: 2425-30, Immunoenzymometric Assay; Momobind Inc., Lake Forest, California, USA) [8].
(5) The homeostasis model assessment-estimated insulin resistance (HOMA-IR) equation was applied to diagnose patients with insulin resistance.

$$\text{HOMA-IR} = \frac{\text{Fasting glucose (mg/dl)} \times \text{fasting insulin (IU/ml)}}{405}.$$

(6) Lipoprotein electrophoresis was performed using Helena cellulose (Pearmont, Texas, USA) acetate strips [9].
(7) Genotyping: determination of *ApoB*-100 genotypes was performed by PCR-restriction fragment length polymorphism (RFLP).

Blood for genotyping was drawn into EDTA-containing receptacles. Genomic DNA was prepared from peripheral blood leukocytes according to the standard procedure and stored at −20°C. To analyze polymorphic sites, we used separate PCR analyses followed by subsequent RFLP analysis. The primer sets used were: F: 5'-CTGAGAGAAGTGTCTTCGAAG-3' and R: 5'-CTCGAAAGGAAGTGTAATCAC-3' for *ECo*RI locus. Briefly, 300 ng of DNA was added to a PCR mixture (final volume of 50 μl) containing 1.5 mmol/l $MgCl_2$, 50 pmol of each primer, 200 mol/l dNTPs, and 1 U of Taq polymerase (Promega, Madison, Wisconsin, USA) in a reaction buffer recommended by Promega. The PCR conditions for the *Eco*RI RFLPs were one cycle at 95°C for 5 min followed by 30 cycles at 98°C for 1 min and 58°C for 1.5 min, with a final elongation at 72°C for 10 min. Negative controls (no DNA added) were included in every PCR run to check for contamination. The PCR-amplified products were digested for 3 h with 5–10 U of

the appropriate *Eco*RI restriction enzyme at 37°C, and the fragments were analyzed using electrophoresis on 2.0% agarose gel. The DNA bands were visualized on a 302 nm UV transilluminator and photographed. The gel was examined for bands of 112 and 510 bp as determined by the molecular weight markers run at the same time. The alleles are named according to the presence or absence of the restriction site [10].

### Statistical analysis

The results were tabulated and statistical analysis was performed using the statistical package for the social sciences (SPSS, version 11.5) [11].

## Results

The studied groups were well matched regarding age and sex. The levels of fasting blood glucose, fasting insulin, and HOMA-IR for insulin resistance were significantly higher in patients (children with different grades of obesity) when compared with controls (Table 1).

The levels of total cholesterol, triglycerides, and LDL-c were significantly higher in patients (children with different grades of obesity) when compared with controls, whereas the level of HDL-c was significantly lower in patients when compared with controls (Table 2).

There was a highly significant difference in the weight and BMI between patients and controls ($P < 0.001$). Moreover, there was a significant difference in BMI between positive and negative polymorphisms (Table 3).

Table 4 shows that there is a significant association between increased fasting insulin as well as increased

**Table 1 Comparison between patient subgroups and the control group regarding fasting blood glucose, fasting insulin, and insulin resistance**

| Tested paramters | Patients (*N* = 40) (group I) | | | Control (*N* = 20) (group II) | *t*-Test/Mann–Whitney *U*-test | *P* value |
|---|---|---|---|---|---|---|
| | Mild obesity (*N* = 10) Subgroup Ia | Moderate obesity (*N* = 10) Subgroup Ib | Severe obesity (*N* = 20) Subgroup Ic | | | |
| FBS (mg/dl) | | | | | | |
| X ± SD | 102.90 ± 1.1 | 99.80 ± 5.67 | 106.70 ± 7.11 | 78.60 ± 6.65 | 3.98[b]4.38[b]12.91[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 86–104 | 89–109 | 91–117 | 70–89 | | |
| Fasting insulin (mIU/ml) | | | | | | |
| X ± SD | 18.59 ± 0.78 | 19.41 ± 2.26 | 21.21 ± 2.19 | 8.19 ± 0.79 | 4.43[b]4.42[b]24.97[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 17.5–19.7 | 17–25 | 17.5–26 | 7–9.10 | | |
| HOMA | | | | | | |
| X ± SD | 4.26 ± 0.25 | 4.77 ± 0.70 | 5.53 ± 0.89 | 2.62 ± 0.72 | 4.41[b]4.41[b]11.33[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 3.9–4.7 | 4.1–6.4 | 3.9–6.7 | 1.4–3.6 | | |

FBS, fasting blood sugar; HOMA, homeostasis model assessment; [a]*t*-Test; [b]Mann–Whitney *U*-test. [1]Mild obesity group versus control group; [2]Moderate obesity group versus control group; [3]Severe obesity group versus control group.

**Table 2 Comparison between patient subgroups and the control group regarding lipid profile**

| Tested paramters | Patient (*N* = 40) (group I) | | | Control (*N* = 20) (group II) | *t*-Test/ Mann–Whitney *U*-test | *P* value |
|---|---|---|---|---|---|---|
| | Mild obesity (*N* = 10) Subgroup 1a | Moderate obesity (*N* = 10) Subgroup 1b | Severe obesity (*N* = 20) Subgroup 1c | | | |
| Cholesterol (mg/dl) | | | | | | |
| X ± SD | 266.0 ± 12.14 | 276.1 ± 26.73 | 306.5 ± 36.72 | 150.0 ± 27.01 | 4.40[b]4.40[b]15.35[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 250–290 | 240–320 | 257–399 | 102–180 | | |
| TG (mg/dl) | | | | | | |
| X ± SD | 177.2 ± 7.24 | 182.1 ± 10.08 | 192.35 ± 23.47 | 103.80 ± 9.0 | 4.41[b]4.41[b]15.75[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 165–192 | 170–199 | 165–274 | 90–119 | | |
| HDL-c (mg/dl) | | | | | | |
| X ± SD | 31.3 ± 1.56 | 32.6 ± 1.71 | 31.8 ± 1.32 | 41.8 ± 4.67 | 4.44[b]4.43[b]9.21[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 30–34 | 30–35 | 30–34 | 38–50 | | |
| LDL-c (mg/dl) | | | | | | |
| X ± SD | 199.26 ± 13.02 | 207.08 ± 26.12 | 236.23 ± 33.38 | 87.44 ± 25.44 | 4.40[b]4.40[b]15.85[a] | <0.001[1]<0.001[2]<0.001[3] |
| Range | 182.4–224 | 172–255 | 190–326 | 43.6–124 | | |

HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; TG, triglyceride; [a]*t*-Test; [b]Mann–Whitney *U*-test; [1]Mild obesity group versus control group; [2]Moderate obesity group versus control group; [3]Severe obesity group versus control group.

HOMA-IR equation (and so between insulin resistance) and positive *Eco*RI polymorphism.

There was a significant increase in serum total cholesterol and LDL-c in patients with positive polymorphism more than in patients with negative polymorphism as illustrated in Table 5.

**Table 3 Comparison between *Eco*RI genotypes with respect to weight, height, and BMI**

| Tested paramters | *Eco*RI genotypes | | *t*-Test | *P* value |
|---|---|---|---|---|
| | +/+ (*N* = 23) | +/– and –/– (*N* = 17) | | |
| Weight (kg) | | | | |
| X ± SD | 77.43 ± 17.51 | 70.47 ± 17.35 | 1.25 | 0.22 |
| Range | 50–107 | 45–101 | | >0.05 |
| Height (m) | | | | |
| X ± SD | 1.38 ± 0.20 | 1.40 ± 0.17 | 0.19 | 0.85 |
| Range | 1.1–1.7 | 1.23–1.57 | | >0.05 |
| BMI | | | | |
| X ± SD | 40.71 ± 5.29 | 36.48 ± 5.05 | 2.55 | 0.01 |
| Range | 35.42–46 | 31.43–41.53 | | <0.05 |

**Table 4 Comparison between *Eco*RI genotypes with respect to fasting blood sugar, fasting insulin, and insulin resistance**

| Tested paramters | *Eco*RI genotypes | | *t*-Test | *P* value |
|---|---|---|---|---|
| | +/+ (*N* = 23) | +/– and –/– (*N* = 17) | | |
| FBS (mg/dl) | | | | |
| X ± SD | 103.65 ± 7.84 | 104.53 ± 12.40 | 0.16 | 0.87 |
| Range | 89–115 | 86–117 | | >0.05 |
| Fasting insulin (mIU/ml) | | | | |
| X ± SD | 20.75 ± 2.38 | 19.23 ± 1.73 | 2.22 | 0.03 |
| Range | 17.5–26 | 17–23.2 | | <0.05 |
| HOMA | | | | |
| X ± SD | 5.28 ± 0.89 | 4.66 ± 0.81 | 2.25 | 0.03 |
| Range | 3.9–6.7 | 4.03–6.6 | | <0.05 |

FBS, fasting blood sugar; HOMA, homeostasis model assessment.

**Table 5 Comparison between *Eco*RI genotypes with respect to lipid profile**

| Tested paramters | *Eco*RI genotypes | | *t*-Test | *P* value |
|---|---|---|---|---|
| | +/+ (*N* = 23) | +/– and –/– (*N* = 17) | | |
| Cholesterol (mg/dl) | | | | |
| X ± SD | 301.78 ± 35.69 | 271.18 ± 24.07 | 3.05 | 0.004 |
| Range | 243–399 | 240–335 | | <0.05 |
| TG (mg/dl) | | | | |
| X ± SD | 187.13 ± 21.79 | 184.47 ± 13.84 | 0.44 | 0.66 |
| Range | 165–274 | 169–225 | | >0.05 |
| HDL-c (mg/dl) | | | | |
| X ± SD | 32.09 ± 1.53 | 31.59 ± 1.50 | 1.02 | 0.31 |
| Range | 30–35 | 30–35 | | >0.05 |
| LDL-c (mg/dl) | | | | |
| X ± SD | 232.27 ± 32.86 | 202.69 ± 22.04 | 3.21 | 0.003 |
| Range | 173.6–326.4 | 172–263 | | <0.05 |

HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; TG, triglyceride.

The *ApoB*-100 *Eco*RI gene polymorphism frequencies among obese patients were significantly changed compared with that in controls. Positive polymorphism was detected in 39 of 40 (97.5%) patients, whereas all controls (100%) showed negative polymorphism (Tables 6 and 7 and Fig. 1).

Concerning lipoprotein electrophoresis, there was a highly significant difference in their patterns between patients and controls. All 20 controls showed normal pattern with normal β-band, whereas all 40 patients had abnormal pattern with increased β-band (Table 7 and Fig. 2).

Finally, the obtained data demonstrated that $E^+/E^+$ and $E^+/E^-$ carriers had significantly higher BMI, fasting glucose, fasting insulin, HOMA-IR, transcription complex, LDL-c, and increased β-band in lipoprotein electrophoresis and significantly lower HDL-c concentrations than $E^-/E^-$ carriers.

## Discussion

Elevated blood pressure, dyslipidemia, and a higher prevalence of factors associated with insulin resistance and type 2 diabetes appear as frequent comorbidities in overweight and obese pediatric population [12].

Overweight children are at risk for various chronic conditions in later life, and this risk may exist even independently of obesity in adult life. Obesity affects almost every organ of the body. Its effects include metabolic syndrome (MS), which is insulin resistance, hyperlipidemia, and hypertension, in addition to mechanical disorders such as osteoarthritis, respiratory problems, sleep apnea, and psychosocial disorders [13].

The MS is a clustering of components that reflects over nutrition, sedentary lifestyles, and resultant excess adiposity. These components include abdominal obesity, insulin resistance, dyslipidemia, and elevated blood pressure [14].

The MS is one of the greatest challenges to public health throughout the world due to its association with major risks for cardiovascular diseases and type 2 diabetes mellitus [15].

It was proven that even one amino acid change in the carboxyl end of the *ApoB* protein can destroy its binding capacity to LDL receptors; it was also shown that *ApoB* was defective in some hyperlipidemic and/or hypercholesterolemic patients in binding to the receptor [6].

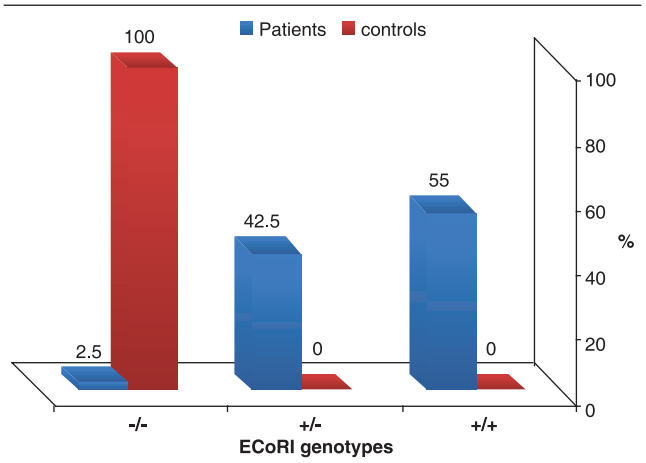
**Table 6 Comparison between patient subgroups and the control group with respect to *Eco*RI genotypes**

| Tested paramters | Patient (*N* = 40) (group I) [*N* (%)] | | | Control (*N* = 20) (group II) [*N* (%)] | $\chi^2$ | *P* value |
|---|---|---|---|---|---|---|
| | Mild obesity (*N* = 10) Subgroup 1a | Moderate obesity (*N* = 10) Subgroup 1b | Severe obesity (*N* = 20) Subgroup 1c | | | |
| *Eco*RI polymorphism | | | | | | |
| Homozygous +/+ | 3 (30.0) | 5 (50.0) | 14 (70.0) | 0 (0.0) | 30.0 | <0.001[1] |
| Heterozygous +/– | 7 (70.0) | 5 (50.0) | 5 (25.0) | 0 (0.0) | 30.0 | <0.001[2] |
| Homozygous –/– | 0 (0.0) | 0 (0.0) | 1 (5.0) | 20 (100) | 36.2 | <0.001[3] |

**Table 7 Comparison between patients and the control group with respect to lipoprotein electrophoresis and *Eco*RI polymorphism**

| Tested paramters | Patient (*N* = 40) [*N* (%)] | Control (*N* = 20) [*N* (%)] | $\chi^2$ | *P* value |
|---|---|---|---|---|
| Lipoprotein electrophoresis | | | | |
| Normal pattern | 0 (0.0) | 20 (100) | 60.0 | <0.001 |
| β-Band | 40 (100) | 0 (0.0) | | |
| *Eco*RI polymorphism | | | | |
| Homozygous +/+ | 22 (55.0) | 0 (0.0) | 55.71 | <0.001 |
| Heterozygous +/– | 17 (42.5) | 0 (0.0) | | |
| Homozygous –/– | 1 (2.5) | 20 (100) | | |

**Figure 1**




Comparison between patients and controls with respect to *Eco*RI polymorphism: only one patient representing 2.5% had homozygous negative polymorphism, whereas 39 patients had positive polymorphism; 22 representing 55% were positive homozygous and 17 representing 42.5% were positive heterozygous polymorphism, whereas all 20 controls representing 100% had negative polymorphism.

The aim of this work was to evaluate the role of *ApoB*-100 *Eco*RI polymorphism on serum lipid parameters and BMI in obese children and its possible relationship with insulin resistance. This is the first study in Egypt to determine the possible relationships of these parameters.

The present study revealed that there was a significant statistical difference in BMI between positive and negative polymorphisms, and hence there was a direct positive relationship between *ApoB*-100 *Eco*RI polymorphism and obesity. This is in agreement with the study by Hu *et al*. [15] who concluded that *Eco*RI restriction sites may serve as potential markers affecting BMI in children from Guangxi [15].

With respect to fasting glucose, fasting insulin, and HOMA-IR, there was a highly significant increase in the mean value of all three parameters in all patients when compared with the mean value of controls.

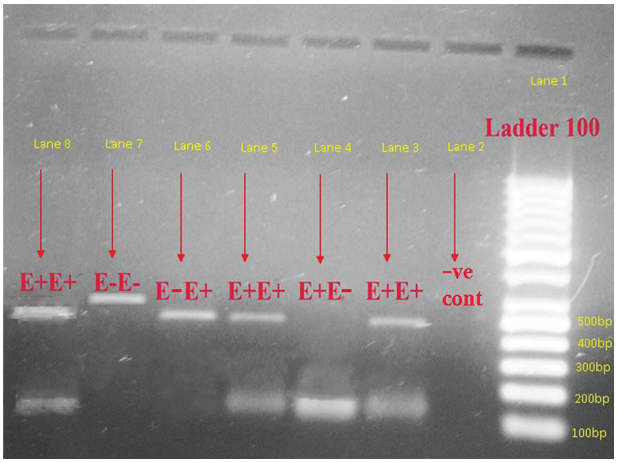
There was a highly positive correlation between obesity, positive *Eco*RI polymorphism, hyperinsulinemia, and insulin resistance, which is a main key in diagnosis of MS, and these results are in accordance with the results of Grundy [16] who found that obesity, MS, and cardiovascular diseases all are direct complications to increased BMI and *ApoB*-100 *Eco*RI polymorphism.

Regarding lipid profile, the obtained data showed that there was a highly significant increase in total cholesterol and LDL-c in patients than in controls, whereas there was a highly significant decrease in HDL-c in patients than in controls.

In our study, we had chosen *ApoB*-100 as a candidate gene of lipid metabolism. In the literature, there were several *ApoB*-100 polymorphisms associated with hyperlipidemia. We studied the *Eco*RI polymorphism at the 4154 position, causing a glutamine to lysine substutition in the 26th exon. Only a few studies have investigated the possible mechanisms whereby the *Eco*RI polymorphism of the *ApoB*-100 gene affects BMI and serum lipids concentration.

Studying LDL kinetics with respect to *ApoB*-100 polymorphism in five different populations, Hu *et al*. [15] observed that, in four of these five populations, the LDL fractional catabolic rate was lower in those carrying the *Eco*RI polymorphism, the difference reaching statistical significance in one population. These data showed that variation in *ApoB*-100 may influence LDL metabolism and that the *Eco*RI polymorphism may influence the LDL catabolic rate [15]. However, Gallagher and Myant [17] did not encourage this hypothesis. Indeed, the major pathway for removal of LDL from the plasma is through binding of *ApoB*-100 on LDL particles to the LDL receptor; however, Gallagher and Myant [17] found no difference

**Figure 2**



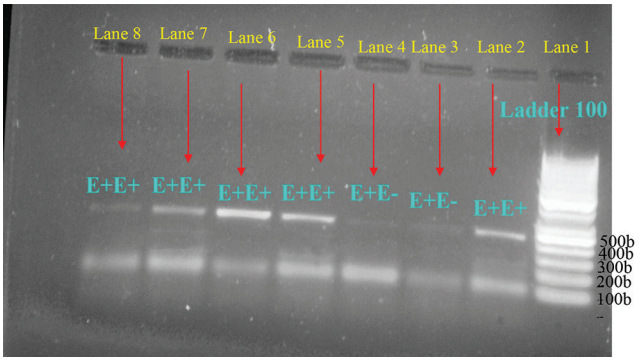

Agarose gel electrophoresis of PCR product after amplification and digestion by restriction enzyme (*Eco*RI). Lane 1, the DNA marker 100 bp (ladder); lane 2, negative control (no DNA was added); lane 4, positive heterozygous polymorphism with one band at 112 bp ($E^+/E^-$ alleles); lane 6, positive heterozygous polymorphism with one band at 510 bp ($E^-/E^+$ alleles); lane 7, homozygous negative polymorphism ($E^-/E^-$ alleles) (no bands neither at 112 bp nor at 510 bp, with an uncut DNA at 600 bp); lane 3, lane 5, and lane 8, positive homozygous polymorphism ($E^+/E^+$ alleles) with two bands at 112 and 510 bp.

between binding affinities to human skin fibroblasts of LDL particles from individuals homozygous for *Eco*RI polymorphism and those from individuals heterozygous for *Eco*RI polymorphism [17].

Pouliot *et al.* [18] investigated whether the *ApoB*-100 *Eco*RI polymorphism influenced the associations described among obesity, regional adipose tissue distribution, and plasma lipoprotein levels in 56 healthy men. After adjusting for age and BMI rate, they observed that total cholesterol levels were significantly higher in heterozygous individuals compared with homozygous negative individuals. Similar to this study, we found increased total cholesterol and LDL-c levels in patients with positive *Eco*RI polymorphism compared with negative polymorphism [18,19].

The obtained data demonstrated that $E^+/E^+$ and $E^+/E^-$ carriers had significantly higher BMI, TC, and LDL-c concentrations than did $E^-/E^-$ carriers. Furthermore, amino acid 4154 is in the C-terminal part of the *ApoB*-100 protein, in a region responsible for lipid association. The polymorphism results in an acidic (Glu) basic (Lys) amino acid substitution, which is nonconservative and has putative effect of *ApoB*-100 polymorphism on BMI and lipid profiles. This assumption is in agreement with that of Timirci *et al.* [20] who found nearly similar results in 90 children and adolescents in Turkey but is in contrast with that of Saha *et al.* [21] who found no effect of *Eco*RI polymorphism on serum lipid levels in healthy Chinese of Singapore [20,21].

**Figure 3**




Agarose gel electrophoresis of PCR product after amplification and digestion by restriction enzyme (ECoRI): Lane 1: Showed the DNA marker 100 bp (ladder). Lane 2, lane 5, lane 6, lane 7 and lane 8 showed: Positive homozygous polymorphism with 2 bands at 112 bp and 510 bp. Lane 3 and lane4 : showed Positive heterozygous polymorphism with only 1 band at 112 bp

## Conclusion

This study revealed a great association between *ApoB*-100 *Eco*RI gene polymorphism and atherogenic hyperlipidemia and insulin resistance in obese pediatric patients. Hence, its detection early in childhood stages may be a useful tool to identify children at great risk to develop chronic metabolic disorders even independent of obesity later on in adult life.

### Conflicts of interest

There are no conflicts of interest.

## References

1 Jobst EE, Enriori PJ, Cowley MA. The electrophysiology of feeding circuits. Trends Endocrinol Metab 2004; **15**:488–499.

2 WHO. WHO consultatiosn report on obesity. Obesity: preventing and managing the global epidemic. WHO technical report series. Geneva: WHO 2000; **894**:1-253.

3 Reaven G, Abbasi F, McLaughlin T. Obesity, insulin resistance, and cardiovascular disease. Recent Prog Horm Res 2004; **59**:207–223.

4 Jebb SA. Aetiology of obesity. Br Med Bull 1997; **53**:264–285.

5 Jolliffe CJ, Janssen I. Vascular risks and management of obesity in children and adolescents. Vasc Health Risk Manag 2006; **2**:171–187.

6 Dedoussis GV. Apolipoprotein polymorphisms and familial hypercholesterolemia. Pharmacogenomics 2007; **8**:1179–1189.

7 Sacks DB. Burtis CA, Ashwood ER, editors. Carbohydrates (Chapter 23). In: Tietz fundamentals of clinical chemistry. 6th ed. USA: Saunders Company 2008:458-459.

8 Park YW, Zhu S, Palaniappan L. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey. Arch Intern Med 2013; **163**:427–436.

9 Jobst EE, Enriori PJ, Cowley MA. The electrophysiology of feeding circuits. Trends Endocrinol Metab 2013; **15**:488-494.

10 Wyatt HR, Wing RR, Hill JO. Long-term weight loss and breakfast in subjects in the National Weight Control Registry. Obes Res 2012; **10**:78–82.

11 SAS Institute. Statistical analytical measures, version 6.11, annual report. North Carolina, USA: SAS Institute; 2015.

12 Must A, Jacques PF, Dallal GE, Bajema CG, Dietz WH. Long term morbidity and mortality of overweight adolescents: a follow-up of the Harvard Growth Study of 1922 to 1935. N Engl J Med 1992; **327**:1350–1355.

13 Manson JE, Willett WC Stampfer MJ, Colditz GA, Hunter DJ,

Hankinson SE, *et al*. Body weight and mortality among women. N Engl J Med 1995; **14**:677–685.

**14** Huang PL. A comprehensive definition for the metabolic syndrome. Dis Model Mech 2009; **2**:231–237.

**15** Gu W, Zhang M, Wen S. Association between the APOB XbaI and EcoRI polymorphisms and lipids in Chinese: a meta-analysis. Lipids Health Dis 2015; **14**:123.

**16** Grundy SM. Inflammation, hypertension, and the metabolic syndrome. JAMA 2003; **290**:3000–3002.

**17** Gallagher JJ, Myant NB. Does the *Eco*RI polymorphism in the human apolipoprotein B gene affect the binding of low density lipoprotein to the low-density lipoprotein receptor? Arterioscler Thromb 1992; **12**:256–260.

**18** Pouliot MC, Despres JP, Dionne FT, Vohi MC, Moorjani S, Prudhomme D, *et al*. *ApoB*-100 gene *Eco*RI polymorphism. Relations to plasma lipoprotein changes associated with abdominal visceral obesity. Arterioscler Thromb 1994; **14**:527–533.

**19** Rigo JC, Vieira JL, Dalacorte RR, Reichert CL. Prevalence of metabolic syndrome in an elderly community: comparison between three diagnostic methods. Arq Bras Cardiol 2009; **93**:85–91.

**20** Timirci O, Darendeliler F, Bas F, Arzu EH, Umit Z, Isbir T. Comparison of lipid profiles in relation to *APOB Eco*RI polymorphism in obese children with hyperlipidemia. In Vivo 2010; **24**:65–69.

**21** Saha N, Tay JS, Humphries SE. Apolipoprotein B-gene DNA polymorphisms (XbaI and *Eco*RI), serum lipids, and apolipoproteins in healthy Chinese. Genet Epidemiol 1992; **9**:1–10.