Am J Hum Genet 37:000–000, 1985

Role of the Apolipoprotein E Polymorphism in Determining Normal Plasma Lipid and Lipoprotein Variation

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SUMMARY

The structural gene locus for apolipoprotein E (apo E) is polymorphic. Three common alleles (ϵ_2, ϵ_3, ϵ_4) code for three major isoforms in plasma and determine six apo E phenotypes that may be identified by isoelectric focusing on polyacrylamide. To establish what fraction of the inherited variation in a normal plasma lipid and lipoprotein profile is attributable to the segregation of the common alleles at the apo E gene locus, we have estimated the average apo E allelic effects on plasma cholestereol (C), triglycerides, very low-density lipoprotein (VLDL)-C, VLDL-apo B, low-density lipoprotein (LDL)-C, LDL-apo B, and high-density lipoprotein (HDL)-C in a representative sample of normolipidemic individuals from Ottawa, Canada. Data from published studies were also analyzed by the same statistical procedures.

As much as 16% of the genetic variance (8.3% of the total variance) for LDL-C could be accounted for by the apo E gene locus. After correction for differences in age, sex, height, and weight, it was found that the ϵ_2 allele lowered and the ϵ_4 allele raised total cholesterol, LDL-C, and LDL-apo B. No other gene has been identified that contributes as much to normal cholesterol variability. Analysis of these data and those of others also indicates that the apo E locus imparts a differential susceptibility to a variety of factors that promote hyperlipidemia. The hypothesis is proposed that the ϵ_2 allele protects

Received August 9, 1984; revised December 4, 1984.

This study was supported in part by grant HL-24489 from the National Institutes of Health, contract DE-AC02-82ER60089 from the Department of Energy, grants MT-5427 from the Medical Research Council of Canada and 6605-2087-52 from the National Health Research and Development Program, and the Quebec Heart Foundation.

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against coronary heart disease (CHD) and, hence, gives a reproductive advantage that is balanced by a predisposition to CHD when the ϵ_2 is combined with a second, independent causative factor to give a reproductive disadvantage. A similar mechanism is proposed for the maintenance of the ϵ_4 allele in the population.

INTRODUCTION

Apolipoprotein E (apo E) is a normal constituent of plasma chylomicrons, very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) in man [1]. Its major source is the liver, but it is also synthesized by the kidney, the adrenals, and monocytes and is present in the cerebrospinal fluid [2–4]. This arginine-rich peptide binds with high affinity to receptors on the surface of liver and extrahepatic cells [5, 6]. As a result, apo E-containing lipoproteins, especially remnants of the triglyceride-rich lipoproteins, are rapidly taken up and catabolized through the process of receptor-mediated endocytosis [1, 7]. Furthermore, the apo E-containing HDL subfraction found in plasma is believed to play an important role in the return of cholesterol from the periphery to the liver (reverse cholesterol transport) [1, 3, 8, 9].

In man, the structural gene locus for apo E is polymorphic [10–12]. Three common alleles, designated ϵ_2, ϵ_3, and ϵ_4, code for three major apo E isoforms, respectively, E2, E3, and E4, and determine six apo E phenotypes that may be distinguished by isoelectric focusing [11–14]. Homozygotes for these alleles (phenotypes E2/2, E3/3, and E4/4) have only one major circulating isoform, whereas heterozygotes (E3/2, E4/2, and E4/3) have two. The protein products of the three alleles differ by an amino acid substitution at one or both of two sites (residues 112 and 158) on the 299 amino acid chain of the apo E molecule [15, 16]. E4 has arginine and E2 cysteine at both sites, while E3 has cysteine at site 112 and arginine at site 158. In addition to these common alleles, rare mutants have also been recently reported [17–19]. Furthermore, apo E circulates in several forms containing differing amounts of sialic acid acquired during post-translational modification [11, 20]. Treatment of plasma with neuraminidase transforms these glycosylated derivatives to the major corresponding isoforms. The three-allele polymorphism for the apo E gene has been demonstrated in all populations studied thus far [13, 21–25]. These studies have also established that the E3 isoform is by far the most common.

There is growing evidence that the apo E locus might be involved in predisposing one to hyperlipidemia. We have recently shown that the ϵ_2 allele is more prevalent in a lipid clinic population than in a group of normolipidemic volunteers [26]. An association with hyperlipidemia and atherosclerotic vascular disease is supported by other studies [24, 25, 27]. The protein specified by the ϵ_2 allele has much lower affinity for the lipoprotein receptor than those specified by the other two alleles [16]. Consequently, individuals homozygous for ϵ_2 (phenotype E2/2) are at increased risk for familial dysbetalipoproteinemia (type

III), a disease where apo E-containing triglyceride- and cholesterol-rich lipoproteins (β-VLDL) accumulate in the plasma and may contribute to the development of premature atherosclerosis [27]. Familial type III and the accumulation of β-VLDL in blood have also been reported in association with the E4/2 [21] and the E3/2 [24] phenotypes, as well as with the absence of apo E from plasma [28]. Factors promoting the development of hypertriglyceridemia in E2/2 individuals such as obesity, pregnancy, oral contraceptives, hypothyroidism, diabetes, estrogen withdrawal, or the presence of another hyperlipidemia gene [1, 21, 27, 29, 30] may also influence the other E2-bearing phenotypes [31]. As for the other alleles, a higher frequency of the ϵ_4 allele has been reported in familial mixed hypertriglyceridemia (type V) [32], but the ϵ_3 allele has yet to be associated with any of the lipid disorders.

Studies by Sing and Orr [33], Moll et al. [34], and Rao et al. [35] have established that about 50% of the variability in normal serum cholesterol levels is attributable to genetic differences among individuals. It remains to be determined what fraction of the inherited variation in a normal plasma lipid and lipoprotein profile is attributable to the segregation of the common alleles at the apo E gene locus. In a recent study of 16 different ago E matings occurring in the families of probands with hyperlipidemia, we noted that the mean cholesterol and LDL-C levels of 46 normolipidemic offspring were higher in the E4 phenotypes (E4/4, E4/2, E4/3) than in the combined remaining phenotypes [14]. Our data indicated that the ϵ_2 allele predisposes to higher triglyceride levels and lower LDL-C concentrations, whereas the ϵ_4 allele is associated with higher cholesterol and LDL-C levels.

We report here an analysis of the effects of the apo E alleles on the quantitative variation in plasma lipids and lipoproteins of a representative sample of normolipidemic individuals from the city of Ottawa, Canada. The estimates of the average allele effects in this sample combined with those estimated from published data demonstrate that as much as 16% of the genetic variance of plasma LDL-C may be associated with allelic differences at the apo E gene locus.

MATERIALS AND METHODS

Sample Definition

Blood samples were obtained after a 12-hr fast from 122 individuals taking part in a longitudinal study of HDL levels in a randomly selected cohort of healthy civil servants aged 20–59 years. They were recruited mainly within the public service in Ottawa from technical, professional, and clerical workers. Known diabetics and persons with blood disorders (hemophilia, anticoagulant therapy) were excluded. The samples were taken during follow-up from subjects whose plasma cholesterol and triglycerides had been normal ($<$ 240 mg/dL and $<$ 150 mg/dL, respectively) at a previous visit. Plasma was separated in the cold and sent on ice to our laboratory. Seventeen subjects were excluded because their plasma cholesterol and/or triglycerides measured in our laboratory exceeded the cutoff points, so that sporadic forms of hyperlipidemia were excluded. The low cutoff points also enhanced the likelihood of excluding genetically determined forms of lipid transport disorders. Three samples were excluded because of technical problems. The remaining 102 subjects comprised 55 men and 47 women with a mean age ($\pm$ SD, range) of 36.1 $\pm$ 8.5 (22–66) years. The height and weight of each individual were

recorded at the time of sampling. The average height, weight, and body-mass index (weight/height^2) for the males were 1.77 m, 75.6 kg, and 24.0, respectively. For the females, the averages, respectively, were 1.62 m, 56.8 kg, and 21.8. These values, as well as the distribution of their lipids and lipoproteins, are similar to those estimated for Canadian adults [36, 37].

Data from population studies already published were used for comparison and statistical analysis. These included blood donors in Marburg, Germany [13]; factory employees in Münster, West Germany [23]; "normal subjects" and hyperlipidemic patients from the greater Washington, D.C., area [24]; blood donors from Christchurch, New Zealand [22]; a representative sample of residents of the Grampian region of Scotland [25]; and hyperlipidemic individuals from greater Montreal [26].

Laboratory Procedures

The apo E phenotype was determined by the method of Bouthillier et al. [14]. Briefly, VLDL are separated from plasma (EDTA 1 mg/ml) by ultracentrifugation for 16 hrs at 105,000 *g* and washed once under the same conditions. An aliquot corresponding to 0.9 mg of triglycerides (about 150 μg protein) is delipidized twice with acetone/ethanol (1 : 1, v/v) and once with diethyl ether, then solubilized in 10 mM Tris-HCl, 8 M urea, and 10 mM dithiothreitol. The apo E isoforms are separated by isoelectric focusing on 7.5% polyacrylamide gel in 8 M urea with 5% ampholines (pH 4–6) at 4°C for 16 hrs at 250 V. The bands are stained with Coomassie Blue G 250, and the tubes in each run are aligned along the apo C bands and photographed. A known phenotype is included in each series for reference. Plasma total cholesterol [38] and triglycerides [39] were measured enzymatically on an automated analyzer (ABA-100, Abbott, Pasadena, Calif.). Plasma lipoprotein cholesterol content [38] was measured after separation of the lipoproteins according to the Lipid Research Clinics protocol [40]. Apo B concentrations were determined by electroimmunoassay (EIA) on total plasma and on the d > 1.006 g/mL ultracentrifugal fraction using the technique of Reardon et al. [41], which includes prior incubation with a bacterial lipase to expose antigenic sites on triglyceride-rich lipoproteins. The addition of lipoprotein-deficient serum (d > 1.21 g/mL) to the LDL standard as suggested by Rosseneu et al. [42] increased the accuracy of the measurement. VLDL-apo B was obtained by subtraction of the LDL-apo B from the total plasma value.

Statistical Methods

Prior to the analysis of the effects of genetic variability at the apo E gene locus for the Ottawa sample, each of the lipid and lipoprotein traits was adjusted by linear regression for variation in age, sex, height, and weight. A one-way analysis of variance was performed on the adjusted lipid and lipoprotein levels to test the null hypothesis that phenotypic variation is unaffected by the apo E locus. Scheffé's procedure for multiple comparisons was used to make specific contrasts among genotypic means. These standard statistical methods are presented in Neter et al. [43]. Allele frequencies (denoted f_{ϵ_i}, $i = 2, 3, 4$) were estimated by the gene counting method. The average effects of the three alleles in the population at large (α_i, $i = 2, 3, 4$) on each age, gender, height, and weight-adjusted trait were estimated according to the formulas:

$$\alpha_2 = \frac{f_{22}\,\overline{\text{E2/2}} + \tfrac{1}{2}f_{23}\,\overline{\text{E2/3}} + \tfrac{1}{2}f_{24}\,\overline{\text{E2/4}}}{f_{\epsilon_2}} - \hat{\mu}$$

$$\alpha_3 = \frac{f_{33}\,\overline{\text{E3/3}} + \tfrac{1}{2}f_{23}\,\overline{\text{E2/3}} + \tfrac{1}{2}f_{34}\,\overline{\text{E3/4}}}{f_{\epsilon_3}} - \hat{\mu}$$

$$\alpha_4 = \frac{f_{44}\,\overline{\text{E4/4}} + \tfrac{1}{2}f_{24}\,\overline{\text{E2/4}} + \tfrac{1}{2}f_{34}\,\overline{\text{E3/4}}}{f_{\epsilon_4}} - \hat{\mu}\ ,$$

where f_{ij} is the expected Hardy-Weinberg frequency and $\overline{E_{i/j}}$ is the average of the trait values for individuals with the $\epsilon_i\epsilon_j$ genotype. The symbol $\hat{\mu}$ denotes the estimate of the population mean. The variance attributable to genotypic differences was computed as

$$\widehat{\sigma_G^2} = \sum_{i=2}^{4} \sum_{j=2}^{4} f_{ij} (\overline{E_{i/j}} - \hat{\mu})^2 .$$

The genetic variance may be subdivided as $\sigma_G^2 = \sigma_A^2 + \sigma_D^2$, where σ_A^2 is the fraction attributable to the average effects of the alleles at the apo E locus and σ_D^2 is the fraction attributable to the nonadditive interactions (dominance) between these alleles. Estimates of these sources of variation were taken to be

$$\widehat{\sigma_A^2} = 2 \sum_{i=2}^{4} f_{\epsilon_i} \alpha_i^2$$

and $\widehat{\sigma_D^2} = \widehat{\sigma_G^2} - \widehat{\sigma_A^2}$.

RESULTS

The relative frequencies of the three apo E alleles in the Ottawa sample are typical of those estimated from other studies of normolipidemic individuals (table 1). In every sample, the genotype frequencies do not deviate significantly from those predicted by the Hardy-Weinberg equilibrium. However, a statistically significant chi-square for the test of heterogeneity among samples (20.52, 10 df, $P < .05$) suggests that there may be population differences. The two large samples from Germany, one a study of blood-bank donors by Utermann et al. [13] and the other a study of factory workers by Menzel et al. [23], have nearly

TABLE 1

ALLELE FREQUENCIES IN SEVERAL POPULATIONS

		RELATIVE FREQUENCY		
	No.	ϵ_2	ϵ_3	ϵ_4
"Normolipidemic" samples:				
Ottawa, Canada [26]	102	.078	.770	.152
Germany [13]	1,031	.077	.773	.150
Germany [23]	1,000	.078	.783	.139
Washington, D.C. [24]	74	.095	.756	.149
Grampian, Scotland [25]	400	.080	.770	.150
Christchurch, New Zealand [22]	426	.120	.720	.160
Weighted mean	3,033	.085	.768	.147
Hyperlipidemic samples*:				
Type IIa	61 (11)†	.050	.743	.207
Type IIb	81 (7)	.124	.753	.123
Type III	16 (4)	.875	.125	.000
Type IV	121 (76)	.149	.694	.157
Type V	39 (4)	.128	.359	.513

* Frequencies are based on pooled sample of patients from Washington, D.C. [24] and Montreal [26].
† No. subjects from Montreal given in parentheses.

identical allele frequencies. The relative frequencies estimated for the smaller Ottawa [26] and Washington, D.C., [24] samples do not differ significantly from those estimated for the German samples, and those for the Grampian region of Scotland [25] are again very similar. However, the estimates given by Wardell et al. [22] for a community in New Zealand are significantly different from the pooled German sample ($\chi^2 = 15.28$, 2 df, $P < .001$).

The higher frequency of ϵ_2, and lower frequency of ϵ_3, in the New Zealand sample is typical of the profile observed in selected subsamples of lipid clinic patients (table 1). The allele frequencies for samples of patients with type III, IV, and V hyperlipidemia, but not types IIa and IIb, are significantly different from the weighted averages of the normolipidemic samples. There is an enrichment of the ϵ_2 allele in type III and type IV. Every type III individual studied had the ϵ_2 allele. In contrast, there is more of the ϵ_4 allele in the subset with type V hyperlipidemia. However, the deviations of the frequencies of the New Zealand sample from the other normolipidemic samples are minor; in fact, there is remarkable homogeneity among populations for the relative frequencies of the three common alleles for the apo E gene locus. The ϵ_3 allele is the most and the ϵ_2 allele the least frequent in every normolipidemic sample studied thus far. Furthermore, in all samples except type III and type V, the rankings of the relative frequencies are the same as for normolipidemic samples.

The Ottawa sample of lipid and lipoprotein data was first analyzed to determine how each phenotype was affected by concomitant variables. Concomitant variation in age, sex, height, and weight combined to account for a fraction of the variance in plasma cholesterol (14.2%), HDL-C (18.8%), LDL-C (14.8%), VLDL-C (11.6%), triglycerides (10.1%), LDL-apo B (11.4%), and VLDL-apo B (3.2%). In each case, adjustment for these measured sources of individual variability resulted in a statistically significant ($P < .01$) reduction in variance.

The value for each adjusted variable, averaged over individuals with the same apo E genotype is shown in table 2. There is statistically significant variability in the average total cholesterol ($P < .09$) and LDL-C ($P < .08$) among genotypes. Since most of the plasma cholesterol is carried in the LDL fraction, it is not surprising that a similar effect for the apo E genotype was observed on LDL-C. For both variables, the E2/2 class has the lowest mean. The average total cholesterol of genotypes carrying the ϵ_2 allele (E2/2, E3/2, E4/2) is 16.2 mg/dL lower than the average of those not having the gene. The average LDL-C is 16.1 mg/dL lower. Both differences are significant at $P < .05$. In contrast, for individuals with the ϵ_4 allele (E4/2, E4/3, E4/4), the total cholesterol is 11.5 mg/dL higher and the LDL-C 9.6 mg/dL higher than for those without it. Both differences are significant at $P < .05$.

There is no evidence in this sample for significant ($P < .10$) variation among apo E genotypes in the average values of HDL-C, VLDL-C, triglycerides, and VLDL-apo B or the ratios of LDL-C to total cholesterol and VLDL-C to triglycerides. The variation of the average LDL-apo B among the six genotypic classes was statistically significant at the .13 level of probability, those with the ϵ_2 allele having a significantly lower average LDL-apo B ($P < .05$). Genotypes with the ϵ_2 allele had an average LDL-apo B 12.1 mg/dL lower than those

TABLE 2

SERUM LIPID LEVELS IN THE SAMPLE OF 102 FROM OTTAWA, ADJUSTED FOR LINEAR AGE, SEX, HEIGHT, AND WEIGHT EFFECTS

	GENOTYPE						PROBABILITY F RATIO	ESTIMATES OF THE POPULATION	
	E4E4	E3E3	E2E2	E4E3	E3E2	E4E2		Mean	Variance
Count	4	63	2	21	10	2	...	...	...
Relative frequency	0.039	0.618	0.020	0.206	0.098	0.020	...	...	...
Variable:									
Total cholesterol	180.3	173.8	136.0	183.5	161.4	178.1	0.09	174.16	732.48
HDL-C	53.3	47.3	47.1	47.3	45.7	45.2	0.93	47.32	130.43
LDL-C	102.9	104.2	73.9	112.8	89.5	109.3	0.08	104.00	602.17
VLDL-C	24.0	22.3	15.0	23.3	26.2	23.5	0.83	22.83	127.56
Triglycerides	65.5	74.4	70.6	70.4	79.3	73.5	0.96	73.60	918.01
LDL-Apo B	86.3	83.9	55.8	86.8	78.4	60.5	0.13	83.03	375.72
VLDL-Apo B	8.5	11.4	5.65	10.5	9.2	18.9	0.56	10.91	66.21
LDL-C/total cholesterol	0.56	0.60	0.55	0.61	0.55	0.61	0.26	0.59	0.0055
VLDL-C/total triglycerides	0.37	0.32	0.19	0.33	0.33	0.32	0.79	0.32	0.0200

without the allele. Subjects carrying the ϵ_4 allele had a 2.41 mg/dL higher average LDL-apo B concentration, but the comparison with those not having the allele was not statistically significant.

Inspection of the genotypic means suggests that the E2/2 class may also have the lowest VLDL-C, LDL-apo B, and VLDL-apo B. Highest values of HDL-C and triglycerides were associated with the E4/4 genotype. However, because of the small numbers of subjects with these phenotypes, the comparisons with the remainder of the sample are not statistically significant and these findings only show a tendency. On the other hand, the consistency of the association of the ϵ_2 and ϵ_4 alleles with significant differences in total cholesterol, LDL-C, and LDL-apo B definitely points to a unique effect of this gene locus on the control of lipid metabolism.

Before considering the average effects of the apo E alleles on lipids and lipoprotein levels, we asked whether the associations of the lipid and apolipoprotein traits were homogeneous among the classes of genotypes. Table 3 presents the estimates of the correlation coefficients for the Ottawa data set. Total cholesterol, LDL-C, and LDL-apo B are strongly correlated. The VLDL-C/total cholesterol correlation, although half as large (.37), is statistically significant, whereas the correlation of total cholesterol and HDL-C is not significantly different from zero. Triglycerides are negatively correlated with HDL-C (−.50) and positively correlated with VLDL-C (.62) and VLDL-apo B (.42). There was no evidence for significant heterogeneity of any of the correlation coefficients between the group of individuals with the ϵ_2 allele (or the ϵ_4 allele) compared with the remainder of the sample. The correlations between the lipid traits are typical of other normolipidemic populations [44].

In table 4, we summarize the estimates of the average effects (α) of the three apo E alleles on each of the lipid and lipoprotein traits. Estimates from the cholesterol, HDL, and triglyceride data published by Menzel et al. [23] are given for comparison. A general tendency is clear in the Ottawa data. The overall influence of the ϵ_2 allele is to decrease total cholesterol and both the cholesterol and the apo B of the LDL fraction, while causing a modest increase (not statistically significant in this sample) in VLDL-cholesterol and triglycer-

TABLE 3

CORRELATION COEFFICIENTS BETWEEN SERUM LIPID AND LIPOPROTEIN TRAITS (OTTAWA) AFTER ADJUSTMENT FOR LINEAR AGE, SEX, HEIGHT, AND WEIGHT EFFECTS

	Total cholesterol	LDL-C	Apo-B LDL	HDL-C	VLDL-C	Apo-B VLDL	Triglycerides
Total cholesterol	1.00	.86	.65	.15	.37	.11	.25
LDL-C	...	1.00	.75	−.17	.08	.09	.23
Apo-B LDL	...	...	1.00	−.23	.17	.05	.27
HDL-C	...	...	...	1.00	−.27	−.29	−.50
VLDL-C	...	...	...	...	1.00	.36	.63
Apo-B VLDL	...	...	...	...	...	1.00	.42
Triglycerides	...	...	...	...	...	...	1.00

NOTE: Absolute values exceeding .327 are significantly different from zero at the .001 level of probability.

TABLE 4

ESTIMATES OF THE AVERAGE EFFECTS (mg/dL) OF THE THREE COMMON APO E ALLELES ON THE SERUM LIPID AND LIPOPROTEIN TRAITS IN THE OTTAWA POPULATION COMPARED TO ESTIMATES DERIVED FROM DATA PRESENTED BY MENZEL ET AL. [23]

	Relative frequency	Average effect of the allele (alpha*)						
		Cholesterol	HDL-C	LDL-C	VLDL-C	Triglycerides	Apo-B LDL	Apo-B VLDL
Ottawa, Canada								
ϵ_2	.078	−12.60	−1.44	−13.06	1.89	3.94	−9.33	−0.56
ϵ_3	.770	−0.29	−0.03	0.01	−0.28	0.36	0.66	0.08
ϵ_4	.152	7.99	0.90	6.68	0.42	−3.86	1.45	−0.11
Munster, Germany†								
ϵ_2	.078	−11.54	−0.10	...	...	+3.13	...	...
ϵ_3	.783	0.34	0.03	...	...	−0.49	...	...
ϵ_4	.139	4.57	−0.10	...	...	1.02	...	...

* See MATERIALS AND METHODS for the estimator of alpha.
† Data not adjusted for age, height, and weight.

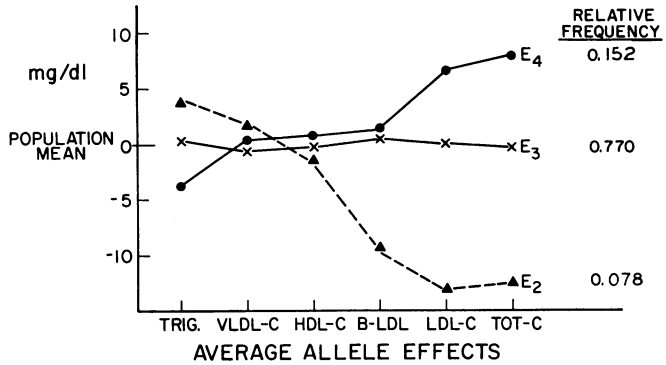

FIG. 1.—Average allele effects

ides. The ϵ_3 allele does not seem to influence any of the seven traits studied. The ϵ_4 allele has an effect opposite to that of the ϵ_2 allele: it significantly increases total cholesterol, LDL-C, and LDL-apo B and is also associated with a small lowering of triglycerides. A graphical representation of these effects is given in figure 1, where a reordering of the traits emphasizes the magnitude of the allele effect on total cholesterol and LDL-C. Estimates (table 4) of the average effects of the apo E alleles on total cholesterol from the data presented by Menzel et al. [23] are very similar to those estimated from the Ottawa sample. An insignificant impact on HDL-C levels and a minor effect on triglycerides are also consistent with the Ottawa findings.

Estimates of the contribution of apo E to the total variation of each of the lipid and lipoprotein phenotypes in the Ottawa population are given in table 5. The formula for the variance attributable to the average effects of the three alleles, σ_A^2, and the deviations due to dominance, σ_D^2, are given under MATERIALS AND METHODS. For each trait, the estimate of the total genotypic variance for the Ottawa population is subdivided into a component ascribable to the average allelic effects presented in table 4 and the remainder due to genotypic differ-

TABLE 5

CONTRIBUTING OF THE APO E LOCUS TO THE TOTAL VARIABILITY OF SERUM LIPID AND LIPOPROTEIN LEVELS IN THE OTTAWA POPULATION

	ESTIMATES OF VARIANCE (%)			
	σ_A^2 allelic effects	σ_D^2 nonallelic effects	Residual	Total
Cholesterol	44.49 (6.1)	5.68 (0.8)	682.31 (93.1)	732.48
HDL-C	0.57 (0.4)	0.68 (0.5)	129.18 (99.1)	130.43
LDL-C	40.33 (6.7)	9.52 (1.6)	552.32 (91.7)	602.17
VLDL-C	0.73 (0.6)	1.29 (1.0)	125.54 (98.4)	127.56
Triglycerides	7.16 (0.8)	0.99 (0.1)	909.86 (99.1)	918.01
LDL-apo B	14.98 (4.0)	8.34 (2.2)	352.40 (93.8)	375.72
VLDL-apo B	0.06 (0.0)	2.27 (3.4)	63.88 (96.6)	66.21

NOTE: Total variability is after correction for age, sex, height, and weight variation.

ences not explained by additivity of the allelic effects. The total genetic variance ($\sigma_A^2 + \sigma_D^2$) associated with the apo E locus as a percentage of the phenotypic variance adjusted for age, sex, height, and weight ranges from less than 1% (triglycerides) to 8.3% (LDL-C). The main effects of the allelic variation of the apo E gene are on the variability of total cholesterol (6.9%), LDL-C (8.3%), and apo B from LDL (6.2%). Assuming that the heritability of plasma total cholesterol level is 50%, the apo E gene may be responsible for as much as 14% of the total polygenic variance. The estimates of the average effects of the alleles (table 4) and the variance (table 5) for each of the traits suggest that the apo E polymorphism primarily affects LDL-C and the associated apo B and, secondarily, the level of triglycerides. Similar estimates of the average effects of the three alleles on total cholesterol collected by Menzel et al. [23] suggest a general effect of this locus on plasma cholesterol variation.

DISCUSSION

Although biometrical studies have established that 50% of the interindividual variability in normal serum cholesterol is associated with polygenic differences [33–35], no single gene locus has previously been shown to make a sizable contribution to the population variance. In a study of a large community (sample size > 9,000), Sing and Orr [45] identified four out of 12 polymorphic genetic blood and serum markers as having a significant effect on cholesterol levels. However, none could be implicated as being physiologically involved in cholesterol metabolism, and together these unlinked genes explained less than 1% of the total variance.

The apo E gene locus in man is the first polymorphic gene to be described that is metabolically involved in the determination of normal interindividual variation in the major plasma lipoprotein fractions and their components. The analysis of the Ottawa sample indicates that as much as 7% of total phenotypic variance (14% of the polygenic variance) for serum cholesterol may be explained by this one gene locus. For LDL-C, 8.3% of the total phenotypic variance is explained by this locus. The impact of this gene on population variability is far greater than the < 0.05% that may be attributed to the segregation of the rare dominant gene responsible for familial hypercholesterolemia (FH) [46]. Taken together, the genetic studies of serum cholesterol are beginning to reveal a genetic architecture responsible for the phenotypic expression of lipid and lipoprotein profiles. There are probably a very small number of loci with rare alleles that have large effects (like FH) and a large number of loci with polymorphic alleles (like apo E and those loci linked to the genetic markers studied by Sing and Orr [45]), each of which has a small effect on the determination of serum values.

It has now been clearly established in several populations that the apo E gene locus is polymorphic for three alleles [10, 11, 13, 14, 21–26]. The least frequent allele is carried by at least 16% of the individuals in the population. Furthermore, it is apparent (table 1) that the relative frequency of each allele is remarkably similar in populations that are geographically isolated in very different ecological settings. What are the forces that are responsible for this similarity

of allele frequencies? Most plant and animal populations are polymorphic for 30%–50% of gene loci coding for structural gene products [47, 48]. The locus coding for apo E is typical of such loci [49, 50]. However, despite the widespread agreement on the ubiquity of polymorphic genetic variability for structural proteins, there are few documented examples that can explain the maintenance of variation. Polymorphic allele frequencies for a gene locus within a Mendelian population may be a consequence of a number of forces operating on the reproduction of the gene pool (see [51] for a review). Two commonly considered mechanisms for the maintenance of a genetic polymorphism are random genetic drift of a mutant allele that is neutral with respect to reproductive fitness and balancing selective forces [52]. The most likely selection schemes are: (1) different fitness values for an allele in different environments, (2) temporally varying environments that select positively for an allele at one time in the life cycle and negatively at another time, and (3) gene-gene interaction that results in the fitness of an allele being determined by allelic variation at a second gene locus. Studies to distinguish these hypotheses have focused on: (1) efforts to understand the geographical distribution of allele frequencies, (2) correlation of allele frequencies with measures of the ecology of the population, and (3) characterization of phenotypic differences that may contribute to fitness differences between alleles. Because the parameters that define population size, migration between populations, mutation rate, and fitness differences are so difficult to measure, little progress has been made in explaining the causes of any but a very few of the known genetic polymorphisms. Information is now becoming available at the gene, physiological, and clinical levels, and the components of fitness that contribute to determining the causes for the apo E polymorphism may therefore be nearer at hand. In fact, the evidence presented here and elsewhere argues that selection may play a significant role in determining the distribution of the three alleles. This evidence comes from the geographical distribution of alleles and their physiological and clinical impact on the individual.

With respect to geographic distribution, if we assume that the apo E alleles have no differential effect on the reproductive fitness of the alternative genotypes, the similarity of relative frequencies for populations as scattered as Germany, Canada, Scotland, and New Zealand would be expected only if (1) there were one large Mendelian population that had recently split up or (2) there were a high degree of gene flow between these populations. Neither of these circumstances obtains. Furthermore, the similarity of the allele frequencies is far greater than is to be expected from the observed heterogeneity among these populations for the allele frequencies of other polymorphic genes (reported by Mourant et al. [53] and considered by Cavalli and Bodmer [54]). Admittedly, we have far too little information on the history of the formation of the populations studied to reject the possible role of random genetic drift completely: the homogeneity of the frequency data strongly suggests that we should explore further the hypothesis that a common force determines differential reproductive fitnesses that are stabilizing the allele frequencies.

A primary physiological effect of the apo E gene locus in the normolipidemic

individual is expressed mainly on the low-density lipoprotein fraction, LDL-C and LDL-apo B being influenced to nearly the same extent. In a smaller study of the normolipidemic offspring of hyperlipidemic parents sampled from the Montreal population, we reported a statistically significant effect of allelic variation at the apo E locus on total serum cholesterol and LDL-C levels [14]. The ϵ_2 allele was associated with lower and the ϵ_4 with higher values of these two variables. These effects are confirmed in the larger randomly selected sample of Ottawa civil servants (tables 2 and 4, fig. 1). That this is a general effect of this gene locus is further supported by the recent report of Menzel et al. [23], who compared lipid levels and frequencies of the apo E phenotypes in a sample of 1,033 normal controls and subjects with documented coronary artery disease. We have estimated the average effects of the apo E alleles (table 4) based on the reported genotypic means and frequencies for the normal controls. The average effect of the ϵ_2 allele was to lower total cholesterol by 11.5 mg/dL relative to the average of the population. The ϵ_4 allele elevated total cholesterol by 4.57 mg/dL. These estimates compare well with the 20 mg/dL difference between the average levels for the two alleles in the Ottawa sample. In both studies, the average effect of the ϵ_3 allele was nil. The estimates of the allele effects on HDL-C and triglycerides from the German sample were also similar to those from the Ottawa sample (table 4).

Relating these physiological effects of the apo E polymorphism to reproductive differences will be difficult. The relationship between physiological adaptation and reproductive fitness is difficult to establish, even for experimental organisms when the gene of interest can be measured and the environmental factors manipulated. To measure the differential reproduction of individuals with different phenotypes, longitudinal data on survival and reproductive success over at least 1 generation will be necessary. The relationships of the apo E alleles to lipid levels and lipid levels to coronary heart disease (CHD) suggest a component of reproductive fitness that may explain, at least partially, the operation of balancing selection that is responsible for maintenance of the three alleles. Comparison of our findings in the Ottawa sample with those from the study of pedigrees related to by a hyperlipidemic proband [14] suggests that the three alleles may be involved in two types of effects on the lipid phenotypes and susceptibility to CHD. Removing the hyperlipidemics from the analysis of pedigrees did not alter the differences among apo E genotypes for total cholesterol and LDL-C whereas a dramatic lowering of the VLDL-C and triglyceride levels for the E2/2 and E2/3 genotypes was observed when hyperlipidemics were not considered. The ϵ_2 has a lowering effect on total cholesterol and LDL-C and ϵ_4 has an elevating effect that is independent of the effect of the elevation of VLDL-C and triglycerides observed in a fraction of the ϵ_2-carrying genotypes with hyperlipidemia. The fact that we observe the same effects on total cholesterol and LDL-C regardless of whether or not we consider those with hyperlipidemia suggests that there is an interaction of a second factor, genetic or environmental, that triggers the susceptible ϵ_2 genotypes to develop hyperlipidemia. The net effect is that the ϵ_2 allele may have a selective advantage in

some individuals and a disadvantage in others. Since the incidence of CHD and other manifestations of atherosclerosis is directly related to the plasma concentration of LDL-C [55], one could infer that subjects with the ϵ_2 allele who have lower levels of this atherogenic lipoprotein gain a selective advantage over the bearer of the ϵ_3 allele. This would confer a relative protection against the development of atherosclerosis only as long as the plasma lipids remain within the normal range and no other atherogenic molecules such as the VLDL are induced by the predisposing factors such as obesity, diabetes, hypothyroidism, or interaction with another hyperlipidemia gene [1, 21, 26, 27, 30, 31, 56]. Indeed, apo E2 isolated from E2/2 subjects with low, normal, or high plasma lipid levels has the same structural and receptor-binding defects [56]. This indicates that it is necessary to overload the homeostasis of lipid metabolism with a second factor if hyperlipidemia is to develop [57]. In 1979, Utermann et al. reported [57] that subjects with the apo E-D and apo E-ND phenotypes (mostly E2/2 and E3/2 in the current nomenclature) had lower total cholesterol concentrations and put forward the hypothesis that this would afford relative protection against atherosclerosis. The lower LDL-cholesterol of the ϵ_2-bearing phenotypes might be explained on the basis of a reduced conversion of VLDL to LDL [58, 59]; the former accumulating only if input into the system is increased. Plasma apo B closely follows the changes observed with LDL-C in the present study (table 2). It is of interest that the low incidence of CHD in Pima Indians has been tentatively ascribed to the maintenance of low levels of plasma cholesterol by a reduced conversion of VLDL-apo B to LDL-apo B [60].

A selective advantage gained by ϵ_4-bearing individuals is more difficult to conceptualize since they have higher LDL-C levels. However, they also tend to have higher HDL-C concentrations, and although only a nonsignificant tendency in this direction is seen in the Ottawa population, it is consistent with the data of Menzel et al. [23] in a larger sample. In addition, small absolute differences in HDL-C may be quite meaningful in relative terms since this represents a small fraction of the total plasma cholesterol. Expressed in percentages, the ϵ_2 allele lowers HDL-C 3.1% and lowers LDL-C 12.5%, whereas the ϵ_4 allele increases HDL-C 1.9% and increases LDL-C 6.4% (table 4). The higher LDL-C in individuals with the ϵ_4 allele might result from a more efficient catabolism of VLDL [58]. Indeed, Gregg et al. [61] recently showed that in contrast to apo E3, apo E4 is distributed differently among the lipoprotein fractions and has an enhanced catabolic rate with a shorter residence time in plasma. This indicates a more rapid clearance of VLDL particles with apo E4. Perhaps this constitutes another selective advantage by preventing the accumulation of atherogenic forms of triglyceride-rich lipoproteins (chylomicron remnants, β-VLDL). This protective mechanism would be operative as long as LDL catabolism is not impaired to the point of raising LDL-C concentrations to levels where the risk of CHD rapidly rises (CHD risk is actually an exponential function of LDL-C concentrations [55]). As with the ϵ_2 allele, the selective advantages of the ϵ_4 allele in normolipidemic individuals (enhanced VLDL catabolism) may be bal-

anced by a selective disadvantage when those carrying the allele have a susceptibility to type V hyperlipidemia. The markedly higher frequency of the ϵ_4 allele in type V (table 1) supports this argument [32].

In conclusion, the work presented here and elsewhere argues that the apo E gene plays a major role in determining interindividual differences in serum cholesterol and predisposition to CHD when the ϵ_2 interacts with a second independent causative factor, which then results in a reproductive disadvantage. A similar association of the ϵ_4 allele with type V hyperlipidemia may also contribute to the balancing of genetically selective forces.

ACKNOWLEDGMENTS

This work was made possible by the generous collaboration of Drs. Joyce Beare-Rogers, Pamela Verdier, and Maurice Collins of the Bureau of Nutritional Sciences in Ottawa, who secured the plasma samples. The technical assistance of Daniel Bouthillier, M.Sc., and Michel Tremblay is also gratefully acknowledged.

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