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# Genetic Influences on Age-Related Change in Total Cholesterol, Low Density Lipoprotein-Cholesterol, and Triglyceride Levels: Longitudinal Apolipoprotein E Genotype Effects

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This study addressed the possible influence of apolipoprotein E (apo E) genotype on age-related changes in total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and triglyceride (TG) levels in older males. Apo E is a component of LDL, is a ligand for the LDL receptor, and apo E genotype has been consistently associated with variation in mean levels of TC and LDL-C, and also appears to influence TG levels. Using male twins followed longitudinally between mean ages of 48 and 63 years, the change in TC, LDL-C, and TG over time for individuals with the $\epsilon 3\epsilon 3$ and the $\epsilon 3\epsilon 4$ genotypes was contrasted. At exam 1 mean TC and LDL-C levels were lower in the $\epsilon 3\epsilon 3$ group than in the $\epsilon 3\epsilon 4$ group, but at exam 3 mean TC and LDL-C levels were significantly higher in the $\epsilon 3\epsilon 3$ group than in the $\epsilon 3\epsilon 4$ group. The rate of change in TC and LDL-C with age differed significantly between $\epsilon 3\epsilon 3$ and $\epsilon 3\epsilon 4$ groups. Results for TG were not statistically significantly. These findings suggest that the apo E genotype effects on risk of coronary artery disease may be age-dependent. This study demonstrates the value of longitudinal studies in refining models for genetic risk factors for disease. 

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## INTRODUCTION

Apolipoprotein E (apo E) is a component of several lipoprotein particle classes and a ligand for lipoprotein receptors. Its role in lipid metabolism as well as its potential contribution to risk of acquiring coronary artery disease (CAD) have been studied extensively [for reviews see Davignon et al., 1988; Mahley, 1988]. In this study we sought to expand the understanding of the effect of aging on the relationship between apo E genotype and total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and plasma triglycerides (TG), important risk factors for CAD.

TC, LDL-C and TG levels, which increase with age in younger adults, have been shown to decline in Caucasian males after age 60 years [NIH, 1982; Hershcopf et al., 1982; Alvarez et al., 1984]. Longitudinal data indicate that part of this decline is an aging effect within individuals which is not explained by changes in environmental correlates [Hershcopf et al., 1982; Newschaffler et al., 1992]. Nevertheless, TC continues to play a significant role in health after the age of 60 years [Metter et al., 1992].

The apo E gene locus on chromosome 19 has three common alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, the protein products of which differ by a single amino acid [Zannis et al., 1981; Cumming and Robertson, 1982]. In samples from a variety of populations, individuals with genotypes containing the $\epsilon 2$ allele tend to have decreased TC and LDL-C levels relative to subjects with the $\epsilon 3\epsilon 3$ genotype. In contrast, $\epsilon 4$-containing genotypes have been associated with increases in these levels [Sing and Davignon, 1985; Utermann et al., 1977, 1984; Wardell et al., 1982; Robertson and Cumming, 1985; Davignon et al., 1988]. The apo E polymorphism has been estimated to be responsible for 4–8% of the variation in age- and sex-adjusted TC and LDL-C levels in the general Caucasian population [Sing and Davignon, 1985; Boerwinkle and Sing, 1987]. The $\epsilon 4$ allele, carried by approximately 23% of Caucasians, has been reported to have an average effect of increasing TC 5–8 mg/dl and LDL-C 7 mg/dl; the $\epsilon 2$ allele has been associated with an average effect of decreasing TC and LDL-C 11–14 mg/dl [Sing and Davignon, 1985]. Meta-analysis [Dallongeville et al., 1992] suggests that both the $\epsilon 2$ and the $\epsilon 4$ alleles may be associated with increases in plasma TG levels, relative to the $\epsilon 3$ allele. Apo E molecules derived from the three alleles vary in their in vitro receptor-binding ability [Mahley, 1983], in their catabolic rates [Demant et al., 1991; Gregg et al., 1986], and in the plasma concentration of apo E protein present in vivo [Utermann, 1985]. The apo E polymorphism effects on lipids and lipoproteins may be mediated by some or all of these differences.

The $\epsilon 4$ allele was found to be associated with earlier age of myocardial infarction (MI) in studies of MI survivors [Cumming and Robertson, 1984; Lenzen et al., 1986] and with angiographic evidence of CAD [Kussi et al., 1989]. Apo E genotype distribution has been found to differ between MI survivors and controls [Cumming and Robertson, 1984]. However, other studies have failed to find an association with presence or severity of CAD [Menzel et al., 1983; Reardon et al., 1985].

Little is known about the effects of variation in apo E genotype on lipid risk factors for CAD in older populations or on rate of change of these risk factors in aging populations. Davignon et al. [1987] did not find apo E polymorphism effects

on TC and LDL-C in octogenarians. The purpose of this study was to examine the effects of genetic variation in apo E on age-related changes in levels of TC and LDL-C using longitudinal data.

## SUBJECTS AND METHODS

### Subjects

The study subjects were participants in the National Heart, Lung, and Blood Institute (NHLBI) Twin Study [Feinleib et al., 1977; Selby et al., 1991], composed of Caucasian male twin pairs, born between 1917 and 1927, all of whom served in the U.S. military. Only the dizygotic (DZ) pairs were considered in the analyses described here. Of 560 DZ pairs originally solicited, 260 participated in clinical examinations between 1969 and 1973, and 183 pairs returned for a second examination between 1980 and 1981. A third examination of 129 of the original 260 pairs was conducted between 1986 and 1987. Of the 260 DZ pairs initially examined, 58 individuals in 55 pairs died between the first and third examinations [Reed et al., 1991].

From all pairs evaluated at the third examination, 100 DZ twin pairs were selected without knowledge of lipid levels or CAD. Apo E genotyping was completed in 197 of the 200 subjects. Eight individuals were excluded: five were under treatment for non-insulin-dependent diabetes and three were treated with lipid-lowering medications at any of the three examinations. Mean ages were 48 years at exam 1, 58 years at exam 2, and 63 years at exam 3. At the third exam, ages ranged from 59 to 70 years.

A subsample of unrelated individuals who attended all three exams was constructed. This assured the independent observations assumed for the statistical tests. Due to small sample sizes in each of the six genotypes, the more frequent $\epsilon 3\epsilon 3$ and $\epsilon 3\epsilon 4$ genotypes were chosen for contrasts. All unrelated $\epsilon 3\epsilon 4$ individuals were included, followed by inclusion of $\epsilon 3\epsilon 3$ individuals who were unrelated both to each other and to the $\epsilon 3\epsilon 4$ individuals. When a pair of twins had the same genotype, the included twin was selected randomly.

### Laboratory Methods

TC was measured by the method of Allain et al. [1974]. LDL-C was estimated, by the equation of Friedewald et al. [1972], only if the measured TGs [Sampson et al., 1985] were less than 400 mg/dl [Warnick et al., 1990]. Apo E genotyping was performed as described by Hixson and Vernier [1990]. Genomic DNA was used as template to polymerase chain reaction (PCR) amplify a 244 bp DNA fragment which contains the variant amino acid residues 112 and 158. The PCR products were digested with the restriction enzyme HhaI and then electrophoresed on an 8% polyacrylamide non-denaturing gel. The genotype was determined from the pattern of restriction fragments.

### Statistical Analyses

Preliminary analyses were performed to describe the sample and to compare it with previous reports. Chi-square goodness-of-fit tests were undertaken to determine whether the number of individuals with each of the six possible genotypes were in proportions consistent with population expectations. In the unrelated subset, repeated

measures analysis of variance (ANOVA) was used to test the hypothesis that the change in TC, LDL-C, or TG levels with age (at exams 1–3) differed between the $\epsilon 3\epsilon 3$ and the $\epsilon 3\epsilon 4$ genotypes. The differences in TC, LDL-C, or TG between exam 1 and exam 3 (level at exam 3 minus level at exam 1) were also contrasted between the $\epsilon 3\epsilon 3$ and $\epsilon 3\epsilon 4$ genotypes using one-way ANOVA.

ANOVA was used to test whether $\epsilon 3\epsilon 3$ vs. $\epsilon 3\epsilon 4$ groups differed in their distribution of TC, LDL-C, or TG within exams 1–3. Because of statistically significant skewness in the distribution of TC, LDL-C, and TG at one or more exams, apo E genotype effects on ln(TC), ln(LDL-C), and ln(TG) were assessed using the subsample of unrelated males. Outliers with TG greater than 500 mg/dl were excluded from all TG analyses. All statistical analyses used the SPSS statistical package [SPSS, 1991].

## RESULTS

The study sample was examined for comparability with lipid levels described in the general population. Table I shows the age-specific estimated mean levels of TC, LDL-C, and TG for Caucasian males from the Lipid Research Clinics Prevalence study [NIH, 1980]; the entire sample of NHLBI twins at each visit; and the sample of 197 individuals for whom apo E genotypes were completed. The TC and LDL-C levels seen in the subset genotyped are similar to those in the general population as well as to those in the larger NHLBI twin sample. Apo E allele frequencies for the 197 individuals were: $f(\epsilon 2) = 0.091$, $f(\epsilon 3) = 0.756$, and $f(\epsilon 4) = 0.152$. These frequencies are not significantly different from those averaged from Caucasian populations: $f(\epsilon 2) = 0.085$ , $f(\epsilon 2) = 0.768$, and $f(\epsilon 4) = 0.147$ [Sing and Davignon, 1985].

As shown in Table II, the mean levels of TC and LDL-C were higher in the $\epsilon 3\epsilon 4$ group than in the $\epsilon 3\epsilon 3$ group at exam 1 and lower at exams 2 and 3. The within

**TABLE I. Mean Age and Lipid Levels (mg/dl) in the (LRC) Sample, the Entire NHLBI Sample, and the NHLBI Subsample in this Study**

| | LRC | All NHLBI twins | This study: all with apo E genotyped |
|---|---|---|---|
| Exam 1 (1969–1973) | | | |
| Age, years (*n*) | 45–49 (327) | 47.9 (1,028) | 48.1 (197) |
| TC | 213.4 | 220.3 (1,023) | 222.5 (195) |
| LDL-C | 143.9 | 143.6 (1,010) | 143.0 (192) |
| TG | 143.4 | 133.1 (1,017) | 137.7 (195) |
| Exam 2 (1980–1981) | | | |
| Age years (*n*) | 55–59 (261) | 57.6 (784) | 57.6 (184) |
| TC | 215.0 | 211.6 (785) | 214.1 (184) |
| LDL-C | 145.8 | 135.1 (764) | 136.0 (174) |
| TG | 134.3 | 161.2 (785) | 168.7 (184) |
| Exam 3 (1986–1987) | | | |
| Age years (*n*) | 60–64 (131) | 63.2 (622) | 63.3 (197) |
| TC | 216.6 | 220.2 (584) | 221.6 (185) |
| LDL-C | 150.4 | 148.2 (550) | 148.0 (168) |
| TG | 130.6 | 147.3 (584) | 161.7 (185) |

TABLE II. Mean ± SD TC, LDL-C, and TG Levels (mg/dl) at Each Exam Stratified by Genotype

| Genotype | N | Exam 1 (mean age 48 years) | Exam 2 (mean age 58 years) | Exam 3 (mean age 63 years) |
|---|---|---|---|---|
| TC | | | | |
| ε3ε3 | 52 | 219 ± 47 | 215 ± 40 | 228 ± 43 |
| ε3ε4 | 25 | 232 ± 29 | 207 ± 39 | 205 ± 33 |
| LDL-C | | | | |
| ε3ε3 | 49 | 151 ± 42 | 141 ± 40 | 154 ± 38 |
| ε3ε4 | 23 | 158 ± 36 | 134 ± 31 | 136 ± 28 |
| TG | | | | |
| ε3ε3 | 49 | 100 ± 52 | 141 ± 70 | 124 ± 73 |
| ε3ε4 | 24 | 124 ± 83 | 151 ± 85 | 122 ± 77 |

exam genotype effects on TC and LDL-C differences are not statistically significant at exams 1 and 2, but at exam 3 the one-sided test that the ε3ε4 mean is greater than or equal to the ε3ε3 mean is rejected for ln(TC) ($P = 0.01$) and ln(LDL-C) ($P = 0.25$). TC and LDL-C are highly correlated with coefficients of 0.88, 0.91, and 0.94 at exams 1–3, respectively. TG levels do not significantly differ between genotypes within each exam, although the higher variance of TG levels results in decreased power to test for differences.

Repeated measures ANOVA detects a significant interaction between age and apo E genotype effects for both TC ($P < 0.001$) and LDL-C ($P = 0.012$). Interaction is not statistically significant for TG ($P = 0.058$), however, this test statistic is marginal, suggesting that the effect may have been significant in a larger sample. The magnitude of changes in TC (diff-TC) and LDL-C (diff-LDL-C) between exams 1 and 3 was dependent on apo E genotype (Fig. 1). Mean diff-TC was 7 mg/dl and diff-LDL-C was 2 mg/dl in the ε3ε3 group; mean diff-TC was −32 mg/dl and diff-LDL-C was −25 mg/dl in the ε3ε4 group. Mean diff-TG was 17 mg/dl in the ε3ε3 group and −36 mg/dl in the ε3ε4 group (Fig. 2). Consistent with the detection of interaction in the repeated measures analysis, the hypotheses that the TC and LDL-C differences were the same in the ε3ε3 and the ε3ε4 genotype groups were rejected both for TC ($P = 0.001$) and for diff-LDL-C ($P = 0.17$), but not for diff-TG ($P = 0.10$). Diff-TC and diff-LDL-C were highly correlated, with a correlation coefficient of 0.86 ($P = 0.001$). Because age distributions at each exam did not differ between the ε3ε3 (mean age 48.1 years at exam 1) and ε3ε4 (mean age 48.3 years) groups, age did not confound these results.

## DISCUSSION

The results of this study support the reversal of the increase in TC and LDL-C associated with the ε3ε4 genotype as the males in this sample aged. A similar decline in TC with age ε3ε4, but not ε3ε3, genotype males aged 53–94 years was seen in a cross-sectional study [Jarvik et al., 1994]. Because 23% of Caucasians are predicted to be ε3ε4, a significant portion of the population may be expected to show this decline.

Absence of the expected apo E genotype effects on TC and LDL-C has been reported in octogenarians [Davignon et al., 1987] and in a sample with mean age

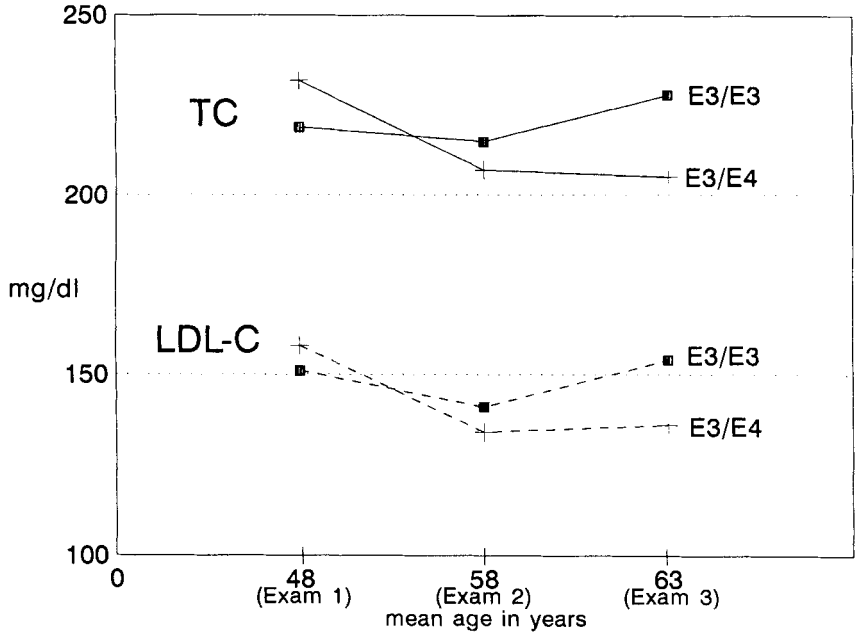


Fig. 1. Longitudinal change in sample mean TC and LDL-C levels for the $\epsilon 3\epsilon 3$ and $\epsilon 3\epsilon 4$ apo E genotype groups.

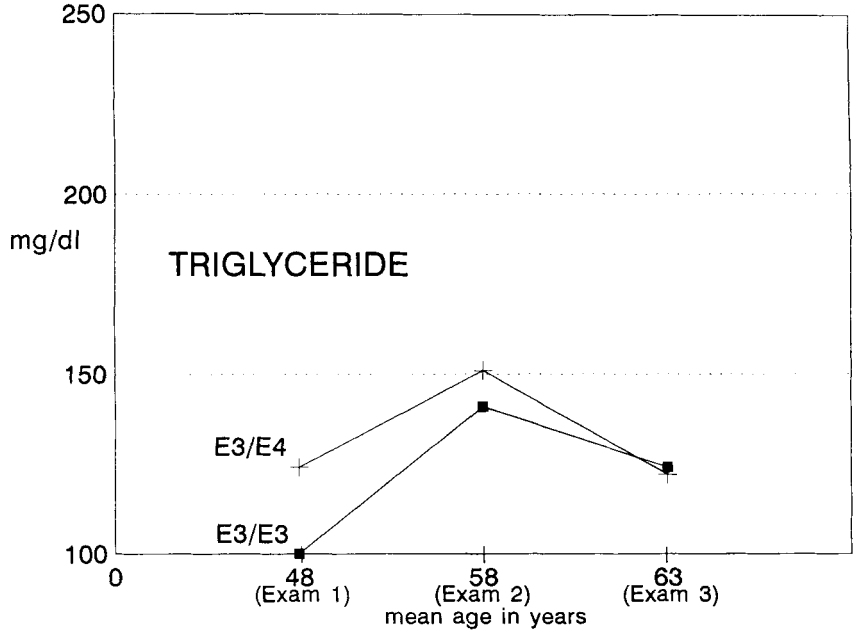


Fig. 2. Longitudinal change in sample mean plasma TG level for the $\epsilon 3\epsilon 3$ and $\epsilon 3\epsilon 4$ apo E genotype groups.

79 years [Jarvik et al., 1994]. Davignon et al. [1987] suggested such results may be due to differential survival—that those individuals with the $\epsilon 4$ allele and increased cholesterol levels had died before the age of 80 years. Differential survival implies a decrease in the relative frequency of the $\epsilon 4$ allele in the elderly. A significant deficit of females, but not males, carrying the $\epsilon 4$ allele was reported by Davignon et al. [1987]. Jarvik et al. [1994] noted a cross-sectional decline in $\epsilon 4$ frequency with increasing age. Decreased $\epsilon 4$ allele frequencies in 65–90-year-old females [Cauley et al., 1993] and in centenarians [Schächter et al., 1994] support differential survival. However, the average effects of the apo E alleles on TC and LDL-C did not differ between the younger and older women [Cauley et al., 1993], as would be expected if their differential survival were based on elevated lipid levels. Differential survival based on apo E genotype may not be solely due to the apo E genotype effects on lipids and lipoproteins. The $\epsilon 4$ allele has been associated with late-onset Alzheimer's disease (AD) [Corder et al, 1993; Yu et al., 1994]. The reduced frequency of the $\epsilon 4$ allele seen in healthy elderly individuals may be due, in part, to development of AD in individuals carrying an $\epsilon 4$ allele.

The age-related change in TC and LDL-C levels associated with the $\epsilon 4$ allele at the third exam cannot be ascribed to such a differential survival effect, since the same individuals were examined over a 15 year period. It is unlikely these results are due to an ascertainment bias, since the mean TC and LDL-C levels in this sample appear typical of population estimates for their age group and the relative frequency of the $\epsilon 4$ allele is similar to that expected. Additionally, the males in the current study are younger at their last exam than the subjects for whom a differential survival effect is suggested [Davignon et al., 1987; Cauley et al., 1993; Schächter et al., 1994, Jarvik et al., 1994].

A previous longitudinal study of apo E genotype effects over 5 years in a sample of mean and women aged 24–67 years did not detect differences in the change in TC with age [Gueguen et al., 1989]. Genotype-dependent differences in the change in TG with weight gain were found. Differences in the length of follow-up and the age and gender distributions of the samples may account for the discordant results. Genetic influences on the reduction of TC and LDL-C in older age would be consistent with the reported inability to ascribe this reduction to changes in weight, diet, or exercise [Hershcopf et al., 1982].

How apo E genotype effects on cholesterol levels might change over time is unclear. As discussed above, the protein products vary in their receptor-binding ability, catabolism, and plasma concentrations. Age-related changes in receptor levels or activity, as reported for postmenopausal women by Arca et al. [1994], or in rates of catabolism or protein synthesis may differentially influence the lipid levels of individuals with different genotypes. If apo E has a role in the pathogenesis of AD, a differential aging effect of the apo E polymorphism may play a role in the expression of the disease. One study found the association of apo E genotype and AD to be dependent on TC levels [Jarvik et al., 1994].

An alternative to a true aging effect is the possibility that the observed decrease in TC and LDL-C in the $\epsilon 3\epsilon 4$, but not the $\epsilon 3\epsilon 3$, genotype represents a genotype-specific response to a general change in the environment over the course of the study. Apo E genotype-dependent changes in lipids in response to dietary changes have been described [Lehtimaki et al., 1992; Jenkins et al., 1993; Uusitupa et al., 1992].

However, in the absence of any decline in the mean TC and LDL-C levels of the $\epsilon3\epsilon3$ group, dietary changes are unlikely to account for the marked decline in the $\epsilon3\epsilon4$ group.

Despite the strong statistical evidence found for the TC and LDL-C effects in this small sample, confirmation of these longitudinal results in an independent sample is desirable, particularly in a sample with sufficient power to test the TG effects and to allow investigation of the $\epsilon2$ allele longitudinal effects. In a cross-sectional study of younger individuals, Reilly et al. [1992] suggest that the regression of TC on age differs between males with the $\epsilon2\epsilon3$ or $\epsilon3\epsilon3$ genotypes.

If the association of the $\epsilon4$ allele with elevations in TC and LDL-C diminishes with age, the impact of the previously reported apo E effects on TC and LDL-C and, thus, on health may actually be limited to younger age groups, at least in males. The results of this study suggest that possible genotype by age interactions should be considered when evaluating the impact of variation in the common alleles at the apo E structural locus on CAD or its risk factors, particularly given the high frequency of the alternative alleles. Changes in the effects of the $\epsilon3\epsilon4$ genotype on TC and LDL-C with age could be one of the confounding factors explaining the discrepancies in studies of the effects of apo E genotype on CAD [Cumming and Robertson, 1984; Lenzen et al., 1986; Kussi et al., 1989; Menzel et al., 1983; Reardon et al., 1985].

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