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The relation of apolipoprotein E polymorphism to multiple cardiovascular risk in children: the Bogalusa Heart Study

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Abstract

Apolipoprotein (apo) E is an important genetic determinant of serum lipoprotein concentrations and coronary artery disease risk. Multiple cardiovascular risk factors in addition to lipoproteins were examined by apoE phenotype in a random subsample (n = 746) of 8–17-year old children from a total community. The apoE2 group (n = 58) carrying E2/2 and E3/2 phenotypes showed lower age-, race- and sex-adjusted mean values of body mass index (BMI: weight/height^2), percent body fat, fasting plasma insulin and LDL cholesterol, and a higher value of HDL cholesterol than the apoE3 group (n = 476) carrying the E3/3 phenotype ($P < 0.01$). In contrast, the apoE4 group (n = 212) carrying E4/4 and E3/4 phenotypes displayed higher values of total cholesterol and LDL cholesterol ($P < 0.01$). Both insulin and BMI, which correlated with each other, showed an association to triglycerides and systolic blood pressure in all three phenotype groups; whereas only BMI associated with LDL cholesterol, total cholesterol to HDL cholesterol ratio and diastolic blood pressure in all three phenotype groups ($P < 0.05$ to $P < 0.0001$). A marked increase in the prevalence of clustering of adverse (top tertile) total cholesterol to HDL cholesterol ratio with increased levels (top tertile) of one or two risk factors (BMI, insulin, and systolic blood pressure) occurred in the apoE3 and apoE4 groups, especially in the latter ($P < 0.01$ to $P < 0.0001$), but not in the apoE2 group. The prevalence of parental history of heart attack and diabetes mellitus among the three phenotype groups paralleled this trend. Thus, the risk status of apoE polymorphism may be associated with a constellation of cardiovascular risk factors in early life.

Keywords: ApoE polymorphism; Cardiovascular risk factors; Children; Obesity; Insulin

1. Introduction

Apolipoprotein E (apoE), in tandem with apoB, plays an important physiologic role in the

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regulation of overall cholesterol homeostasis and concentration of lipoproteins in serum [1,2]. In humans, the structural gene locus for apoE is polymorphic [3,4]. Three common alleles, e2, e3 and e4, determine the six apoE phenotypes E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4. Compared with the E3/3 phenotype, phenotypes E3/2 and E2/2 are associated with lower and E4/4 and E4/3 with higher serum total cholesterol, low-density lipoprotein (LDL) cholesterol, and apoB concentrations [5–8]. Isoforms of apoE and related phenotypes differ in terms of their influence on cell surface receptor (apoE and apoB/E receptors) binding and catabolism of apoB-containing lipoproteins, and intestinal absorption of dietary cholesterol, mechanisms that likely govern variations in serum lipoprotein concentrations [9–13].

Recently, the relationship between the polymorphism at the apoE gene locus and coronary artery disease (CAD) and other atherosclerotic vascular diseases has received a great deal of attention based on the differential impact of these genetic variants on LDL concentrations [14–19]. However, results from recent studies have suggested that apoE genotypic effects on coronary artery disease may not be mediated entirely by changes in atherogenic lipoprotein concentrations [20–22]. Furthermore, evidence has been provided prospectively that allelic variation in the gene coding apoE may act as a predictor of death from coronary heart disease [23]. Since atherosclerosis is a multifaceted disorder influenced by a constellation of interrelated risk factors, the question arises as to whether apoE polymorphism affects other processes or risk factors related to cardiovascular disease.

Population-based studies, including ours, indicate that the effect of allelic variation at the apoE gene locus of serum lipoprotein concentration seen in adults is already apparent in childhood [24–26]. The present study examines the relation of apoE polymorphism to multiple cardiovascular risk in children sampled from a total community. Besides lipoproteins, the risk factor variables include insulin, adiposity, blood pressure, and parental history of heart attack and diabetes mellitus.

2. Methods

2.1. Population

The Bogalusa Heart Study is a long-term epidemiologic study of cardiovascular risk factors from birth through young adulthood in the biracial community (65% white and 35% black) of Bogalusa, Louisiana. The study population was derived from a cross-sectional survey of children (n = 2559) aged 8–17 years, representing 85% of all eligible individuals. A random subsample of children (n = 892) was selected for apoE phenotyping. Fifty percent of blacks were randomly selected within each age and sex group, and a random sample of white children was then selected by age and sex to provide equal age-race-sex distribution.

2.2. General examinations

Subjects were instructed to fast 12 h prior to venipuncture, and compliance was determined by interview on the morning of the examination.

Replicate measurements of height, weight and skinfold thickness (triceps and subscapular) were obtained, and mean values used in analyses. The body mass index (BMI), defined as body weight in kilograms divided by squared height in metres, was used as a measure of overall adiposity. The percent body fat was calculated from the race- and sex-specific regression equation involving age and the sum of triceps and subscapular skinfolds, derived from body density data on children and adolescents [27]. Replicate systolic and diastolic blood pressure levels were recorded as the first and fourth Korotkoff phases, respectively. The mean of six readings was used in the analyses.

Parental history of heart attack and diabetes mellitus was obtained through questionnaires. Verification of parental histories were not performed.

2.3. Laboratory analyses

Cholesterol and triglyceride concentrations were measured with a Technicon AutoAnalyzer II (Technicon Instrument, Tarrytown, NY) accord-

ing to protocols developed by the Lipid Research Clinics Program [28]. Serum concentrations of very-low-density lipoprotein (VLDL) cholesterol, LDL cholesterol, and high-density lipoprotein (HDL) cholesterol were determined by a combination of heparin-calcium precipitation and agar-agarose gel electrophoresis [29]. The laboratory is monitored for quality control by a surveillance program of the Centers for Disease Control and Prevention in Atlanta, GA.

ApoE phenotyping was performed directly in serum using a modification [24] of the method of Havekes et al. [30], which is based on isoelectric focusing of delipidated serum followed by immunoblotting using rabbit antihuman apoE antiserum. Serum samples that were kept frozen at −70°C after lipid and lipoprotein analyses were sent to Helsinki, Finland (Dr. Ehnholm) for apoE phenotyping.

Plasma immunoreactive insulin was measured by a commercial radioimmunoassay kit (Phadebas, Pharmacia Biotech Inc., Piscataway, NJ). Plasma glucose was determined by a glucose oxidase method using the Beckman Instant Glucose Analyzer (Beckman Instruments, Palo Alto, CA).

2.4. Statistical analysis

Individuals were excluded from data analyses concerning physiologic variables on the basis of non-fasting status ($n = 104$), apoE4/2 phenotype ($n = 22$), and any missing data on phenotype ($n = 5$), physiologic variables ($n = 24$), and anthropometric measurements ($n = 6$). In addition, individuals ($n = 11$) whose reported fasting insulin or glucose values were above the 99th percentile of the distribution were considered outliers or potentially non-fasting and excluded from data analyses. The remaining children were categorized into the following three phenotype groups because homozygous phenotypes E2/2 ($n = 5$) and E4/4 ($n = 22$) comprised of too few individuals: (1) apoE2 group ($n = 58$) carrying the E2/2 and E3/2 phenotypes, (2) apoE3 group ($n = 476$) carrying the E3/3 phenotype, and (3) apoE4 group ($n = 212$) carrying the E4/3 and E4/4 phenotypes. Individuals with the E4/2 phenotype were not included because they carry an allele that is common to either the apoE2 group or apoE4 group.

Statistical analysis was performed using the SAS software package [31]. Insulin values were logarithmically transformed due to a highly skewed distribution. An analysis of covariance was used to compare the mean values of body fatness measures and physiologic measures among the three phenotype groups; race, sex, age, age^2, and age^3 were included as covariates. Post hoc comparisons of study variables among the phenotype groups were determined by repeated t-tests with a selected significance level of $P < 0.01$ to compensate for inflated error rate. The prevalence of parental history of cardiovascular disease among children in different apoE phenotype groups was compared by a chi-square test.

The association of BMI and insulin with physiologic variables was examined by Spearman correlation coefficients, before and after controlling for age, race and sex. To determine the clustering of multiple cardiovascular risk factors among children in different apoE phenotype groups, age-, race- and sex-specific tertiles of the total sample were obtained for total cholesterol to HDL cholesterol ratio, insulin, systolic blood pressure and BMI. The prevalence of clustering was calculated as the percentage of participants in the top tertiles of selected variables. A z-test was used to compare the observed with the expected prevalence of clustering under the null hypothesis that no associations existed between the selected variables [32].

3. Results

The distribution of apoE phenotype and allele frequencies as well as serum lipid and lipoprotein concentrations of six phenotypes in this population of children have been previously described in depth [25]. Briefly, as in other populations [5,33], the e3 allele was most common in all the four race-sex groups. The allele distribution showed a significant race difference with blacks having a lower frequency of the e3 allele and higher frequencies of both e2 and e4 alleles than whites (data not shown).

Table 1
Serum levels (mean ± S.E.) of lipoprotein variables in children by ApoE phenotype group

Variable†	ApoE phenotype group		
	E2 (n = 58)	E3 (n = 476)	E4 (n = 212)
Cholesterol (mg/dl)			
Total*	161.2 ± 3.7	164.2 ± 1.3	172.4 ± 1.9[a]
VLDL	8.1 ± 0.9	8.7 ± 0.3	9.0 ± 0.5
LDL*	79.4 ± 2.9[a]	89.7 ± 1.0	99.2 ± 1.5[a]
HDL*	73.7 ± 2.6[a]	65.8 ± 0.9	63.8 ± 1.4
Triglycerides (mg/dl)	66.6 ± 4.2	65.3 ± 1.5	68.3 ± 2.2

†Age-, race- and sex-adjusted values.
*Variation among phenotype groups, $P < 0.001$.
[a]Different from E3 group, $P < 0.01$.

Covariates (age-, race- and sex-) adjusted mean concentrations of serum lipoprotein variables are given in Table 1 for each of the three apoE phenotype groups. Total cholesterol, LDL cholesterol and HDL cholesterol values varied among the three phenotype groups ($P < 0.001$). The apoE2 group had lower values of LDL cholesterol and higher values of HDL cholesterol when compared with the apoE3 group ($P < 0.01$). On the other hand, the apoE4 group displayed higher values of total cholesterol and LDL cholesterol when compared with the apoE3 group ($P < 0.01$).

Age-, race-, and sex-adjusted mean values of fasting plasma insulin, percent body fat and BMI are displayed in Fig. 1 according to apoE phenotype group. Mean values of all three variables differed among the apoE phenotype groups ($P < 0.01$). Compared to the apoE3 group, the apoE2 group showed consistently lower values for insulin, percent body fat, and BMI ($P < 0.01$). Mean values of subscapular skinfold (E2:9.9 mm, E3:12.4 mm, and E4:11.2 mm) and triceps skinfold (E2:13.5 mm, E3:15.8 mm, and E4:14.5 mm) were also lower in the apoE2 group than in the apoE3 group ($P < 0.05$ to 0.01). Other variables such as age, plasma glucose, and blood pressure (both systolic and diastolic) did not differ significantly among the three phenotype groups (data not shown). Essentially the same results were obtained when data were analyzed without adjusting for age, race and sex.

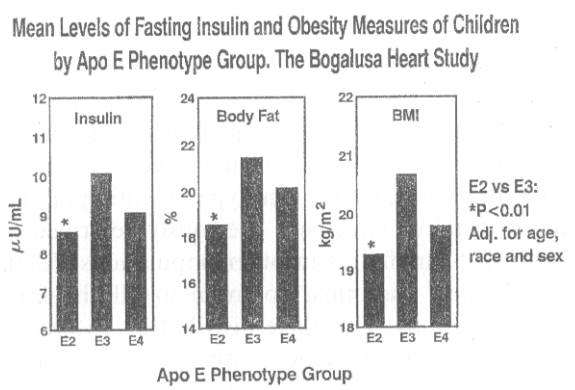

Fig. 1. Mean levels of insulin and obesity measures of children by apoE phenotype group.

Relation of fasting insulin and BMI to selected cardiovascular risk factor variables by apoE phenotype group are given in Table 2 and Table 3, respectively, in terms of partial correlation coefficients adjusted for age, race and sex. Correlations were not uniform among the three phenotype groups. Insulin correlated positively with VLDL cholesterol only in the apoE3 and apoE4 groups, with total cholesterol to HDL cholesterol ratio only in the apoE3 group, with diastolic blood pressure only in the apoE3 and apoE4 groups, and with triglycerides, systolic blood pressure, and BMI in all three phenotype groups ($P < 0.05$ to $P < 0.0001$). BMI correlated positively with total cholesterol and VLDL cholesterol only in the apoE3 and apoE4 groups, and with triglycerides, LDL cholesterol, total

Table 2
Relation of fasting insulin to cardiovascular risk factor variables in children by ApoE phenotype group

Insulin vs	ApoE phenotype group		
	E2 (n = 58)	E3 (n = 476)	E4 (n = 212)
Cholesterol			
Total	0.10 (0.14)†	0.06 (0.06)	0.07 (0.10)
VLDL	0.26[a] (0.21)	0.34[d] (0.30[d])	0.31[d] (0.23[c])
LDL	0.09 (0.05)	0.07 (0.07)	0.12 (0.09)
HDL	−0.04 (0.11)	−0.07 (−0.06)	−0.10 (−0.04)
Total/HDL	0.10 (0.02)	0.13[b] (0.12[b])	0.15[a] (0.10)
Triglycerides	0.60[d] (0.56[d])	0.32[d] (0.31[d])	0.30[d] (0.24[c])
Systolic blood pressure	0.45[d] (0.44[c])	0.29[d] (0.32[d])	0.27[d] (0.28[d])
Diastolic blood pressure	0.22 (0.18)	0.21[d] (0.18[d])	0.17[b] (0.16[a])
BMI	0.61[d] (0.54[d])	0.50[d] (0.46[d])	0.46[d] (0.43[d])

†Spearman correlation coefficients; values in parentheses were adjusted for age, race and sex.
[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$; [d]$P < 0.0001$.

cholesterol to HDL cholesterol ratio, systolic blood pressure, and diastolic blood pressure in all three phenotype groups ($P < 0.05$ to $P < 0.0001$).

The clustering of adverse total cholesterol to HDL cholesterol ratio with increased levels of BMI, insulin, and systolic blood pressure is shown as two-variable clustering in Fig. 2 and three-variable clustering in Fig. 3 according to the apoE phenotype group. The ratio of total cholesterol to HDL cholesterol was included as a measure of dyslipidemia, since this ratio, considered as the best predictor of CAD, reflects both atherogenic and antiatherogenic lipoproteins [34]. If there was no association between these risk factors, one would expect 11.1% individuals with two-variable clustering, and 3.7% individuals with three-variable clustering by chance alone. As can be seen, a marked increase in the prevalence of clustering of the two or three risk factors occurred in the apoE3 and apoE4 groups, but not in the apoE2 group. Furthermore, the prevalence of clustering of adverse total cholesterol to HDL cholesterol ratio with other risk factors was highest in the apoE4 group (two-variable clustering: 14.5–18.2%, $P < 0.01$; three-variable clustering: 8.4–9.4%, $P < 0.0001$).

The prevalence of parental history of heart attack and diabetes mellitus by apoE phenotype group is shown in Fig. 4. Compared to the apoE3 group, the apoE2 group had lower prevalence of parental history of heart attack (8.8% vs. 1.9%, $P < 0.08$) and diabetes mellitus (10.4% vs. 2.0%, $P < 0.05$). Although not significantly different from the apoE3 group, the apoE4 group tended to have the highest prevalence of parental history of heart attack (9.7%) and diabetes mellitus (13.6%).

4. Discussion

The present study demonstrates that apoE polymorphism relates not only to serum lipoprotein concentrations in childhood, as previously reported [24–26], but to a constellation of interrelated cardiovascular risk factors as well. These observations are derived from randomly selected free-living children from a total community. The association of apoE polymorphism with serum total cholesterol and LDL cholesterol concentrations noted in the study population is consistent with that of other population studies showing higher values with phenotypes E4/4 and E4/3 and lower values with phenotypes E3/2 and E2/2 compared to those of phenotype E3/3 [5–8].

Obesity measures and fasting plasma insulin concentrations of our study population varied among the apoE phenotype groups, with individuals carrying the apoE2 isoform having signifi-

Table 3
Relation of adiposity to cardiovascular risk factor variables in children by ApoE phenotype group

BMI vs	ApoE phenotype group		
	E2 (n = 58)	E3 (n = 476)	E4 (n = 212)
Cholesterol			
Total	0.07 (0.09)†	0.09^{a} (0.15^{c})	0.04 (0.15^{a})
VLDL	0.22 (0.15)	0.27^{d} (0.21^{d})	0.26^{d} (0.14^{a})
LDL	0.31^{a} (0.28^{a})	0.13^{b} (0.18^{d})	0.15^{a} (0.20^{c})
HDL	−0.24 (−0.10)	-0.10^{a} (−0.04)	-0.22^{c} (−0.07)
Total/HDL	0.38^{b} (0.27^{a})	0.19^{d} (0.17^{d})	0.24 (0.15^{a})
Triglycerides	0.47^{d} (0.41^{d})	0.24^{d} (0.23^{d})	0.21^{b} (0.12^{a})
Systolic blood pressure	0.64^{d} (0.63^{d})	0.48^{d} (0.49^{d})	0.48^{d} (0.49^{d})
Diastolic blood pressure	0.45^{c} (0.41^{c})	0.29^{d} (0.28^{d})	0.29^{d} (0.29^{d})

†Spearman correlation coefficient; values in parentheses were adjusted for age, race and sex.
$^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$.

cantly lower values than those carrying only the apoE3 isoform. Earlier studies in a rather small number (n = 63) of selected women did not show such a relationship [35,36]. Regarding blood pressure, the levels remained similar among the apoE phenotype groups in our study population, although an earlier report in a selected group of adults noted higher systolic blood pressure in subjects with E4/4 and E4/3 phenotypes than those with E3/3 phenotype [37]. The biologic basis for these observations is not known. Further studies in other populations are needed to confirm the present findings.

The adverse impact of excess adiposity and hyperinsulinemia on blood pressure and serum lipoprotein variables are well-known in adults and children alike [38–43]. Furthermore, excess adiposity is related to insulin resistance and its attendant hyperinsulinemia [38,44]. In the present study, although the bivariate relationships of these variables were in the expected directions, only certain variables showed significant correlations in all three phenotype groups. For example, both insulin and adiposity, which correlated significantly with each other, showed significant correlation to triglycerides and systolic blood pressure in all three phenotype groups. On the other hand, only adiposity correlated significantly with LDL cholesterol, total cholesterol to HDL cholesterol ratio, and diastolic blood pressure in all three phenotype groups.

The potential of CAD risk depends on a constellation of risk factors that are interrelated to some extent. Clustering of adverse levels of lipoprotein, blood pressure, adiposity, and insulin, which occurs frequently in both children [45,46] and adults [47,48] has been termed Syndrome X [49]. Insulin is considered to play a pathogenic role in this multiple metabolic disorder [49]. Furthermore, evidence has been provided for the genetic transmission of this disorder in individuals with dyslipidemic hypertension [50]. In the present study, no significant clustering of multiple risk factors related to Syndrome X occurred among those carrying the apoE2 isoform. In contrast, clustering of these multiple risk factors was markedly high in other apoE phenotype groups, especially among those carrying the apoE4 isoform. Whether this trend becomes pronounced in adulthood, especially among those carrying the apoE4 isoform, is not clear.

Although bivariate relationships among the risk factor variables occurred in varying degrees within all three apoE phenotype groups, the consistently low LDL cholesterol, adiposity, and insulin coupled with high HDL cholesterol values noted in carriers of apoE2 isoform may account for the lack of clustering of adverse lipoprotein levels with other risk factors in this group. On the other hand, the situation is just the opposite in apoE3 and apoE4 groups, especially the latter in which the adverse lipoprotein levels are relatively more common.

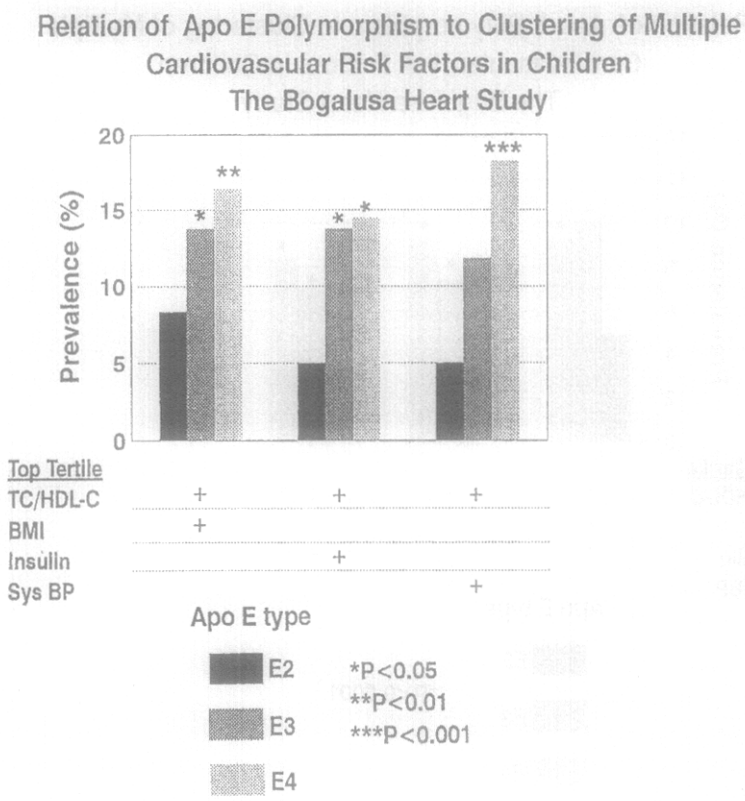

Fig. 2. Relation of apoE polymorphism to clustering of adverse (top tertile) total cholesterol to HDL cholesterol ratio with increased levels (top tertile) of BMI, insulin or systolic blood pressure.

In the present study, the prevalence of parental history of heart attack and diabetes mellitus among the three phenotype groups corresponded with the trend of multiple risk factors clustering in the offspring, with the prevalence being lowest in carriers of apoE2 isoform and highest in carriers of apoE4 isoform. This is consistent with the growing body of evidence showing an anti-atherogenic role for the e2 allele and an atherogenic role for the e4 allele [5,14–23]. Parental history may be a surrogate measure of future risk of morbidity in these children, given the familial nature of cardiovascular disease. Although offspring studies have a decreasing power to investigate genotype-disease relationships, the association of the apoE polymorphism with parental history of heart attack has been shown in young adults of different European populations, the e4 allele being more frequent and the e2 allele less frequent among offspring of affected fathers than among control subjects [51].

Recent studies have strongly suggested that the effect of apoE genotype on CAD may not be simply attributable to its effect on lipoprotein concentrations [20–22]. Results from the present study might provide clues in this regard. It is tempting to speculate that apoE genotype may regulate multiple metabolic conditions related to syndrome X, thereby affecting a constellation of cardiovascular risk factors early in life. Clearly, further studies are needed in this direction.

Acknowledgements

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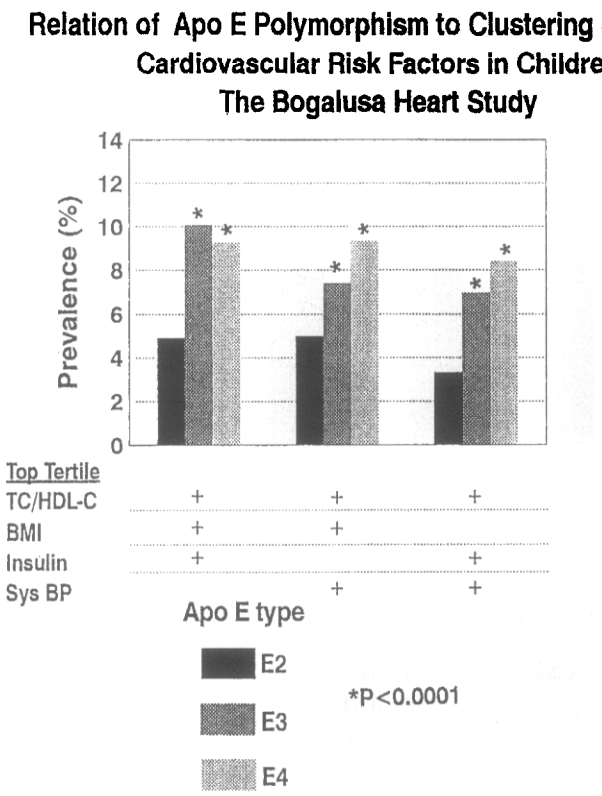

Fig. 3. Relation of apoE polymorphism to clustering of adverse (top tertile) total cholesterol to HDL cholesterol ratio with increased levels (top tertile) of BMI and insulin or BMI and systolic blood pressure or insulin and systolic blood pressure.

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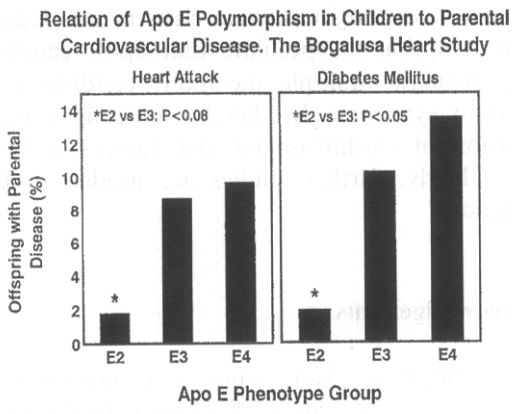

Fig. 4. Relation of apoE polymorphism in children to the prevalence of parental history of heart attack and diabetes mellitus.

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