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Original Paper

A Common *APOE* Polymorphism Is an Independent Risk Factor for Reduced Glomerular Filtration Rate in the Spanish RENASTUR Cohort

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Key Words
APOE polymorphisms · Type 2 diabetes mellitus · Glomerular filtration rate · Renal function

Abstract
Objective: *APOE* gene variants may contribute to the risk of chronic kidney disease. Our aim was to determine whether the common *APOE*-ε2/ε3/ε4 polymorphism is associated with a reduced estimated glomerular filtration rate (eGFR) in the RENASTUR population, a cohort of elderly individuals from the region Asturias (northern Spain). ***Methods:*** A total of 743 Spanish Caucasians aged 55–85 years were genotyped for the *APOE*-ε2/ε3/ε4 polymorphism. Individuals with a previous diagnosis of renal disease were not eligible for the study. Participants with a documented history of type 2 diabetes mellitus (T2DM) or hypertension or who were receiving antidiabetic or antihypertensive drugs were classified as diabetics and hypertensives. The eGFR was calculated using the Modification of Diet in Renal Disease formula, and those with an eGFR <60 ml/min/1.73 m^2 (n = 91) were considered as having impaired renal function. The effect of alleles and genotypes on clinical (hypertension, T2DM) and analytical findings was statistically determined. ***Results:*** In addition to age and T2DM, *APOE*-ε2 was significantly associated with an eGFR <60 ml/min/1.73 m^2 (p = 0.002; OR = 2.30). This association remained statistically significant after correction for multiple variables. Although the effect of the *APOE*-ε2 allele on the eGFR was observed both among diabetics and nondiabetics, the significance was stronger in the T2DM group. ***Conclusion:*** The *APOE*-ε2 allele is a genetic risk factor for impaired renal function among healthy elderly Spanish individuals.

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Introduction

Chronic kidney disease (CKD) is a multifactorial disorder with both acquired (nongenetic) and inherited (genetic) risk factors contributing to define the risk [1]. Previous studies have analyzed the effect of a common *APOE* polymorphism defined by three alleles (*APOE*-ε2/ε3/ε4) on the risk of developing impaired renal function, manifested as a reduced glomerular filtration rate (GFR) [2–13]. While some authors concluded that the ε2 allele was associated with a higher risk of CKD compared with the ε3 allele, others failed to confirm this association. The ε2 allele has also been linked to macroalbuminuria and worse kidney function in diabetics, whereas the ε4 allele has been shown to be protective against reduced glomerular filtration and diabetic nephropathy [3, 10, 12]. The association between the *APOE* polymorphism and low GFR and CKD could be explained by differences in the ability to clear lipids in the kidney, which might result in glomerular damaging and impaired filtration [14–16].

The aim of this study was to determine whether the common *APOE*-ε2/ε3/ε4 polymorphism was associated with a reduced GFR in the RENASTUR population, a cohort of elderly healthy Caucasian individuals from Asturias (northern Spain).

Methods

Study Population and Data Collection

This study was approved by the Ethical Committee of the Hospital Universitario Central de Asturias (HUCA). The RENASTUR cohort consisted of 743 apparently healthy individuals aged 55–85 years who were all Caucasian and lived in the region of Asturias (northern Spain; total population: 1 million). They were chosen from the general population to evaluate the renal function in healthy elderly subjects and were recruited through the Primary Health Care Center of the city of Oviedo. Individuals with a history of renal disease were not eligible for the study. Briefly, we used a computer program to randomly select the social security ID of 1,200 individuals who were asked by telephone to participate in the study. A total of 743 (62%) of these individuals agreed, signed an informed consent, and were interviewed and evaluated by a qualified physician (table 1).

Age, sex, and smoking (ever/never smoked) were self-reported. Body mass index (BMI) was calculated by weight and height measured at the examination, where also systolic and diastolic blood pressure values were obtained. Individuals with a documented history of type 2 diabetes mellitus (T2DM) or hypertension or who were receiving antidiabetic or antihypertensive drugs were classified as diabetics and hypertensives, respectively. The biochemical profiles of all participants were obtained from fasting blood samples collected by venipuncture. Dyslipidemia was considered to be present in participants with total cholesterol >240 mg/dl, triglycerides >200 mg/dl, or high-density lipoprotein (HDL) cholesterol <40 mg/dl or who had a prior diagnosis of dyslipidemia or were receiving lipid-lowering treatment. The estimated GFR (eGFR) was calculated using the Modification of Diet in Renal Disease formula [17].

APOE Genotyping

The three *APOE*-ε2/ε3/ε4 alleles are defined by two single nucleotide polymorphisms, 3937T/C and 4075C/T (rs429358 and rs7412, respectively; http://www.ensembl.org), that result in two amino acid changes at protein positions 112 (Cys in ε3 and ε2, and Arg in ε4) and 158 (Arg in ε3, Cys in ε2, and Arg in ε4). DNA was obtained from 5 ml of blood leukocytes, and the *APOE* genotypes were determined through a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, as previously reported [18, 19]. To confirm the accuracy of this genotyping method, PCR fragments from several individuals with each of the RFLP genotypes were sequenced using BigDye chemistry and an ABI3130 automated system (Applied Biosystems).

Statistical Analysis

Fisher's exact and Student's t tests were used to compare categorical and continuous variables between the groups, respectively. Variables that were significantly associated with eGFR in the univariate analysis were included in the multivariate logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (CI) were also calculated. p values <0.05 were considered statistically significant.

Table 1. Main characteristics of the total RENASTUR cohort and of individuals with an eGFR <60 and ≥60 ml/min/1.73 m^2

Main characteristics	Total (n = 743)	eGFR <60 ml/min/1.73 m^2 (n = 91)	eGFR ≥60 ml/min/1.73 m^2 (n = 652)	p values
Age, years	72 (51–85)	74 (56–81)	71 (51–85)	0.006
Male gender	312 (42)	45 (49)	267 (41)	0.12
Smokers	93 (13)	15 (16)	78 (12)	0.41
Weight, kg	75 (43–160)	77 (54–155)	73 (43–160)	0.10
BMI	29.25 (17.40–46.90)	29.67 (22.24–46.90)	29.22 (17.40–45.83)	0.62
Total cholesterol, mg/dl	220 (38–335)	209 (38–321)	219 (71–335)	0.06
LDL cholesterol, mg/dl	133 (10–249)	127 (48–239)	131 (10–249)	0.06
HDL cholesterol, mg/dl	56 (5–276)	55 (5–276)	60 (25–158)	0.05
Triglycerides, mg/dl	105 (8–470)	116 (8–436)	106 (39–470)	0.07
Dyslipidemia	226 (30)	33 (36)	193 (30)	0.20
Serum creatinine, mg/dl	0.81 (0.41–76)	1.27 (0.71–76)	0.77 (0.41–1.40)	<0.001
Albuminuria (albumin/creatinine), mg/g	1.69 (0.30–1,520)	4.79 (0–1,525)	1.60 (0–561)	0.001
Hypertensives	207 (28)	32 (35)	175 (27)	0.10
Diabetics (type II)	176 (24)	32 (35)	144 (22)	0.007
APOE genotypes				
23	107 (14)	22 (24)	85 (13)	0.002*
33	515 (69)	52 (57)	463 (71)	
34/44	114 (15)	17 (19)	97 (15)	
24	7 (<1)	0	7 (1)	
APOE alleles				
2	114 (8)	22 (12)	92 (7)	
3	1,246 (84)	142 (78)	1,104 (85)	
4	127 (9)	18 (10)	109 (8)	

Values are mean (range) or n (%). * p = 0.002 (*APOE* genotypes 23 vs. 33); OR = 2.30; 95% CI = 1.33–3.99.

Results

Table 1 summarizes the main characteristics of the entire RENASTUR cohort as well as of those patients with an eGFR ≥60 ml/min/1.73 m^2 (n = 652) and <60 ml/min/1.73 m^2 (n = 91). In the univariate analysis, the following variables were significantly associated with an eGFR <60 ml/min/1.73 m^2: older age (p < 0.001), T2DM (p = 0.007), and the *APOE*-ε23 genotype (p = 0.002). In the multivariate logistic regression analysis, age, diabetes, and the *APOE* genotype remained independent risk factors for an eGFR <60 ml/min/1.73 m^2 (table 2). Compared to the most common ε33 genotype, the frequency of *APOE*-ε23 was significantly higher in the eGFR <60 ml/min/1.73 m^2 group (p = 0.002; OR = 2.30, 95% CI = 1.33–3.99).

Although the effect of *APOE*-ε23 on eGFR was observed among both diabetics and nondiabetics, the significance was stronger in the T2DM group (table 3). In the non-T2DM group, the frequencies of *APOE*-ε23 were 20 and 13% (eGFR <60 and ≥60 ml/min/1.73 m^2, respectively; p = 0.02) compared to 31 and 13% among the T2DM participants (p = 0.008).

Compared to the *APOE*-ε33 and -ε34/44 groups, the *APOE*-ε23 group had lower total cholesterol (p = 0.04) and low-density lipoprotein (LDL) cholesterol levels (p = 0.01), but higher triglyceride levels (p = 0.04; table 4). Dyslipidemia was more frequent among *APOE*-ε4 allele carriers (p = 0.002). No significant difference in the frequency of T2DM and hypertension was found between the genotypes.

Table 2. Univariate and multivariate analysis for individuals with an eGFR <60 versus ≥60 ml/min/1.73 m^2

	Univariate analysis p values/OR (95% CI)	Multivariate analysis p values/OR (95%CI)
Age (years)	0.003/0.93 (0.89–0.97)	0.001/0.906 (0.85–0.90)
Weight (kg)	0.305/1.34 (0.76–2.35)	
BMI	0.149/0.98 (0.97–1.005)	
Total cholesterol (mg/dl)	0.045/1.007 (1–1.015)	0.84/1 (0.99–1.01)
LDL cholesterol (mg/dl)	0.099/1.007 (0.99–1.01)	
HDL cholesterol (mg/dl)	0.477/1.006 (0.99–1.022)	
Triglycerides (mg/dl)	0.164/0.99 (0.99–1)	
Dyslipidemia	0.20/1.35 (0.85–2.14)	
Uric acid (mg/ml)	0.206/0.98 (0.97–1)	
Diabetes	0.007/1.88 (1.18–3.01)	0.02/2.08 (1.09–4.68)
Smoker	0.895/1.06 (4.30–2.62)	
Hypertension	0.10/1.48 (0.92–2.36)	
Albuminuria rate	<0.001/3.73 (1.78–7.81)	0.099/2.24 (0.86–5.83)
APOE genotype (23 vs. 33)	0.002/2.30 (1.33–3.99)	0.015/2.52 (1.19–5.33)

Table 3. *APOE* genotypes in diabetics and nondiabetics with an eGFR <60 and ≥60 ml/min/1.73 m^2

	No T2DM <60 ml/min/1.73 m^2	No T2DM ≥60 ml/min/1.73 m^2	T2DM <60 ml/min/1.73 m^2	T2DM ≥60 ml/min/1.73 m^2
22	0	0	0	0
23	12 (20)	66 (13)	10 (31)	19 (13)
24	0	4	0	3
33	36 (60)	359 (71)	17 (53)	104 (72)
34	11 (18)	75 (15)	5 (16)	17 (12)
44	1	4	0	1

Values are n (%). p values: 0.02 (No T2DM) and 0.008 (T2DM).

Table 4. Main characteristics of the entire RENASTUR cohort according to *APOE* genotypes

	22/23 (n = 107)	33 (n = 515)	34/44 (n = 114)	p value
Age, years	74 (52–81)	73 (54–81)	74 (54–81)	0.81
Weight, kg	75.5 (47–110)	76 (52–166)	73 (46–163)	0.57
BMI	29.75 (18.59–47.78)	29.35 (18.22–43.97)	28.42 (21–46.87)	0.24
Total cholesterol, mg/dl	206 (97–313)	215 (71–321)	217 (18–335)	0.04
LDL cholesterol, mg/dl	118 (52–210)	133 (67–239)	137 (41–470)	0.01
HDL cholesterol, mg/dl	59 (34–143)	51 (5–276)	57 (26–102)	0.60
Triglycerides, mg/dl	109.5 (40–436)	99 (8–382)	106.5 (42–470)	0.04
Dyslipidemia (yes)	18 (17)	164 (32)	44 (39)	0.002
T2DM (yes)	32 (28)	121 (23)	23 (20)	0.007
Hypertension (yes)	36 (33)	141 (27)	30 (26)	0.46
eGFR <60 ml/min/1.73 m^2	22 (19)	52 (10)	17 (15)	0.002

Values are median (min–max) or n (%).

Discussion

The main finding of our study is that there exists a significant association between the *APOE*-ε2 allele and impaired renal function measured as a decreased GFR (<60 ml/min/1.73 m^2). The ε2 allele has been linked to the risk of developing nephropathy in T2DM as well as to the progression of renal disease in type 1 diabetics and in patients with several renal diseases, and has also been shown to be a predictor of GFR in apparently healthy individuals [2–9]. Compared to the ε3 protein isoform, apoE-ε2 binds less effectively to apoE and LDL receptors, and this could result in hepatic upregulation of LDL receptors and enhanced clearance of LDL [20–22]. This was in agreement with the significantly lower median LDL value among our ε23 participants (table 4).

A recent report did not find significant associations of *APOE* alleles with kidney function among older adults in the Cardiovascular Health Study (CHS) [13]. Compared to *APOE*-ε3 homozygotes, *APOE*-ε2 carriers in the CHS also showed lower total cholesterol and LDL cholesterol levels as well as higher triglyceride values. However, our participants had higher mean BMI (29.25 vs. 26.30), and the frequency of T2DM was also higher in our cohort (24 vs. 14%). Since the effect of *APOE*-ε2 on reduced eGFR was higher among diabetics, the different rate of T2DM (and other risk factors for reduced eGFR) could explain, in part, the discrepancies between the studies.

The pathway by which apoE acts on the kidney and the ε2 isoform predisposes to lower eGFR has not been elucidated yet. Mean triglyceride values are significantly higher among ε2 carriers, and this would promote the accumulation of cholesteryl esters by human mesangial cells, leading to changes in the mesangial matrix and impaired renal function [23, 24]. In support of this hypothesis is the fact that the ε2 allele has been found to be overrepresented among patients with lipoprotein glomerulopathy, which is characterized by an abnormal accumulation of lipids in the glomerular capillary lumen [25, 26]. At least one study has reported an increased expression of ApoE in glomerular lesions of patients with diabetic nephropathy, and carriers of the ε2 allele show increased protein expression and glomerular hypertrophy [27]. In addition, ApoE has an immunomodulatory effect (through the reduction of immune stimulatory proteins and T-cell activation) and promotes cell regeneration – two mechanisms that could explain the association between the ε2 isoform and mesangial cell proliferation/diminished filtration surface [14, 28, 29].

Finally, the association observed between *APOE* variants and impaired renal function could also be seen in cardiorenal disease [30]. The *APOE*-ε4 allele has been widely considered proatherogenic, and carriers of this isoform would have an increased risk of developing coronary artery disease and suffering ischemic episodes. Due to its lipid-lowering effect, the ε2 allele has been considered protective against coronary artery disease. Impaired renal function/CKD has been related to the risk of suffering heart failure [31]. Recent studies have identified associations between gene variants and heart failure which could be explained by an effect on renal function [32]. These risk variants could predispose individuals who suffered episodes that damage the heart, such as myocardial infarction, to heart failure. To interpret the effect of *APOE* on heart failure, we should consider that ε2 is reduced (and ε4 increased) among patients with ischemic episodes. Thus, any study with the aim to determine the role of ε2 on heart failure should take into account its effect on renal function and atherosclerosis.

In conclusion, we report an association between the *APOE*-ε2 allele and reduced eGFR among elderly Spanish Caucasians.

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Disclosure Statement

None of the authors have competing interests related to this work.

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