Am. J. Hum. Genet. 49:338–349, 1991

The Apolipoprotein E Polymorphism: A Comparison of Allele Frequencies and Effects in Nine Populations

D. Michael Hallman,* Eric Boerwinkle,* Nilmani Saha,† Christoph Sandholzer,‡ Hans Jürgen Menzel,‡ Albert Császár,‡ and Gerd Utermann‡

*Center for Demographic and Population Genetics, University of Texas Health Science Center, Houston; †Division of Genetics, National University Hospital, Singapore; and ‡Institute for Medical Biology and Human Genetics, University of Innsbruck, Innsbruck

Summary

Application of uniform methods for measuring the apolipoprotein (apo) E polymorphism and plasma cholesterol levels in nine populations (Tyrolean, Sudanese, Indian, Chinese, Japanese, Hungarian, Icelandic, Finnish, and Malay) revealed significant heterogeneity among them in apo E type frequencies and mean cholesterol levels. The major apo E types in all populations were E3/2 (frequency range from 7.0% in Indians to 16.9% in Malays), E3/3 (frequency range from 39.8% in Sudanese to 72.1% in Japanese), and E3/4 (frequency range from 11.3% in Japanese to 35.9% in Sudanese). Mean cholesterol levels ranged from 144.2 mg/dl in the Sudanese to 228.5 mg/dl in the Icelandics. Two-way analysis of variance of the effect of population and apo E type on cholesterol levels showed no significant interaction effect, indicating that the effects of apo E type on cholesterol levels do not differ significantly among the populations. The overall average excess for the $\varepsilon 2$ allele was -14.12 mg/dl (range -31.63 to -8.82 mg/dl); for the $\varepsilon 3$ allele, 0.04 mg/dl (range -1.87 to 1.58 mg/dl; and for the $\varepsilon 4$ allele, 8.14 mg/dl (range -1.71 to 13.31 mg/dl). Despite the apparent heterogeneity in these values, especially for the $\varepsilon 4$ allele, comparison of the average excesses by a method of repeated sampling with random permutations revealed no significant difference in effects among populations. These data indicate that a given apo E allele acts in a relatively uniform manner in different populations despite differences in genetic background and environmental factors.

Introduction

Apolipoprotein E (apo E) is a serum glycoprotein consisting of 299 amino acid residues which is found in circulating chylomicrons, chylomicron remnants, very-low-density lipoproteins, intermediate-density lipoproteins, and high-density lipoproteins. apo E is thought to play a regulatory role in lipid metabolism through the cellular uptake of apo E–bearing lipoproteins by two classes of receptors, an apo E–specific remnant receptor and the low-density lipoprotein (LDL) receptor (Mahley 1988; Beiseigel et al. 1989; Kowal et al. 1989).

Received November 2, 1990; revision received February 21, 1991.

Address for correspondence and reprints: Dr. Eric Boerwinkle, Center for Demographic and Population Genetics, University of Texas Health Science Center, Houston, TX 77225.

0002-9297/91/4902-0010$02.00

Three common apo E variants distinguishable by isoelectric focusing have been described and designated E2, E3, and E4 (Utermann et al. 1977; Bouthillier et al. 1983). These are coded for by three alleles ($\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$, respectively; Zannis et al. 1982). The isoforms show differences in amino acid residues at two sites, residues 112 and 158 (Rall et al. 1982). In all populations studied thus far, apo E3 has been the predominant isoform, but the relative proportions of the three isoforms have shown variation among populations (reviewed in Davignon et al. 1988; Sepehrnia et al. 1989).

Numerous studies of the effects of the apo E polymorphism on lipid metabolism have shown that individuals with at least one $\varepsilon 2$ allele tend to have lower levels of total plasma cholesterol than do individuals who are homozygous for the $\varepsilon 3$ allele, while individuals with at least one $\varepsilon 4$ allele tend to have higher levels of total plasma cholesterol than do $\varepsilon 3$ homozygotes

(Utermann et al. 1979; for a review, see Davignon et al. 1988). Estimates of the average effects of each allele on cholesterol levels in different populations have generally shown consistency in the direction of these effects across populations, though variations in the magnitude of the effects seem to exist (Sing and Davignon 1985; Boerwinkle and Sing 1987; Sepehrnia et al. 1989).

In several studies (Schneider et al. 1981; Hui et al. 1984), apo E2 has been reported to have a reduced binding affinity for LDL (B/E) receptors, which may in turn lead to the accumulation of apo E–containing lipoproteins (Utermann 1985). apo E4 has been reported to have a faster rate of clearance from plasma than does apo E3 (Gregg et al. 1986). Boerwinkle and Utermann (1988; also see Davignon et al. 1988) proposed a model in which the apo E polymorphism differentially affects plasma cholesterol levels through its effect on LDL receptor regulation. In the model, presence of the ε2 allele leads to receptor up-regulation and increased LDL clearance, and presence of the ε4 allele leads to receptor down-regulation and decreased LDL clearance. However, the role of apo E in cholesterol metabolism has not been completely elucidated, and Palmer et al. (1986) reported that apo E phenotype did not significantly improve prediction of parameters of whole-body cholesterol metabolism.

Current consensus is that apo E does affect plasma cholesterol levels. However, given the extent of variation in mean plasma cholesterol levels in different populations (Keys 1980; International Collaborative Study Group 1986), questions remain as to whether the biological effects of the different apo E alleles on plasma cholesterol levels are the same across different populations. Despite the general consistency in the direction of effects found in previous studies, it is difficult to assess whether the magnitude of effects is the same when previously published results from different populations are compared, since sampling procedures and exclusion criteria (e.g., see Sing and Davignon 1985; Boerwinkle et al. 1987), laboratory methods (e.g., see Asakawa et al. 1985; Sing and Davignon 1985) and analytical techniques (e.g., see Ehnholm et al. 1986; Boerwinkle et al. 1987) vary across studies. In addition, most studies have measured effects in populations of western European descent, although Japanese (Asakawa et al. 1985; Eto et al. 1986; Kobori et al. 1988), Chinese (Wang 1986), and Nigerian (Sepehrnia et al. 1989) populations have been reported on. In the present study, nine populations of different ethnicity from widely varying locations were sampled, all lipid and apolipoprotein measurements were made under standardized conditions in one laboratory, and all analyses were carried out in a similar manner. While the groups studied showed wide variations in cholesterol levels and apo E allele frequencies, we found no evidence that the effects of the apo E polymorphism on plasma cholesterol levels were significantly different among the populations.

Material and Methods

A. Population Samples

Serum samples from Tyrolean, Hungarian, Chinese, Malay, and Indian individuals were collected from unrelated blood bank donors. The Chinese, Malay, and Indian samples were all collected in Singapore. Japanese serum samples were collected from doctors at the Kyushu University School of Medicine, from other doctors and pharmacists in the same area, and from students at Fukuoka University. Finnish samples were collected from personnel of the National Public Health Institute and from healthy volunteers (Ehnholm et al. 1986). The Icelandic samples were from subjects participating in the MONICA Study (WHO MONICA Project 1988). The Sudanese samples were from black volunteers in Khartoum.

B. Laboratory Methods

apo E phenotypes were determined by isoelectric focusing followed by immunoblotting with anti–apo E and by SDS-PAGE followed by immunoblotting with anti–apo E (Menzel and Utermann 1986). The latter method allows discrimination of the apo E2 (arginine 158 to cysteine 158) isoform from apo E3 and E4 as well as from rare apo E2 variants (Utermann et al. 1984). Total plasma cholesterol was determined enzymatically by using a commercially available kit (Boehringer Mannheim, Germany).

C. Statistical Analyses

Allele frequencies were estimated by the gene-counting method. A χ^2 test for goodness of fit was used to test whether observed allele frequencies agreed with those expected under the hypothesis of Hardy-Weinberg equilibrium. The technique of χ^2 decomposition (Freeman 1987) was used to test for differences in apo E type distributions among populations. Linear regression analysis was used to adjust total plasma cholesterol for the effect of age in all groups. One- and two-way analyses of variance (Scheffé 1959) were used to test the effects of sample group and apo E type on cholesterol levels. Equality of variances among groups was tested by the method of Levene (1960); where variances were unequal, the modified F statistic of Brown and Forsythe (1974) was used in assessing significance. No differences in results were found when the Brown-Forsythe technique, as compared with standard analysis-of-variance methods, was used. Unbalanced cells in the analyses of variance were adjusted for by the standard parametric method (Herr 1986; see also Kutner 1974; Speed and Hocking 1976). Power for the analyses of variance was determined using the noncentral F distribution (Pearson and Hartley 1951).

apo E allele effects were calculated by the formula

$$\alpha_i = \frac{F_{ii}\mu_{ii} + (1/2)\sum_{i \neq j} F_{ij}\mu_{ij}}{F_i} - \mu. \ ,$$

where α_i is the average excess of the ith allele (Falconer 1985; Templeton 1986), j is the other alleles at the locus, μ_{ij} is the mean cholesterol level of the ijth genotype, $\mu.$ is the overall group cholesterol mean, F_{ii} is the observed frequency of the homozygous ii class, F_{ij} is the observed frequency of the heterozygous ij classes, and F_i is the allele frequency of the ith allele. This formula is modified from that presented by Boerwinkle and Sing (1987) in that observed frequencies within genotype classes are used, rather than expected frequencies under the hypothesis of Hardy-Weinberg equilibrium. The proportion of phenotypic variability attributable to the apo E polymorphism was estimated by the formula given by Boerwinkle et al. (1987), though, again, observed rather than expected frequencies were used in the calculations. Homogeneity of the average excesses of apo E alleles among groups was tested by the method of repeated sampling with random permutations of group membership, as described by Boerwinkle and Sing (1987; also see Efron 1982; Templeton et al. 1988). The test statistic used is given by

$$g(\alpha) = \sum_k \sum_j (\alpha_{jk} - \alpha_{j}.)^2 \ ,$$

where α_{jk} is the average excess of the jth allele in the kth group and $\alpha_j.$ is the average excess of the jth allele over all groups. An estimated distribution of the statistic under the null hypothesis of no difference of allele average excesses among groups was obtained by a permutation method. Repeated calculations of $g(\alpha)$ were carried out, with observations randomly reassigned to groups at each repetition. In the present case, 7,500 permutations were found to produce a stable distribution and critical value (data not shown). If the value of $g(\alpha)$ from the original observations exceeded the value of the distribution at the percentile corresponding to the desired significance level, the hypothesis of no difference among groups was rejected.

Results

Six individuals had rare apo E types; four (E2/1, E3/1, E3/1, and E3/5) were among the Tyroleans, and two (E3/5 and E4/5) were among the Sudanese. These individuals were included in estimates of allele frequencies and in tests of allele frequencies but were excluded from further analyses. For one individual from India, apo E type was not known; this individual was excluded from all analyses.

The distribution of apo E types among groups was significantly heterogeneous (likelihood-ratio [LR] $\chi^2 = 161.8$, 72 df; $P < .01$). Table 1 shows the distribution of the common phenotypes (those without ε1 or ε5 alleles) in all sample groups. The most common type in all of the groups was E3/3, with E4/3 being the next most common type in all except the Japanese and Malay groups, in which the E3/2 type was the second most common. The relative frequency of the E3/2 type was highest in the Japanese (72.1%) and lowest in the Sudanese (39.8%). The relative frequency of the E4/3 type was highest in the Sudanese (35.9%) and lowest in the Japanese (11.3%). The relative frequency of the E3/2 type was highest in the Malays (16.9%) and lowest in the Indians (7.0%). The E2/2 and E4/2 types were comparatively rare in all groups. The E4/4 type was relatively uncommon in six of the nine groups, but its frequency approached 9% in the Sudanese and 6% in the Finns.

The nine samples were grouped into six a priori categories on the basis of ethnicity, in order to investigate further the differences in apo E type distributions. The European populations were split into two groups on the basis of linguistic relationships (Ruhlen 1987), with the Tyrolean and Icelandic samples forming one group, the Finnish and Hungarian samples another. Among the Asian populations, the Chinese and Japanese samples formed one group; the Indian and Malay samples were each treated separately. The Sudanese sample also formed a group by itself. The frequency distribution of apo E types among these six groups showed significant heterogeneity (LR χ^2 = 108.9, 25 df; $P < .01$). Decomposition of this χ^2 showed that the Sudanese differed significantly from all other samples combined in apo E type distribution (LR χ^2 = 36.2, 5 df; $P < .01$). The Chinese and Japanese differed significantly from the remaining groups combined (LR χ^2 = 39.2, 5 df; $P < .01$). The Finns and Hungarians differed significantly from a final homogeneous group composed of the Tyroleans, Icelandics, Indians, and Malays (LR χ^2 = 19.6, 5 df; $P < .01$). Figure 1 summarizes the results of these comparisons in graphical form.

Table 2 shows the estimated allele frequencies for the different populations, including the pooled groupings resulting from the χ^2 decomposition presented above. As expected, the most common allele in each population was ε3. The next most common allele was ε4, in all populations except the Chinese and Japanese, in which the ε2 allele was slightly more common than the ε4. The ε2 and ε4 alleles were almost equally common in the Malays. The frequency of the ε2 allele was lowest in the Indians (.046) and highest in the Malays (.114). The frequency of the ε3 allele was lowest in the Sudanese (.619) and highest in the Japanese (.846). The frequency of the ε4 allele was lowest in the Chinese and Japanese (both .074) and highest in the Sudanese (.291). Each of the groups was in Hardy-Weinberg equilibrium, except the Hungarians (χ^2 = 14.617, 3 df; $P < .05$) and the Sudanese (χ^2 = 20.467, 6 df; $P < .05$). However, the Hungarian result was due almost entirely to a relative excess of E2/2 subjects (n = 4), while the Sudanese result was due almost entirely to a single E4/5 subject. Adjusting for small cell frequencies in the Hungarians by combining the E2/2, E4/2, and E4/4 subjects removed the significance of this result (χ^2 = 1.601, 1 df; $P > .05$). Similarly, combining the E2/2, E2/5, E3/5, E4/5, and E5/5 subjects in the Sudanese removed the significance of that result (χ^2 = 4.990, 2 df; $P > .05$). Thus, it is likely that the initial significance of these differences was due to chance.

The column marked "TOTAL" in table 1 shows the overall means and SDs for cholesterol levels in each population. Analysis of variance revealed significant variation among the populations in mean total cholesterol levels, which ranged from a low of 144.2 mg/dl in the Sudanese to a high of 228.5 mg/dl in the Icelandics (F = 63.5, 8 and 1,867 df; $P < .0001$). The Chinese, Japanese, and Malays had similar mean cholesterol values, with the Malays lowest at 182. 4 mg/dl and the Chinese highest at 189.3 mg/dl. The mean total cholesterol level in the Indians (197.5 mg/dl) did not differ significantly from that in the Chinese, Japanese, and Malays. The four European populations all had mean cholesterol values >210 mg/dl. The mean total cholesterol level in Tyroleans did not differ from that in the Hungarians and Finns but did differ from that in the Icelandics. The Sudanese differed from all other groups in mean total cholesterol level. The variances in cholesterol levels among groups also differed significantly (Levene's F = 5.3, 8 and 1,867 df; $P < .0001$), with the Sudanese and the Far Eastern populations showing much less variability among individuals than did the remaining populations. As shown in table 1, the low-variance populations had cholesterol SDs clustered around 33 mg/dl and tended to have relatively low mean cholesterol values; the remaining populations all had SD ⩾ 40 mg/dl and tended to have relatively high mean cholesterol values. The Spearman rank correlation of cholesterol means and SDs for all populations was .78.

Table 1 shows the cholesterol means and SDs for each of the apo E types within each population. To facilitate comparisons, the deviations of the apo E type-specific mean cholesterol levels from the population-specific levels are also shown. In all populations except the Hungarians, the mean for the E3/2 type showed a negative deviation from the population mean; only in the Malays was this negative deviation <9 mg/dl. The Hungarians showed a positive deviation of 3.3 mg/dl. The deviations for the E3/3 type showed no clear positive or negative trends; however, they all fell within ±4 mg/dl of the corresponding group means. The deviations for the E4/3 type were positive in all populations except the Sudanese (−0.6 mg/dl) and the Malays (−3.2 mg/dl). The Tyroleans showed the greatest positive deviation (13.3 mg/dl), while the Icelandics showed the smallest (6.7 mg/dl). Comparisons with the E2/2, E4/2, and E4/4 types are difficult to make because of the small numbers of these types.

Results of two-way analyses of variance, with population, apo E type, and an interaction term as independent variables and with cholesterol as the dependent variable, are shown in table 3. Analyses were performed using all apo E types and also using only the most common types (E3/2, E3/3, and E4/3). In both cases, population and apo E type were significant but the interaction term was not, indicating that the effect of apo E type on cholesterol level was not significantly different among the different populations.

The average excess for each apo E allele on choles-

Table 1

Apo E Phenotype Counts, Relative Frequencies, Total Serum Cholesterol Means, SDs, and Within-Phenotype Deviations from Population Means, by Population

Population and Measure	Value For Phenotype						Total
	E2/2	E3/2	E4/2	E3/3	E4/3	E4/4	
Tyrolean:							
n	6	62	10	300	81	10	469
%	1.3	13.2	2.1	64.0	17.3	2.1	100.0
Mean	198.9	200.4	219.3	209.5	224.2	229.2	211.3
SD	17.7	39.9	55.9	39.7	37.8	41.3	40.1
Deviation	−12.4	−10.9	8.0	−1.8	12.9	17.9	
Icelandic:							
n	0	19	6	111	43	6	185
%	.0	10.3	3.2	60.0	23.2	3.2	99.9
Mean	. . .	208.1	209.4	229.5	235.2	248.7	228.5
SD	. . .	26.7	46.1	47.6	39.4	30.4	43.8
Deviation	. . .	−20.4	−19.1	1.0	6.7	20.2	
Finnish:							
n	1	20	3	95	72	12	203
%	.5	9.9	1.5	46.8	35.5	5.9	100.1
Mean	177.7	206.1	198.6	214.3	224.9	240.6	218.2
SD	.0	56.7	36.8	48.7	37.3	61.2	47.0
Deviation	−40.5	−12.1	−19.6	−3.9	6.7	22.4	
Hungarian:							
n	4	16	2	132	46	2	202
%	2.0	7.9	1.0	65.3	22.8	1.0	100.0
Mean	170.8	228.9	184.4	218.7	226.7	307.3	220.9
SD	18.0	34.7	15.6	42.0	41.0	14.4	42.1
Deviation	−50.1	8.0	−36.5	−2.2	5.8	86.4	
Chinese:							
n	3	29	2	132	22	2	190
%	1.6	15.3	1.1	69.5	11.6	1.1	100.2
Mean	152.9	180.0	199.2	190.3	198.9	200.8	189.3
SD	33.6	32.6	.0	32.0	38.8	5.5	33.1
Deviation	−36.4	−9.3	9.9	1.0	9.6	11.5	
Japanese:							
n	2	44	3	230	36	4	319
%	.6	13.8	0.9	72.1	11.3	1.3	100.0
Mean	162.6	176.1	166.8	186.6	193.7	188.4	185.6
SD	30.9	30.1	20.9	32.8	36.5	33.4	33.0
Deviation	−23.0	−9.5	−18.8	1.0	8.1	2.8	
Indian:							
n	1	10	1	97	31	2	142
%	.7	7.0	.7	68.3	21.8	1.4	99.9
Mean	118.5	167.2	247.4	197.8	206.9	203.2	197.5
SD	.0	18.8	.0	36.8	59.2	30.1	43.0
Deviation	−79.0	−30.3	49.9	.3	9.4	5.7	
Sudanese:							
n	1	10	5	41	37	9	103
%	1.0	9.7	4.9	39.8	35.9	8.7	100.0
Mean	74.4	132.3	156.6	146.3	142.5	155.3	144.2
SD	.0	28.5	61.4	31.1	32.1	37.8	34.0
Deviation	−69.8	−11.9	12.4	2.1	−1.7	11.1	

(*continued*)

Table 1 (continued)

Population and Measure	Value For Phenotype E2/2	E3/2	E4/2	E3/3	E4/3	E4/4	Total
Malay:							
n	3	20	1	71	19	4	118
%	2.5	16.9	.8	60.2	16.1	3.4	99.9
Mean	144.8	180.8	203.6	185.0	180.2	179.1	182.4
SD	36.9	33.6	.0	33.0	28.9	32.1	32.6
Deviation	−37.6	−1.6	21.2	2.6	−2.2	−3.3	
Combined populations:							
n	21	230	33	1209	387	51	1,931
%	1.1	11.9	1.7	62.6	20.0	2.6	99.9
Mean	165.0	189.9	198.4	201.2	210.0	211.5	201.4
SD	37.2	40.9	49.9	42.1	47.7	53.6	44.1
Deviation	−36.4	−11.5	−3.0	−.2	8.6	10.1	

NOTE—Means, SDs, and deviations are in mg/dl.

terol is shown in table 4, for each population and for all populations combined. The effect of the ε3 allele is close to zero overall. This is to be expected, since ε3 is the most common allele in all the populations; individuals who carry this allele contribute most to the overall mean cholesterol value. The effect of the ε2 allele is negative in all populations, ranging from − 8.82 mg/dl in the Malays to − 31.63 mg/dl in the Indians, with a mean value, over all populations, of − 14.12 mg/dl. The effect of the ε4 allele is positive in all populations except the Malays. The range of effects of the ε4 allele is less than that of the ε2 allele, varying from − 1.71 mg/dl in the Malays to 13.31 mg/dl in the Tyroleans, with a mean value, over all populations, of 8.14 mg/dl. Despite the apparent heterogeneity of effects in the different populations, particularly with the ε4 allele, the average excess of each allele did not differ significantly among populations when tested by the permutation procedure described in the Material and Methods section ($g(\alpha) = 601.498$; $P > .05$).

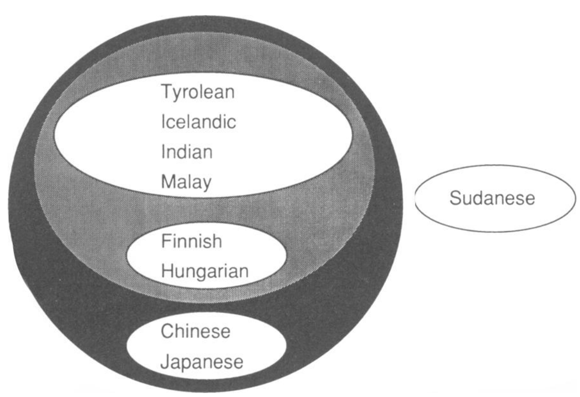

Figure 1 Graphical representation of results of χ^2 decomposition. Beginning with all populations, groups defined by a priori criteria were split off and tested against the remaining populations. Further details of the procedure are given in the Material and Methods section.

Discussion

apo E allele frequencies were highly heterogeneous among the populations studied, though some clustering was detected. The Chinese and Japanese formed one homogeneous group, with both populations showing higher frequencies of the ε3 allele and lower frequencies of the ε4 allele than did the other populations. The high frequency of the ε3 allele in both populations has been reported in previous studies (Asakawa et al. 1985; Tsuchiya et al. 1985; Eto et al. 1986; Wang 1986). The frequency of the ε2 allele in the Japanese sample here (.080) is higher than ε2 frequencies reported in earlier studies, which ranged from .018 to .038, and the frequency of the ε4 allele is somewhat lower (.074 vs. .086–.129; see Asakawa et al. 1985; Tsuchiya et al. 1985; Eto et al. 1986; Kobori et al. 1988). The range of allele frequencies across these studies is wide enough to leave some doubt as to whether sampling variation or population substructure is the cause. There is insufficient evidence to favor either alternative at present. As shown in figure 1, the Malays and Indians, despite their Asian origins,

Table 2

Estimated Relative Frequencies and SDs of Apol E Alleles

Group	Estimated Relative Frequency (SD) of apo E Allele				
	ε1	ε2	ε3	ε4	ε5
Individual populations:					
Tyrolean	.003 (.002)	.090 (.009)	.789 (.013)	.117 (.010)	.001 (.001)
Icelandic	. . . (. . .)	.068 (.013)	.768 (.022)	.165 (.019)	. . . (. . .)
Finnish	. . . (. . .)	.062 (.012)	.695 (.023)	.244 (.021)	. . . (. . .)
Hungarian	. . . (. . .)	.064 (.012)	.807 (.020)	.129 (.017)	. . . (. . .)
Chinese	. . . (. . .)	.097 (.015)	.829 (.019)	.074 (.013)	. . . (. . .)
Japanese	. . . (. . .)	.080 (.011)	.846 (.014)	.074 (.010)	. . . (. . .)
Indian	. . . (. . .)	046 (.012)	.827 (.022)	.127 (.020)	. . . (. . .)
Sudanese	. . . (. . .)	.081 (.019)	.619 (.034)	.291 (.032)	.001 (.002)
Malay	. . . (. . .)	.114 (.021)	.767 (.028)	.119 (.021)	. . . (. . .)
Groupings from χ^2 decomposition:					
Tyrolean, Icelandic, Indian, and Malay	.002 (.001)	.082 (.006)	.788 (.010)	.129 (.008)	.001 (.001)
Finnish and Hungarian	. . . (. . .)	.063 (.009)	.751 (.015)	.186 (.014)	. . . (. . .)
Chinese and Japanese	. . . (. . .)	.086 (.009)	.840 (.011)	.074 (.008)	. . . (. . .)
All populations	.001 (.0004)	.079 (.004)	.784 (.007)	.135 (.006)	.001 (.0004)

Table 3

Two-Way Analysis-of-Variance Results for Population and for Apo E Type Affecting Cholesterol Levels

Source	All Phenotypes			E3/2, E3/3, and E4/3 Phenotypes Only		
	Mean Square	df	*F*	Mean Square	df	*F*
Population	97,284.5	8	65.1[a]	57,980.3	8	39.2[a]
apo E type	18,203.7	5	12.2[a]	15,366.0	2	10.4[a]
Interaction	1,178.1	40	.8	1,327.9	16	.9
Error	1,494.2	1,822		1,477.5	1,746	

[a] Significant at $\alpha = .01$.

Table 4

Average Excesses of apo E Alleles in Different Populations

Population	Average Excess of apo E Allele (mg/dl)		
	ε2	ε3	ε4
Tyrolean	−8.85	−.99	13.31
Icelandic	−20.09	.35	6.80
Finnish	−15.28	−1.87	9.63
Hungarian	−13.30	−.59	10.34
Chinese	−13.48	.66	9.87
Japanese	−11.14	.57	5.51
Indian	−31.63	.21	10.06
Sudanese	−11.52	−.04	3.36
Malay	−8.82	1.58	−1.71
Overall	−14.12	.04	8.14

grouped with the Tyroleans and Icelandics; this combined group differed significantly from a group composed of the Finns and Hungarians.

In all four European populations the ε3 allele was most frequent, followed by ε4 and then ε2. This is in agreement with all previous studies among Caucasians, except one in Nancy, France, in which the ε2 and ε4 alleles were equally frequent (Boerwinkle et al. 1987). However, the Europeans clearly did not form a homogeneous group. The Finns and, to a lesser extent, the Icelandics had elevated frequencies of the ε4 allele, compared with the other two populations, and the Tyroleans had a higher frequency of the ε2 allele, compared with the other three. The Finns and Hungarians differed from the other European groups (as well as from the Malays and Indians) in the χ^2 decomposition analysis, and linguistic evidence would place these two populations closer to one another than to the other two European populations (Ruhlen 1987). However, the Finns and Hungarians show the lowest and highest frequencies, respectively, for the ε3 allele among the Europeans and so may not form a genetically homogeneous group. That these populations may be less closely related genetically than the evidence of a common language origin would suggest has been indicated previously (Harding and Sokal 1988).

The Sudanese, with relatively low ε3 and high ε4 frequencies, appear different from all other populations. The high frequency of the ε4 allele (.291) reported here for the Sudanese accords very well with the only other result thus far reported for an African population: .296 among Nigerian blacks (Sepehrnia et al. 1989). However, the Sudanese show a higher frequency of the ε2 allele (.083) than do the Nigerians (.027).

It is tempting to speculate whether there exists a relationship between the frequencies of the apo E alleles and the prevalence of coronary heart disease (CHD) across populations. The Japanese and Chinese, two populations with traditionally low rates of CHD, had the lowest frequency of the ε4 allele in the populations considered here. Conversely, the high frequency of the ε4 allele in the Finns has led to speculation that this allele is contributing to the increased prevalence of CHD in this population (Sing and Moll 1989). However, the Sudanese had the highest frequency of the ε4 allele in any of the populations studied here, and, while population-based studies in Sudan are lacking, CHD is known to be uncommon in other black African populations (Williams 1971; Falase et al. 1972; Shaper 1974). It should be noted that Nigerians, who have also been shown to have a high frequency of the ε4 allele (Sepehrnia et al. 1989), have a relatively low frequency of CHD (Williams 1971; Falase et al. 1972). Clear relationships between CHD prevalence and the frequency of the ε2 allele also are not evident. The data presented here and those reviewed by Davignon et al. (1988) do not show a trend between an elevated frequency of the ε2 allele and decreased prevalence of CHD. Thus, the relationship between apo E allele frequencies and CHD may be suggestive but is not convincing. apo E has been found to play a diverse and increasingly important role in normal human physiology (Mahley 1988). We suggest that selective pressures on phenotypic traits unrelated to CHD, in combination with stochastic processes, have given rise to the observed apo E allele frequency differences among populations.

The average excesses of the apo E alleles within populations were generally consistent with the average effects reported in previous studies (reviewed in Davignon et al. 1988), despite significant differences in mean cholesterol levels among populations. All populations showed an overall cholesterol-lowering effect for the ε2 allele, and all except the Malays showed a cholesterol-raising effect for the ε4 allele. The seemingly anomalous cholesterol-lowering effect of the ε4 allele in the Malays was not of sufficient magnitude to produce a statistically significant difference in allele effects among the different groups, by either test procedure employed. However, the power of the *F* test used to detect significant interaction effects was approximately .50; thus, with the data shown in table 1, a biologically real interaction effect would have a 50% chance of going undetected.

Both genotype-specific mean cholesterol levels and allele frequencies are used in calculating average effects or excesses (Falconer 1985; Templeton 1986). In addition, the analysis-of-variance results shown in table 3 are frequency dependent, and apo E allele frequencies are significantly different among groups. An attempt to remove the effects of frequency from the comparisons among populations is shown in figure 2. This shows extensive overlap in the standard errors of cholesterol means among populations within the common apo E phenotypes, indicating that even such apparently extreme results as the mean cholesterol level for the Indian E3/2 subjects are not really different from the E3/2 cholesterol means in other populations. The unexpectedly high cholesterol level for the Hungarian E3/2 type might seem to indicate that the ε2 allele is acting differently in Hungarians; however, the Hungarian E2/2 and E4/2 subjects had much lower cholesterol levels than did the Hungarian E3/3 subjects, producing the expected average excess for the ε2 allele.

In the model proposed by Boerwinkle and Utermann (1988), the ε4 allele tends to raise cholesterol levels because apo E4–containing lipoproteins are internalized faster by the liver, leading to down-regulation of LDL receptors and to an increase in plasma LDL levels. In ε4 homozygotes, higher levels of dietary cholesterol would result in greater LDL receptor down-regulation. Utermann (1987) has suggested that the tendency of the ε4 allele to raise cholesterol levels may be increased when the diet is high in

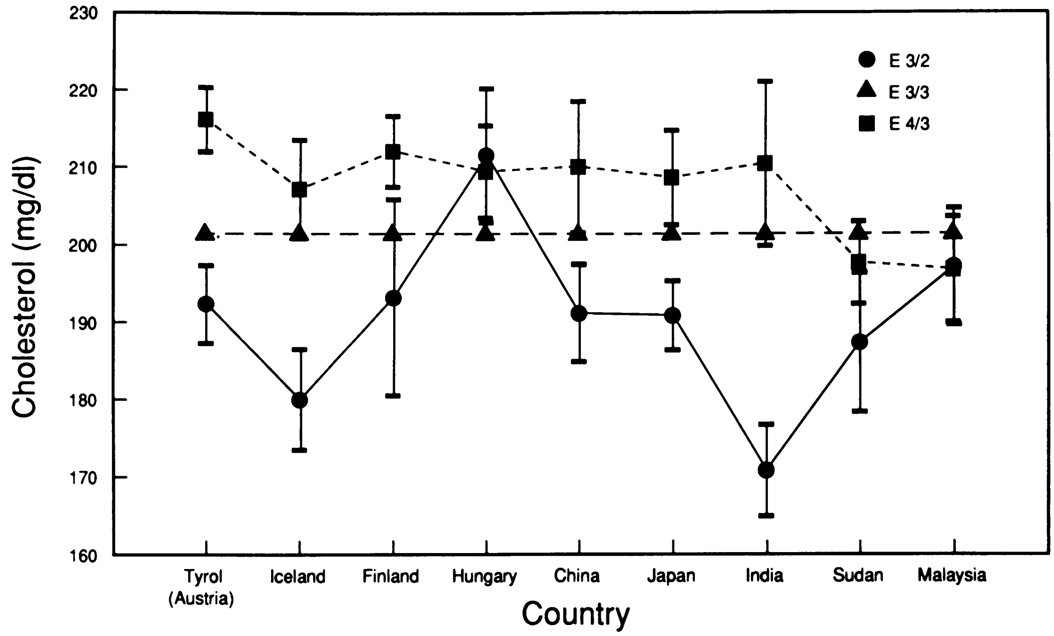

Figure 2 Phenotype-specific cholesterol means (± 1 SEM for E3/2 and E4/3 phenotypes), by population. Individual cholesterol values were adjusted so that apo E3/3 mean cholesterol values in each population are equal to the overall mean cholesterol value for all subjects.

cholesterol. A study in Nigerians did not support this hypothesis (Sepehrnia et al. 1989). The present study offers some support, in that the elevating effect of the ε4 allele on cholesterol tends to be less in some populations in which diets low in fat and animal products are typical—the Malays, Sudanese, and Japanese—and greater in some populations in which diets high in fat and animal products are typical—the Tyroleans and Finns. However, the cholesterol-elevating effect of the ε4 allele is high in the Chinese despite diets that, compared with Western diets, are low in fat and animal products (Tan et al. 1984), and the ε4 effects in the Icelandics and Hungarians are moderate despite diets typically higher in fat and animal products (FAO 1983, 1984, 1987). Results of studies in which lipid measurements were made before and after dietary intervention, to test for interactions between diet and apo E type, have been mixed: Tikkanen et al. (1990) found such interaction; Xu et al. (1990) did not. Further studies of this type in different populations are needed to clarify the combined role of diet and apo E in affecting serum cholesterol levels.

Investigations of the role of the apo E polymorphism in affecting dietary response represent one way of studying genotype × environment interaction. In agriculture, where such interaction has been studied most, it is not considered operationally significant unless genotypes change ranks in different environments or unless the response curves intersect (Gregorius and Namkoong 1986). In human genetics, such a definition is overly demanding, because the range of known environment influences is often restricted compared with those available for study in an experimental setting. In humans, three separate study designs have been used to investigate the interaction between the apo E polymorphism and a second stratum. The first compares the gene's effects among populations and is the design used in the present study. The second compares the effects of the apo E polymorphism among strata within a population. For example, Shriver et al. (1991) have reported that the effects of the apo E polymorphism are the same in non-insulin-dependent diabetics as they are in a random sample from the same population, and studies of the influence of gender on apo E effects have indicated either no differences between the sexes (Boerwinkle et al. 1987) or slight differences in magnitude but not direction (Robertson and Cumming 1985; Lehtimäki et al. 1990). The third and most powerful design compares apo E effects in a sample of individuals first in one environment and then in another. For example, Xu et al. (1990) have reported that there is no significant interaction between the apo E polymorphism and diet as they both affect lipid, lipoprotein, and apolipoprotein levels. The response of lipid levels to a low-fat diet was similar for each of the three common apo E genotypes. Evidence from all three types of studies indicates that the well-known effects of the apo E polymorphism, with the ε2 allele tending to lower cholesterol levels and the ε4 allele tending to raise them, are robust to a large number of genetic and environmental influences.

Acknowledgments

We thank Drs. Gunnar Sigurdsson (Reykjavik City Hospital, Iceland), Taizo Sato (Fukuota University, Japan), and Carolin Lackner for providing plasma samples. We are indebted to Linda Fineder and Ulrike Scheidle for skillful technical assistance and to Dr. Ranajit Chakraborty for advice on the power calculations. This work was supported by Fonds zur Foerderung der Wissenschaftlichen Forschung grant S-4610 to G.U., and by National Institutes of Health grant HL-40613 to E.B.

References

Asakawa J, Takahashi N, Rosenblum BB, Neel JV (1985) Two-dimensional gel studies of genetic variation in the plasma proteins of Amerindians and Japanese. Hum Genet 70:222–230

Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK (1989) The LDL-receptor-related protein, LRP, is an apolipoprotein E–binding protein. Nature 341:162–164

Boerwinkle E, Sing CF (1987) The use of measured genotype information in the analysis of quantitative phenotypes in man. III. Simultaneous estimation of the frequencies and effects of the apolipoprotein E polymorphism and residual polygenetic effects on cholesterol, betalipoprotein and triglyceride levels. Ann Hum Genet 51:211–226

Boerwinkle E, Utermann G (1988) Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. Am J Hum Genet 42:104–112

Boerwinkle E, Visvikis S, Welsh D, Steinmetz J, Hanash S, Sing CF (1987) The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, betalipoprotein and triglyceride levels in a sample of unrelated individuals. Am J Med Genet 27:567–582

Bouthillier D, Sing CF, Davignon J (1983) Apolipoprotein E phenotyping with a single gel method: application to the study of informative matings. J Lipid Res 24:1060–1069

Brown MB, Forsythe AB (1974) The small sample behavior of some statistics which test the equality of several means. Technometrics 16:129–132

Davignon J, Gregg RE, Sing CF (1988) Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1–21

Efron B (1982) The jackknife, the bootstrap and other resampling plans. Society for Industrial and Applied Mathematics. Philadelphia

Ehnholm C, Lukka M, Kuusi T, Nikkila E, Utermann G (1986) Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. J Lipid Res 27:227–235

Eto M, Watanabe K, Ishii K (1986) Reciprocal effects of apolipoprotein E alleles (ε2 and ε4) on plasma lipid levels in normolipidemic subjects. Clin Genet 29:477–484

Falase AO, Cole TO, Osuntokun BO (1972) Myocardial infarction in Nigerians. Trop Geogr Med 25:147–150

Falconer DS (1985) A note on Fisher's 'average effect' and 'average excess.' Genet Res 46:337–347

FAO (1983) Review of food consumption surveys, 1981 (household food consumption by economic groups). Food and Agriculture Organization of the United Nations, Rome

——— (1984) Food balance sheets: 1979–1981 average. Food and Agriculture Organization of the United Nations, Rome

——— (1987) The fifth world food survey. Food and Agriculture Organization of the United Nations, Rome

Freeman DH (1987) Applied categorical data analysis. Marcel Dekker, New York

Gregg RE, Zech LA, Schaefer EJ, Stark D, Wilson D, Brewer HB (1986) Abnormal in vivo metabolism of apolipoprotein E_4 in humans. J Clin Invest 78:815–821

Gregorius N-R, Namkoong G (1986) Joint analysis of genotype and environment effects. Theor Appl Genet 72:413–422

Harding RM, Sokal RR (1988) Classification of the European language families by genetic distance. Proc Natl Acad Sci USA 85:9370–9372

Herr DG (1986) On the history of ANOVA in unbalanced factorial designs: the first 30 years. Am Stat 40:265–270

Hui DY, Innerarity TL, Mahley RW (1984) Defective hepatic lipoprotein receptor binding of β-very low density lipoproteins from type III hyperlipoproteinemic patients: importance of apolipoprotein E. J Biol Chem 259:860–869

International Collaborative Study Group (1986) Metabolic epidemiology of plasma cholesterol: mechanisms of variation of plasma cholesterol within populations and between populations. Lancet 2:991–996

Keys A (1980) Seven countries: a multivariate analysis of death and coronary heart disease. Harvard University Press, Cambridge, MA, pp 121–135

Kobori S, Nakamura N, Uzawa H, Schichiri M (1988) Influence of apolipoprotein E polymorphism on plasma lipid and apolipoprotein levels, and clinical characteristics

of type III hyperlipoproteinemia due to apolipoprotein E phenotype E2/2 in Japan. Atherosclerosis 69:81–88
Kowal RC, Herz J, Goldstein JL, Esser V, Brown MS (1989) Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. Proc Natl Acad Sci USA 86:5810–5814
Kutner MH (1974) Hypothesis testing in linear models (Eisenhart model I). Am Stat 28:98–100
Lehtimäki T, Moilanen Tm, Viikari J, Åkerblom HK, Ehnholm C, Rönnemaa T, Marniemi J, et al (1990) Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6-year follow-up study. J Lipid Res 31:487–495
Levene H (1960) Robust tests for equality of variance. In: Olkin I, Ghurye SG, Hoeffding W, Madow WA, Mann HB (eds) Contributions to probability and statistics. Stanford University Press, Stanford, CA, pp 278–292
Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240: 622–630
Menzel H-J, Utermann G (1986) Apolipoprotein E phenotyping from serum by western blotting. Electrophoresis 7:492–495
Palmer RH, Nichols AV, Dell RB, Ramakrishnan R, Lindgren FT, Gong EL, Blum CB, et al (1986) Lack of relationship in humans of the parameters of body cholesterol metabolism with plasma levels of subfractions of HDL or LDL, or with apoE isoform phenotype. J Lipid Res 27: 637–644
Pearson ES, Hartley HO (1951) Charts of the power function for analysis of variance tests, derived from the noncentral F-distribution. Biometrika 38:112–130
Rall SC, Weisgraber KH, Mahley RW (1982) Human apolipoprotein E: the complete amino acid sequence. J Biol Chem 257:4171–4178
Robertson FW, Cumming AM (1985) Effects of apoprotein E polymorphism on serum lipoprotein concentration. Arteriosclerosis 5:283–292
Ruhlen M (1987) A guide to the world's languages, vol 1: Classification. Stanford University Press, Stanford, CA
Scheffé H (1959) The analysis of variance. J Wiley, New York
Schneider WJ, Kovanen PT, Brown MS, Goldstein JL, Utermann G, Weber W, Havel RJ, et al (1981) Familial dysbetalipoproteinemia: abnormal binding of mutant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits, and cows. J Clin Invest 68:1075–1085
Sepehrnia B, Kamboh MI, Adams-Campbell LL, Bunker CH, Nwankwo M, Majumder PP, Ferrell RE (1989) Genetic studies of human apolipoproteins. X. The effect of the apolipoprotein E polymorphism on quantitative levels of lipoproteins in Nigerian blacks. Am J Hum Genet 45: 586–591
Shaper AG (1974) Coronary heart disease. In: Shaper AG, Hutt MSR, Feffar Z (eds) Cardiovascular disease in the tropics. British Medical Association, London, pp 148–159
Shriver MD, Boerwinkle E, Hewett-Emmett D, Hanis CL (1991) Frequency and effects of the apolipoprotein E polymorphism in Mexican-American non-insulin-dependent diabetics. Diabetes 40:334–337
Sing CF, Davignon J (1985) Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 37:268–285
Sing CF, Moll PP (1989) Genetics of variability of CHD risk. Int J Epidemiol 18 [Suppl 1]: S183–S195
Speed FM, Hocking RR (1976) The use of the R( )-notation with unbalanced data. Am Stat 30:30–33
Tan PY, Ng TB, Saha N (1984) Dietary intakes of three ethnic groups of Singapore. Proc Nutr Soc 43:135A
Templeton AR (1986) The general relationship between average effect and average excess. Genet Res 49:69–70
Templeton AR, Sing CR, Kessling A, Humphries S (1988) A cladistic analysis of phenotype associations with haplotypes inferred from restriction endonuclease mapping. II. The analysis of natural populations. Genetics 120:1145–1154
Tikkanen MJ, Huttunen JK, Ehnholm C, Pietinen P (1990) Apolipoprotein E_4 homozygosity predisposes to serum cholesterol elevation during high fat diet. Arteriosclerosis 10:285–288
Tsuchiya S, Yamanouchi Y, Onuki M, Yamakawa K, Miyazaki T, Taya T, Kondo I, et al (1985) Frequencies of apolipoprotein E5 and E7 in Japanese. Jpn J Hum Genet 30:271–278
Utermann G (1985) Genetic polymorphism of apolipoprotein E—impact on plasma lipoprotein metabolism. In: Crepaldi G, Tiengo A, Baggio G (eds) Diabetes, obesity, and hyperlipidemias. III. Proceedings of the 4th European Symposium on Metabolism. Elsevier, Amsterdam, pp 1–27
——— (1987) Apolipoprotein E polymorphism in health and disease. Am Heart J 113:433–440
Utermann G, Hees M, Steinmetz A (1977) Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. Nature 269:604–607
Utermann G, Pruin N, Steinmetz A (1979) Polymorphism of apolipoprotein E. III. Effect of a single polymorphic gene locus on plasma lipid levels in man. Clin Genet 15: 63–72
Utermann G, Weisgraber KH, Weber W, Mahley RW (1984) Genetic polymorphism of apolipoprotein E: a variant form of apolipoprotein E2 distinguished by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. J Lipid Res 25:378–382
Wang K (1986) Studies of human apolipoprotein E genetic isoforms and their phenotypes among the Chinese population. Acta Acad Med Sinicae 8:198
WHO MONICA Project (1988) Geographical variation in

the major risk factors of coronary heart disease in men and women aged 35–64 years. World Health Stat Q 41: 115–140
Williams AO (1971) Coronary atherosclerosis in Nigeria. Br Heart J 33:95–100
Xu C-F, Boerwinkle E, Tikkanen MJ, Huttunen JK, Humphries SE, Talmud PJ (1990) Genetic variation at the apolipoprotein gene loci contribute to response of plasma lipids to dietary change. Genet Epidemiol 7:261–275
Zannis VI, Breslow JL, Utermann G, Mahley RW, Weisgraber KH, Havel RJ, Goldstein JL, et al (1982) Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes. J Lipid Res 23:911–914