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Influence of apolipoprotein E polymorphisms on serum creatinine levels and predicted glomerular filtration rate in healthy subjects

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Abstract

Background. There are conflicting results regarding the effect of apolipoprotein (ApoE) polymorphisms on the progression of a variety of renal diseases. However, there are no data on the possible effect of the ApoE alleles on serum creatinine levels and predicted glomerular filtration rate (GFR) in healthy subjects.

Methods. 290 apparently healthy individuals were studied. ApoE genotyping was performed by the polymerase chain reaction; the Modification of Diet in Renal Disease equation (MDRD) predicted the GFR.

Results. ApoE2 was associated with lower levels of total cholesterol, low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol, as well as with higher levels of triglycerides in our population. Furthermore, the ApoE2 allele was associated with increased serum creatinine levels compared with both the E3 and E4 alleles (1.04 ± 0.13 *vs* 0.92 ± 0.13 *vs* 0.88 ± 0.11 mg/dl, respectively, $P = 0.0077$), while the MDRD-predicted GFR was decreased in ApoE2 carriers compared with both E3 and E4 carriers (80.3 ± 10.2 *vs* 88.1 ± 9.6 *vs* 89.3 ± 9.7 ml/min/1.73 m^2, respectively, $P = 0.031$). These observations remained significant statistically even if the effect of ApoE polymorphisms on age- and body-mass index-adjusted serum creatinine and MDRD-predicted GFR was separately analysed in both men and women. Although, ApoE4 carriers tended to exhibit lower levels of serum creatinine and higher values of predicted GFR compared with the E3 carries, these differences did not reach statistical significance.

Conclusions. ApoE2 allele seems to be associated with increased serum creatinine levels and decreased MDRD-predicted GFR in healthy subjects.

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Introduction

Apolipoprotein E (ApoE), which is a 34 200 kDa protein consisting of 299 amino acids, plays a major role in the metabolism of lipids and lipoproteins [1]. It is found in chylomicrons, chylomicrons remnants, very low-density lipoproteins (VLDL), VLDL remnants, and in a subfraction of the high-density lipoproteins (HDL), serving as a ligand for their receptor-mediated catabolism, via the low-density lipoprotein (LDL) receptor (ApoB100/E) and the other ApoE receptors. The ApoE gene, located on the chromosome 19q13.2, has three common alleles, ε2, ε3 and ε4, coding for the three main isoforms of the ApoE protein: E2 ($Arg^{158} \rightarrow Cys$), E3 (parent isoform), and E4 ($Cys^{112} \rightarrow Arg$). Therefore, there are six common ApoE polymorphisms: ApoE3/3, ApoE4/4, ApoE2/2, ApoE3/2, ApoE4/2 and ApoE4/3. ApoE isoforms differ in their receptor binding ability with ApoE4 having the maximum binding capacity, while ApoE2 is defective in its binding ability to the ApoE receptors [2]. Consequently, ApoE polymorphisms are major determinants of serum lipid levels in the general population; ApoE2 has a cholesterol-lowering effect, while ApoE4 has a cholesterol-raising effect compared with ApoE3 [2].

Beyond the known influence of ApoE polymorphisms on serum lipid profile, on the pathogenesis of atherosclerosis and the development of neurodegenerative disorders, ApoE also exerts a major role in the pathogenesis and the progression of a variety of renal diseases, as well as in the atherosclerotic complications associated with them (recently reviewed in [3]). However, there is much discrepancy in the literature concerning the possible ApoE polymorphism-mediated

predisposition to the deterioration of renal function and the development of end-stage renal disease in both diabetic and non-diabetic, as well as in paediatric and adult patients [3]. Some studies have provided evidence that ApoE2 allele carries such a genetic risk [4–10], whereas others have shown that the ApoE4 allele is a risk factor for the progression of renal failure [11–13]. On the other hand, some studies have provided evidence that the ApoE4 allele protects from the progression of diabetic nephropathy in type 2 diabetics [9,14]. Furthermore, the ApoE4 allele has been associated with reduced postoperative increase in serum creatinine levels after cardiac coronary bypass surgery, whereas there was no difference in the preoperative serum creatinine concentrations among the different ApoE allele groups [15]. The ApoE4 allele has been associated with better creatinine clearance in kidney transplanted patients [16], whereas the ApoE2 allele has been reported to be a negative predictor of creatinine clearance in type 1, but not in type 2 diabetic patients [6]. In fact, there are experimental data for a protective role of kidney ApoE, with the ApoE2 isoform being less protective against mesangial cell proliferation than the ApoE3 and ApoE4 isoforms [17].

However, there are no studies concerning the possible effect of ApoE polymorphisms on the levels of serum creatinine and glomerular filtration rate (GFR) in healthy individuals without renal disease. Thus, we undertook the present study to test the hypothesis that ApoE polymorphisms could influence serum creatinine levels and predicted GFR values in healthy subjects.

Subjects and methods

Study population

The study population consisted of 290 apparently healthy consecutive individuals of Caucasian origin, who underwent a regular check-up in our outpatient internal medicine clinic. Before inclusion in the study, these subjects were screened for the absence of hypertension (blood pressure >140/90 mmHg or use of antihypertensive drugs), diabetes mellitus (fasting serum glucose >126 mg/dl), cardiovascular disease (negative patient history and normal rest electrocardiogram), renal dysfunction [serum creatinine >1.4 mg/dl (124 μmol/l)], and microalbuminuria [albumin:creatinine ratio ≥22 (in men) or ≥31 (in women) mg albumin/g creatinine in a random urine sample]. Furthermore, none of these individuals were receiving drugs known to affect serum lipid profile or renal function (lipid lowering drugs, β-blockers, diuretics, contraceptives, etc.). All participants gave informed consent for genetic analysis and the ethics committee of our university hospital approved the protocol.

Laboratory measurements

In all participants blood samples were obtained after a 14-h overnight fast for gene genotype detection, as well as for the determination of serum laboratory parameters. Blood samples were centrifuged for 30 min (3600 *g*) and then the serum was separated and stored at −80°C for analysis of laboratory parameters.

Concentrations of total cholesterol and triglycerides were determined enzymatically on the Olympus AU600 clinical chemistry analyser (Olympus Diagnostica, Hamburg, Germany). HDL-cholesterol (HDL-C) was determined in the supernatant, after precipitation of the ApoB-containing lipoproteins with dextran sulphate-Mg^{2+} (Sigma Diagnostics, St Louis, MO, USA). LDL-cholesterol (LDL-C) was calculated using the Friedewald formula, while the non-HDL-C was calculated by the equation: non HDL-C = total cholesterol − HDL-C.

Finally, serum creatinine levels were determined using the Jaffé method [kinetic alkaline picrate reaction, normal range 0.7–1.4 mg/dl (62–124 μmol/l)], while serum albumin and serum urea nitrogen were measured according to the bromcresol green and enzymatic urease methods, respectively.

ApoE genotyping

DNA was extracted from the whole blood specimens according to standard procedures. ApoE genotyping was performed as described by Hixson and Vernier. Polymerase chain reaction (PCR) was used to amplify a 244-bp sequence of the ApoE gene, including the two polymorphic sites. The PCR products were then digested with the restriction enzyme *Hha*I and the different genotypes were detected after electrophoresis on 8% non-denaturing polyacrylamide gels, treated with ethidium bromide and visualized in ultra-violet radiation. Subjects were classified as ApoE2 carriers if they had the ApoE2/3 or the ApoE2/2 genotype, ApoE3 carriers if they had the ApoE3/3 genotype, and ApoE4 carriers if they had the ApoE4/3 or the ApoE4/4 genotype. Subjects with the ApoE4/2 ($n = 3$) were excluded from the study due to their small number and the difficulties in their classification. Thus, 287 subjects were finally included in the analysis.

Estimation of GFR

GFR was predicted by a recently described formula, which was developed based on the data derived from the Modification of Diet in Renal Disease (MDRD) study in patients with renal dysfunction [18]:

$$\text{Predicted GFR in ml/min/1.73 m}^2 = 170 \times (\text{serum creatinine in mg/dl})^{-0.999} \times (\text{age in years})^{-0.176} \times (\text{serum urea nitrogen in mg/dl})^{-0.170} \times (\text{albumin in g/dl})^{+0.318} (\times 0.762 \text{ if female}) (\times 1.180 \text{ if black}).$$

Additionally, the MDRD equation has been shown to be more precise and accurate for predicting GFR in healthy adults, compared with the Cockcroft–Gault formula [19]. It should be mentioned that the laboratory methods used for the determination of serum levels of creatinine, albumin and urea nitrogen in our study were the same as those used in the MDRD study. Moreover, the MDRD formula has been validated in our institution compared with the estimation of the GFR by the renal clearance of ^{125}I-iothalamate. The comparison revealed that the MDRD equation is more precise and accurate in predicting GFR in both healthy subjects and patients with renal failure than the Cockcroft–Gault formula (data not shown).

Statistical analysis

Statistical analysis was performed with STATISTICA 6.0 statistical software. The effect of the ApoE gene polymorphisms on laboratory parameters was tested using the one-way analysis of variance (one-way ANOVA) followed by the LSD test (in case of significant effects) for multiple pairwise comparisons, except for serum triglycerides, where the Kruskal–Wallis ANOVA median test was used followed by the Mann–Whitney *U* test for pairwise comparisons because of their skewed distribution. Furthermore, the effect of ApoE polymorphisms on the serum creatinine and MDRD-predicted GFR values was adjusted for age and body-mass index (BMI) by the analysis of covariance (ANCOVA) in both men and women. Finally, multiple linear regression analysis was performed to test the effect of ApoE polymorphisms and other factors (sex, age and BMI) on serum creatinine levels.

Results

Table 1 demonstrates the clinical and laboratory characteristics of the study population. ApoE gene frequencies were in Hardy–Weinberg equilibrium and they were not different from those reported in other European populations.

Table 2 demonstrates the effect of ApoE alleles on the laboratory parameters of our subjects. The three groups were well matched with regard to age, sex ratio, smoking habits and BMI. In agreement with other reports, ApoE2 carriers had the lowest levels of total cholesterol, LDL cholesterol and non HDL cholesterol, and the highest levels of serum triglycerides compared with the ApoE3 and ApoE4 carriers, while there was no difference in the levels of the HDL cholesterol and fasting serum glucose. Importantly, ApoE2 carriers had the highest serum creatinine concentration [1.04 ± 0.13 mg/dl (92.0 ± 11.5 μmol/l)] compared with both ApoE3 carriers [0.92 ± 0.13 mg/dl (81.0 ± 11.5 μmol/l), $P = 0.009$ by the LSD test] and ApoE4 carriers [0.88 ± 0.11 mg/dl (77.8 ± 9.7 μmol/l), $P = 0.001$ by the LSD test], while there was no significant difference in

Table 1. Clinical and laboratory characteristics of the study population

	Healthy subjects
Number of subjects	287[a]
Sex (male/female)	146/141
Age (years)	59.5 ± 17.2
Smoking habit (yes/no)	78/209
BMI (kg/m^2)	26.2 ± 3.1
T-CHOL (mmol/l) (mg/dl)	5.2 ± 0.84 (201.8 ± 32.7)
TRG (mmol/l) (mg/dl)	1.2 ± 0.6 (102.8 ± 54.1)
HDL-C (mmol/l) (mg/dl)	1.4 ± 0.3 (52.3 ± 10.8)
LDL-C (mmol/l) (mg/dl)	3.5 ± 0.8 (135.1 ± 31.6)
Non-HDL-C (mmol/l) (mg/dl)	3.9 ± 1.0 (150.3 ± 37.4)
Fasting serum glucose (mmol/l) (mg/dl)	5.0 ± 0.4 (92.4 ± 8.0)
Serum creatinine (μmol/l) (mg/dl)	81.3 ± 11.5 (0.92 ± 0.13)
Serum urea nitrogen (mmol/l) (mg/dl)	5.5 ± 1.5 (15.5 ± 4.1)
Albumin (g/l) (g/dl)	41 ± 4 (4.1 ± 0.4)
MDRD-predicted GFR (ml/min/1.73 m^2)	87.9 ± 9.9
Systolic blood pressure (mmHg)	132.3 ± 6.7
Diastolic blood pressure (mmHg)	82.5 ± 6.7
Frequencies of ε3/ε4/ε2 (%) alleles	78.3/13.0/8.7

Values are expressed as mean ± SD. BMI, body-mass index; T-CHOL, total cholesterol; TRG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-HDL cholesterol; MDRD, modification of renal disease equation; GFR, glomerular filtration rate.
[a]The three ApoE4/2 individuals were excluded from the study.

Table 2. Effect of ApoE polymorphisms on serum creatinine levels and MDRD-predicted GFR in the study population (the three ApoE4/2 subjects were excluded from the analysis)

	E2 allele ($n = 50$) ApoE2/3, ApoE2/2	E3 allele ($n = 162$) ApoE3/3	E4 allele ($n = 75$) ApoE3/4, ApoE4/4	*P*
Age (years)	59.3 ± 10.2	59.1 ± 11.1	59.9 ± 18.1	NS
Sex (male/female)	26/24	82/80	38/37	NS
Smoking (yes/no)	14/36	44/118	20/55	NS
BMI (kg/m^2)	26.1 ± 2.8	26.2 ± 3.0	26.3 ± 2.9	NS
Serum creatinine (μmol/l) (mg/dl)	92.0 ± 11.5[a,b] (1.04 ± 0.13)[a,b]	81.0 ± 11.5 (0.92 ± 0.13)	77.8 ± 9.7 (0.88 ± 0.11)	0.0077
MDRD-predicted GFR (ml/min/m^2)	80.3 ± 10.2[a,b]	88.1 ± 9.6	89.3 ± 9.7	0.031
Fasting serum glucose (mmol/l) (mg/dl)	5.09 ± 0.40 (92.6 ± 7.6)	5.08 ± 0.50 (92.5 ± 8.3)	5.02 ± 0.44 (91.3 ± 8.0)	NS
T-CHOL (mmol/l) (mg/dl)	4.9 ± 0.8[a,b] (187.8 ± 31.3)[a,b]	5.3 ± 0.9 (203.5 ± 35.0)	5.7 ± 0.8[c] (219.5 ± 29.1)[c]	0.02
TRG (mmol/l) (mg/dl)	1.60 ± 1.00[a,b] (143.4 ± 88.5)[a,b]	1.10 ± 0.50 (98.2 ± 48.6)	1.15 ± 0.60 (101.6 ± 55.1)	0.03
HDL-C (mmol/l) (mg/dl)	1.33 ± 0.25 (51.4 ± 9.7)	1.36 ± 0.28 (52.4 ± 10.7)	1.34 ± 0.26 (51.7 ± 10.1)	NS
LDL-C (mmol/l) (mg/dl)	3.0 ± 0.6[a,b] (115.0 ± 24.8)[a,b]	3.5 ± 0.9 (134.7 ± 34.5)	3.8 ± 0.7[c] (145.3 ± 27.5)[c]	0.001
Non-HDL-C (mmol/l) (mg/dl)	3.6 ± 0.8[a,b] (140.1 ± 32.1)[a,b]	3.9 ± 1.0 (152.1 ± 42.0)	4.4 ± 0.8[c] (169.6 ± 31.2)[c]	0.001

Values are expressed as mean ± SD. BMI, body-mass index; T-CHOL, total cholesterol; TRG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-HDL cholesterol; MDRD, modification of renal disease equation; GFR, glomerular filtration rate. Values were compared using one-way ANOVA followed by LSD test for pairwise comparisons in case of significant results, except for TRG, where the Kruskal–Wallis median test was used followed by the Mann–Whitney *U* test for pairwise comparisons. NS, not significant.
[a]$P < 0.05$ compared with E3 allele carriers.
[b]$P < 0.05$ compared with E4 allele carriers.
[c]$P < 0.05$ compared with E3 allele carriers.

serum creatinine levels between the ApoE3 and ApoE4 carriers ($P = 0.21$ by the LSD test). The above association remained significant statistically even after adjustment of serum creatinine levels for age and BMI (Figure 1). A multiple linear regression analysis taking into account the ApoE alleles, sex, age and BMI recognized that sex (beta $= -0.60$, $P = 0.00000$), BMI (beta $= 0.18$, $P = 0.02$) and ApoE alleles (beta $= -0.17$, $P = 0.012$) significantly affected serum creatinine concentration.

Furthermore, ApoE2 allele carriers exhibited the lowest values of MDRD-predicted GFR (80.3 ± 10.2 ml/min/1.73 m^2) compared with both ApoE3 carriers (88.1 ± 9.6 ml/min/1.73 m^2, $P = 0.035$ by the LSD test) and ApoE4 carriers (89.3 ± 9.7 ml/min/1.73 m^2, $P = 0.02$ by the LSD test), while there was no significant difference in MDRD-predicted GFR between ApoE3 and ApoE4 carriers. This association remained significant statistically even after adjustment of predicted GFR values for age and BMI (Figure 1).

Finally, Table 3 shows the age- and BMI-adjusted effect of ApoE polymorphisms on serum creatinine and MDRD-predicted GFR levels separately in men and women. The above-described association of ApoE2 allele with higher serum creatinine concentrations and lower MDRD-predicted GFR values remained significant statistically and to the same extent in both male and female subgroups, and this association persisted even after correction for age and BMI.

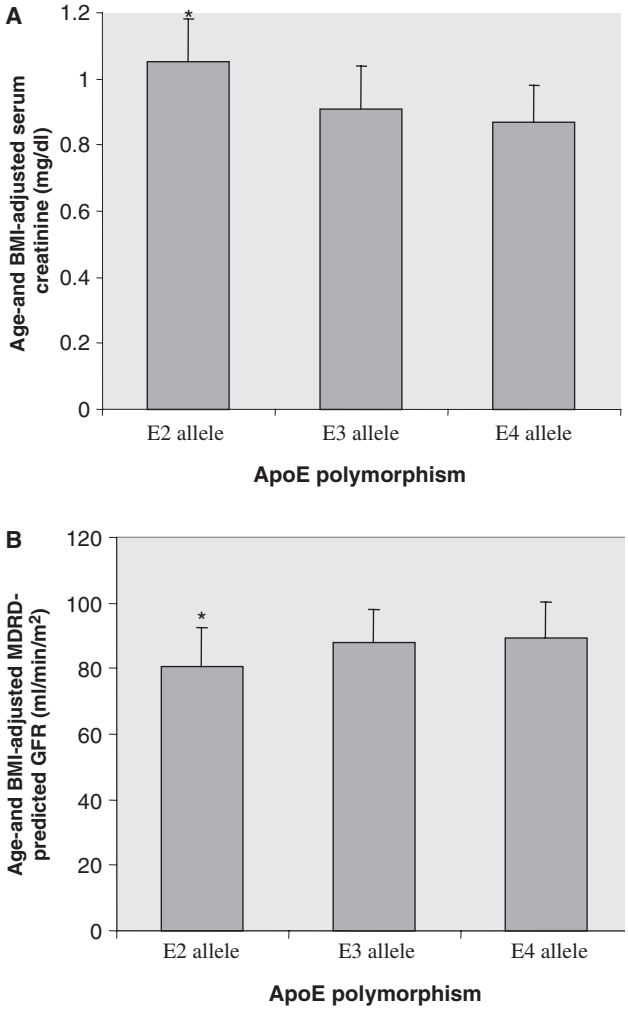

Fig. 1. Bar graphs showing the overall effect of ApoE polymorphism on the age- and BMI-adjusted serum creatinine levels (**A**) and MDRD-predicted GFR (**B**) in the whole study population. Values are expressed as mean ± SD. BMI, body-mass index; MDRD, modification of renal disease equation; GFR, glomerular filtration rate. Values were adjusted for age and BMI using the analysis of co-variance (ANCOVA) with age and BMI treated as covariates, followed by LSD test for pairwise comparisons in case of significant results. *$P < 0.05$ for comparison between the E2 group with either the E3 or the E4 group. To convert serum creatinine in mg/dl to μmol/l, multiply by 88.4.

Discussion

Our study provides for the first time evidence that the polymorphisms of ApoE, independently of gender or other known factors, may significantly affect serum creatinine levels and predicted GFR in apparently healthy individuals, with the ApoE2 allele being associated with increased serum creatinine levels and decreased MDRD-predicted GFR compared with the E3 and E4 alleles.

There is only one report in the literature concerning the effect of ApoE polymorphisms on serum creatinine levels. This study did not reveal any difference in serum baseline pre-operative creatinine among the different ApoE allele groups in surgical coronary bypass patients [15]. However, the study population consisted of patients with coronary artery disease, including diabetics, while there was no report on the prevalence of hypertension or dyslipidaemia in these patients. Additionally, ApoE2 has been associated with decreased creatinine clearance in type 1, but not in type 2 diabetics [6], while ApoE4 was linked with better creatinine clearance in kidney transplanted patients [16].

Our study suggests that ApoE2 allele may have an unfavourable effect on renal function, and the serum creatinine levels and the MDRD-predicted GFR in healthy individuals reflect this. This observation is in good agreement with the above mentioned study in diabetic patients [6], and with studies that have shown that the ApoE2 allele is a genetic risk factor for the progression of renal disease in type 1 diabetics [5,6], in type 2 diabetics [7–9], in patients with IgA nephropathy [10] and in patients with different renal diseases [4]. However, other studies have failed to show any association between ApoE polymorphisms and diabetic nephropathy in type 1 diabetics [20]. The reasons for these conflicting results are poorly understood, but may be related to ethnic differences, different study protocols, sampling errors, different prevalence of ApoE alleles among the studied populations, or to interactions with other genetic or environmental factors [3].

Table 3. Effect of ApoE polymorphisms on serum creatinine levels and MDRD-predicted GFR after adjustment for sex, age and BMI

	E2 allele ($n=26$) ApoE2/2, ApoE2/3	E3 allele ($n=82$) ApoE3/3	E4 allele ($n=38$) ApoE4/3, ApoE4/4	*P*
Male subjects ($n=146$)				
Age- and BMI-adjusted serum creatinine levels (μmol/l) (mg/dl)	$106.0\pm8.8^{a,b}$ $(1.20\pm0.10)^{a,b}$	90.2 ± 7.9 (1.02 ± 0.09)	85.7 ± 7.0 (0.97 ± 0.08)	0.009
Age- and BMI-adjusted MDRD-predicted GFR (ml/min/m^2)	$83.4\pm10.3^{a,b}$	89.2 ± 9.2	91.2 ± 7.0	0.04
	E2 allele ($n=24$) ApoE2/2, ApoE2/3	E3 allele ($n=80$) ApoE3/3	E4 allele ($n=37$) ApoE4/3, ApoE4/4	*P*
Female subjects ($n=141$)				
Age- and BMI-adjusted serum creatinine levels (μmol/l) (mg/dl)	$79.6\pm7.9^{a,b}$ $(0.90\pm0.09)^{a,b}$	70.7 ± 7.9 (0.80 ± 0.09)	68.9 ± 5.3 (0.78 ± 0.06)	0.01
Age- and BMI-adjusted MDRD-predicted GFR (ml/min/m^2)	$72.2\pm12.9^{a,b}$	83.7 ± 9.5	86.5 ± 13.7	0.03

Values are expressed as mean ± SD. BMI, body-mass index; MDRD, modification of renal disease equation; GFR, glomerular filtration rate. Values were compared using analysis of co-variance (ANCOVA) with age and BMI treated as co-variates, followed by LSD test for pairwise comparisons in case of significant results.
[a] $P<0.05$ compared with E3 allele carriers.
[b] $P<0.05$ compared with E4 allele carriers.

ApoE4 carriers in our healthy population tended to have the lowest serum creatinine levels and the highest MDRD-predicted GFR, although there were not significant differences from E3 carriers. However, this is in keeping with the studies that have shown a protective effect for the ApoE4 allele from the deterioration of renal function in type 2 diabetics [9,14], and from acute renal impairment in post-bypass surgical patients [15], as well as with the study that showed a better renal function in ApoE4 kidney transplanted patients [16], and with our findings showing a significantly lower prevalence of the ApoE4 allele in patients with end-stage renal disease compared with the healthy population (7.3 *vs* 13.0%, respectively, $P<0.01$). However, other studies have related the ApoE4 allele with the progression of renal disease in diabetic patients [12,13] and in renal transplant recipients [11].

As it was anticipated, ApoE polymorphisms significantly affected serum lipid levels in our participants; ApoE2 had a cholesterol-lowering effect, while ApoE4 had a cholesterol-raising effect compared with the ApoE3 allele. This cholesterol-lowering effect of ε2 allele can be seen in almost all studies involving healthy subjects [2]. Because of the failure of ApoE2 isoform to bind effectively to the LDL and the ApoE receptors, less cholesterol from the chylomicrons, the VLDL and their remnants enters the hepatocytes, resulting in an up-regulation of the LDL receptors, which in turn lowers serum ApoB-containing lipoproteins [2]. Moreover, LDL clearance is probably enhanced due to the higher affinity of the LDL particles for the LDL receptor compared with the remnant lipoproteins carrying the defective ApoE2 [2]. On the other hand, the ApoE4-induced increase of total and LDL cholesterol levels is attributed to an increase in the intestinal absorption of dietary cholesterol, and to a down-regulation of LDL receptors in the surface of hepatic cells. The latter results from an increased delivery of cholesterol to the hepatic cells owing to the enhanced interaction of ApoE4-containing remnants and the ApoE receptors. Furthermore, the ApoE genotype can influence the location of ApoE in lipoproteins, with the E4 isoform being preferentially located in the VLDL and with the E3 and E2 isoforms in HDL. Finally, ApoE2 was associated with higher serum triglycerides in our healthy individuals, probably due to the delayed receptor-mediated clearance of the triglyceride-rich lipoproteins.

The underlying mechanisms for the observed association between E2 allele and higher creatinine concentrations, as well as lower predicted GFR values are not well understood. First, ApoE2 is associated with higher levels of triglycerides, and thus higher concentrations of triglyceride-rich lipoproteins in our healthy population. An increase of triglyceride-rich lipoproteins could stimulate the accumulation of cholesteryl esters by human mesangial cells (HMCs) leading to a change in the properties of the mesangial matrix and glomerulosclerosis, thus impairing renal function. In fact, triglyceride-rich lipoproteins from ApoE2 diabetic patients enhanced cholesteryl ester accumulation by HMCs significantly more than those from ApoE3 patients [9]. Furthermore, ApoE2/2-induced type III hyperlipoproteinaemia is closely associated with glomerular lipidosis or a lipoprotein glomerulopathy-like disease [3]. On the other hand, the ApoE4 isoform has been reported to be more effective in modulating the direct uptake and conversion of remnant lipoproteins to LDL, leading to decreased levels of triglyceride-rich lipoproteins, thus preserving renal function [14]. Secondly, ApoE has been shown to inhibit mesangial cell proliferation induced by cytokines or LDL in experimental models [17]. In these

experiments, ApoE2 was found to be the least effective isoform independent of the presence of hyperlipidaemia. Moreover, the ApoE2 isoform has also been shown to have no significant antiproliferative effect on smooth muscle cells [17]. Therefore, the inability of ApoE2 to regulate mesangial expansion due to different stimuli may result in restriction of glomerular capillary luminal volume, which in turn diminishes filtration surface [6]. This mechanism may partially explain the observed unfavourable effect of ApoE2 allele on renal function. Thirdly, ApoE2 may not be the truly unfavourable gene, but it may be in linkage disequilibrium with another renal harmful gene. This hypothesis, however, was rejected in another study, which examined the effect of ApoE polymorphisms on the development of diabetic nephropathy in type 1 diabetics [5].

On the other hand, the observed association between the ApoE polymorphism and the levels of serum creatinine and the creatinine-based MDRD-predicted GFR could be the consequence of a mechanism unrelated to the renal function, for instance creatinine metabolism. Yet, there are no literature data to support this hypothesis.

One limitation of our study is that the determination of serum creatinine was performed using the Jaffé kinetic method, which is less accurate compared with the enzymatic methods for creatinine measurement due to the presence of non-creatinine chromogens. However, since the Jaffé method was also used in the MDRD study [18], measurement of creatinine with another laboratory method could have resulted in inaccurate results regarding the use of the MDRD-derived formula. Moreover, no medical condition known to interfere with creatinine assay in the Jaffé method (i.e. diabetic ketoacidosis, administration of certain cephalosporins, lipaemia or jaundice) was present in any of our study subjects. Finally, the Jaffé method used in our laboratory has been compared with an enzymatic method for creatinine determination (creatinine deiminase, Randox, Mauguio, France). We found a high correlation between the two methods ($r = 0.99$), when considering creatinine concentrations ranging from 0.70 to 11.3 mg/dl (62–1.000 μmol/l). In fact, all creatinine values in our study were within this range.

Additionally, our study participants consisted of apparently healthy individuals visiting our outpatient clinic for a regular medical check-up. However, this population may not be representative of a general healthy population, since their mean age was 60 years old, and their mean BMI was in the overweight range. Therefore, the results obtained from our study should be carefully extrapolated in the general population.

In conclusion, we provide for the first time data on the possible effect of ApoE polymorphisms on serum creatinine levels and predicted GFR values in apparently healthy subjects; the ApoE2 allele seems to be associated with the highest creatinine concentrations and the lowest predicted GFR values in these healthy individuals. Further larger prospective studies using more accurate measurements of renal function are necessary to better clarify the association between ApoE polymorphisms and renal disease.

Conflict of interest statement. None declared.

References

1. Mahley RW. Apolipoprotein E. Cholesterol transport protein with expanding role in cell biology. *Science* 1988; 240: 622–630
2. Utermann G. Apolipoprotein E polymorphism in health and disease. *Am Heart J* 1987; 113: 433–440
3. Liberopoulos E, Siamopoulos K, Elisaf M. Apolipoprotein E and renal disease. *Am J Kidney Dis* 2004; 43: 223–233
4. Oda H, Yorioka N, Ueda C, Kushihata S, Yamakido M. Apolipoprotein E polymorphism and renal disease. *Kidney Int* 1999; 56 [Suppl]: S25–S27
5. Araki SI, Moczulski DK, Hanna L, Scott LJ, Warram JH, Krolewski AS. APOE polymorphisms and the development of diabetic nephropathy in type 1 diabetes: results of case-control and family-based studies. *Diabetes* 2000; 49: 2190–2195
6. Werle E, Fiehn W, Hasslacher C. Apolipoprotein E polymorphism and renal function in German type 1 and type 2 diabetic patients. *Diabetes Care* 1998; 21: 994–998
7. Eto M, Horita K, Morikawa A *et al*. Increased frequency of apolipoprotein E2 allele in non-insulin dependent diabetic (NIDDM) patients with nephropathy. *Clin Genet* 1995; 48: 288–292
8. Ha SK, Park HS, Kim KW *et al*. Association between apolipoprotein E polymorphism and macroalbuminuria in patients with non-insulin dependent diabetes mellitus. *Nephrol Dial Transplant* 1999; 14: 2144–2149
9. Eto M, Saito M, Okada M *et al*. Apolipoprotein E genetic polymorphism, remnant lipoproteins, and nephropathy in type 2 diabetic patients. *Am J Kidney Dis* 2002; 40: 243–251
10. Yorioka N, Nishida Y, Oda H, Watanabe T, Yamakido M. Apolipoprotein E polymorphism in IgA nephropathy. *Nephron* 1999; 83: 246–249
11. Roussos L, Floren CH, Carlson J, Svensson PJ, Wallmark A, Ekberg H. Increased prevalence of apolipoprotein E3/E4 genotype among Swedish renal transplant recipients. *Nephron* 1999; 83: 25–30
12. Boize R, Benhamou PY, Corticelli P, Valenti K, Bosson JL, Halimi S. ApoE polymorphism and albuminuria in diabetes mellitus: a role for LDL in the development of nephropathy in NIDDM? *Nephrol Dial Transplant* 1998; 13: 72–75
13. Lehtinen S, Rantalaiho V, Wirta O *et al*. Apolipoprotein E gene polymorphism, hypercholesterolemia and glomerular filtration rate in type 2 diabetic subjects: a 9-year follow-up study. *J Biomed Sci* 2003; 10: 260–265
14. Kimura H, Suzuki Y, Gejyo F *et al*. Apolipoprotein E4 reduces risk of diabetic nephropathy in patients with NIDDM. *Am J Kidney Dis* 1998; 31: 666–673
15. Chew STH, Newman MF, White WD *et al*. Preliminary report on the association of apolipoprotein E polymorphisms, with postoperative peak serum creatinine concentrations in cardiac surgical patients. *Anesthesiology* 2000; 93: 325–331
16. Bayes B, Pastor MC, Lauzurica R *et al*. Apolipoprotein E alleles, dyslipemia and kidney transplantation. *Transplant Proc* 2002; 34: 373
17. Chen G, Paka L, Kako Y, Singhal P, Duan W, Pillarisetti S. A protective role for kidney apolipoprotein E. Regulation

of mesangial cell proliferation and matrix expansion. *J Biol Chem* 2001; 276: 49142–49147
18. Levey AS, Bosch JP, Lewis JB *et al*. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; 130: 461–470
19. Lin J, Knight EL, Hogan ML, Singh AK. A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease. *J Am Soc Nephrol* 2003; 14: 2573–2580
20. Onuma T, Laffel LMB, Angelico MC, Krolewski AS. Apolipoprotein E genotypes and risk of diabetic nephropathy. *J Am Soc Nephrol* 1996; 7: 1075–1078

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