Apolipoprotein E gene polymorphism in Greek children with nephrotic syndrome.

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ABSTRACT: Apolipoprotein E (ApoE) genotypes were determined for children with nephrotic syndrome (NS) (study group, N=17) and control group (N=31). In controls, ε3/ε3 was prominent. In study group, ε2 allele was present in contrast to controls. Hypercholesterolemia seems related to apoE genes polymorphism. Genotyping could predict the clinical course of NS.

Key Words: ApoE, Genotype, Nephrotic syndrome, Children.

INTRODUCTION

Nephrotic syndrome (NS) is characterized by severe hyperlipidemia, with high cholesterol, triglycerides and low-density cholesterol levels[1]. Lipid abnormalities have been linked to the progression of nephropathy in these patients[2], and it has been supported that LDL-apheresis or lipid-lowering medications can act favourably on nephrotic hypoalbuminemia or albuminurea[3]. Apolipoprotein E (apoE) is a 35-kDa plasma protein, which is synthesized in the liver and plays essential role in lipid metabolism[4]. Moreover, it contributes in renal protection by regulating mesangial cell proliferation and matrix expansion[5]. There are three major isoforms of apoE, known as apoE2, apoE3 and apoE4, products of three alleles (ε2, ε3 and ε4, respectively) of a single gene on chromosome 19. Three homozygous phenotypes of apoE (apoE2/2, E3/3 and E4/4) and three heterozygous phenotypes (apoE2/3, E4/3 and E2/4) can arise from these alleles[6]. It is already well known that apoE genotype variations can affect lipoprotein levels[7]. In specific, E2 homozygous phenotype has been linked to familial dysbetalipoproteinemia[8].

In childhood, NS lipid abnormalities are mostly characterized by hypercholesterolemia and, less frequently, by hypertriglyceridemia. Studies have shown that apoE polymorphism is associated with renal disease[9] and has been considered a possible prognostic factor for the course of childhood NS[10]. In the present study, frequencies of apoE genotypes and alleles in Greek children with NS have been evaluated and correlated to the degree of their hyperlipidemia, as long as no such study has been made yet.

MATERIAL AND METHODS

Forty six children (mean age 6.26±3.2 years) were included in the study, either admitted in our department or examined in the Outpatient Clinic of our hospital. All laboratory assays were performed in the Laboratory for Lipids and Cardiovascular Disease Prevention from Childhood of the 2nd Pediatric Department. The children were divided into two groups: the study group, which consisted of 15 children with diagnosed NS and no hypertension or diseases such as hepatitis B or C, and the control group, of 31 age-matched children with no NS or other chronic disease. Diagnosis of NS was made according to the ISKDC criteria[11]. In the study group, only one child had new onset of NS, whereas 11 had episodes of relapse and 3 had a history of NS in the last five years. Written informed consent

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to participation in the study was obtained from all patients and controls.

Blood samples were collected after overnight fast. Serum was separated and stored at -20°C. Total cholesterol and triglycerides serum levels were determined using a biochemical analyzer (Hitachi 911, Roche Diagnostics GmbH, D-68298 Mannheim). Genomic DNA from whole blood was obtained by the use of a kit (QIAamp Blood DNA purification kit, Qiagen, USA). The apoE gene polymorphisms were detected by typical polymerase chain reaction (PCR) and subsequent reaction fragment length polymorphism (RFLP) techniques[12,13], with the use of the following primers (Invitrogen):

5′ - TCCAAGGAGCTGCAGGCGGCGCA - 3′ (23 bp, Tm 68°C) (*Forward*) and

5′ - GCCCCGGCCTGGTACACTGCCA - 3′ (22 bp, Tm 68°C) (*Reverse*).

Thermo cycler PTC200 (MJ Research) was used for the purpose of the study.

Statistical analysis of the results was performed using SPSS statistical package (v.13). The χ^2-test was used to evaluate agreement with Hardy-Weinberg equilibrium and differences between the two groups. Multiple regression analysis was used in order to investigate possible relation of apoE genotypes to levels of total cholesterol and triglycerides. Statistical significance was considered for *P* value less than 0.05.

RESULTS AND DISCUSSION

The results are summarized in Table 1. Both groups were in Hardy-Weinberg equilibrium. In specific, among the 15 children of the study group, 3 (20.00%) had genotype ε2/3, 10 (66.66%) had ε3/3 and 2 (13.33%) had ε3/4. Among the 31 children of the control group, 28 (90.30%) had ε3/3 and 3 (9.70%) had ε3/4. The apoE genotype distribution of the study group was not statistically significantly different when compared with the healthy controls ($P > 0.05$), although ε3/3 frequency was lower in study than in control group. Nevertheless, ε2 seems to be present only in children with NS. These results come in accordance with the results of other studies, in which ε3/3 is the predominant genotype in different healthy populations all over the world[14] as well as in populations of children with NS[10,15].

The study of genotypes and alleles according to gender in children with NS showed that girls' genotype (n=4) was ε3/3, whereas between boys (n=11), 3 of them (27.27%) had ε2/3, 6 (54.55%) had ε3/3 and 2 (18.18%) had ε3/4. Most likely, in the control group all girls (n=17) had ε3/3 and genotype distribution in boys (n=14) was: 11 (78.60%) with ε3/3 and 3 (21.40%) with ε3/4. This results lead us to the conclusion that ε2 allele can be present in male children population with NS and not in healthy male children.

The determination of total cholesterol serum levels in children with NS showed the following results (mean ± SD): for ε3/3 genotype values were 356.0 ± 46.2mg/dL, for ε2/3 421.0 ± 45.2 mg/dL and for ε3/4 440.0 ± 44.3 mg/dL (mg_{TC}/dL=38.5 x mmol/L), showing increased serum total cholesterol levels in the presence of ε2 or ε4 allele. In refer to triglycerides serum levels in the study group, the results were the following: for ε3/3 genotype 260.7 ± 157.0 mg/dL, for ε2/3 335.5 ± 74.3mg/dL and for ε3/4 245.0 ± 44.2 mg/dL (mg_{TG}/dL=87.7 x mmol/L). These lead us to the conclusion that the presence of ε4 in children with NS is mainly associated with severe hypercholesterolemia, as it has been shown before[1], rather than hypertriglyceridemia. Multiple regression analysis showed that total cholesterol serum levels are borderly significantly correlated ($P = 0.058$) with apoE polymorphism, whereas serum levels of triglycerides didn't present any significant correlation with apoE genotypes ($P = 0.884$).

The present study has the limitation of the small number of children participating. That may be attributed to the reduced number of incidents of NS in childhood, in recent years in our country. Nevertheless, the results can lead to some primary conclusions on apoE polymorphism in Greek children with NS and on the relation between severity of their hyperlipidemia and their apoE genotype. This needs to be further investigated in larger patient groups, in order to improve prognosis and management and, consequently, the course of their disease.

Abbreviations

NS: Nephrotic syndrome

ApoE: Apolipoprotein E

Table 1. Frequencies of apolipoprotein E genotypes and alleles in study and control groups.

	Study group (n=15)		**Control group (n=31)**	
Genotypes	**n**	**%**	**n**	**%**
ε2/2	0	0.00	0	0.00
ε2/3	3	20.00	0	0.00
ε2/4	0	0.00	0	0.00
ε3/3	10	66.66	28	90.30
ε3/4	2	13.33	3	9.70
ε4/4	0	0.00	0	0.00
Alleles				
ε2	3	10.00	0	0.00
ε3	25	83.40	59	95.20
ε4	2	6.60	3	4.80

Πολυμορφισμός του γονιδίου της απολιποπρωτεΐνης Ε σε παιδιά με νεφρωσικό σύνδρομο στην Ελλάδα.

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ΠΕΡΙΛΗΨΗ Προσδιορίσθηκαν οι γονότυποι της απολιποπρωτεΐνης Ε (apoE) σε παιδιά με νεφρωσικό σύνδρομο (ΝΣ) (ομάδα ελέγχου, Ν=17) και σε ομάδα μαρτύρων (Ν=31). Στους μάρτυρες επικρατούσε ο ε3/ε3. Στην ομάδα ελέγχου, βρέθηκε το ε2 αλλήλιο σε αντίθεση με τους μάρτυρες. Συμπερασματικά, η διερεύνηση του γονότυπου μπορεί να βοηθήσει στην πρόγνωση του ΝΣ.

Λέξεις κλειδιά: ApoE, γονότυπος, Νεφρωσικό σύνδρομο, Παιδιά.

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