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Apolipoprotein E polymorphism in hemodialysed patients and healthy controls

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Abstract

The possible association between the end-stage renal disease (ESRD) and apolipoprotein E (APOE) polymorphism was found in some, but not all studies. We have analysed the *APOE* genotypes in 995 haemodialysed patients (cases) and a sample of 6242 healthy individuals (controls) in the Czech Republic. There was a statistically significant difference in the frequencies of *APOE* alleles between the cases and controls, with more carriers of the *APOE2* allele in ESRD patients (15.9%) than in controls (12.2%) (p=0.005). The odds ratio of ESRD for the *APOE2* allele, compared with *APOE3E3* homozygotes, was 1.37 (95% confidence interval 1.13-1.67). The strength of the association increased with the time spent on haemodialysis: the odds ratios of all-cause ESRD in patients dialysed for 8 or more years, 1-8 years and less than one year (non-survivors) were 1.27 (0.94-1.71), 1.41 (1.09-1.81) and 1.94 (0.88-4.18), respectively. This study suggests that the *APOE2* allele is a possible genetic risk factor for all-cause ESRD in Caucasians.

Keywords

Apolipoprotein E; end-stage renal disease; polymorphism

Introduction

End-stage renal disease (ESRD) is a serious health problem worldwide, with more than one million of patients requiring renal replacement therapy (Moeller *et al.* 2002), and the number of ESRD patients continues to grow. Development of ESRD is a function of both genetic and non-genetic factors. While a number of non-genetic factors have already been identified (e.g. hypertension, obesity, smoking, inadequate activation of angiotensin-renin system) (Gross and Amann, 2004, Leblanc *et al.* 2005), the genetic factors influencing renal failure are poorly understood (Nordfors *et al.* 2005).

Lipid abnormalities patients with ESRD are common, and these abnormalities may further contribute to the progression of renal disease. The most important genetic determinant of plasma cholesterol is the gene for apolipoprotein E (*APOE*, gene ID 348, OMIM acc No.

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107741). ApoE is a component of triglyceride-rich lipoproteins and a subfraction of high density lipoproteins. There are three common *APOE* variants (E2 [Arg158 → Cys], E3 and E4 [Cys112 → Arg]), and almost thirty *APOE* mutations (Hubacek *et al.* 2000). The different functional properties of the *APOE* isoforms result in an elevated plasma cholesterol in *APOE4* allele carriers, while the lowest plasma concentrations of cholesterol are found in carriers of the *APOE2* allele (Davignon *et al.* 1988). However, as the frequencies of individual alleles vary between different populations, mainly due to differences in ethnic background (Hubacek *et al.* 2000, Davignon *et al.* 1988, Gerdes *et al.* 1992, Svobodova *et al.* 2007), findings on the associations between genotypes and disease in one population cannot be automatically extrapolated to all other populations.

It is known that the APOE is expressed in the kidney tissue but the exact role of apolipoprotein E in the kidney remains unclear. Animal experiments suggest that apolipoprotein E has a protective function, possibly as an autocrine regulator of mesenginal expansion and kidney function (Chen *et al.* 2001). In addition, many human *APOE* mutations have been detected in the patients with different types of kidney disease and they may independently contribute to kidney dysfunction (for review see Hubacek *et al.* 2000, Liberopoulos *et al.* (2004). It is therefore plausible that the common *APOE* variants may play a significant role in kidney disease development.

Several previous studies, using different research designs, have analysed *APOE* variants in ESRD patients. These studies, reviewed by Liberopoulos *et al.* (2004), suggest that the carriers of the *APOE2* allele have higher risk of ESRD development but the results so far are not conclusive. The major limitation of the previous studies relates to their low statistical power due to relative small numbers of subjects included in these studies. The comparison of studies is also complicated by the fact that there are many different causes of the ESRD.

In the present study, we have examined the relative frequencies of *APOE* genotypes in patients with all-cause ESRD and compared them with healthy controls drawn from a large population sample of healthy individuals. As the present study is much larger than previous investigations, it should provide a statistically more reliable test of the hypothesis that all-cause ESRD is associated with the *APOE* genotype.

Materials and Methods

We have genotyped 995 (out of 1014 collected, 565 males and 449 females,) adult patients (mean age 66.9 ± 12.7 years, CR 97.8%), who were undergoing haemodialysis for at least three months in 27 haemodialysis units across all over the Czech Republic; the centres were selected primarily on the basis of their willingness to participate in the study (Hubacek *et al.* 2007). The most common verified causes of end stage renal failure was diabetic nephropathy (N = 102), chronic tubulointerstitial nephropathy (N = 99) and nephrosclerosis based on hypertension (N = 39). Unfortunately, approximately 40% of the patients with chronic renal failure did not undergo renal biopsy and it was therefore not possible to specify the primary cause of the renal disease. We were therefore unable to analyse of the role of *APOE* polymorphism and ESRD with regard to the primary disease. Patients were further classified into three groups according to the time spend on the hemodialysis (HD) treatment (> 8 years, 1 - 7 years and those who survived for less than one year). Limited follow up of the patients shows that there was no conversion to the peritoneal dialysis; 78 (7.8%) underwent kidney transplantation.

As healthy controls, we used participants in a large cohort study, the Czech part of the international project Health, Alcohol, Psychological factors in Eastern Europe (the HAPIEE study [Peasey *et al.* 2006]). The participants of the HAPIEE study were randomly selected

from population registers of 7 towns. We have genotyped 6,627 individuals (3,049 males and 3,578 females, mean age 58.3 ± 7.1 years). All patients and healthy controls were of Caucasian ethnicity.

Genomic DNA was isolated from whole blood using the salting out method (Miller *et al.* 1988). Genotyping of *APOE* was performed as described by Hixson and Vernier (1990). Because of the low numbers of *APOE2E2* and *APOE4E4* homozygotes, the subjects were divided in three groups for the statistical analysis: (1) carriers of the allele *APOE2* (*APOE2E2* and *APOE3E2* individuals), (2) *APOE3E3* homozygotes, and (3) carriers of the allele *APOE4* (*APOE4E4* and *APOE4E3* individuals). Subjects with *APOE4E2* genotypes (17 persons, [1.7%] from ESRD patients, 130 [2.1%] healthy controls) were excluded from the analysis because of the uncertainty about appropriate pooling with other genotypes. The statistical analysis used Chi-square and Fisher exact tests.

Results and Discussion

In both cases and controls, the most common genotype was *APOE3E3* genotype (frequency in population controls 66.1%, table 1). Both in cases and controls, the genotype frequencies were in agreement with Hardy Weinberg equilibrium. There was a statistically significant difference in the frequencies of the *APOE* genotype between patients with all-cause ESRD and healthy controls (table 1), with more carriers of the *APOE2* allele in all-cause ESRD patients than in healthy controls (15.9 % versus 12.2 %, odds ratio 1.37, 95% confidence interval 1.13-1.67, p=0.005). For the three most common diagnosed causes of ESRD available in our data, the *APOE2* allele frequencies were 13.9% for diabetic nephropathy, 12.5% for chronic tubulointerstitial nephropathy, and 12.8% for nephrosclerosis based on hypertension; none of these frequencies differed significantly from that among controls. In addition, there were no significant differences in the frequencies of the *APOE* genotypes by history of ischemic heart disease or diabetes mellitus. The relative frequency of the *APOE2* allele carriers increased as the survival on HD treatment decreased (table 2); the odds of dying within one year was almost twice higher among carriers of at least one *APOE2* allele than among persons with *APOE3E3* genotype (p for trend 0.001).

Our finding of the association between *APOE2* and increased risk of all-cause ESRD is supported by three observations. First, the frequencies of the *APOE* genotypes in our study population were similar to those found in the neighbouring European populations (Gerdes *et al.* 1992). Second, the association was stronger for disease with shorter survival, which might reflect a biological gradient by the severity of the disease (although the primary disease was not known). The presence of the *APOE2* allele could potentially affect survival on hemodyalisis treatment and perhaps influence the development of MIA (malnutrition-inflammation-atherosclerosis) syndrome (Pecoits-Filho *et al.* 2002). Finally, the observed association between *APOE* genotype and ESRD are also consistent with some (although not all) previous reports. Case-control studies by Oda *et al.* (1999) and Horita *et al.* (1994) in Japan (using cases with or without non-insulin-dependent diabetes mellitus, NIDDM) also found higher allele frequency of *APOE2* in ESRD patients than in healthy population.

We are aware that the literature is not entirely consistent. For example, a study by Roussos *et al.* (2004) also suggested the involvement of *APOE* in renal disease but in their study the implicated allele was *APOE3E4* genotype in a subgroup of ESRD patients with NIDDM; however, they did not observe a higher percentage of *APOE2* in their cases, compared to controls. Other studies failed to detect any significant differences in *APOE* genotypes between patients with kidney disease and controls (Feussner *et al.* 1992, Guz *et al.* 2000, Arikan *et al.* 2007). However, these studies were very small, with less than 100 cases per

study; given their low statistical power and the relatively weak association between ESRD and *APOE* genotype, false negative results are the most likely explanation.

A major limitation of this study is the lack of information on the primary disease in most of the ESRD patients. It was not possible to analyse the role of the APOE in patients with different primary diagnoses and to establish whether the effect of APOE is specific to a particular primary cause.

In conclusion, in this study, the largest on the relationship between all-cause ESRD and *APOE* genotype to date, we found a significantly higher frequency of the *APOE2* allele carriers in all-cause ESRD patients in comparison to healthy controls, and this association was stronger in one-year non-survivors. The results suggest that the *APOE2* allele is a possible genetic risk factor for ESRD development in Caucasians.

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Table 1

***APOE* genotypes in all-cause ESRD patients and healthy controls and odds ratios (95% confidence interval) for all-cause ESRD for the +*APOE2* or +*APOE4* allele carriers vs. *APOE3E3* homozygotes.**

	Cases N (%)	Controls N (%)	Odds ratio (95% CI
+ *APOE2* allele	158 (15.9%)	760 (12.2%)	1.37 (1.13-1.67)
APOE3E3	625 (62.8%)	4128 (66.1%)	1.0 (reference)
+ *APOE4* allele	195 (19.6%)	1224 (21.7%)	1.05 (0.88-1.26)

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Table 2

***APOE* genotypes and odds ratio of all-cause ESRD patients for the *+APOE2* carriers vs. *APOE3-3* homozygotes, stratified by time spend on haemodialysis treatment.**

Time spend on haemodialysis	*+ APOE2*	*APOE3E3*	*+APOE4*	Odds ratio (*+E2* vs. *E3E3*) (95% CI)
>8 years	60 (15.3%)	257 (65.5%)	75 (19.1%)	1.27 (0.94-1.71)
1-7 years	88 (16.3%)	340 (62.8%)	113 (20.9%)	1.41 (1.09-1.81)
1 year non-survivors	10 (22.2%)	28 (62.2%)	7 (15.6%)	1.94 (0.88-4.18)