

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 ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$). 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 $\epsilon 2$ 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 $\epsilon 2/\epsilon 2$ and $\epsilon 4/\epsilon 4$ carriers presenting with the lowest and highest Lp(a) plasma concentrations, respectively (1).

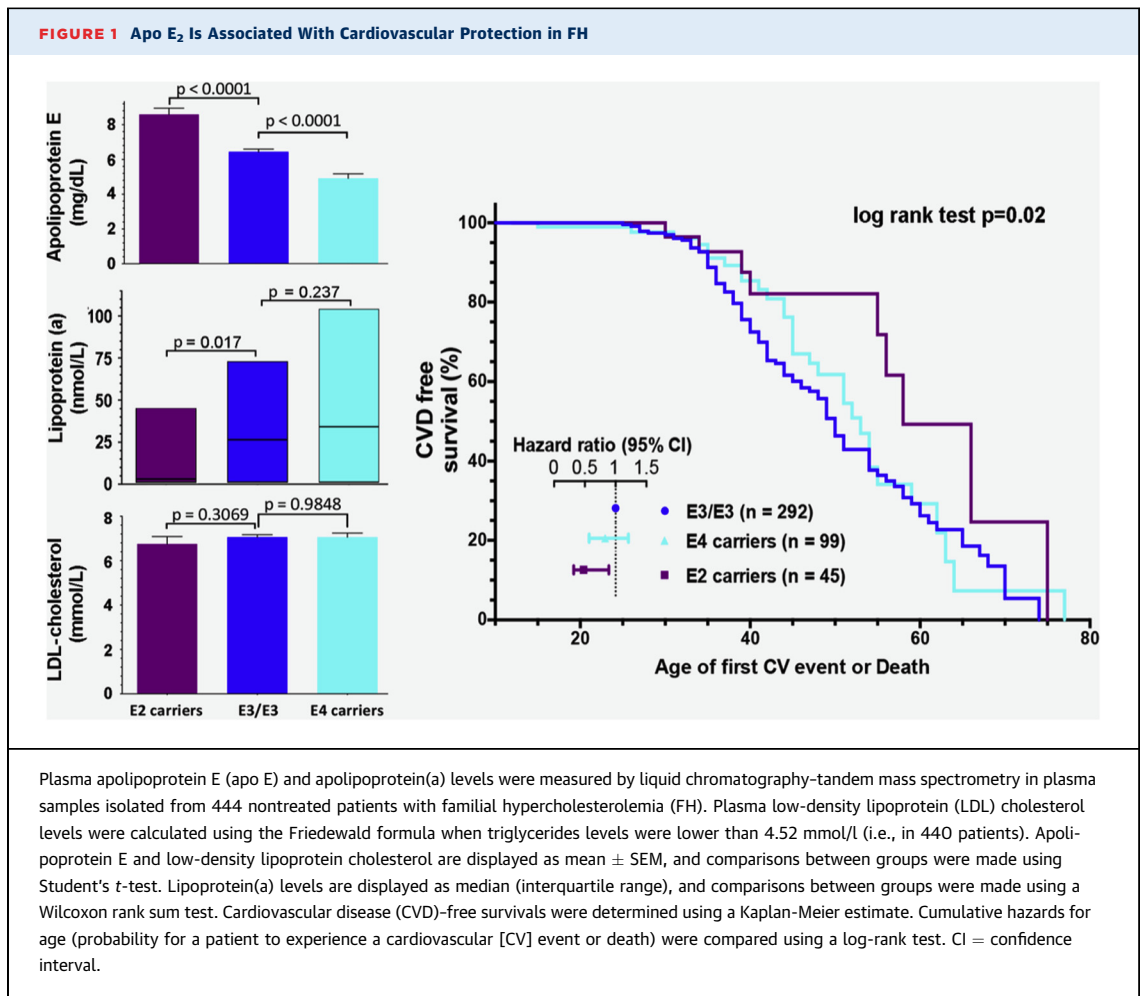
Lp(a) contains a highly polymorphic signature protein, apolipoprotein(a) [apo(a)], bound to the apolipoprotein B100 (apo B100) of an LDL particle. The proatherogenic properties of Lp(a) are mediated by its LDL moiety rich in cholesterol, as well as by its elevated content in proinflammatory oxidized 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 \pm 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 $\epsilon 3/\epsilon 3$ carriers ($n = 292$) and served as the control group carriers. The remaining patients were $\epsilon 3/\epsilon 4$ ($n = 79$), $\epsilon 2/\epsilon 3$ ($n = 42$), $\epsilon 4/\epsilon 4$ ($n = 20$), $\epsilon 2/\epsilon 4$ ($n = 8$), or $\epsilon 2/\epsilon 2$ ($n = 3$). The $\epsilon 2/\epsilon 4$ carriers were excluded from all subsequent analyses. Compared with $\epsilon 3/\epsilon 3$, $\epsilon 2$ carriers (i.e., $\epsilon 2/\epsilon 3$ and $\epsilon 2/\epsilon 2$) had increased plasma apo E levels, whereas $\epsilon 4$ carriers (i.e., $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$) 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, $\epsilon 2$ carriers had significantly reduced Lp(a) concentrations (median 3 nmol/l [interquartile range (IQR): 0 to 45 nmol/l]) compared with $\epsilon 3/\epsilon 3$ homozygotes (median 27 nmol/l [IQR: 0 to 73 nmol/l]). Lp(a) concentrations were slightly but not significantly increased in $\epsilon 4$ carriers (median 34 nmol/l [IQR: 0 to 104 nmol/l]) compared with the $\epsilon 3/\epsilon 3$ 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 $\epsilon 2$ carriers, 28% of $\epsilon 3/\epsilon 3$ carriers, and 25% of $\epsilon 4$ 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 IV₂ repeats in humans (1 to more than 40) (3), we verified that the number of kringle IV repeats on apo(a) was similar in $\epsilon 3/\epsilon 3$, $\epsilon 2$, and $\epsilon 4$ carriers, on average at a mean \pm 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 E₂ is associated with reduced Lp(a) (1). Whether LDLR or LRP1 plays a role in that process is unknown. The mechanism by which apo E₂ specifically reduces Lp(a) clearly remains to be established. The percentage of patients with FH who had CVD was significantly less in $\epsilon 2$ carriers than



in $\epsilon 3/\epsilon 3$ carriers (20% vs. 37%; $p = 0.021$) and intermediate in $\epsilon 4$ carriers (31%).

The reduced rate of CVD in $\epsilon 2$ 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 $\epsilon 2$ allele compared with $\epsilon 3/\epsilon 3$ carriers (hazard ratio: 0.48 [95% confidence interval (CI): 0.32 to 0.89]; $p = 0.02$). In contrast, patients carrying one $\epsilon 4$ allele had similar CVD-free survival time as $\epsilon 3/\epsilon 3$ (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 $\epsilon 4$ carriers may relate to the similar levels of Lp(a) observed in $\epsilon 4$ carriers and $\epsilon 3/\epsilon 3$ 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 E₂ confers a significant degree of cardioprotection. Unlike non-FH subjects, who have variable effects of apo E genotypes on LDL cholesterol (1), apo E₂ 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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