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Letters

Reduced Lipoprotein(a) Associated With the Apolipoprotein E2 Genotype Confers Cardiovascular Protection in Familial Hypercholesterolemia

There are 3 isoforms of apolipoprotein E (apo E) in humans (ε2, ε3, and ε4). They differ by single amino acid substitutions that variably affect their affinity for the low-density lipoprotein receptor (LDLR) and for the LDLR-related protein (LRP1), with ε2 having the weakest binding to these receptors (1). The plasma levels of lipoprotein(a) [Lp(a)], a highly atherogenic LDL-like lipoprotein species, are influenced by the polymorphism of apo E, with ε2/ε2 and ε4/ε4 carriers presenting with the lowest and highest Lp(a) plasma concentrations, respectively (1).

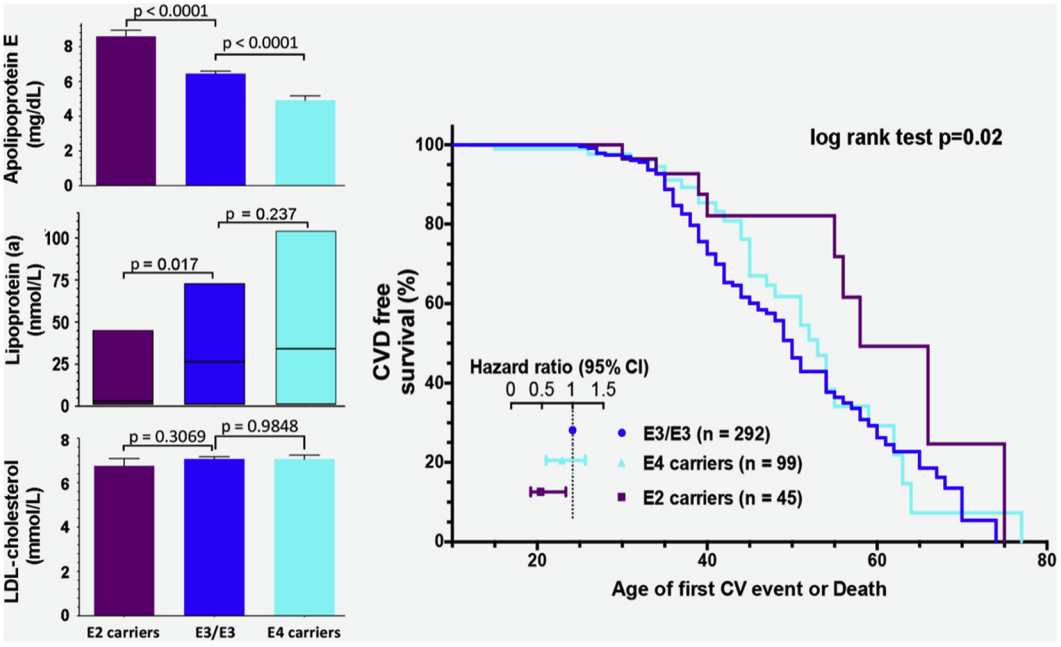
Lp(a) contains a highly polymorphic signature protein, apolipoprotein(a) [apo(a)], bound to the apolipoprotein B100 (apo B100) of an LDL particle. The proatherogenic properties of Lp(a) are mediated by its LDL moiety rich in cholesterol, as well as by its elevated content in proinflammatory oxidized phospholipids, and by the antifibrinolytic effects of its plasminogen-like apo(a) moiety. Elevated Lp(a) is an independent predictor of cardiovascular disease (CVD), particularly for patients with familial hypercholesterolemia (FH) (2,3).

To evaluate whether the modulation of Lp(a) levels by apo E isoforms affects CVD risk, we assessed apo E and apo(a) concentrations, as well as polymorphisms, in a cohort of patients at very high cardiovascular risk by liquid chromatography-tandem mass spectrometry (4). These parameters were measured in plasma samples collected at the first visit from 444 nontreated heterozygous patients with FH (all genetically confirmed carriers of a single mutated *LDLR* allele) attending the lipid clinic at Groote Schuur Hospital in Cape Town, South Africa (5). Their mean ± SD plasma levels of total cholesterol (8.9 ± 2.0 mmol/l), triglycerides (1.5 ± 0.9 mmol/l), high-density lipoprotein (HDL) cholesterol (1.3 ± 0.2 mmol/l), LDL cholesterol (7.0 ± 1.9 mmol/l), apo A-I (96 ± 28 mg/dl), apo B (155 ± 50 mg/dl), apo E (6.4 ± 2.8 mg/dl), and apo(a)/Lp(a) (52 ± 48 nmol/l) were typical of a heterozygous FH phenotype. One-third (32.9%) of these patients had experienced at least 1 cardiovascular event before the initial visit.

The majority of these patients were ε3/ε3 carriers (n = 292) and served as the control group carriers. The remaining patients were ε3/ε4 (n = 79), ε2/ε3 (n = 42), ε4/ε4 (n = 20), ε2/ε4 (n = 8), or ε2/ε2 (n = 3). The ε2/ε4 carriers were excluded from all subsequent analyses. Compared with ε3/ε3, ε2 carriers (i.e., ε2/ε3 and ε2/ε2) had increased plasma apo E levels, whereas ε4 carriers (i.e., ε3/ε4 and ε4/ε4) had reduced plasma apo E levels (**Figure 1**). LDL cholesterol levels were similar in all groups (**Figure 1**), as well as non-HDL cholesterol levels (not shown). In contrast, ε2 carriers had significantly reduced Lp(a) concentrations (median 3 nmol/l [interquartile range (IQR): 0 to 45 nmol/l]) compared with ε3/ε3 homozygotes (median 27 nmol/l [IQR: 0 to 73 nmol/l]). Lp(a) concentrations were slightly but not significantly increased in ε4 carriers (median 34 nmol/l [IQR: 0 to 104 nmol/l]) compared with the ε3/ε3 control group (**Figure 1**). The detection limit for apo(a) measurement by liquid chromatography-tandem mass spectrometry was 2 nmol/l (6). All patients with apo(a) levels lower than this limit were reported as having Lp(a) concentrations of 0 nmol/l. Thus, 48% of ε2 carriers, 28% of ε3/ε3 carriers, and 25% of ε4 carriers had Lp(a) levels lower than the detection limit. Given that Lp(a) levels are strongly influenced by the polymorphism of apo(a) resulting from the presence of a variable number of kringle IV_2 repeats in humans (1 to more than 40) (3), we verified that the number of kringle IV repeats on apo(a) was similar in ε3/ε3, ε2, and ε4 carriers, on average at a mean ± SD of 21.7 ± 7.2, 20.6 ± 8.6, and 20.4 ± 7.0 (p = 0.196), respectively, thus ruling out a chance finding.

These results confirm specifically in patients with FH a landmark observation made in the general population showing that apo E_2 is associated with reduced Lp(a) (1). Whether LDLR or LRP1 plays a role in that process is unknown. The mechanism by which apo E_2 specifically reduces Lp(a) clearly remains to be established. The percentage of patients with FH who had CVD was significantly less in ε2 carriers than

FIGURE 1 Apo E_2 Is Associated With Cardiovascular Protection in FH



Plasma apolipoprotein E (apo E) and apolipoprotein(a) levels were measured by liquid chromatography–tandem mass spectrometry in plasma samples isolated from 444 nontreated patients with familial hypercholesterolemia (FH). Plasma low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald formula when triglycerides levels were lower than 4.52 mmol/l (i.e., in 440 patients). Apolipoprotein E and low-density lipoprotein cholesterol are displayed as mean ± SEM, and comparisons between groups were made using Student's *t*-test. Lipoprotein(a) levels are displayed as median (interquartile range), and comparisons between groups were made using a Wilcoxon rank sum test. Cardiovascular disease (CVD)-free survivals were determined using a Kaplan-Meier estimate. Cumulative hazards for age (probability for a patient to experience a cardiovascular [CV] event or death) were compared using a log-rank test. CI = confidence interval.

in ε3/εε3 carriers (20% vs. 37%; p = 0.021) and intermediate in ε4 carriers (31%).

The reduced rate of CVD in ε2 carriers remained significant after adjustment for age, sex, tobacco use, body mass index, diabetes, HDL cholesterol, hypertension, and LDLR mutation status. We next performed a Kaplan-Meier estimate to determine the CVD-free survival time according to apo E genotypes (Figure 1). The CVD-free survival time was significantly longer in patients with FH carrying one ε2 allele compared with ε3/ε3 carriers (hazard ratio: 0.48 [95% confidence interval (CI): 0.32 to 0.89]; p = 0.02). In contrast, patients carrying one ε4 allele had similar CVD-free survival time as ε3/ε3 (hazard ratio: 0.84 [95% CI: 0.57 to 1.21]; p = 0.35). It is far-fetched to speculate that the apparent lack of increase in CVD in ε4 carriers may relate to the similar levels of Lp(a) observed in ε4 carriers and ε3/ε3 homozygotes in the present study.

These results are unique in that we investigated patients at very high cardiovascular risk, and none of these patients were taking lipid-lowering medication at the time of their first visit (5), we were able to demonstrate that apo E_2 confers a significant degree of cardioprotection. Unlike non-FH subjects, who have variable effects of apo E genotypes on LDL cholesterol (1), apo E_2 did not significantly reduce LDL in our FH cohort, thus indicating that the cardioprotection observed here stems, at least in part, from the lowering of Lp(a).

There are some limitations to our study, in particular the lack of data on the ethnic background of our patients. However, our results clearly underpin that Lp(a) should be measured systematically in all patients with FH, given that with progressively more effective pharmacological LDL reduction, Lp(a) becomes a strong predictor of CVD (3) and, as such, an important driver of residual CVD risk.

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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