Apolipoprotein E Gene Polymorphism Is Related to Metabolic Abnormalities, But Does Not Influence Erythrocyte Membrane Lipid Composition or Sodium-Lithium Countertransport Activity in Essential Hypertension

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The aim of this study was to analyze the influence of the apolipoprotein E (apoE) gene polymorphism on insulin resistance and plasma lipid composition of essential hypertensive patients. A secondary objective was to analyze if differences regarding plasma lipids had an effect on the erythrocyte membrane lipid composition and the activity of the erythrocyte membrane sodium-lithium countertransport. We studied 128 untreated nondiabetic essential hypertensive patients enrolled from our outpatient clinic. We considered as hyperinsulinemic all subjects having more than 80 mU/L of plasma insulin 120 minutes after a 75-g oral glucose intake. The number of hyperinsulinemic subjects among carriers of the ε4 allele was higher that in ε4 noncarrier subjects (13 of 19 *v* 45 of 109, *P* < .05; odds ratio [OR], 3.08; confidence interval [CI], 0.99-10.57). Plasma insulin at baseline and plasma insulin and glucose at 120 minutes after overload was higher in carriers of the ε4 allele (respectively, 17.5 ± 6.9 *v* 12.4 ± 4.9 mU/L, *P* < .01; 111.9 ± 39.9 *v* 88.7 ± 48.2, *P* < .05; and 143.8 ± 29.3 *v* 121.2 ± 30.8 mg/dL, *P* < .005). Subjects with the ε4 allele had a plasma lipid profile more atherogenic than those without this allele. This profile was mainly characterized by higher levels of low-density lipoprotein (LDL) cholesterol (150.1 ± 31.2 *v* 133.0 ± 34.3 mg/dL, *P* < .05) and very–low-density lipoprotein (VLDL) triglycerides (134.7 ± 85.5 *v* 99.2 ± 68.8 mg/dL, *P* < .05) and by lower levels of high-density lipoprotein (HDL) cholesterol (41.8 ± 10.7 *v* 50.0 ± 14.7 mg/dL, *P* < .05). There were no differences between groups regarding erythrocyte membrane cholesterol or phospholipids composition and sodium-lithium countertransport (SLC) activity.

APOLIPOPROTEIN E (apoE) is a component of several classes of triglyceride-rich plasma lipoproteins. ApoE exhibits genetic polymorphism with 6 common apoE genotypes as a result of a single apoE gene locus with 3 common alleles designated ε2, ε3, and ε4, and their gene products are, respectively, E2, E3, and E4. There are 3 homozygous genotypes ε4/ε4, ε3/ε3, and ε2/ε2 and 3 heterozygous genotypes ε4/ε3, ε3/ε2, and ε4/ε2. The apo E3 isoform is the normal form of the protein, and the ε3/ε3 genotype is the most frequently found in every population studied so far.[1,2] In normotensive adults[3] and children,[4] population-based studies have shown that subjects carrying the ε4 allele have higher plasma levels of total and low-density lipoprotein (LDL) cholesterol than subjects with other genotypes. In agreement with this, the ε4 allele was more frequently found in subjects having myocardial ischemia[5,6] or an ischemic stroke[7,8] than in controls.

Both hypertension and insulin resistance are closely related to plasma lipid abnormalities.[9] A previous study has shown that the apo ε4/* genotype frequency is increased in essential hypertension.[10] Recently, Dembinska-Kiec et al[11] have found an increase in the E4/E3 phenotype frequency in hyperinsulinemic patients compared with normoinsulinemic subjects. To date, the plasma lipoprotein profile in hypertensive subjects with or without hyperinsulinemia and according to the apoE genotype has not been well studied.

Moreover, it has been previously reported that plasma lipoproteins and cell membrane lipid composition are related in hypertension, and that erythrocyte membrane lipid composition or distribution modifies the cell membrane sodium-lithium countertransport (SLC) activity.[12-16] The SLC is a well-known marker of predisposition to essential hypertension[17] that seems to be related to a worsened plasma lipid profile.[18] The possible influence of the apoE genotype on these parameters is not known.

Our aim in this study was to examine the influence of the apo ε4/* genotype (subjects carrying the ε4 allele) on insulin resistance, plasma lipoprotein profile, erythrocyte lipid composition, and SLC activity in essential hypertension and to study the prevalence of hyperinsulinemia in carriers of this allele. Therefore, we have studied 128 essential hypertensive patients who were classified as ε-4 carriers or ε-4 noncarriers. Our hypothesis is that ε-4 carriers as compared with ε-4 noncarriers, have more prevalence of hyperinsulinemia and a more atherogenic plasma lipoprotein profile, which might alter the lipid composition of erythrocyte membrane and increase the activity of the SLC.

MATERIALS AND METHODS

Patients

A total of 128 (male/female: 71/57) mild to moderate essential hypertensive patients attending our outpatient clinic were studied. We considered mild to moderate hypertension to stages I and II of the VI report of the Joint National Committee (JNC).[19] They were free of target organ damage and were not taking any medication with effects on serum lipids or blood pressure. There were no women on birth control pills or estrogen replacement hormones. Subjects diagnosed with diabetes mellitus or impaired glucose tolerance, those having causes of secondary hyperlipidemia, and those with a family history of primary hyperlipidemia, were excluded. All patients had documented stable weights for at least 3 weeks and consumed at least 150 g carbohydrates daily for 3 days before inclusion.

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Submitted January 6, 2000; accepted June 9, 2000.

Supported by a Grant No. FIS 96-0447.

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0026-0495/01/5002-0026$35.00/0

doi:10.1053/meta.2001.19429

Plasma Glucose, Insulin, and Lipid Measurements

Blood samples were drawn after an overnight fast of 12 hours, and they were separated by ultracentrifugation at 40,000*g* at 4°C, during 18 hours. The very–low-density lipoprotein (VLDL) fraction was obtained from the supernatant and its composition of cholesterol and triglycerides was measured by conventional enzymatic methods. Next, total high-density lipoprotein (HDL) and HDL_3 (HDL_2 was calculated by subtracting HDL_3 from total HDL) was obtained by precipitation with addition of polyethylene glycol (Quantolip inmuno-GMBH, Heidelberg, Germany) in the undernatant, and their composition in cholesterol and triglycerides was measured. The composition of the LDL was then obtained by subtracting the content of cholesterol and triglycerides of total HDL from that of the undernatant.

Additionally, a 75-g oral glucose tolerance test was performed. After oral glucose overload, blood samples were collected for measurements of plasma glucose and insulin at 0 and 120 minutes. Plasma glucose was measured by conventional enzymatic methods and plasma insulin by radioimmunoassay.

According to a previously described limit,[20] subjects having plasma levels of insulinemia at 120 minutes after oral glucose overload higher than 80 mU/L were considered as hyperinsulinemics.

ApoE Genotype Determination

The apoE polymorphism was genotyped using polymerase chain reaction (PCR) by amplification of the polymorphic fragment of the apoE gene and digestion of the PCR product with the restriction enzyme Cfol according to the method of Wenhan et al.[21]

Membrane Lipid Measurements

Total lipids were extracted from 1 mL packed erythrocytes with 18 mL isopropanol:chloroform 11:7 (vol:vol) containing 0.45 mmol/L 2,6-di-ter-p-cresol (BHT) as an antioxidant.[22] The lipid extract was dried in a N_2 stream.

Cholesterol and phospholipid concentrations were determined by means of the Iatroscan technique.[23] The Iatroscan MK-5 (Iatron Laboratories, Tokyo, Japan), equipped with a flame ionization detector, scanner, and integrator (Iatrocorder TC-11, sensitivity 10 mV), was used in combination with Chromarod S, having a precoated active silica thin layer.

SLC Activity

Li^+-stimulated maximal efflux rate was measured according to the technique described by Canessa et al.[17] Briefly, erythrocytes (20% hematocrit level) were incubated for 1 hour at 37°C in a Li^+-loading solution containing 75 mmol/L Li_2 CO_3, 10 mmol/L glucose, and 120 mmol/L morpholino-propansulfonic acid–Tris buffer (MOPS-TRIS) (pH 7.4 at 37°C) and then washed 4 times with 110 mmol/L $MgCl_2$. After this procedure, the red blood cell Li^+ content was measured (a mean value of 6.2 ± 0.3 mmol/L cells was obtained), and 2 aliquots of 1 mL packed cells were then incubated for 1 hour at 37°C in an iso-osmolar sodium-enriched medium (150 mmol/L NaCl) or in a sodium-free medium (85 mmol/L sucrose, 75 mmol/L $MgCl_2$), both containing 10 mmol/L glucose, 0.1 mmol/L ouabain, and 10 mmol/L MOPS-TRIS (pH 7.4 at 37°C). The SLC maximal efflux rate was estimated as the differences between the Li^+ effluxes into sodium-enriched and sodium-free media. External Li^+ concentrations were measured after centrifugation in the supernatants by atomic absorption spectophotometry (Perkin-Elmer 460, Norwalk, CT).

Statistical Analyses

Whenever the variance was not homogeneous, the differences between groups were compared using a nonparametric test (Mann-Whitney *U* test).[24] Categorical variables (insulin resistance and ε4 carriers) were analyzed using the χ^2 test with Yates correction when needed. The odds ratio (OR) of being hyperinsulinemic in the ε4 carrier group was calculated. The Epi-Info 6.0 (Centers for Disease Control, USA) and SPSS 9.00 (SPSS Inc, Cary, NC) statistical software were used.

RESULTS

The apoE genotype and allele distribution are shown in Table 1. There were no statistically significant differences between subjects carrying the ε4 allele (ε3/ε4 or ε4/ε4) and those without the ε4 allele regarding age, body mass index, male/female ratio, and systolic or diastolic blood pressure (Table 2).

The number of hyperinsulinemic subjects among carriers of the ε4 allele was higher than in ε4 noncarrier subjects (13 of 19 *v* 45 of 109, $P < .05$; OR, 3.08; confidence interval [CI], 0.99-10.57). In agreement with this, plasma insulin at baseline and plasma insulin and glucose at 120 minutes after overload were higher in carriers of the ε4 allele than in the other subjects (Table 3).

Plasma lipids and lipoprotein profile were more atherogenic in patients with the ε4/* genotype (Table 4) than in the other subjects. Finally, cholesterol and phospholipid composition of erythrocyte membrane and also SLC activity was similar in both groups (Table 5).

DISCUSSION

Hyperlipidemia is more common in hypertensive than in normotensive subjects.[25] The high prevalence of hyperinsulinemia in hypertensives might explain this phenomenon.[26] Because plasma insulin influences lipoprotein lipase activity,[27] insulin-resistant subjects have increased plasma levels of VLDL triglycerides and a decreased amount of HDL cholesterol.[28] However, the mechanisms leading to the increased plasma levels of total cholesterol and LDL cholesterol, commonly observed in hypertensive patients, are not well understood.

Population-based studies have shown that subjects carrying the apo E4/* phenotype have increased levels of both total and LDL cholesterol.[3,4] On the other hand, the prevalence of carriers of ε4 allele seems to be increased in hypertensive patients.[10,11] Consequently, it might be hypothesized that the plasma lipoprotein profile observed in hypertension might be

Table 1. ApoE Genotype and Allele Frequency of the Hypertensive Patients (n = 128)

Genotype	No. of Subjects (%)
ε3/ε3	92 (71.9)
ε2/ε3	15 (11.7)
ε3/ε4	16 (12.5)
ε2/ε4	0 (0)
ε4/ε4	3 (2.3)
ε2/ε2	2 (1.6)
Allele	Frequency
ε-2	0.075
ε-3	0.839
ε-4	0.086

Table 2. Characteristics of the Studied Population

	ε-4 Noncarriers (n = 109)	ε-4 Carriers (n = 19)
Age (yr)	45.5 ± 11.5	44.5 ± 7.5
Sex (male/female)	60/49	11/8
Body mass index (kg/m^2)	27.4 ± 2.6	26.1 ± 3.1
Systolic blood pressure (mm Hg)	151.2 ± 11.3	149.6 ± 9.8
Diastolic blood pressure (mm Hg)	95.3 ± 6.7	96.1 ± 6.2

NOTE. Data are means ± SD.

influenced by hyperinsulinemia and also by a higher number of subjects with the apo ε-4/* genotype.

Nevertheless, up to date, only one study analyzed simultaneously insulin resistance and apoE isoform distribution in 60 hypertensive patients.[11] In agreement with us, these investigators observed an increased E4/E3 phenotype frequency in hyperinsulinemic hypertensive than in normoinsulinemic hypertensive patients. Regarding plasma lipids, when comparing hypertensives with and without hyperinsulinemia, they found an increase of total triglycerides and VLDL triglycerides in the former group. However, total cholesterol levels were similar in both groups, and cholesterol levels of lipoproteins were not measured. Finally, they determined apoE isoforms by isoelectric focusing of delipidated VLDL fraction and not by PCR (therefore, measuring phenotypes and not genotypes) and obtained a distribution of apoE phenotypes in hypertensive patients with a high frequency (32%) of E4/E2 isoforms and no one individual with the E3/E2 or E2/E2 isoforms (apparently, in disagreement with the Hardy-Weinberg equilibrium). We have now studied a larger sample of essential hypertensive patients. They were genotyped for the apoE allele polymorphism by PCR and were classified as hyperinsulinemic or normoinsulinemic. We observed an apoE genotype and allele frequency in agreement with the Hardy-Weinberg equilibrium (Table 1) and not substantially different from that obtained by other investigators[29] in subjects living in our own country.

We have found a higher number of hyperinsulinemics in hypertensive patients with the ε4 allele than in those without this allele. In agreement with this, patients with the ε4/* genotype had higher plasma levels of insulin at baseline and insulin and glucose at 120 minutes after overload. Moreover, we have observed a plasma lipoprotein profile more atherogenic in carriers of the ε4 allele than in the other subjects (Table 4). This was mainly characterized by higher plasma levels of LDL cholesterol and a lipid profile similar to that observed in the insulin resistance syndrome. This means lower levels of HDL cholesterol and an increase of VLDL triglycerides.[27,28] The absence of differences in plasma levels of total cholesterol might be explained by the decreased amount of HDL cholesterol in subjects carrying the ε4 allele.

In nonhypertensive populations, the mentioned decrease of HDL cholesterol in carriers of the ε4 allele is uncommonly observed. However, a meta-analysis of studies including healthy, diabetic, hyperlipidemic or obese subjects (14,799 individuals from 45 population samples and 17 different countries) reported a significant decrease of HDL cholesterol (LDL cholesterol was not measured) and an increase of triglycerides (VLDL fraction was not measured) in subjects with the E4/E3 phenotype, the most common among carriers of the ε4 allele.[30] Therefore, even in nonhypertensive populations, when the size of the sample increases, the ε4 allele seems to be related to a plasma lipid profile, similar to that observed by us.

Finally, because different influences among plasma lipids, cell membrane lipids, and transmembrane ion transporters have been described in healthy subjects,[31] diabetes,[32,33] or hypertension,[12-16] we measured cholesterol and phospholipid composition of erythrocyte membrane and also the activity of the ion carrier SLC. We did not observe differences regarding cell membrane lipid composition or activity of the erythrocyte

Table 3. Plasma Glucose and Insulin at Baseline and at 120 Minutes After an Oral 75 g Glucose Overload

	ε-4 Noncarriers (n = 109)	ε-4 Carriers (n = 19)
Glucose at baseline (mg/dL)	95.8 ± 11.8	99.4 ± 12.1
Glucose at 120 min (mg/dL)	121.2 ± 30.8	143.8 ± 29.3*
Insulin at baseline (mU/L)	12.4 ± 4.9	17.5 ± 6.9†
Insulin at 120 min (mU/L)	88.7 ± 48.2	111.9 ± 39.9‡

NOTE. Values are means ± SD.
* $P < .005$.
† $P < .01$.
‡ $P < .05$.

Table 4. Plasma Lipid and Lipoproteins

	ε-4 Noncarriers (n = 109)	ε-4 Carriers (n = 19)
Total cholesterol	221.4 ± 54.0	237.4 ± 46.3
HDL-cholesterol	50.0 ± 14.7	41.8 ± 10.7*
HDL_2-cholesterol	11.4 ± 11.3	8.8 ± 8.4
HDL_3-cholesterol	38.5 ± 11.4	32.6 ± 7.4
LDL cholesterol	133.0 ± 34.3	150.1 ± 31.2*
VLDL cholesterol	23.8 ± 22.7	32.8 ± 21.6
Total triglycerides	155.7 ± 104.0	201.1 ± 121.8
HDL triglycerides	20.7 ± 15.6	19.1 ± 8.1
LDL triglycerides	36.3 ± 22.3	45.7 ± 23.8
VLDL triglycerides	99.2 ± 68.8	134.7 ± 85.5*
VLDL-Tg/HDL-c	2.4 ± 2.5	3.7 ± 3.4*
LDL-c/HDL-c	3.0 ± 1.4	3.8 ± 1.3†

NOTE. Values are mg/dL.
Abbreviations: VLDL-Tg/HDL-c, VLDL-triglycerides/HDL-cholesterol ratio, LDL-c/HDL-c, LDL-cholesterol/HDL-cholesterol ratio. Values are means ± SD.
* $P < .05$.
† $P < .01$.

Table 5. Cholesterol and Phospholipid Composition and SLC Activity in Erythrocyte Membrane

	ε-4 Noncarriers (n = 109)	ε-4 Carriers (n = 19)
Cholesterol (%)	48.3 ± 6.4	48.1 ± 5.3
P-ethanolamine (%)	18.1 ± 3.9	18.6 ± 2.7
P-choline (%)	17.9 ± 2.9	18.3 ± 3.5
Sphingomyelin (%)	15.0 ± 3.8	15.3 ± 3.8
SLC (mmol/L cell/h^{-1})	0.38 ± 0.15	0.35 ± 0.12

NOTE. Values are mean ± SD.
Abbreviations: P, phosphatidyl; SLC, sodium-lithium countertransport.

membrane SLC among ε4 carriers and non-ε4 carriers. Thus, indicating that the impaired plasma lipoprotein profile observed in subjects with the ε4/* genotype does not seem to influence the mentioned parameters in the erythrocyte membrane. In conclusion, we have observed that hypertensive patients with the apoE ε4/* genotype have more parameters associated with insulin resistance and a plasma lipoprotein profile more atherogenic than other hypertensives, and this was mainly characterized by an increased LDL cholesterol and VLDL triglycerides and by a decrease of HDL cholesterol. These differences do not influence the studied variables in cell membrane. Nevertheless, further studies are needed to explore these issues.

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