Apolipoprotein E gene polymorphism and polycystic ovary syndrome patients in Western Anatolia, Turkey

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Abstract

Purpose Dyslipidemia, cardiovascular disease and hypertension are more frequently seen in patients with PCOS than in normal patients. We aimed at evaluating the distribution of Apo E alleles that can influence cardiovascular risk of the PCOS patients and control subjects.

Methods In this study, 129 young women with PCOS and 91 healthy women were included. In all subjects we performed hormonal, biochemical and Apo E genetic analysis.

Results The Apo E3 allele was found at a significantly higher frequency in the PCOS patient group compared with the control group. The Apo E2 allele was found at a significantly higher frequency in the control group compared with the patient group with PCOS.

Conclusions Although there were genotype and allele differences between control and patient groups in this study, no statistically significant change was determined in lipid and other cardiovascular risk factors in connection with allele and genotype.

Abbreviations

ApoE	Apolipoprotein E
PCOS	polycystic ovary syndrome
BMI	body mass index
CI	confidence interval
CV	coefficient(s) of variation
MLRA	Multiple Logistic Regression Analysis
CVD	cardiovascular disease
DHEAS	dehydroepiandrosterone sulphate
E ₂	17 β -estradiol
Hcy	homocysteine
17-OHP	17-hydroxyprogesterone
P	progesterone
PRL	prolactin
T	testosterone
FSH	follicle stimulating hormone
LH	luteinizing hormone
HOMA-IR	homeostasis model of assessment insulin resistance

Keywords Apolipoprotein E gene · Cardiovascular risk factors · Hyperlipidemia · Polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine malfunctions, which typically occur with chronic anovulation and hyperandrogenism [1]. Almost 7% of women at reproductive age are diagnosed with PCOS [2].

Dyslipidemia, cardiovascular disease and hypertension are more frequently seen in patients with PCOS than in normal patients [3–5].

The disorder has also been reported to be associated with an increase in sub-clinical atherosclerotic disease [5].

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Apolipoproteins that form the protein part of the lipoproteins are important in lipid metabolism. It regulates the transport and redistribution of lipoproteins in blood as a cofactor. Apolipoprotein E (ApoE) is a 34,200 kDa polymorphic glycoprotein consisting of 299 amino acids [6]. Three common alleles of ApoE are E2, E3, E4 code for three isoforms E2, E3 and E4 in exon 4 ApoE genes. These isoforms result in six common phenotypes, which are E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4 [7]. Relation of ApoE2 to the threat of atherosclerosis is controversial. ApoE4 is connected with decreased longevity, increased plasma lipid and ApoB levels and higher prevalence of cardiovascular illness and also of Alzheimer's [8, 9].

Addition of genetic risk factors (such as abnormality of Apo E, PPAR, and TPA-PAI) to increasing technology and inactivity increases the human susceptibility to cardiovascular disease in the future. To date, there is no data available for Turkish subjects in the genetic polymorphism of ApoE and its relation with increased cardiovascular risk factors in PCOS patients. In this study, we aimed at evaluating the distribution of ApoE alleles that can influence cardiovascular risk of the Turkish PCOS patients and control subjects.

Materials and methods

Patients

In this study, 129 young women with PCOS and 91 healthy women were included. PCOS was defined by Rotterdam PCOS consensus criteria [10].

Patients who had Diabetes Mellitus (diagnosed by 75 g Oral Glucose Tolerance Test), hyperprolactinemia, congenital adrenal hyperplasia (diagnosed with the adrenocorticotrophic hormone stimulation test), thyroid disorders, Cushing's syndrome, hypertension, hepatic or renal dysfunction were excluded from the study. At study entry, all subjects underwent venous blood drawing for complete hormonal assays, lipid profile, glucose, and insulin. All blood samples were obtained during early follicular phase of the spontaneous or progesterone-induced menstrual cycle in the morning between 8 AM and 9 AM respectively after an overnight fasting, and while resting in bed.

Biochemical assay

Serum concentrations of hs-CRP were determined by an immunonephelometric assay (N-hs-CRP; Dade Behring, Izmir, Turkey); intra- and inter-assay CVs were 1.72 and 2.80%, respectively. Serum total cholesterol, LDL and HDL cholesterol, triglyceride, aspartate aminotransferase (AST),

alanine aminotransferase (ALT), and -glutamyltransferase (GGT) were measured by Olympus AU 2,700 automated analyzer (Toshiba, Tokyo, Japan). Plasma insulin concentrations were determined by Immulite 2000 that uses two-site chemiluminescent immunometric assay. Insulin resistance was calculated by using homeostasis model assessment insulin resistance index (HOMA-IR) [11] according to the following formula:

$$\text{HOMA-IR} = \frac{\text{fasting serum insulin (mU/ml)} \times \text{fasting plasma glucose (mmol/l)}}{22.5}$$

ApoE gene genotyping

Genomic DNA was extracted from peripheral leukocytes of the subjects using the High Pure PCR Template Preparation Kit (Roche Applied Science). For the detection of the presence of the three ApoE, E alleles E2, E3 and E4 (codon 112 and 158) were analyzed by the commercial LightCycler ApoE Mutation Detection Kit (Roche Diagnostics, Mannheim, Germany).

All experiments were carried out on LightCycler™ Instrument (Roche Applied Science, Germany) according to the protocols provided by manufacturer. Polymorphic alleles were identified by specific melting temperature (T_m) of the resulting amplicons. In the case of ApoE, codon 112 and 158 were analyzed simultaneously and therefore two T_m values were obtained for each allele: for E2, these were 56°C and 57.5°C; for E3, 56°C and 66°C; and for E4, 62.5°C and 66°C respectively.

Statistical analysis

SPSS 14.0 for Windows (SPSS Inc. Chicago, USA) was used for statistical analysis of the results. $P < 0.05$ values were accepted as statistically significant. The characteristics of the patients with PCOS and the mean plasma homocystein, glucose, and insulin, dehydroepiandrosterone sulphate, 17β-estradiol, homocystein, 17-hydroxyprogesterone, prolactin, testosterone levels between the two clinical groups were compared by Student's *t* test for unpaired data and between and within the different groups (E2/E3, E3/E3, E3/E4) of ApoE genotypes with the ANOVA. To determine which of the parameters, for which Student's *t* test found a statistically significant difference between patient and control groups, was more strongly associated with PCOS, we performed univariate logistic regression analysis. Differences in the genotype distribution between different groups were assessed by logistic regression analysis of heterogeneity. All results were expressed as means ± SD.

Results

Biochemical and hormonal parameters of the patients with PCOS and the control group are seen in Table 1. The levels of total cholesterol ($p=0.004$), LDL-cholesterol ($p=0.023$), triglyceride ($p=0.004$), fasting glucose ($p<0.001$), fasting insulin ($p<0.001$), homocystein ($p<0.001$), fibrinogen ($p<0.001$), FSH, LH, 17-OHP and free-testosterone ($p<0.001$) in patients with PCOS were determined to be statistically significantly high. Statistically significant difference was not determined for age and BMI between both groups. Again, no statistically significant difference was determined between control and patient groups for DHEAS, estradiol, total testosterone, prolactin, HDL-cholesterol, and hs-CRP levels.

ApoE genotype distribution in patient and control groups is seen in Table 2. There is no patient or healthy subject with E2/E2 and E4/E4 genotypes in our study. E2/E3 and E3/E3 genotypes were determined to be higher in control group ($p<0.001$) and E3/E3 was determined to be higher in the group of patients with PCOS ($p<0.001$, OR: 6.34, CI: 2.93–13.74). However, E3/E4 genotype was determined to be at similar levels in patient and control groups ($p=0.07$, OR: 1.94, CI: 0.67–5.66). The ApoE3 allele showed a significantly higher frequency in PCOS patient group (91.8%) when compared with control group (75.6%) ($p<0.001$). The ApoE2 allele showed a significantly higher frequency in the control group (16.7%) when compared with patient group with PCOS (4.3%) ($p<0.001$). In this study, ApoE4 allele frequency was not statistically significantly different in patient and control groups ($p>0.513$).

Metabolic and hormonal levels were analyzed in patients with PCOS according to different allele situations (E2, E3, E4) of Apo E. (Table 3). As a result of this analysis, a relation was found only between Apo E alleles and prolactin and LH levels. LH levels were found to be low in patients with PCOS in the existence of E3 and E4 allele ($p=0.018$). However, prolactin levels was statistically lower in the ApoE4 allele-carrying PCOS patients ($p=0.046$).

Discussion

In the present study, we compared the frequency of the ApoE gene polymorphism in PCOS patients and healthy controls living in Western Turkey. Women with PCOS are more prone to have metabolic syndrome than healthy women [12]. Additionally, different markers of atherosclerosis, such as fibrinogen, high sensitive C-reactive protein, homocysteine, oxidative stress markers, PAI-1, TPA, carotid intimae-media thickness, and echocardiographic findings have also been found to be changed [13–16]. Dyslipidemia is very common and includes elevated triglyceride levels and low high-density lipoprotein (HDL) cholesterol concentrations in PCOS patients [17].

Dyslipidemia may be the most common metabolic abnormality in PCOS, although the type and extent of the findings have been variable. PCOS patients have an atherogenic lipid profile characterized by lower high-density lipoprotein (HDL) cholesterol and/or HDL₂ cholesterol levels, and higher triglyceride and low-density lipoprotein (LDL) cholesterol levels than the age- and

Table 1 Clinical characteristics of patients and controls

Characteristic	Patients * $n=129$	Control* $n=91$	$p^{\&}$
Age (years)	24.58 $\pm$ 4.61	25.48 $\pm$ 3.38	0.115
BMI (k/m ²)	24.47 $\pm$ 4.64	24.2 $\pm$ 3.31	0.65
Total-cholesterol (mg/dl)	199.86 $\pm$ 38.82	187.33 $\pm$ 13.77	0.004
LDL-C (mg/dl)	117.75 $\pm$ 29.35	110.31 $\pm$ 11.40	0.023
HDL-C (mg/dl)	56.76 $\pm$ 13.89	57.33 $\pm$ 4.49	0.705
Triglycerides (mg/dl)	122.34 $\pm$ 55.27	115.09 $\pm$ 24.07	0.004
Fasting Glucose (mg/dl)	91.43 $\pm$ 7.55	88.08 $\pm$ 3.96	< 0.001
Fasting insulin (mIU/ml)	12.16 $\pm$ 9.07	4.71 $\pm$ 1.17	< 0.001
Homocysteine (μ mol/L)	2.63 $\pm$ 1.60	1.33 $\pm$ 0.22	< 0.001
FSH (mIU/ml)	5.43 $\pm$ 1.77	4.17 $\pm$ 1.24	< 0.001
LH (mIU/ml)	6.53 $\pm$ 3.68	4.18 $\pm$ 0.99	< 0.001
Estradiol (pg/ml)	34.90 $\pm$ 20.83	39.02 $\pm$ 32.35	0.254
DHEA-S (μ g/dl)	225.92 $\pm$ 89.32	222.29 $\pm$ 42.84	0.721
17-OHP (ng/ml)	1.72 $\pm$ 0.87	0.90 $\pm$ 0.31	< 0.001
Total-Testosterone (ng/ml)	0.99 $\pm$ 1.36	1.07 $\pm$ 0.69	0.632
Free-Testosterone (pg/ml)	3.39 $\pm$ 1.80	1.52 $\pm$ 0.40	< 0.001
Prolactine (pmol)	17.08 $\pm$ 7.27	15.51 $\pm$ 4.49	0.07
Fibrinogen (mg/dl)	367.00 $\pm$ 75.07	277.12 $\pm$ 44.07	< 0.001
hs-CRP(mg/dl)	0.41 $\pm$ 0.63	0.29 $\pm$ 0.22	0.730

*mean $\pm$ SD

$\&$ 2-tailed t test

$P<0.05$ values were accepted as statistically significant

Table 2 Distrubution of ApoE haplotypes and genotypes

Haplotypes/ Genotypes	Patients*	Control*	p ^{&}	OR ^{&}	95% CI ^{&}
E2	11 4.3%	30 16.7%	< 0.001		R
E3	235 91.8%	136 75.6%	< 0.001	0.212	0.103–0.437
E4	10 3.9%	14 7.8%	0.513	0.513	0.177–1.490
E2/E3	11 8.6%	31 33.3%	< 0.001		R
E3/E3	108 83.6%	46 51.1%	< 0.001	6.34	2.93–13.74
E3/E4	10 7.8%	14 15.6%	0.072	1.94	0.67–5.66

P<0.05 values were accepted as statistically significant

*% frequency

[&] Logistic regression analysis

weight-matched control women [18]. Thus, the presence of abdominal obesity, insulin resistance and dyslipidemia predispose women with PCOS to cardiovascular diseases (CVDs) [19].

Another study, found that E4 carriers carry a higher risk of hyperuricaemia and postprandial hypertriglyceridaemia after a fat excess in patients with metabolic syndrome [35]. Some researchers described a connection between E4 variant and increased fasting plasma glucose levels and plasma insulin levels [20, 21]. In our study, E4 allele frequency was determined to be similar in patient and control groups. Our results demonstrate a statistically significant increase in triglyceride, LDL-cholesterol and total cholesterol levels of

the PCOS patients when compared to those of controls. However, no statistically significant difference was found in LDL-cholesterol, total cholesterol, triglyceride, and HDL-cholesterol levels in E3, E4, and E2 alleles of ApoE gene. Some researchers presented similar study and they demonstrated similar results in patients with PCOS from Finland [22]. On the other hand, in Western Anatolia ApoE4 allele frequency (3.9%) was lower than that of Finland (17.2%) in women with polycystic ovary syndrome.

Increasing evidence suggests that atherosclerosis is a chronic inflammatory process and due to this fact the markers of the inflammatory response such as CRP and fibrinogen might be useful in assessing the risk of cardiovascular disease [23]. Women's Health Study has shown that CRP is a strong independent risk factor for cardiovascular diseases in females [24]. Endothelial dysfunction and high concentrations of high-sensitivity CRP were detected even in normal-weight women with PCOS [25].

No statistical correlation was found between different alleles of Apo gene and homocysteine, fibrinogen, and hs-CRP in our current study. The Asian people usually have lesser *apo* ϵ 4 frequency than European people. The APOE4 allele has also been connected to increased plasma LDL-C concentrations in healthy subjects [26, 27]. The negative effects of lifestyle factors can interact with genetic factors (e.g. ApoE4), therefore increasing cardiovascular risk [28, 29]. These values are similar to those in other populations, such as Italians (ApoE4: 9.4%) and the French (ApoE4: 12.0%) [30, 31]. In our study, the ApoE2 allele frequency in western Turkish healthy population (16.7%) was found to be higher than in Caucasian (8.0%) and Greek populations (8.1%) [32, 33].

Table 3 Biochemical and Hormonal parameters between ApoE haplotypes in patient group

	Haplotypes and parameters (Mean $\pm$ SD)			
	E2	E3	E4	<i>P</i> value
Total-cholesterol (mg/dl)	188.20 $\pm$ 40.13	200.36 $\pm$ 39.43	207.40 $\pm$ 30.45	0.503
LDL- cholesterol (mg/dl)	111.20 $\pm$ 29.32	118.12 $\pm$ 29.82	121.00 $\pm$ 25.76	0.712
HDL- cholesterol (mg/dl)	59.00 $\pm$ 16.18	55.66 $\pm$ 12.37	66.00 $\pm$ 22.68	0.067
Triglycerides (mg/dl)	110.50 $\pm$ 45.34	125.00 $\pm$ 54.90	106.90 $\pm$ 69.22	0.468
Fasting Glucose (mg/dl)	92.70 $\pm$ 6.91	91.03 $\pm$ 7.32	94.30 $\pm$ 10.37	0.360
Basal Insulin (mIU/ml)	11.20 $\pm$ 7.61	12.18 $\pm$ 9.56	13.10 $\pm$ 5.32	0.893
Homocysteine(μ mol/L)	2.73 $\pm$ 1.62	2.57 $\pm$ 1.61	3.13 $\pm$ 1.49	0.569
FSH (mIU/ml)	5.48 $\pm$ 1.80	5.54 $\pm$ 1.74	4.19 $\pm$ 1.80	0.069
LH (mIU/ml)	8.50 $\pm$ 5.16	6.29 $\pm$ 3.33	6.98 $\pm$ 5.00	*0.018
Estradiol (pg/ml)	38.20 $\pm$ 20.70	33.53 $\pm$ 19.79	46.00 $\pm$ 29.43	0.167
DHEA-S (μ g/dl)	247.11 $\pm$ 127.18	222.39 $\pm$ 86.19	240.30 $\pm$ 78.69	0.597
17-OHP (ng/ml)	1.98 $\pm$ 1.25	1.74 $\pm$ 0.85	1.29 $\pm$ 0.51	0.177
Total-Testosterone (ng/ml)	0.86 $\pm$ 0.29	1.04 $\pm$ 1.48	0.63 $\pm$ 0.20	0.503
Free-Testosterone (pg/ml)	3.30 $\pm$ 1.53	3.48 $\pm$ 1.84	2.61 $\pm$ 1.52	0.343
Prolactine(pmol)	18.36 $\pm$ 10.55	17.33 $\pm$ 7.09	13.00 $\pm$ 3.09	*0.046
Fibrinogen (mg/dl)	343.81 $\pm$ 72.34	369.50 $\pm$ 74.70	365.70 $\pm$ 85.13	0.560

**P*<0.05 values were accepted as statistically significant

The occurrence of the ApoE4 allele may therefore contribute to the variation in the risk of these diseases such as coronary artery disease, hyperlipidemia, type 2 diabetes, metabolic syndrome, and polycystic ovary syndrome across populations. ApoE4 allele frequency was not determined to be higher in patients with PCOS than the controls in our study.

The ApoE3 allele is the most common allele in each population [34]. The ApoE3 allele showed a significantly higher frequency in the PCOS patient group when compared with the control group ($p < 0.001$). E3/E3 genotype of ApoE gene in patients with polycystic ovary syndrome was found to be statistically significantly higher than that of healthy control group in this study. But no relation was found between ApoE gene E3/E3 and lipid parameters and cardiovascular risk factors such as hs-CRP, fibrinogen, and homocysteine. However, there is a need for long-term prospective studies for explaining the relation between ApoE3 allele elevation and reduction of cardiovascular disease risk in patients with PCOS.

Although there was a genotype and allele difference between control and patient group in this study, no statistically significant change was determined in lipid and other cardiovascular risk factors in connection with allele and genotype. ApoE gene polymorphism has no effect on cardiovascular risk factors such as lipid and hs-CRP, fibrinogen, and homocysteine in Turkish patients with PCOS.

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