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# Influence of the apolipoprotein E ε4 allele on human embryonic development

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**Abstract**

Human apolipoprotein E (apoE) exists in three major isoforms encoded by distinct alleles (APOE ε2, ε3 and ε4) and has important functions in nerve development and repair. Inheritance of the ε4 allele is a major risk factor for the development of Alzheimer's disease. To investigate the role of APOE polymorphisms in embryonic development, we analyzed the APOE genotypes of 81 spontaneously aborted embryos and 110 adult controls using a solid-phase minisequencing technique. The ε4 allele was significantly less frequent in the spontaneous abortion group than in the control group ($P = 0.009$), while the frequency of ε3 was significantly increased ($P = 0.005$), suggesting that ε4 may have protective effects during embryogenesis. These protective effects might counterbalance the deleterious age-related effects of the ε4 allele in natural selection. 

*Keywords*: Apolipoprotein E; Isoform; Polymorphism; Spontaneous abortion; Foetal; Embryonic development

Apolipoprotein E (apoE) is a constituent of plasma lipoproteins, and is essential in the redistribution of lipids between cells, by mediating the uptake of lipoproteins through interaction with specific receptors [10]. Human apoE exists in three major isoforms (E2, E3 and E4) encoded by distinct alleles (ε2, ε3 and ε4) and is expressed at highest levels in liver and brain [10]. E2 and E4 differ from the most common isoform, E3, by a single cysteine or arginine change at amino acids 112 and 158 of the 299 amino acid peptide chain [17]. These amino acid substitutions modify the binding affinity of apoE to its receptors and have a major impact on total and LDL-cholesterol levels in the serum. E4 binds with equal or slightly greater affinity than E3, whereas E2 binds with only 2% of the affinity of E3 under certain conditions [18]. The different APOE alleles have been extensively studied in relation to diverse human age-related diseases including cardiovascular and neurodegenerative disorders. Inheritance of the ε4 allele is a major risk factor for the development of Alzheimer's disease [4,12] and is associated with various degrees of risk of dyslipidaemia and coronary heart disease [19]. In spite of the fact that apoE plays a key role in lipid metabolism and nerve development and repair, there are no reports on the role of the different APOE alleles in human embryonic development. In this report, we present the first investigation of the association of the major APOE polymorphisms and spontaneous abortion. We analyzed DNA samples from 81 spontaneously aborted human embryos and 110 adult controls for the APOE ε2, ε3 and ε4 alleles and genotypes by using a solid-phase minisequencing technique [2]. This technique directly detects the six common APOE genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4) and is not sensitive to silent polymorphisms.

The study was approved by the Ethics Committee at the

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Table 1
APOE allele frequencies for control and spontaneous abortion groups

| APOE allele | Observed frequency | | Odds ratio (95% CI) | $P^a$ |
|---|---|---|---|---|
| | Control group ($n = 110$; 220 alleles) | Spontaneous abortion group ($n = 81$; 162 alleles) | | |
| *ε2* | 0.077 | 0.043 | 0.539 (0.214–1.36) | 0.205 |
| *ε3* | 0.832 | 0.932 | 2.78 (1.35–5.71) | 0.005 |
| *ε4* | 0.091 | 0.025 | 0.253 (0.083–0.773) | 0.009 |

[a] By Fisher's Exact test.

University Hospital of Heraklion and written informed consent was obtained from the relatives of all participants. The study group consisted of 81 foetal tissue samples from spontaneous abortions, obtained from the Department of Obstetrics and Gynecology, Medical School, University of Crete, Heraklion. Foetal death occurred between the sixth and twentieth week of pregnancy (10.5 ± 2.8), with the majority (87.5%) occurring earlier than the twelfth week. The control group consisted of 110 DNA samples from healthy blood donors from Crete.

The foetal tissue material was preserved at −70 °C after its collection. Genomic DNA was extracted from the frozen tissues as well as from the control blood specimens as previously described [5]. The study and control materials were analyzed for the APOE alleles and genotypes by the solid-phase minisequencing method as previously described [2], and compared by a two-tailed Fisher's Exact test. This method was considered preferable whenever the expected number in any cell was less than five and for consistency, all results reported here are based on Fisher's Exact test. Statistical significance was defined as $P < 0.05$. Odds ratios and 95% confidence intervals (CI) were calculated according to Altman [1]. All analyses were performed using SYSTAT (SPSS, Inc., Chicago, IL).

The APOE ε2, ε3 and ε4 allele frequencies are presented in Table 1. The ε4 allele frequency was significantly decreased (2.5 versus 9.1%, $P = 0.009$) while the ε3 allele frequency was significantly increased (93.2 versus 83.2%, $P = 0.005$) in the spontaneous abortion group. The ε2 allele frequency was not significantly different between control and spontaneous abortion groups. The odds ratios, a measure of the relative risk of spontaneous abortion between embryos with or without a given allele, were 2.78 (95% CI, 1.35–5.71) and 0.253 (95% CI, 0.083–0.773) for the ε3 and ε4 alleles, respectively. Notably, the APOE allele frequencies found in our control group agree well with those reported in other Southern European populations [3,8,15]. The APOE genotype distributions for the control and spontaneous abortion groups are presented in Table 2. Only four of 81 spontaneously aborted embryos had ε3/ε4 genotypes as compared with the control group in which 17 of 110 individuals had ε3/ε4 genotypes (4.9 versus 15.5%, $P = 0.033$). There was a higher prevalence of ε3/ε3 genotypes in the spontaneous abortion group compared with the control group ($P = 0.004$). No ε2/ε2, ε2/ε4 or ε4/ε4 genotypes were detected in the spontaneous abortion group, whereas the control group displayed one ε2/ε4 genotype and one ε4/ε4 genotype.

In conclusion, we found a decreased frequency of the ε4 allele in the spontaneous abortion group, suggesting that this allele may have beneficial effects during embryogenesis. The increased frequency of the ε3 allele may indicate that this allele has a negative effect on foetal survival. No additional information could be extracted from the genotype distributions than that obtained from the allele frequencies. Future studies with larger sample sizes will reveal if specific genotypes are favoured and if the ε4 allele has a dose-effect on reducing the risk of spontaneous abortion.

Table 2
APOE genotype distributions for control and spontaneous abortion groups[a]

| APOE genotype | Observed frequency | | Odds ratio (95% CI) | $P^b$ |
|---|---|---|---|---|
| | Control group ($n = 110$) | Spontaneous abortion group ($n = 81$) | | |
| *ε2/ε2* | NO | NO | ND | ND |
| *ε2/ε3* | 0.145 | 0.086 | 0.556 (0.213–1.45) | 0.264 |
| *ε2/ε4* | 0.009 | NO | ND | ND |
| *ε3/ε3* | 0.682 | 0.864 | 2.97 (1.38–6.40) | 0.004 |
| *ε3/ε4* | 0.155 | 0.049 | 0.284 (0.090–0.901) | 0.033 |
| *ε4/ε4* | 0.009 | NO | ND | ND |

[a] NO, not observed; ND, not determined.
[b] By Fisher's Exact test.

Although numerous hypotheses have been advanced, it remains unclear how APOE ε4 increases the risk of neurodegenerative and cardiovascular diseases. It is clear that apoE plays an important role in lipid transport and metabolism and that there are phenotypic differences between the different APOE genotypes with regard to differential lipoprotein receptor-binding activity [18]. In addition, recent reports indicate that different apoE protein isoforms have differential effects on neuronal plasticity and survival [9,16]. Compared with the E3 protein isoform, E4 appears not to support maintenance of healthy neurites and neuronal cells [9,16]. However, other studies have shown potentially beneficial effects of the E4 isoform on neuron survival. In a recent study, it was reported that E4, but not E3, activates an extracellular signal-regulated kinase cascade that results in activation of cAMP-response element-binding protein and induction of many different genes, including the cell-protective gene *Bcl*-2 [11]. Our results indicating that APOE polymorphisms may influence embryonic survival further emphasize the biological significance of this gene.

The association of the ε4 allele with age-related diseases illustrates to some extent the idea that deleterious alleles are not selected against if they act late in life, when the effects of natural selection are weaker. However, its deleterious effects are probably spread out over the last decades of life. The data presented in this investigation suggest that the negative effects of the ε4 allele may be counterbalanced by beneficial influences during embryogenesis in order to account for its stable frequency in adult populations. It should also be noted that the ε4 allele is considered by many to be the ancestral form of the APOE gene, as it has been shown to resemble APOE genes found in primates, cows, pigs, rats, mice and guinea pigs at the ε3/ε4 polymorphic site [6,7]. Linkage disequilibrium studies also reached the same conclusion using adjacent gene mapping analyses in distant human populations [14]. Interestingly, it has been shown that the prevalence of the ε4 allele is quite high in East African populations [13], particularly in the Tutsi and Zairian populations [20] for which the ε4 allele is not considered a risk factor for Alzheimer's disease. It is thus very tempting to speculate that the human acquisition and retention of the ε3 allele in the course of the last few millenniums may have to do with an environmental determinant of survival, which may have somewhat disappeared or diminished due to migration and/or changes in nutrition that took place as humans migrated out of Africa.

Our results warrant additional investigations. They need to be confirmed in larger studies. The mechanism by which the ε4 allele might promote foetal survival needs to be established. Moreover, it is not excluded that linkage disequilibrium with polymorphisms in neighbouring genes may complicate the picture.

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