apoE4 allele and the natural history of cardiovascular risk factors

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Methods to analyze longitudinal changes in cardiovascular risk factors are performed over a median follow-up time of 7 yr (maximum 14.3 yr) for men. Longitudinal changes in these CV risk factors were analyzed by linear mixed-effects models. The prevalence of the apoE4 allele was 25.5%. apoE4 was independently associated with no change in those without apoE4 in the 6th age-decade over 10 yr. No significant effect of apoE4 on longitudinal changes in total or HDL-cholesterol, triglycerides, or blood pressures was observed. In conclusion, apoE4 influences fasting plasma glucose and its changes over time in men.

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Table 1. Characteristics of study population at baseline

<table>
<thead>
<tr>
<th></th>
<th>apoE4− (n = 228)</th>
<th>apoE4+ (n = 78)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>58±16</td>
<td>58±17</td>
<td>0.99</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.7±3.2</td>
<td>26.3±4.9</td>
<td>0.25</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>186±33.2</td>
<td>193±59.0</td>
<td>0.12</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>42.9±10.6</td>
<td>41.6±10.3</td>
<td>0.31</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dl</td>
<td>119.4±30.7</td>
<td>126.7±34.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>115.0±74.8</td>
<td>120.0±71.6</td>
<td>0.60</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>124.5±19.2</td>
<td>128.9±18.2</td>
<td>0.06</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>79.0±9.9</td>
<td>80.5±10.5</td>
<td>0.24</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>94.0±11.8</td>
<td>96.5±11.9</td>
<td>0.09</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>45.5±14.7</td>
<td>48.4±13.7</td>
<td>0.10</td>
</tr>
<tr>
<td>Fasting plasma glucose mg/dl</td>
<td>95.4±12.3</td>
<td>100.7±15.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. apoE4, apolipoprotein E4; BMI, body mass index; HDL and LDL, high- and low-density lipoprotein, respectively; SBP, DBP, and MBP, systolic, diastolic, and mean blood pressure, respectively; PP, pulse pressure.

BMI was determined as body weight (kg)/height squared (m²). Total and fractionated cholesterol, triglycerides, and plasma glucose were determined from a fasting blood sample drawn from the antecubital vein. The concentrations of plasma triglycerides and total cholesterol were determined by an enzymatic method (ABA-200 ATC Biochromatic Analyzer; Abbott Laboratories, Irving, TX). The concentration of HDL-cholesterol was determined by a dextran sulfate-magnesium precipitation procedure. The concentration of glucose was determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). LDL-cholesterol concentrations were estimated by the Friedewald formula. CV risk factors were measured at each visit. Thus repeated measurements of CV risk factors were available and were used for the purpose of the present study.

Statistical analysis. All analyses were performed with SAS statistical software (version 8.1). Continuous variables were expressed as means ± SD and categorical variables as proportions. An unpaired t-test was used for comparison between groups at first visit for continuous variables and the χ² test to compare categorical variables. Hardy-Weinberg equilibrium was tested by a χ² test.

Linear mixed-effects (3, 39) regression models were used to analyze the effects of apoE4 on the longitudinal changes in traditional CV risk factors. The mixed-effects regression model easily accommodates unbalanced, unequally spaced observations (15) and, consequently, is an ideal tool for analyzing longitudinal changes in BLSA data (34). Separate models were employed to determine the longitudinal changes of each CV risk factor.

In the mixed-effects model, the longitudinal changes in CV risk factors are represented by the follow-up time (Time and Time²) and its interactions. Cross-sectional differences across age are represented by terms involving age at first examination (FAge) and their interaction with other variables. The variables included in the models were FAge, Time, Time², apoE4, BMI, and the following interaction terms: FAge × Time, apoE4 × FAge, apoE4 × Time, and apoE4 × FAge × Time. For each variable, the mixed-effects model calculates a coefficient, which represents the covariate-adjusted contribution of this variable to the longitudinal changes in the CV risk factor (dependent variable). A significant interaction of apoE4 with Time indicates significant differences in the longitudinal changes of the specific CV risk factor between the apoE4 groups. A statistically significant interaction term between FAge, Time, and apoE4 for a given risk factor indicates that the changes in this risk factor over time differed by apoE4 status and that these changes varied according to age at first visit. To reduce the effect of the multicollinearity that is introduced into the model by the inclusion of higher-order interaction terms involving FAge and Time, these variables were centered at 63.3 and 4.8 yr, respectively.

Three random terms are included in the model: intercept, Time, and Time². These random effects allow each participant’s beginning value to vary from the population average (intercept) and the longitudinal trajectory to vary from the population average longitudinal trajectory for both the linear (Time), and quadratic (Time²) terms. These random effects are assumed to have an unstructured covariance matrix, and the errors are assumed to be independent with constant variance.

A number of methods were applied to assess whether a mixed-effects model was providing an adequate fit for these data. A plot of the standardized residuals vs. predicted values was constructed for each model, a plot of the semi-varogram of the standardized residuals was constructed to check whether the mixed-effects models adequately model the serial correlation in the data, and the correlations between the observed and predicted values were computed.

To test for secular drifts in the values of the traditional CV risk factors, subjects were divided into two groups based on the date of their first visit, and the effects of secular trends, apoE4, and

Table 2. Predictors of longitudinal changes in CV risk factors, as estimated from linear mixed-effects regression models

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol</th>
<th>HDL-Cholesterol</th>
<th>Triglycerides</th>
<th>SBP</th>
<th>DBP</th>
<th>PP</th>
<th>FBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>135.6</td>
<td>61.1</td>
<td>40.1</td>
<td>113.7</td>
<td>66.2</td>
<td>67.4</td>
<td></td>
</tr>
<tr>
<td>FAge</td>
<td>0.030 (0.124)</td>
<td>0.048 (0.037)</td>
<td>-0.499 (0.282)</td>
<td>0.621 (0.061)</td>
<td>0.041 (0.032)</td>
<td>0.571 (0.043)</td>
<td>0.049 (0.055)</td>
</tr>
<tr>
<td>Time</td>
<td>-1.244 (0.230)</td>
<td>-0.101 (0.064)</td>
<td>-1.080 (0.594)</td>
<td>1.780 (0.147)</td>
<td>0.453 (0.086)</td>
<td>1.216 (0.117)</td>
<td>-0.057 (0.191)</td>
</tr>
<tr>
<td>Time²</td>
<td>-0.063 (0.042)</td>
<td>0.035 (0.012)</td>
<td>-0.212 (0.091)</td>
<td>0.020 (0.023)</td>
<td>-0.010 (0.015)</td>
<td>0.014 (0.022)</td>
<td>-0.086 (0.033)</td>
</tr>
<tr>
<td>FAge × Time</td>
<td>-0.023 (0.014)</td>
<td>0.003 (0.004)</td>
<td>-0.041 (0.035)</td>
<td>0.036 (0.009)</td>
<td>-0.002 (0.005)</td>
<td>0.035 (0.007)</td>
<td>-0.014 (0.009)</td>
</tr>
<tr>
<td>BMI</td>
<td>1.806 (0.361)</td>
<td>-0.751 (0.106)</td>
<td>6.306 (0.824)</td>
<td>0.824 (0.180)</td>
<td>0.667 (0.101)</td>
<td>1.900 (0.134)</td>
<td>1.302 (0.053)</td>
</tr>
<tr>
<td>apoE4</td>
<td>4.994 (4.140)</td>
<td>-0.242 (1.232)</td>
<td>4.460 (4.974)</td>
<td>2.113 (2.078)</td>
<td>0.380 (1.098)</td>
<td>1.709 (1.494)</td>
<td>5.015 (1.921)</td>
</tr>
<tr>
<td>apoE4 × FAge</td>
<td>0.142 (0.236)</td>
<td>0.095 (0.070)</td>
<td>0.054 (0.541)</td>
<td>-0.030 (0.117)</td>
<td>0.010 (0.062)</td>
<td>-0.055 (0.084)</td>
<td>0.216 (0.108)</td>
</tr>
<tr>
<td>apoE4 × Time</td>
<td>-0.665 (0.496)</td>
<td>-0.060 (0.140)</td>
<td>0.005 (0.302)</td>
<td>-0.034 (0.180)</td>
<td>-0.051 (0.186)</td>
<td>0.005 (0.251)</td>
<td>0.812 (0.310)</td>
</tr>
<tr>
<td>apoE4 × FAge × Time</td>
<td>-0.026 (0.027)</td>
<td>-0.002 (0.006)</td>
<td>-0.063 (0.071)</td>
<td>0.001 (0.017)</td>
<td>-0.003 (0.010)</td>
<td>0.008 (0.014)</td>
<td>0.042 (0.017)</td>
</tr>
</tbody>
</table>

FAge, age at first visit; Time and Time², follow-up time; FBG, fasting blood glucose. Mathematical expressions of the mixed-effects model solution for each (CV) risk factor can be derived from the table. In these models, FAge is centered at 63.3 yr and Time is centered at 4.8 yr [by subtracting the means for FAge (63.3) and Time (4.8), respectively] to reduce the effect of the multicollinearity introduced to the model by including higher-order and interaction terms involving FAge and Time. For example, by subtracting the mean from terms such as FAge and Time, the new, transformed, centered variables will be less correlated with each other than the original, uncentered variables. The new transformed variables will also change the interpretation of the lower-order terms in the model (15, 39). For example, the estimate of the intercept or constant term in the model can be interpreted as the average of each CV risk factor’s value at the mean for the centered values and at a value of 0 for the uncentered variables. *P < 0.05; †P < 0.01; ‡P < 0.001.
their interaction were compared with analysis of covariance (ANCOVA).

RESULTS

The prevalence of apoE4 in the excluded subjects was 26.2%. The 306 remaining subjects formed the basis of this investigation. The prevalence of apoE4 was 25.5%. Genotype frequencies were in Hardy-Weinberg equilibrium.

At their first BLSA visit, men with and without apoE4 were of similar age and had similar DBP, MBP, PP, BMI, triglycerides, and total and HDL-cholesterol (Table 1). Men with apoE4 had higher values of SBP and LDL-cholesterol than those without apoE4, although the difference did not attain the threshold for statistical significance. Men with apoE4 showed, on average, 5 mg/dl higher fasting plasma glucose than those without apoE4 ($P < 0.05$).

The median follow-up time was 7 yr (maximum 14.3 yr). The mean number of follow-up measurements per subject was 4.8 ± 1.7 (median 5). Fifteen subjects (3.5%) had a single measurement.

**Association of apoE4 genotype with longitudinal changes in CV risk factors.** The main objective of the present study was to evaluate for differences in the longitudinal changes in CV risk factors among the apoE4 groups. This was performed with
mixed-effects linear regression models. The output of these models are mathematical equations, which can be constructed by using the coefficients in Table 2. A separate model was constructed for each CV risk factor, and the coefficients for each variable in these models are listed in Table 2. The plots of the standardized residuals vs. predicted values (not shown) do not suggest that there are any major problems with the mixed-effects models that we have fitted, although a few of the plots exhibit a slight increase in variation of the standardized residuals with increasing predicted value. This was not deemed to be of sufficient magnitude to require us to complicate the analyses further. The semi-variogram plot of the standardized residuals (not shown) all appear to fluctuate randomly around 1, suggesting that the covariance structure employed in the mixed-effects models is appropriately modeling the serial correlation in the data. The results are also illustrated graphically in Fig. 1, where the predicted longitudinal changes for the eight CV risk factors (Fig. 1, A–H) are shown for three starting ages and according to apoE4 status.

apoE4 and longitudinal changes in plasma lipids. Total cholesterol levels decreased over time and increased with increasing BMI (Fig. 1A and Table 2). Similar results were noted for LDL-cholesterol (Fig. 1B; not in Table 2). HDL-cholesterol levels varied inversely with BMI (Fig. 1C and Table 2) and did not show a linear trend over time. Triglyceride levels varied directly with BMI and decreased in a quadratic manner over time (Fig. 1D and Table 2).

Neither apoE4 nor any of the interaction terms involving apoE4 was significantly associated with any of the four lipid variables.

apoE4 and longitudinal changes in BP. Similarly, neither apoE4 nor any of the interaction terms involving apoE4 was significantly associated with any of the three BP variables. SBP and PP at the initial observation were higher at older age and increased with time, and this longitudinal trend was more pronounced the older the starting age. A higher SBP, but not PP, was associated with a higher BMI (Fig. 1, E and F, and Table 2). DBP increased with increasing BMI (Table 2), and there was a mild trend for DBP to increase over time (Fig. 1G).

apoE4 and longitudinal changes in fasting plasma glucose. The factors influencing fasting plasma glucose are shown in Table 2. Fasting plasma glucose was higher in those subjects with higher BMI and increased over time. As noted above, the presence of apoE4 was associated with a higher plasma glucose on the first BLSA visit (P < 0.05; Table 1). The statistically significant interaction term between FAge and apoE4 indicates that differences in plasma glucose by apoE4 status were larger in those subjects who entered the study at an older age. The statistically significant interaction term between Time and apoE4 suggests that the change in fasting plasma glucose over time was greater in subjects with apoE4. The statistically significant interaction term between FAge, Time, and apoE4 means that the change in fasting plasma glucose over time differed by apoE4 status and that the differences in fasting plasma glucose changes over time between the apoE4 groups differed by the starting age (greater with higher age) (Fig. 1H). Thus the effects of apoE4 appear to be both age and time dependent. Figure 2 illustrates the relative predicted changes over time, in fasting plasma glucose for three starting ages, by apoE4 status.

Because fasting glucose at baseline was slightly higher in men with apoE4, and because it is known that higher fasting glucose levels predict future increases in fasting glucose, an additional mixed-effects model that included fasting glucose at baseline as an independent variable was constructed. As expected, fasting glucose at baseline was a significant independent predictor of changes in fasting glucose over time. However, a significant interaction between apoE4, FAge, and Time indicated that the presence of apoE4 affects longitudinal changes in fasting glucose independently of the level of fasting glucose on the first visit.

Correlation between observed and predicted data. The correlations of observed values and predicted values (derived from the mixed-effects models) for total cholesterol, HDL-cholesterol, SBP, and fasting blood glucose were, respectively, 0.86, 0.92, 0.86, and 0.89. They were similar in those with and without apoE4. These results indicate a high predictive power for the final mixed-effects models.

Secular drift in traditional CV risk factors. To exclude the possibility that the observed differences in the changes in the CV risk factors might be due to secular drifts in the measurements that might have occurred over time, the study cohort was divided into two subgroups according to the date of their first visit.

A secular trend effect was observed for total and LDL-cholesterol (P < 0.01) and for SBP and DBP (P < 0.05 for both). No significant difference (P = 0.78) was observed for fasting plasma glucose. ANCOVA evaluating the effects of secular groups and apoE4 status showed that the interaction term between secular groups and apoE4 status was not significant for any of these risk factors. This suggests that secular trends did not interfere with the effects of apoE4 on the studied parameters in this population.

DISCUSSION

Several animal and human studies have provided evidence supporting the link between apoE and atherosclerosis (see Ref. 8 for review). apoE was first ascribed a role in receptor-mediated clearance of plasma lipoproteins and thus its role in the development of atherosclerosis was experimentally linked.
to hypercholesterolemia and hypertrygliceridemia (40). Subsequently, the association between apoE and atherosclerosis was found to be independent of lipoprotein changes (4, 19). Macrophages lacking apoE were found to contribute to atherosclerosis in the absence of significant changes in lipoproteins (31). Enhanced oxidative stress and LDL-oxidation in the context of apoE4 were also described (11, 17).

Because of the polymorphic nature of apoE in humans, its role in the development of atherosclerotic process is more complex than in animals. Several studies have documented a link between apoE4 and an increased risk of coronary disease (6, 12, 22, 33). Our recent study (33) showed that the increased risk of coronary events in subjects with apoE4 was independent of cholesterol levels. Thus interactions more complex than, and independent of, lipid levels are expected to occur between apoE and traditional CV risk factors.

The present study is the first to analyze the effects of apoE4 on the longitudinal changes in traditional CV risk factors in adults. We employed mixed-effects statistical models because they are the preferred method for analyzing repeated-measures data (15), especially datasets, such as the BLSA, that have unequally spaced and unequal number of repeated measurements for each individual. The strength of these models is that they have greater flexibility to model time effects, as they can properly handle missing data, and they have the structure necessary to account for the correlation between repeated measurements made on the same subject.

Our observation that the apoE4 allele was not associated with significantly higher levels of LDL-cholesterol in the BLSA cohort differs from older studies reporting an association between apoE4 and higher LDL-cholesterol both in normal subjects and in patients with coronary artery disease (22). However, two recent studies did not find differences in the levels of cholesterol between subjects with and without apoE4 (1, 29). Similarly, in an analysis of a larger BLSA sample, we did not find significant differences in lipid levels between these two groups (33).

The observation in the present study that apoE4 is associated with higher fasting glucose and more rapid change over time is novel. Recently, a study reported that plasma insulin (at baseline and 120 min after a glucose load) and glucose (120 min after a glucose load) were higher in apoE4 carriers than in non-apoE4 carriers (37). Similar findings were observed in subjects with Alzheimer’s disease (5). Conversely, no differences in the proportion of apoE alleles across increasing quintiles of insulin resistance were observed in the Framingham Offspring Study (27). Although the results of the latter study were based on a cross-sectional analysis, our longitudinal observations are consistent with the lack of a significant association between apoE4 and features of the insulin resistance syndrome with the exception of blood glucose levels in men.

We cannot exclude the possibility that the higher levels of fasting glucose at first visit observed in subjects with apoE4 are the result of an inherited genotype (apoE4?) over the life span rather than a sort of “selection bias” of our study population. Our longitudinal analysis, confirming the significant interaction between the presence of the apoE4 allele and a greater increase in fasting glucose over time, even after adjustment for baseline glucose levels, is in support of the first hypothesis.

It is conceivable that the higher risk for CV events in subjects with apoE4 may, in part, have been related to their slightly higher SBP and LDL-cholesterol at baseline. However, the trend and the slope of change in SBP and LDL-cholesterol were similar between subjects with and without the apoE4 allele. Furthermore, in a previous analysis involving a larger subgroup of the BLSA population, we found that the increased risk for CV events in subjects with the apoE4 allele was independent of any interaction between the apoE4 allele and LDL levels (33). Conversely, the elevation in fasting glucose levels over time observed in the present study may, in part, explain the increased risk for CV events associated with the apoE4 allele. A recent study showed a continuous positive association between fasting glucose and CV events (25). Thus a 1 mmol/l lower fasting glucose was associated with an ~20% decreased risk of coronary or cerebrovascular events (25). Elevated fasting glucose may increase the risk of CV events through vascular lesions (elevated carotid intima media thickness) (38) and/or through increased likelihood of developing type 2 diabetes (2).

Potential mechanisms that link apoE4 to increasing glucose levels remain speculative. Studies in patients with Alzheimer’s disease suggest that apoE4 may modulate the structure and conformation of β-amyloid (13, 42). Recently, attention has been called to the relation between amyloid within the islets of the pancreas and the progression from normal glucose levels to impaired fasting glucose and to diabetes mellitus (16, 21). The amyloid deposition within the pancreatic islets may thus represent the mechanism that links apoE4 to the progressive increases in fasting plasma glucose with age (time from birth) and with time (follow-up time from first BLSA visit).

This may be one of the mechanisms by which apoE4 is associated with atherosclerosis. The higher fasting glucose levels in apoE4 subjects may start a vicious cycle, i.e., apoE increases glucose levels, which in turn increase the glycation of apoE (23, 24). In addition, it has been shown that pancreatic islets isolated from transgenic mice producing human islet amyloid polypeptide quickly develop islet amyloid deposition when incubated with a high glucose concentration (9) and that the degree of amyloid deposition correlates positively with the ratio of serum glucose to serum insulin (20). We suggest that the expression of the apoE alleles may contribute to initiating and/or regulating the deposition of amyloid.

REFERENCES