

Apolipoprotein E alleles in women with pre-eclampsia

N Makkonen, S Heinonen, M Hiltunen, S Helisalml, A Mannermaa, P Kirkinen

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.

(*J Clin Pathol* 2001;54:652-654)

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 baseline 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/

Department of
Obstetrics and
Gynecology, Kuopio
University Hospital,
70211 Kuopio, Finland
N Makkonen
S Heinonen
P Kirkinen

Chromosome and
DNA Laboratory of the
Division of Diagnostic
Services, Kuopio
University Hospital
M Hiltunen
S Helisalml
A Mannermaa

Correspondence to:
Dr Heinonen
seppo.heinonen@kuh.fi

Accepted for publication
16 January 2001

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%
<i>Genotype frequencies</i>			
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.

- 1 Roberts JM, Redman CWG. Pre-eclampsia: more than pregnancy induced hypertension. *Lancet* 1993;341:1447-51.
- 2 Rodgers GM, Taylor RN, Roberts JM. Pre-eclampsia is associated with a serum factor cytotoxic to human endothelial cells. *Am J Obstet Gynecol* 1988;159:908-14.
- 3 Barton JR, Hiett AK, O'Connor WN, et al. Endomyocardial ultrastructural findings in preeclampsia. *Am J Obstet Gynecol* 1991;165:389-91.
- 4 Jonsdottir L, Arngrimsson R, Geirsson RT, et al. Death rates from ischemic heart disease in women with a history of hypertension in pregnancy. *Acta Obstet Gynecol Scand* 1995;74:772-6.
- 5 Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am J Obstet Gynecol* 1996;175:1365-70.
- 6 Hannaford P, Ferry S, Hirsch S. Cardiovascular sequelae of toxemia of pregnancy. *Heart* 1997;77:154-8.
- 7 Kaaia R, Tikkanen M, Viinikka L, et al. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. *Obstet Gynecol* 1995;85:353-6.
- 8 Sattar N, Bendoric A, Berry C, et al. Lipoprotein subfraction concentration in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet Gynecol* 1997;89:404-8.
- 9 Miettinen T. Impact of apoE phenotype on the regulation of cholesterol metabolism. *Ann Med* 1991;23:181-6.
- 10 Nagy B, Rigo J, Jr, Fintor L. Apolipoprotein E alleles in women with severe pre-eclampsia. *J Clin Pathol* 1998;51:324-5.
- 11 Australian Society for the Study of Hypertension in Pregnancy. Management of hypertension in pregnancy: consensus summary. *Med J Aust* 1993;158:700-2.
- 12 Davey DA, MacGillivray I. The classification and definition of the hypertensive disorders of pregnancy. *Am J Obstet Gynecol* 1988;158:892-8.
- 13 Brown MA, de Swiet M. Classification of hypertension in pregnancy. *Baillieres Clin Obstet Gynaecol* 1999;13:27-39.
- 14 Vandenplas S, Grobler-Rabie A, Brebner K, et al. Blot hybridization of genomic DNA. *J Med Genet* 1984;21:164-72.
- 15 Tsukamoto K, Watanabe T, Matsushima T, et al. Determination by PCR-RFLP of apoE genotype in a Japanese population. *J Lab Clin Med* 1993;121:598-602.
- 16 Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 1990;31:545-8.
- 17 Miller J. Dyslipoproteinaemia of liver disease. *Baillieres Clin Endocrinol Metab* 1990;4:807-32.



Is your paper being cited?

CiteTrack service

CiteTrack will alert you by email whenever new content in the *Journal of Clinical Pathology* or a participating journal is published that matches criteria you want to track

Topics: Tell CiteTrack which words or subjects to watch for in new content

Authors: Be alerted whenever key authors you are following publish a new paper

Articles: Know whenever a paper of interest to you is referenced by another paper

www.jclinpath.com



Apolipoprotein E alleles in women with pre-eclampsia

N Makkonen, S Heinonen, M Hiltunen, et al.

J Clin Pathol 2001 54: 652-654

doi: 10.1136/jcp.54.8.652

Updated information and services can be found at:

<http://jcp.bmj.com/content/54/8/652.full.html>

References

These include:

This article cites 17 articles, 4 of which can be accessed free at:

<http://jcp.bmj.com/content/54/8/652.full.html#ref-list-1>

Article cited in:

<http://jcp.bmj.com/content/54/8/652.full.html#related-urls>

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections

[Molecular genetics](#) (253 articles)

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>