The Apolipoprotein E Gene and Taq1A Polymorphisms in Childhood Obesity

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Obesity is a multifactorial disease that is influenced by genetic and environmental factors. The apolipoprotein E (*Apo E*) polymorphism has been reported to influence some lipid profile abnormalities associated with obesity in childhood. In this study, the relationship between the *Apo E* gene and Taq1A polymorphisms with childhood obesity has been studied. Regarding the *Apo E* genotypes, e3/4 was the most frequent in both the patient and control groups. Further, there was a significance between the *Apo E* genotypes with low density lipoprotein and total cholesterol levels. However, no relationship was found between the Taq1A polymorphism and obesity. In conclusion, polygenic inheritance should be kept in mind when dealing with childhood obesity.

Introduction

OBESITY IS A MULTIFACTORIAL disease that is influenced by genetic and environmental factors. Although most genes that contribute to this predisposition are still unknown, some recently discovered single gene defects have provided insight into the hereditary nature of body weight (Ranadive and Vaisse, 2008).

Apolipoprotein E (*Apo E*) exhibits three protein isoforms (e2, e3, and e4) encoded by three haploalleles at codon positions 112 and 158 (Fulton *et al.*, 2009). The e2 allele is determined by cysteine at positions 112 (Cys) and 158 (Cys); the e3 allele is distinguished by cysteine at position 112 (Cys) and arginine at position 158 (Arg); and the e4 allele is associated with arginine at positions 112 (Arg) and 158 (Arg). These alleles result in six different isoprotein forms: e2/2, e2/3, e3/3, e2/4, e3/4, and e4/4 (Belkovets *et al.*, 2001).

The *Apo E* polymorphism has been reported to influence some lipid profile abnormalities associated with obesity in childhood (Guerra *et al.*, 2003). The e2 allele is significantly associated with lower mean levels of low density lipoprotein (LDL)-C, whereas the e4 allele is associated with higher levels of LDL-C (Sanghera *et al.*, 1996).

Dopamine D2 receptors in the central nervous system are involved in the regulation of feeding (Southon *et al.*, 2003). Dopamine D2 receptors are reported to be reduced in obese individuals (Stice *et al.*, 2008). The Taq1A polymorphism has two alleles denoted: A1 (T allele) and A2 (C allele). Recently, Taq1A was shown to reside in a neighboring gene called ankyrin-containing kinase 1 (Davis *et al.*, 2008). In addition, PET studies have found that individuals with at least one A1 allele

of the Taq1A polymorphism evidenced 30–40% fewer D2 receptors than those with the A2/A2 allele (Stice *et al.*, 2008).

In this study, we studied the relationship between the *Apo E* gene and Taq1A polymorphisms with regard to childhood obesity.

Materials and Methods

Patients

The study population was composed of 46 obese and 50 control patients recruited from the Gazi University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey. The study protocol was approved by the local ethics committee. Written informed consent was obtained from all subjects.

Obesity was defined as a body mass index (BMI) at or above the 95th percentile for children of the same age and sex. Blood samples for the measurement of glucose, total, LDL, and high density lipoprotein-cholesterol and triglycerides were obtained after an overnight fast.

Genotyping

Genomic DNA was extracted from blood samples taken from the patients and controls using an extraction kit (EZ-DNA Genomic DNA Isolation Kit; Biological Industries, Kibbutz, Israel) according to the manufacturer's instructions. Taq1A genotyping was performed according to the primers in Table 1. The polymerase chain reaction products were digested with the restriction enzyme Taq1 overnight at 65°C. There was no restriction site for the A1 allele, whereas the A2

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TABLE 1. THE POLYMERASE CHAIN REACTION PRIMERS, ANNEALING TEMPERATURES, AND RESTRICTION ENZYMES

	PCR primers	Annealing	Restriction enzyme
<i>Apo E</i>	F 5'-CTG GGC GCG GAC ATG GAG GAC GT-3' R 5'-GAT GGC GCT GAG GCC GCG CTC G-3'	65°C	HhaI (CfoI)
Taq1A	F 5'-CCG TCG ACC CTT CCT GAG TGT CAT CA-3' R 5'-CCG TCG ACG GCT GGC CAA GTT GTC TA-3'	62°C	TaqI

Apo E, Apolipoprotein E; PCR, polymerase chain reaction.

allele had a restriction site (Grandy *et al.*, 1993). *Apo E* genotyping was also carried out using the primers described in Table 1. The polymerase chain reaction products were digested with the restriction enzyme HhaI (CfoI) overnight at 37°C. The e2, e3, and e4 alleles were determined as follows: no HhaI restriction site for the e2 allele; one HhaI restriction site at position 158 for the e3 allele; and two HhaI restriction sites at positions 112 and 158 for the e4 allele (Belkovets *et al.*, 2001).

Statistical analysis

The normality of the distribution of the continuous variables was analyzed using Shapiro–Wilk's test, and Levene's test was used to assess the homogeneity of the variances in the different groups. Normally distributed groups with homogeneous variances were compared by one-way analysis of variance. Then, the Duncan test was used for multiple comparisons. Parametric test assumptions were not available for some variables, so the two group comparisons of those variables were performed with the Mann–Whitney U test. More than two group comparisons were performed using the Kruskal–Wallis one-way analysis of variance by rank test, and then multiple comparisons between pairs of groups were carried out using the Dunn test. Categorical variables were analyzed by Pearson's χ^2 test. Data analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0; SPSS, Chicago, IL). A *p*-value of <0.05 was considered significant.

Results

The Taq1A polymorphism, *Apo E* genotypes, and allelic frequencies in both patients and controls are given in Tables

TABLE 2A. APOLIPOPROTEIN E GENOTYPES IN THE PATIENT AND CONTROL GROUPS

	Patients (n = 38)	Control (n = 42)
e2/2	–	2 (4.8)
e2/3	2 (5.3)	–
e2/4	12 (31.6)	4 (9.5)
e3/3	8 (21)	9 (21.4)
e3/4	16 (42.1)	26 (61.9)
e4/4	–	1 (2.4)

TABLE 2B. APOLIPOPROTEIN E ALLELE FREQUENCIES IN THE PATIENT AND CONTROL GROUPS

	e2	e3	e4	Total
Obese	14 (18.42%)	34 (44.74%)	28 (36.84%)	76
Control	8 (9.52%)	44 (52.38%)	32 (38.10%)	84

2a, b, and 3. Regarding the *Apo E* genotypes, the most frequent genotypes were found to be e3/4 and e2/4 in obese children, whereas e3/4 and e3/3 were most common in the control group. There was no significance regarding the Taq1A polymorphism (Table 3).

Discussion

Three homozygous (e2/2, e3/3, and e4/4) and three heterozygous (e2/3, e2/4, and e3/4) phenotypes have been described and e3/3 is reported to be the most common phenotype, with a rate of occurrence of over 50% in all ethnic groups and 60–65% in Caucasians (Parlier *et al.*, 1997). The frequency of the *Apo e2/2* genotype is reported to be lower in the Japanese population (0.14%) than in the Caucasian populations (0.72%). Further, in Caucasian populations approximately 5% of *Apo e2/2* subjects are assumed to develop type III hyperlipoproteinemia (Eto *et al.*, 2002). In this study, the e3/4 genotype was common in both the patient and control groups.

Studies indicated the patterns of change in total cholesterol and LDL-C varied by *Apo E* genotype (Sanghera *et al.*, 1996; Fulton *et al.*, 2009). Further, we found significance between the *Apo E* genotypes with LDL-C and total cholesterol levels (*p* = 0.003 and *p* = 0.006, respectively). However, no relationship was found between fasting glucose level and body weight.

Apo E is reported to be an important genetic determinant of serum lipoprotein concentrations and coronary artery disease risk (Srinivasan *et al.*, 1996). E4 carriers of *Apo E* were shown to have higher total and LDL cholesterol levels adjusted according to age, sex, BMI, and insulin sensitivity (Johansson *et al.*, 2009). Nascimento *et al.* (2009) reported that e4 carriers presented with a worse lipid profile than e2 and e3/3 carriers. In our study, we found a relationship between LDL cholesterol and total cholesterol levels with the e2/4 genotype (*p* < 0.05). In addition, the e2/3 genotype was related to BMI (*p* = 0.048).

TABLE 3. TAQ1A POLYMORPHISM IN THE PATIENT AND CONTROL GROUPS

	Patients (n = 45)	Control (n = 49)	<i>p</i>
<i>Genotypes</i>			
A1A1	2	3	ns
A1A2	42	45	
A2A2	1	1	
<i>Alleles</i>			
A1	0.51	0.52	ns
A2	0.49	0.48	

ns, nonsignificant.

The presence of the A1 allele is not reported to be related to body weight (Nisoli *et al.*, 2007), which was also indicated in our study. In addition, the Taq1A polymorphism is unlikely to be a common cause of obesity in Nauruan and Australian subjects (Southon *et al.*, 2003). Further, the Taq1A polymorphism was not associated with blood lipids such as cholesterol, high density lipoprotein, and LDL cholesterol and triglycerides (Noble *et al.*, 1994). Our findings also confirmed these results.

There was a significant association between BMI and the presence of the A1 allele of Taq1A polymorphism for men and women (Comings *et al.*, 1996). However, we could not find a relationship between BMI and the Taq1A polymorphism.

In conclusion, polygenic inheritance should be kept in mind when dealing with childhood obesity. Further, *Apo E* genotypes influence the risk of dyslipidemia and should be taken into account in the management and follow-up of these patients.

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