Apolipoprotein E alleles in women with pre-eclampsia

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Abstract

Aims—To investigate the frequency of three apolipoprotein E (apoE) alleles among women with pre-eclampsia.

Methods—The presence of the three most common apoE alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) was determined by polymerase chain reaction–restriction fragment length polymorphism in two groups of women: healthy pregnant women ($n = 91$) and pregnant women with a diagnosis of pre-eclampsia ($n = 133$). In addition, the frequencies of the alleles in the general population in this area are presented for comparison.

Results—The frequency of the apo $\epsilon 4$ allele was 18.4% among women with pre-eclampsia and 18.7% among healthy pregnant women (Fisher's exact test; $p = 0.941$), which is close to the rate in the general population in this area (19%). None of the apolipoprotein E genotypes was significantly over-represented, and homozygous genotype $\epsilon 4$ was not associated with more severe clinical disease than were the other genotypes.

Conclusion—The observed profiles of allele and genotype frequencies confirm an equilibrium state between apoE polymorphism and pre-eclampsia and suggest that apoE does not play a major role in the development of pre-eclampsia.

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Keywords: apolipoprotein E; gene polymorphism; pre-eclampsia

The aetiology of pre-eclampsia is unknown, but endothelial cell injury and altered endothelial cell function play a pivotal role,¹ and may be central to the clinical diversity and pathophysiological processes of the syndrome. It has been proposed that a poorly perfused placenta is the origin of a humoral factor that affects maternal systemic function, directly or indirectly, by activating endothelial cells, with resultant vascular injury.² This process might also result in a tendency to develop ischaemic heart disease later in life.¹⁻⁶

The lipid profiles of pregnant women who subsequently develop pre-eclampsia are characterised by increased values of triglyceride rich lipoproteins and cholesterol.⁷ These are potential contributors to the endothelial dysfunction seen in pre-eclampsia.⁸ It is well known that cholesterol absorption efficiency from the intestine increases whereas bile acid secretion by the liver falls in the allelic order: $\epsilon 2 < \epsilon 3 < \epsilon 4$.⁹ Apolipoprotein E (apoE; especially the $\epsilon 4$ allele) is an established genetic

marker for dyslipidaemia⁸ and it plays an important role in lipid metabolism in non-pregnant women. Results published by Nagy *et al* suggested that apo $\epsilon 2$ alleles were more frequent in patients with pre-eclampsia than in controls and that, therefore, this particular allele might increase the risk of developing pre-eclampsia.¹⁰ Clinically, this finding was unexpected and it raises the question of whether the observed association occurred by chance in a relatively small number of patients and whether the result can be reproduced in another population. The aim of our study was to investigate the possible role of apoE in pre-eclampsia by determining the frequency of apoE alleles and genotypes using the polymerase chain reaction (PCR) combined with restriction fragment length polymorphism (RFLP) analysis

Patients and methods

Written approval for our study was obtained from the ethics committee of Kuopio University Hospital. Informed consent was obtained from all patients and documented.

Information was collected retrospectively from 133 pre-eclamptic pregnancies and from 91 control women who delivered at Kuopio University Hospital between January 1994 and December 1998. Forty one familial cases and 92 sporadic cases were investigated. To ensure homogeneity of the genetic background, the controls originated from a regional population and were enrolled by random selection in this case control study.

Hypertensive complications of pregnancy were classified as advocated by the US National Institute of Health working group on hypertension in pregnancy. Pre-eclampsia was defined as the development of hypertension and new onset proteinuria (> 300 mg of urinary protein in 24 hours) in women with no proteinuria at base line. For those with a base-line diastolic pressure below 90 mm Hg, hypertension was defined as a rise of at least 25 mm Hg, measured on two consecutive occasions at least 24 hours apart. The comparison of different classification systems has shown that it is probably better to split *de novo* hypertension along these lines—"pre-eclampsia" and "gestational hypertension" (The International Society for the Study of Hypertension in Pregnancy, 1988)—rather than to use the confusing terms "mild" and "severe" pre-eclampsia (Australian Society for the Study of Hypertension in Pregnancy, 1993), the former applying only to raised blood pressure.¹¹⁻¹³ Women with chronic hypertension were excluded from our study.

DNA was extracted from peripheral blood lymphocytes using a standard phenol/

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Table 1 Apolipoprotein E allele frequencies and genotype among women with pre-eclampsia and healthy pregnant controls, and in the general population

Allele frequencies	Pre-eclamptic women (%)	Controls (%)	Population (%)
ϵ 2	14/133 (5.3%)	11/91 (6.0%)	4%
ϵ 3	203/133 (76.3%)	137/133 (75.3%)	77%
ϵ 4	49/133 (18.4%)	34/133 (18.7%)	19%
Genotype frequencies			
2/2	0/133 (0%)	0/91 (0%)	0.3%
2/3	11/133 (8.3%)	10/91 (11.0%)	5.4%
2/4	3/133 (2.3%)	1/91 (1.1%)	1.8%
3/3	79/133 (59.4%)	54/91 (59.3%)	58.7%
3/4	34/133 (25.6%)	19/91 (20.9%)	30.6%
4/4	6/133 (4.5%)	7/91 (7.7%)	3.2%

chloroform extraction method.¹⁴ ApoE genotypes were analysed using PCR-RFLP (HhaI) as described previously^{15 16}; 400 ng of genomic DNA was used for PCR. The reaction volume of 50 μ l contained 25 pmol each primer, 200 mmol of each dNTP (Promega, Madison, Wisconsin, USA), 50 mM KCl, 10 mM Tris/HCl (pH 9.0), 1.5 mM MgCl₂, 0.1% Triton X-100, 0.052% dimethyl sulfoxide (DMSO) and 0.5 U of Taq DNA polymerase (Promega).

A volume of 18 μ l of a 299 bp PCR fragment was digested with HhaI (New England Biolabs, Beverly, Maryland, USA) using 8 U of enzyme at 37°C for at least three hours. Digested fragments were analysed on a 0.5 mm 10% non-denaturing polyacrylamide gel containing 5% glycerol. After 120 minutes of electrophoresis at 400 V the DNA fragments were visualised by ethidium bromide staining. The fragment sizes from polymorphic HhaI sites after cleavage were as follows; E2/E2: 91 bp and 83 bp; E3/E3: 91 bp, 48 bp, and 35 bp; E4/E4: 72 bp, 48 bp, and 35 bp; E3/E2: 91 bp, 83 bp, and 48 bp; E4/E2: 91 bp, 83 bp, 72 bp, and 48 bp; E4/E3: 91 bp, 72 bp, and 48 bp. Heterozygote E4/E2 was used as a positive control and a blank sample as a negative control. From the PCR results, the genotypes and allele frequencies were calculated. Statistical analysis was carried out using Fischer's exact test with SPSS 8.0 software and significance was defined as $p < 0.05$. ApoE genotypes were found to be in exact Hardy-Weinberg equilibrium in both patient and control groups.

Results

The mean (SD) maternal ages in the study and control groups were 28.8 (6.4) and 28.7 (5.4) years, respectively (NS). The mean (SD) gestational age at the development of pre-eclampsia was 31.7 weeks (3.5). When only the first degree relatives of the index patients were taken into account, positive family history was reported by 30 affected women, who had six affected sisters and 28 affected mothers.

Table 1 presents the apoE genotypes and allele frequencies. Population frequencies were derived from the same geographical area and they were based on more than 1500 tested subjects in the general population, including both women and men. The frequency of the apo ϵ 4 allele was 18.4% among pre-eclamptic cases and 18.7% among healthy pregnant women ($p = 0.941$), which in turn corresponded well to the population frequencies (19%), as expected. None of the apoE

genotypes was significantly over-represented. Affected women were then divided into subgroups according to the number of ϵ 4 alleles: no ϵ 4 allele (genotypes 2/3, 3/3), one ϵ 4 allele (genotypes 2/4, 3/4), and two ϵ 4 alleles (genotype 4/4). Using the general population as a reference, the 2/3, 2/4, 3/4, and 4/4 genotypes were not associated with an increased risk of pre-eclampsia. Women with the ϵ 4/4 genotype constituted 4.5% of all affected women and 7.7% in the control group. The clinical course of pre-eclampsia, as regards the onset and severity of the disease, among affected women with the ϵ 4/4 genotype was comparable with that of those women with pre-eclampsia and other genotypes. Furthermore, there were no observed differences between sporadic and familial cases with pre-eclampsia (data not shown). Collectively, an equilibrium state between apoE alleles and genotypes was established in both women with pre-eclampsia and controls.

Discussion

Although pre-eclampsia is a multifactorial disease, it has a general tendency towards familial clustering, suggesting that genetic factors might play an important role.¹⁰ ApoE is an established genetic marker of dyslipidaemia and atherosclerosis (especially apo ϵ 4) and it plays an important role in lipid metabolism.¹⁷ Theoretically, the apo ϵ 4 allele could also be associated with the hyperlipidaemia observed in pre-eclamptic women in pregnancy before the onset of the disease, and the risk of cardiovascular disorders later in life could also be related to these metabolic changes.³ Unexpectedly, it has been reported that there is a higher frequency of apo ϵ 2 alleles in women with pre-eclampsia than in those experiencing a healthy pregnancy.¹⁰ To evaluate whether this association is seen across populations, in our study we applied a multi-allele polymorphism approach to this gene in women affected by pre-eclampsia. Collectively, in our study there was no evidence that apoE alleles might represent a risk factor with regard to pre-eclampsia.

The frequency of the ϵ 4 allele was not significantly higher among women with pre-eclampsia than healthy pregnant women or the general population. Accordingly, the results of our study confirm that there was an overall equilibrium state between the apoE genotypes and alleles in both healthy pregnant women and those with pre-eclampsia. The frequency of the apo ϵ 4/4 genotype was not significantly increased among affected women. Furthermore, affected pregnant women with the homozygous apo ϵ 4/4 genotype had an equally good pregnancy outcome as affected women with different allele combinations. This last finding suggests that there is no link between apoE genotype and pre-eclampsia. The association described previously between apo ϵ 2 and severe pre-eclampsia¹⁰ does not exist across populations.

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