Apolipoprotein E Genotype and Cholesterogenesis in Polygenic Hypercholesterolemia

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We studied 22 normal-weight patients with polygenic hypercholesterolemia (PH), of which 11 (two males and nine females) had the apolipoprotein (apo) E3/4 genotype and 11 (one male and 10 females) the E3/3 genotype. The two groups were comparable for age, body mass index, total and low-density lipoprotein (LDL) cholesterol levels. The diagnosis of PH was made on the basis of clinical assessment, the criteria being type IIa hypercholesterolemia without tendon xanthomas and/or family history and clinical criteria indicative of familial hypercholesterolemia and/or familial combined hyperlipidemia. To avoid the influence of the habitual individual diet on cholesterogenesis, daily urinary mevalonic acid (MVA) excretion, an index of whole-body cholesterol synthesis, was evaluated in the steady-state condition while patients were on a low-fat, low-cholesterol diet for at least 3 months. Urinary MVA excretion rates were 2.52 ± 0.8 μmol/24 h in E3/4 patients, significantly higher ($P < .001$) than in E3/3 patients (1.38 ± 0.6 μmol/24 h). This is the first evidence of a higher rate of cholesterogenesis in PH patients carrying the ε4 allele versus the ε3 allele under a standardized lipid-lowering diet. We conclude that the higher rate of cholesterogenesis in PH patients with the ε4 allele might partly explain the interindividual differences in response to treatment with cholesterol synthesis inhibitors such as statins.

APOLIPOPROTEIN E (apo E) plays a central role in lipoprotein metabolism, being a major protein constituent of chylomicrons, very–low-density lipoproteins, their remnant particles, and high-density lipoproteins (HDLs).[1] In humans, apoE is a polymorphic protein with three major isoforms, apo E2, E3, and E4, which are under the control of three independent codominant alleles.[2] Genetic variation in apo E accounts for 5% to 15% of the variance in low-density lipoprotein (LDL) cholesterol levels in the general population.[1] In most if not all human populations, it has been found that individuals with apo E4 display high levels of plasma total and LDL cholesterol, whereas those with apo E2 show the opposite pattern.[1,3] Studies performed with sterol balance under hospital conditions have demonstrated that the association of the ε4 allele with elevated plasma levels of LDL cholesterol can be explained by a number of mechanisms: a high cholesterol absorption efficiency, a low LDL–apo B removal rate, or a low cholesterol synthesis rate. In contrast, subjects with the ε2 allele have lower plasma cholesterol levels, low cholesterol absorption efficiency, a high LDL–apo B removal rate, and a high cholesterol synthesis rate.[4,5] Values for these measures in apo E3/3 subjects are between these two categories. These data would suggest that enhanced cholesterol absorption in individuals with the apo ε4 allele overloads hepatocytes with cholesterol; this, in turn, reduces hepatic cholesterol synthesis and LDL–apo B/E receptor activity, resulting in reduced LDL receptor-mediated degradation and increased LDL cholesterol concentration.[6]

However, other studies aimed at evaluating the effects of apo E isoforms on the cholesterol synthesis rate yielded conflicting results, suggesting the lack of a clear-cut relationship between these two factors.[7,8] The different results might be partly attributed to the different methods used to assess cholesterogenesis and to the characteristics of the population sample.

Polygenic hypercholesterolemia is the most common cause of increased cholesterol in Western populations. It is currently considered a heterogeneous clinical entity that results from multiple interactions between genetic and environmental factors.[9] Among these factors, apo E polymorphism and the cholesterol synthesis rate might have a role.

Mevalonic acid (MVA) is the immediate product of hepatic hydroxymethyl glutaryl–coenzyme A (HMG-CoA) reductase activity. The plasma MVA concentration has been demonstrated to be a good indicator of the in vivo rate of cholesterol synthesis in man, and it correlates with the sterol balance,[10] incorporation of deuterated water,[11] and HMG-CoA reductase activity in hepatocytes.[12] Urinary excretion of MVA varies in parallel with the plasma concentration in normal subjects.[13] Based on these characteristics, MVA has the potential to produce the most accurate information about the relationships between apo E isoforms and in vivo cholesterogenesis. Against this background, we decided to use MVA to study the effects of the two most common apo E genotypes (E3/3 and E3/4) on cholesterol synthesis in patients with polygenic hypercholesterolemia (PH).

SUBJECTS AND METHODS

Patients

From 65 consecutive patients at our lipid clinic with the diagnosis of PH, we found 11 normal-weight subjects (two males and nine females) with the E3/4 genotype. The mean age was 60.9 ± 8.2 years, the body mass index was 24.1 ± 1.6 kg/m^2, and total and LDL cholesterol were 7.61 ± 0.4 and 5.35 ± 0.4 mmol/L, respectively. Subsequently, we selected 11 PH patients (one male and 10 females) with the E3/3 genotype matched for age, body mass index, and total and LDL cholesterol (Table 1). The diagnosis of PH was made on the basis of clinical assessment, the criteria being type IIa hypercholesterolemia defined in accordance with the World Health Organization statement,[14] the absence of tendon xanthomas, the presence of hypercholesterolemia in less than 10% of first-degree relatives, and the absence of other than type IIa phenotypes in first-degree relatives with hyperlipidemia.[9] The females were all postmenopausal. None of the patients had gastrointestinal, thyroid, liver, or renal disease or diabetes mellitus; none used lipid-lowering medication or any other agent known to affect lipid metabolism from at least 2 months. All patients were maintained on an isocaloric diet containing 60% carbohydrate, 25% fat (total cholesterol

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Table 1. Subject Characteristics for Apo E Genotypes 3/3 and 3/4

Characteristic	Apo E 3/3	Apo E 3/4
No. of subjects	11	11
Age (yr)	62.2 ± 6.4	60.9 ± 8.2
Sex (M/F)	1/10	2/9
Body mass index (kg/m²)	24.2 ± 1.6	24.1 ± 1.2
Total cholesterol (mmol/L)	7.74 ± 0.8	7.61 ± 0.4
HDL cholesterol (mmol/L)	1.32 ± 0.3	1.42 ± 0.3
Triglycerides (mmol/L)	1.72 ± 0.6	1.64 ± 0.4
LDL cholesterol (mmol/L)	5.59 ± 0.7	5.35 ± 0.4
Creatininuria (mmol/24 h)	7.25 ± 1.2	8.23 ± 2.0
Daily urinary MVA excretion (μmol/24 h)	1.38 ± 0.6	2.52 ± 0.8*

*$P < .001$.

<300 mg/d, monounsaturated fatty acids 10%, polyunsaturated fatty acids 5%, and saturated fatty acids 5%), and 15% protein for at least 3 months before entering the study. During this run-in period, lipid parameters and body weight were assessed every 2 weeks. Compliance to diet was assessed by a 3-day recall questionnaire administered by a trained dietician every month. Patients were studied in the steady-state condition, ie, when no variations in body weight and lipid parameters occurred over the previous 30-day period. The mean baseline follow-up period on the diet before steady state was reached was 98.5 ± 12 days.

Blood was collected after an overnight fast for measurement of total cholesterol, HDL cholesterol, and triglyceride levels. Patients were then instructed to collect a 24-hour urine sample for measurement of daily urinary MVA excretion. Creatininuria was used as an index to evaluate whether urine had been properly collected.

Lipid and Apolipoprotein Assays

Serum cholesterol and triglyceride levels were measured enzymatically using commercially available test kits (Boehringer, Mannheim, Germany). Serum HDL cholesterol was determined after precipitation of apo B–containing lipoproteins with phosphotungstic acid, using the same cholesterol kit. Serum LDL cholesterol was calculated according to the Friedewald formula.[15]

Apo E Genotyping

Apo E genotyping was performed on DNA extracted from leukocytes using the restriction isotyping procedure on polymerase chain reaction–amplified fragments as described by Hixson and Vernier.[16]

MVA Assay

The 24-hour urinary excretion of MVA was quantified with our own method as previously described.[17] Briefly, after addition of 2-^{3}H-mevanolactone (MVL) to each sample as an internal standard, MVA was converted to MVL using Dowex 50 (H^+) and then extracted into CH_2Cl_2:PrOH (9:1 vol/vol). After purification with a Lc-Si silica cartridge and conversion back to the acid form, MVA was esterified to the 3,5-bis(trifluoromethyl)benzyl ester and the trimethylsilyl derivative prepared using bis(trimethylsilyl)-trifluoroacetamide. Derivative samples were reconstituted in *n*-octane for gas chromatography (GC)–mass spectrometry (MS). The GC column was routed into the ion source of a VG Biotech-Quattro mass spectrometer (FISONS, Manchester, UK). The amount of derived MVA was measured by chemical ionization-MS using CH_4 as a reactant gas and operating the spectrometer in the single-ion mode.

Statistical Analysis

Values are expressed as the mean ± SD. The significance of differences between means was calculated by Student's two-tailed unpaired *t* test using the Statview statistical package (Abacus Concepts, Berkeley, CA).

RESULTS

Lipid parameters in the two groups of patients are reported in Table 1. Urine creatinine values were in the normal range in both groups, thus indicating that 24-hour urine had been properly collected. Mean urinary MVA excretion was 1.38 ± 0.6 and 2.52 ± 0.8 μmol/24 h in patients with the apo E3/3 and apo E3/4 genotype, respectively ($P < .001$). Significance was maintained when MVA excretion values were divided by creatininuria values (193.7 ± 90 and 315.0 ± 116.7 nmol MVA/mmol creatinine in E3/3 and E3/4, respectively, $P < .01$).

DISCUSSION

To our knowledge, this is the first study to evaluate the influence of apo E genotype on cholesterol synthesis in patients with PH. Previous studies performed in healthy men suggested a decreased cholesterol synthesis in carriers of the apo ε4 allele.[5,6] However, in other studies, healthy adults with different apo E phenotypes had similar cholesterol synthesis rates. A large study performed on 160 Dutch nuclear families demonstrated that plasma levels of lathosterol, an indicator of whole-body cholesterol synthesis, did not differ among the various apo E phenotypes in adults. Moreover, lathosterol levels were higher (rather than lower) in children with the E3/4 phenotype versus E3/3 and E3/2.[7] Others have measured cholesterol synthesis as the rate of deuterium uptake into plasma free cholesterol, and did not find any influence of apo E genotype on the sterol synthesis rate in normolipidemic individuals.[8]

The diverging results might be related to the different methods used to assess cholesterol synthesis rather than to genotyping versus phenotyping evaluation of apo E polymorphism. MVA excretion values have a wide interindividual variability, ranging from 1 to 3 μmol/24 h with a mean of 1.8 ± 0.3 in a sample of adult subjects studied in our laboratory. These values, which are in keeping with those reported from other laboratories,[18,19] were obtained in subjects following a balanced diet with a normal cholesterol content. In our study performed in patients with PH, subjects with the E3/4 genotype have a higher cholesterogenesis than those with the E3/3 genotype. Our data parallel the results found in a subset of younger subjects with a wide range of cholesterol levels.[7] Since previous studies of lipoprotein kinetics have demonstrated a reduced LDL–apo B fractional catabolic rate in healthy ε4 carriers,[5] we may speculate that the higher MVA excretion in our PH patients carrying an ε4 allele is the consequence of a decreased LDL-receptor activity and would indicate a major role of these receptors in modulating cellular cholesterogenesis in these patients.

Another point to be considered is the diet. Several studies indicate that the ε4 allele is associated with a greater sensitivity to dietary manipulations, although many inconsistencies have been reported. The reasons for these heterogeneous results in dietary intervention studies are difficult to identify, and the clinical relevance of these experiments is that the apo E phenotype may not help to predict the cholesterol-lowering effects of the diet.[1,6] It has recently been demonstrated that the

cholesterol-raising effect of apo E4 increased with an increasing dietary intake of cholesterol and saturated fatty acids, thus suggesting that diet is a major factor interacting with apo E.[20] To avoid influences due to dietary changes, we chose to study patients under a standardized low-fat, low-cholesterol diet (step I diet recommended by the National Cholesterol Education Program), which represents the first therapeutic measure in the treatment of hypercholesterolemia.[21] Notably, in a study of lipoprotein kinetics in healthy men from Finland, shifting from a normal diet to a low-fat, low-cholesterol diet reduced cholesterol absorption and plasma cholesterol and increased cholesterogenesis and the LDL–apo B fractional catabolic rate. The largest increase in cholesterol synthesis was observed in $\epsilon 4$ carriers.[22] This result is in keeping with our own data in PH patients.

Subjects with PH are candidates for treatment with lipid-lowering agents such as HMG-CoA reductase inhibitors (or statins). A wide interindividual variation in treatment responses to statins has been observed in a number of studies.[23] Among several mechanisms possibly involved, differences in the cholesterol synthesis rate may have a role. The potential clinical relevance of the relationship between the apo E genotype and cholesterol synthesis rate rests on the possibility to predict a response to treatment with statins. In patients with heterozygous familial hypercholesterolemia (FH), lovastatin and simvastatin were less effective in patients with the E3/4 phenotype. However, this trend was significant only at certain time points or doses or in a given gender.[24-26] Studying 154 cases of PH, Ojala et al[27] described smaller reductions in LDL cholesterol in patients with the E3/4 phenotype compared with those with the E3/3 phenotype only on a 20-mg dose of lovastatin, an effect that vanished when the dose was doubled. Finally, a recent analysis of a large angiographic trial performed on 320 hypercholesterolemic subjects showed no differences in the percentage of the LDL cholesterol decrease after pravastatin administration in apo E3 and apo E4 subjects (-27% and -26%, respectively).[28]

In conclusion, our study provides preliminary evidence that PH patients carrying the $\epsilon 4$ allele have a higher rate of cholesterogenesis than those with the E3/3 genotype. We believe that assessing relationships between apo E genotype and cholesterogenesis may help to elucidate some regulatory mechanisms of cholesterol metabolism in PH. Furthermore, the higher cholesterogenesis in PH patients with the $\epsilon 4$ allele might partly explain the interindividual differences in response to treatment with cholesterol synthesis inhibitors such as statins.

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