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Genetic Analysis of Plasma Sitosterol, Apoprotein B, and Lipoproteins in a Large Amish Pedigree with Sitosterolemia

T. H. BEATY,[1] P. O. KWITEROVICH, JR.,[2] M. J. KHOURY,[1] S. WHITE,[2] P. S. BACHORIK,[2] H. H. SMITH,[2] B. TENG,[3] AND A. SNIDERMAN[3]

SUMMARY

We previously reported the finding of phytosterolemia, xanthomatosis, and hyperapobetalipoproteinemia (hyperapoB) in five siblings in a large Amish pedigree ascertained through a 13-year-old boy who died suddenly from advanced coronary atherosclerosis. Here, we present further analyses of the plasma levels of the plant sterol, sitosterol, of low density (beta) lipoprotein (LDL) sterol, and of LDL B protein. Of 254 relatives and spouses of the proband, 90.5% were examined. A series of genetic models were explored using a pedigree analysis where parameters reflecting frequency, transmission, and penetrance of putative genotypes were examined simultaneously using a maximum likelihood approach. Segregation analysis of the sitosterol levels showed that the phenotype of sitosterolemia was controlled by a rare autosomal recessive gene. There was also significant familial correlation in plasma sitosterol levels that was attributed to a polygenic component under a mixed model but could also be due to shared environments such as diets. The recessive model was supported by our finding that the plasma sitosterol levels in the parents and in six children born to three of the five sitosterolemics were less than 1 mg/dl, well within the normal range.

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[1] Department of Epidemiology, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD 21205.

[2] Department of Pediatrics, Lipid Research-Atherosclerosis Unit, Johns Hopkins Medical Institutes, Baltimore, MD 21205.

[3] Cardiovascular Reserch Unit, Royal Victoria Hospital, McGill University, Montreal H3G 1Y6, Canada.

The phenotype of hyperapoB is based on an elevated level of LDL B protein in the presence of a norma! LDL cholesterol level (low LDL sterol to LDL B ratio). For both LDL sterol and LDL B, a polygenic model showed a slightly greater improvement in ln likelihood than did the Mendelian single locus model when both were compared to a sporadic model. Similar results were obtained for sterol levels of high density (alpha) lipoprotein (HDL) sterol. When segregation analysis was performed using the ratio of LDL sterol to LDL B, the Mendelian single locus model gave a slightly better fit to the data than did the polygenic model. While the analyses presented here provided unequivocal evidence for the recessive phenotype of phytosterolemia, we also identified a possible single gene factor that could account for the major portion of the strong familial aggregation in the ratio of LDL sterol to LDL B, and to a lesser extent LDL B. However, there is clear evidence of familial aggregation for these traits in this pedigree beyond that due to Mendelian components.

INTRODUCTION

Sitosterolemia is a rare genetic disorder of lipid and lipoprotein metabolism in which there is greatly increased intestinal absorption of dietary plant sterols that are only minimally absorbed in normal individuals [1–9]. In sitosterolemia, plasma levels of total plant sterols are elevated (range 13–37 mg/dl) and constitute 7%–16% of the total plasma sterols [1]. Sitosterol is the plant sterol that is predominately elevated (range 8–27 mg/dl), while the levels of campesterol and stigmasterol are much lower. In normal individuals, the plasma concentration of sitosterol is very low (range 0.3–1.7 mg/dl) and represents $<1\%$ of the total plasma sterol [1]. The plasma levels of total and low density lipoprotein (LDL) sterol remain normal or moderately elevated in sitosterolemia [1–9]. Each of the 16 reported cases developed tendon xanthomas before the age of 10 years, and atherosclerosis of the coronary arteries, aorta, and aortic valve (producing aortic stenosis) occurred as early as the second decade of life [1–9].

Hyperapobetalipoproteinemia (hyperapoB) is a lipoprotein phenotype characterized by an elevated plasma level of the major apoprotein of LDL, LDL B, in the presence of a normal level of LDL cholesterol [10–12]. HyperapoB is due to the presence of an increased number of LDL particles that are smaller, denser, and relatively depleted in cholesterol and enriched in LDL B [12]. Thus, the elevated LDL B level in hyperapoB phenotype is often accompanied by a low ratio of LDL cholesterol to LDL B. HyperapoB is strongly associated with coronary artery disease [10–14]. In an Amish pedigree, and the original family described by Bhattacharyya and Connor [2], we previously found the hyperapoB phenotype in patients with sitosterolemia and xanthomatosis and in their first-degree relatives without sitosterolemia [5].

Limited information is available about the inheritance of sitosterolemia [1].

Few of the offspring of sitosterolemic patients with xanthomas have been studied, but the presence of normal sitosterol levels in several pairs of parents indicated that the disorder may be inherited as a recessive trait. Finally, the data that indicated a relationship between the phenotype of hyperapoB and sitosterolemia were limited to studies in two nuclear families mentioned above.

Here, we present further analyses of a large Amish pedigree designed to identify possible Mendelian mechanisms controlling the plasma levels of sitosterol and the major carrier of plasma sterol, LDL, measured as both LDL sterol and LDL B. The dataset presented here also permitted us to examine HDL sterol. These data were used to examine several models of inheritance including a Mendelian single locus mechanism, a polygenic model of inheritance, and a mixed model including independent Mendelian and polygenic components.

METHODS

Sampling

A large Amish pedigree located in Lancaster County, Pennsylvania, was ascertained through a 13-year-old boy who died suddenly and on postmortem was found to have advanced atherosclerosis and xanthomatous plaques in one ankle [5]. Parents and sibs of the proband were examined, and five of 12 sibs had tendon and tuberous xanthomas and sitosterolemia [5]. Three sitosterolemic sibs and both parents of the proband showed clinical evidence of coronary artery disease [5]. The eldest sib with sitosterolemia subsequently died suddenly at age 39. On postmortem examination, significant aortic stenosis and coronary atherosclerosis of the right anterior descending coronary artery were found in this sib. There was no evidence of myocardial infarction, however. Of the 264 living relatives of the proband (fig. 1), 91% (240 individuals) were examined and a blood sample obtained, including six children of the sitosterolemic patients.

All members of the pedigree were instructed to continue their usual diet and to fast for at least 12 hrs before blood samples were drawn. Blood was collected using disodium EDTA (1.5 mg/ml) as the anticoagulant, and aliquots of plasma were used for the lipid and lipoprotein analyses. Separate aliquots were sealed in sterile vials and carried on wet ice to the Montreal Laboratory for LDL B analyses or stored at −20°C for future analyses of the plant sterols. Ten individuals were excluded from analyses because they were not fasting at least 12 hrs at the time blood was drawn, they were or had been pregnant within the last 3 months, or they were breast feeding an infant at the time.

Measurement of Plasma Lipid and Lipoprotein Levels

The total sterol content of plasma and the lipoprotein fractions were measured colorimetrically as described [15]. The method measures both cholesterol and plant sterols [1]. The quantitative lipid and lipoprotein data are expressed as plasma total sterol and lipoprotein sterol. The proportion of the total sterol that was plant sterol was determined separately (see below). The plasma levels of total sterol and triglycerides and the concentrations of sterol in very low density (prebeta) lipoproteins (VLDL), LDL, and HDL were determined using methods of the Lipid Research Clinics (LRC) Program [15]. Briefly, HDL sterol was determined in the supernatant following the precipitation of LDL and VLDL with heparin sulfate and manganese chloride, and VLDL and LDL sterol levels were measured following preparative ultracentrifugation of a separate aliquot of plasma.

Measurement of LDL B Protein

The content of LDL B protein in unfractionated plasma was measured in the Montreal laboratory by radial immunodiffusion (RID) as described [10, 16]. This assay permits the measurement of LDL B without interference from VLDL B [16].

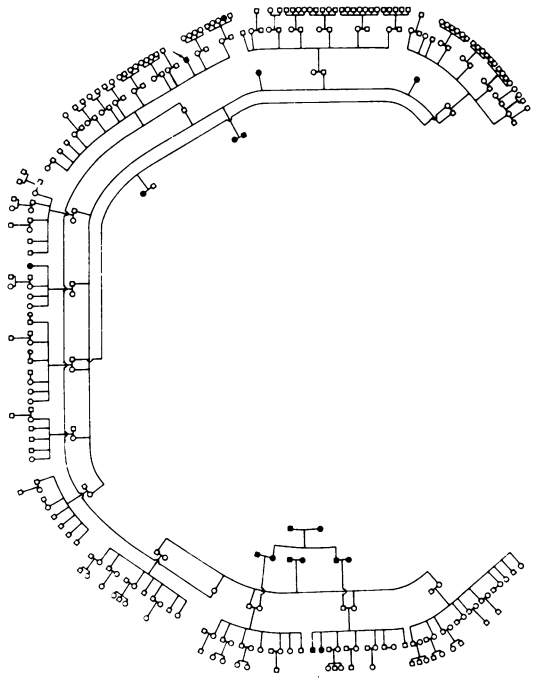

FIG. 1.—Amish pedigree ascertained through the sudden death of a 13-year-old boy. Dead individuals are noted *by the solid symbols*.

Measurement of Plasma Plant Sterols

Plasma samples from 238 members were analyzed for the presence of plant sterols by gas-liquid chromatography (GLC) using the method of Ishikawa et al. [17] with minor modifications. These data are expressed as sitosterol levels (mg/dl) since this was the only plant sterol present in sufficient quantities in normal plasma to be detected by this method. Briefly, plasma, which had been frozen, was thawed thoroughly and 250 μl taken for analysis. Lipids in plasma were saponified for 15 min at 80°C with tetramethylammonium hydroxide isopropanol containing an internal standard (5 α cholestane). The samples were then extracted with tetrachloroethylene-methyl butyrate and partitioned with water. The lower phase was dried under nitrogen, silylated, redried, taken up in ethyl acetate, and an aliquot injected into the gas chromatograph. GLC was performed at 245°C using a 3% OV-17 column and a nitrogen flow of 30 cc/min. The area under the curves for 5 α cholestane, cholesterol, and sitosterol were determined. Purified standards of cholesterol and sitosterol were subjected to GLC with each group of samples studied. Aliquots of a plasma pool from a known phytosterolemic and from a normal human pool were also used for similar analysis during each GLC run.

Data Analysis

Age and sex effects. The effect of age and sex on ln-transformed plasma levels of sitosterol was examined and found to be nonsignificant. Lipoprotein sterol levels were ln-transformed and regressed on age, age^2, age^3, and sex to adjust for the effects of these covariates. No interaction between age and sex were statistically significant in these data. This adjustment procedure accounted for 13.4% of the observed variation in ln(LDL sterol), 14.9% of the

observed variation in ln(LDL B), and 9.5% of the observed variation in ln(HDL sterol) (only age and age^2 terms were used here). Residual values were computed for ln(LDL sterol), ln(HDL sterol), and ln(LDL B) for use in segregation analysis. The ratio of LDL sterol to LDL B was also computed and used in a segregation analysis, but since no significant association with either age or sex was seen in these data, this ratio was used directly.

Segregation analysis. A series of genetic models were examined on this large pedigree using the pedigree analysis package (PAP) where parameters reflecting frequency, transmission, and penetrance of putative genotypes were estimated simultaneously using a maximum likelihood approach [18]. A series of models was examined, including a sporadic model, a polygenic model, a Mendelian single-locus model, and a mixed Mendelian model with both major gene and polygenic effects. Comparisons between hierarchical models were made by comparing their maximum ln-likelihoods. Under Wilkes' theorem, twice this difference will asymptotically approach a chi-square distribution with degrees of freedom equal to the difference in the number of parameters estimated in the two models. Since here the sampling units are pedigrees and the data used here is a single pedigree, we hesitate to rely strictly on the likelihood ratio statistic to test hypotheses. Therefore, we provide the relative ln-likelihood of each model compared to the minimal, sporadic model for each series of analyses.

We fit a hierarchical series of models, beginning with the sporadic model where only an overall mean and variance were estimated and all pedigree members were assumed to be independently sampled from a single normal distribution. Under this minimal model, the phenotype X can be written as $X = \mu + e$, where the error term e represents unobserved factors leading to the observed degree of variation about the overall mean μ. Next, a polygenic model of inheritance was fit, again with a common mean and variance estimated, but, in addition, some correlation was allowed among relatives. Specifically, this polygenic model states that the phenotype X is $X = \mu + g + e$, where the genetic factors influencing the trait are commonly assumed to be normally distributed deviations about the mean with variance σ_g^2 and the unobserved error term e absorbs the remaining variation. Thus, the total phenotypic variation is the sum of these two components (i.e., $\sigma^2 = \sigma_g^2 + \sigma_e^2$). Under this model, the covariance between two relatives (or correlation if dealing with standardized variables) is solely determined by their degree of kinship, that is, for any two pedigree members I and J: $\text{Cov}(X_i, X_j) = 2\phi_{ij}\sigma_g^2$, where ϕ_{ij} is the kinship coefficient between I and J. When analyzing data using this polygenic model, the proportion of variation in the observed phenotype that can be attributable to differences in genes, that is, the heritability ($= \sigma_g^2/\sigma^2$) is estimated, along with the mean and the total variance.

As an alternative genetic model, a Mendelian single locus model was fit to the data. Here, the underlying model is $X = \mu_i + e$, where μ_i ($i = 1, 2, 3$) is the genotypic mean for the three genotypes in a two-allele system, and the error term e is assumed to be normally distributed about these means with some constant variance, that is, e is assumed to be independent among relatives. While this model corresponds to a Mendelian mechanism and is thus more

biologically meaningful, it does require specification of more parameters than the polygenic model. Here, the frequency of the one allele, the three genotypic means, and the common variance about these means must be estimated. Furthermore, the Mendelian model is not a subset of the polygenic model or vice versa, so strict comparisons are difficult. To contrast these two competitors, we also examined a third model that contains both types of genetic factors. This mixed model allows both a single locus component and an independent polygenic component to influence the phenotype, that is, $X = \mu_i + g + e$, where all three components are assumed to be independent. The parameters to be estimated under this model include: three single locus genotypic means (μ_1, μ_2, and μ_3), the allele frequencies under Hardy-Weinberg equilibrium, the normal variation about these genotypic means ($\sigma_g^2 + \sigma_e^2$), and the proportion of this variation that can be attributed to shared independently segregating genes affecting the phenotype in an additive manner (i.e., the ratio $\sigma_g^2/\sigma_g^2 + \sigma_e^2$). From this, the heritability can be calculated. The algorithm for computing the likelihood functions of these separate models of inheritance [19] and the algorithms for estimating these parameters have been described elsewhere [20].

RESULTS

Of the 230 relatives whose plasma was analyzed by GLC, 217 (117 males and 100 females) had data on sitosterol and met the criteria for inclusion (i.e., were fasting and were not pregnant or nursing at the time). The distribution of ln-transformed sitosterol (after scaling away from the origin) in these 217 individuals is shown in figure 2. The peak at left end of this distribution represents

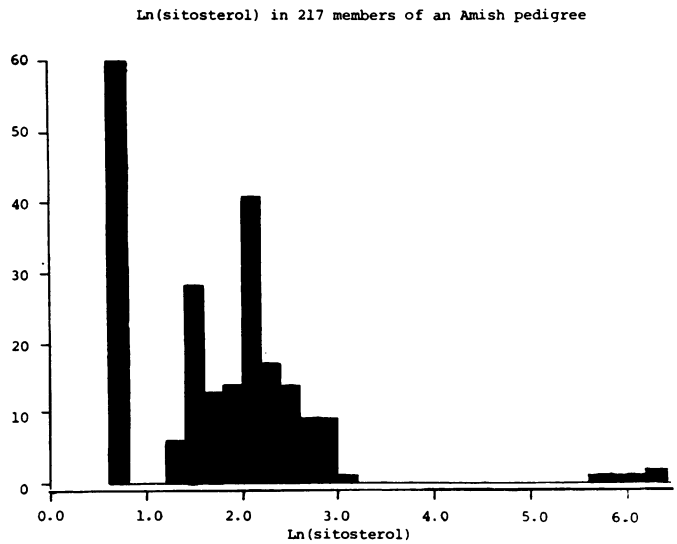

FIG. 2.—Distribution of ln(sitosterol) in 217 members of an Amish pedigree. Sitosterol values in mg/dl were scaled by a factor of 10 and shifted by 2 U away from the origin before ln-transformation. Thus, the median sitosterol value (0.6 mg/dl) corresponds to 2.08 on this scale.

TABLE 1

SEGREGATION ANALYSIS OF ln(SITOSTEROL) IN 217 RELATIVES IN AN AMISH PEDIGREE

MODEL	ESTIMATED PARAMETERS				RELATIVE ln L
	Mean	Standard deviation	Frequency	Heritability*	
Sporadic	1.80	.968	...	...	0
Polygenic	1.67	.775	...	.361	7.57
Recessive Mendelian	1.70	.716	.937	...	50.62
	1.70	"			
	6.04	"			
Recessive mixed	1.69	.482	.937	.471	66.36
	1.69	"			
	6.17	"			

* In this and subsequent tables, heritability is computed as the ratio of polygenic variance to the total variance ($\sigma_g^2/\sigma_g^2 + \sigma_{mg}^2 + \sigma_e^2$).

both those with only trace amounts of sitosterol (46 individuals) and those in whom no sitosterol was detected at all. Results of fitting these data are presented in table 1. The relative ln likelihoods shown in the last column of the table are the difference in ln likelihoods between each model and the sporadic model. In general, if there is any familial aggregation of the trait, one must expect some improvement in ln likelihood of either the polygenic or the Mendelian model compared to the minimal sporadic model. Table 1 clearly shows that the recessive Mendelian model gives a far better fit to the data than the sporadic model or even than the polygenic model for ln(sitosterol) data. This is not surprising since the high levels of sitosterol clustered in a single sibship. The estimated gene frequency in this Mendelian model strongly suggests that the sitosterolemia seen in this pedigree is caused by a rare recessive gene. Although not shown here, a codominant model was also examined and was not significantly better than the recessive model.

A generalized single locus model was also examined on this pedigree, where the probability of transmitting the putative normal allele was estimated in addition to the parameters of the recessive mixed model listed in table 1. The likelihood function of this generalized model failed to maximize within the permissible range for these probabilities (the estimated probability of the putative normal homozygote hit the upper boundary at 1.0, while that for the putative recessive homozygote hit the lower boundary at 0). When only the transmission probability of the heterozygote was estimated (along with the other parameters listed in the last row of table 1), its maximum likelihood estimate (MLE) was .41 ± .11, which was not significantly different from the Mendelian expectation of .5.

This recessive model is supported by our findings in the parents of the sitosterolemia and in the six children born to three of the five sitosterolemics: five of six children had only trace amounts of sitosterol and the plasma levels in the remaining child of a sitosterolemic and both parents were < 1 mg/dl, well

TABLE 2

SEGREGATION ANALYSIS OF RESIDUAL ln(LDL B) IN 230 RELATIVES IN AN AMISH PEDIGREE

MODEL	ESTIMATED PARAMETERS				RELATIVE ln L
	Mean	Standard deviation	Frequency	Heritability	
Sporadic	4.74	.253	...	...	0
Polygenic	4.65	.145	...	.659	27.16
Mendelian	4.56	.174	.862	...	26.93
	4.91	"			
	5.05	"			
Mixed	4.59	.125	.909	.430	31.60
	4.93	"			
	4.90	"			

within the normal range. However, there is significant familial correlation in ln(sitosterol) levels over and above the effects of this rare allele, as demonstrated by the further increase in the relative ln likelihood seen in a recessive mixed model. A statistically significant polygenic component is seen in this model ($\chi^2 = 31.5$ with 1 d.f., $P < .001$), which accounts for 47.1% of the variability in ln(sitosterol) after the difference in genotypic means is considered.

The results of fitting these models on residual ln(LDL B), residual ln(LDL sterol), residual ln(HDL sterol), and the ratio of LDL sterol to LDL B levels in 230 members of this Amish pedigree with lipoprotein data (124 males and 106 females) are shown in tables 2–5. Table 2 shows the results of fitting these models on residual ln(LDL B), and we do see that both the polygenic model and the Mendelian model result in an improvement in the ln likelihood over the sporadic model, the former model giving a slightly greater improvement than the latter. By subtracting the relative ln likelihood of the Mendelian model from that for the mixed model, we can obtain a test statistic for the null hypothesis that there is no genetic variation in the distribution of residual ln(LDL B), that is, $\sigma_g^2 = 0$. Twice this difference is an approximate chi-square test statistic and was 9.34 with 1 d.f. ($P = .002$), which allows us to reject this null hypothesis. A similar test statistic computed by subtracting the relative ln likelihood of the polygenic and mixed models was 8.88 with 3 d.f. ($P = .03$), which allows rejection of the null hypothesis that there is no Mendelian component, but not as strongly. Thus, the most parsimonious model for the control of residual ln(LDL B) is the mixed model where 43% of the variation in residual ln(LDL B) is attributed to unobserved polygenic factors in this Amish pedigree and 29% is attributed to a Mendelian component.

Table 3 shows the results of a similar analysis on residual ln(LDL sterol). Here again the polygenic model shows a slightly greater improvement in ln likelihood compared to the sporadic model than does the Mendelian single locus model, and the null hypothesis of no polygenic component was rejected (the test statistic was 4.60 with 1 d.f., $P = .03$). The null hypothesis of no

TABLE 3

SEGREGATION ANALYSIS OF RESIDUAL ln(LDL-STEROL) IN 230 RELATIVES IN AN AMISH PEDIGREE

MODEL	ESTIMATED PARAMETERS				RELATIVE ln L
	Mean	Standard deviation	Frequency	Heritability	
Sporadic	4.87	.314	...	...	0
Polygenic	4.74	.182	...	.655	26.29
Mendelian	4.62	.211	.811	...	25.71
	4.98	"			
	5.29	"			
Mixed	4.62	.191	.815	.190	28.01
	4.94	"			
	5.22	"			

Mendelian component could not be rejected for this trait (the test statistic was 3.4 with 3 d.f.). Essentially, these two competing models gave very similar fits to these data, as judged by the relative ln likelihoods. However, the variation attributed to the additive genetic component (65.5% ± 11.0) under the polygenic model was slightly greater than the computed variation due to the putative major gene (σ_{mg}^2 = 45.8%) under the simple Mendelian model.

Table 4 shows the results of segregation analysis using the ratio of LDL sterol to LDL B. Here, the Mendelian single locus model gave a slightly better fit to the data than did the polygenic model, as judged by the relative ln likelihood (table 4). Under this Mendelian model, three genotypic means were estimated and they suggest a codominant expression of the putative allele for a low ratio of LDL sterol to LDL B. Comparisons among these models favor the mixed model since both the Mendelian component and the polygenic component were statistically significant (the test statistic for the former was 12.6 with 3 d.f., P = .006, and was 11.6 with 1 d.f., P = .0006, for the latter). Under this

TABLE 4

SEGREGATION ANALYSIS OF THE RATIO OF LDL STEROL TO LDL B PROTEIN IN 230 RELATIVES IN AN AMISH PEDIGREE

MODEL	ESTIMATED PARAMETERS				RELATIVE ln L
	Mean	Standard deviation	Frequency	Heritability	
Sporadic	1.15	.189	...	...	0
Polygenic	1.11	.128	...	.559	12.10
Mendelian	1.08	.151	.867	...	12.56
	1.23	"			
	1.64	"			
Mixed	1.08	.114	.705	.105	18.38
	1.27	"			
	1.89	"			

TABLE 5

SEGREGATION ANALYSIS OF RESIDUAL ln-HDL STEROL IN 230 RELATIVES IN AN AMISH PEDIGREE

MODEL	ESTIMATED PARAMETERS				RELATIVE ln L
	Mean	Standard deviation	Frequency	Heritability	
Sporadic	3.94	.236	...	...	0
Polygenic	3.94	.176	...	.472	7.48
Mendelian	3.43	.175	.245	...	9.35
	4.10	"			
	3.83	"			
Mixed	3.46	.143	.260	.357	14.74
	3.96	"			
	3.98	"			

mixed model, the putative Mendelian component accounted for 80% of the variation in the ratio of LDL sterol to LDL B, with an additional 10.5% attributable to polygenic factors.

Table 5 shows results of a similar series of models evaluated using residual ln(HDL sterol). Although the estimated genotypic means for the mixed model would suggest a dominant gene influencing residual ln(HDL sterol) levels, the polygenic component accounted for slightly more of the total variation in residual ln(HDL sterol). The most parsimonious model for these data was a mixed model where 35.6% of the total variation in residual ln(HDL sterol) is attributed to polygenic factors and 28.8% was attributable to a single Mendelian locus.

DISCUSSION

The analysis of sitosterol levels in this large pedigree clearly suggests sitosterolemia is an autosomal recessive trait. The findings presented here, especially the exact parameter estimates, must be viewed with caution, however, because this single large pedigree is not a representative sample of the Amish population since it was ascertained through a proband with premature coronary artery disease. The proband died suddenly, and thus no information was available on his sitosterol or lipoprotein values. To properly consider the effects of ascertainment in this situation, it would be necessary to conduct a bivariate analysis of coronary artery disease and each of the quantitative measures presented here. However, complete medical histories were not available for the members of this pedigree so this type of analysis is not possible. In an effort to assess the impact of ascertainment on the recessive mixed model shown in table 1, we arbitrarily assigned the proband an ln-sitosterol equal to the mean of the sitosterolemic group seen in figure 2. The likelihood of the recessive mixed model was then maximized after conditioning on this assigned value. Final parameter estimates were changed very little under this ascertainment correction, except for the estimated gene frequency which was closer to 1.0, emphasizing the rarity of the putative sitosterolemic allele.

The level of inbreeding in this pedigree due to the reproductive isolation of

the Amish population will lead to a deficiency in the number of heterozygotes relative to a random mating population, so the estimated allele frequency shown in table 1 must be interpreted with extreme caution for this reason also. The Amish population in Lancaster, Pennsylvania, was founded by a relatively small group of individuals and has been reproductively isolated since its beginning. This fact results in a nonzero background level of inbreeding in current Amish population. Based on genealogical data covering this population for more than 10 generations, the mean inbreeding coefficient of the first 2 generations of this pedigree was estimated at .002752 [21]. When this low level of inbreeding was considered in the computation of genotypic frequencies for founders of this pedigree, there was a negligible impact on the estimated genotypic means and standard deviation shown in table 1.

It is also important to realize that the apparent high level of polygenic variation in the recessive mixed model for ln(sitosterol) may not reflect just genetic differences among relatives, but could also be due to shared environmental factors influencing sitosterol levels. Diet is one important factor that could lead to a level of familial correlation that could mimic polygenic variation, although no data on diet was available in this study. The fact that the sitosterol levels were significantly correlated between spouses ($r = .30 \pm .14$) suggests a relation between environment (perhaps diet) and the level of sitosterol. While the plasma level of sitosterol in these relatives is low, median value was 0.6 mg/dl, these analyses indicate that perhaps dietary intake of plant sterols does influence the subsequent plasma levels despite minimal absorption in normal individuals. This tenet is compatible with studies of Mellies et al. [22] who found that normal infants fed formulas containing high amounts of plant sterols from vegetable oil developed considerable amounts of sitosterol in their plasma (up to 9 mg/dl). Further, these same workers showed that sitosterol was detected in the aorta of infants fed vegetable oil-rich formulas [23].

The studies of sterol and apoprotein levels in LDL showed a high level of familial aggregation in this Amish pedigree and provided evidence of direct Mendelian control of the ratio of LDL sterol to LDL B and, to a lesser extent, of LDL B to HDL sterol. The putative Mendelian component for the ratio of LDL sterol to LDL B accounted for 80% of the variation in this trait.

No evidence of a Mendelian component for LDL sterol itself, such as that seen with the LDL receptor mutants in familial hypercholesterolemia (FH) [24], was detected in this pedigree. The estimated heritabilities presented here, however, must also be interpreted with some degree of caution because of the background level of inbreeding. Using the inbreeding value for the founders of this pedigree and the estimated heritabilities from table 5, one can estimate a baseline heritability in a hypothetical random mating population from which the founders of the Amish were drawn [25]. However, this adjustment results in a very minor change in the final estimators; for example, the estimated heritability for residual ln(LDL sterol) was 65.5% from this pedigree, while the approximate heritability in the absence of inbreeding would be 65.6%. Thus, any effect of inbreeding is much less than the error variance from this analysis.

The association of the hyperapoB phenotype with sitosterolemia was sug-

gested in our original report of part of this pedigree [5]. There the definition of hyperapoB was based on an elevated level of LDL B protein in the presence of normal LDL cholesterol level. The ratio of LDL sterol to LDL B in those with the hyperapoB phenotype is usually between 1.0 and 1.2 but may vary from 0.6 to 1.3 [26]. In normal individuals, the ratio of LDL sterol to LDL B may vary from 1.0 to 1.8, leading to overlap in the distributions of the ratio between normals and those with hyperapoB. Further, some patients, such as those with FH, have an elevated LDL B but a normal or even elevated ratio of LDL sterol to LDL B, leading to an overlap in the distributions of LDL B levels between those with hyperapoB and those with FH. Since the phenotype of hyperapoB as now used implies the presence of an elevated number of LDL particles of abnormal composition, use of either an LDL B level alone, or the ratio of LDL sterol to LDL B protein alone, will not permit an optimal definition of the hyperapoB phenotype. Further work is in progress to develop analyses that will allow the concomitant assessment of the increase in the number of LDL particles and their altered composition.

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REFERENCES

1. Salen G, Shefer S, Berginer M: Familial diseases with storage of sterols other than cholesterol: cerebrotendinous xanthomatosis and sitosterolemia with xanthomatosis, in *Metabolic Basis of Inherited Disease*, 5th ed, edited by Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS, New York, McGraw-Hill, 1983, pp 713–730
2. Bhattacharyya AK, Connor WE: β-Sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. *J Clin Invest* 53:1033–1043, 1974
3. Shulman RS, Bhattacharyya AK, Connor W, Fredrickson DS: β-Sitosterolemia and xanthomatosis. *N Engl J Med* 294:482–483, 1976
4. Miettinen TA: Phytosterolaemia, xanthomatosis and premature atherosclerotic arterial disease: a case with high plant sterol absorption, impaired sterol elimination and low cholesterol synthesis. *Eur J Clin Invest* 10:27–35, 1980
5. Kwiterovich PO Jr, Bachorik PS, Smith HH, et al.: Hyperapobetalipoproteinemia in two families with xanthomas and phytosterolaemia. *Lancet* 1:466–469, 1981
6. Salen G, Ahrens EH Jr, Grundy SM: The metabolism of β-sitosterol in man. *J Clin Invest* 49:952–967, 1970
7. Salen G, Kwiterovich PO Jr, Shefer S, et al.: Increased plasma cholestanol and 5 α-saturated plant sterol derivatives in subjects with sitosterolemia and xanthomatosis. *J Lipid Res* 26:203–209, 1985
8. Lin HJ, Wang C, Salen G, Lam KC, Chan TK: Sitosterol and cholesterol metabolism in a patient with sitosterolemia and xanthomatosis. *Metabolism* 32:126–133, 1983
9. Whitington GL, Ragland JB, Sabesin SM, Kuiken LB: Neutral sterolemia and xanthomatosis (abstr.). *Circulation* 60:Part II:33, 1979
10. Sniderman A, Shapiro S, Marpole D, et al.: The association of coronary atherosclerosis and hyper*apo*betalipoproteinemia (increased protein but normal cholesterol content in human plasma low density lipoprotein). *Proc Natl Acad Sci USA* 77:604–608, 1980

11. SNIDERMAN A, WOLFSON C, TENG B, FRANKLIN F, BACHORIK P, KWITEROVICH P: Association of hyperapobetalipoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. *Ann Int Med* 97:833–839, 1982
12. TENG B, THOMPSON GR, SNIDERMAN AD, FORTE TM, KRAUSE RM, KWITEROVICH PO JR: Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinemia, normolipidemia and familial hypercholesterolemia. *Proc Natl Acad Sci USA* 80:662–666, 1983
13. BRUNZELL JD, SNIDERMAN AD, ALBERS JJ, KWITEROVICH PO JR: Apoproteins B and A-1 and coronary artery disease in humans. *Arteriosclerosis* 4:79–83, 1984
14. CAMPEAU L, ENJALBERT M, LESPERANGE J, ET AL.: The relation of risk factors to the development of atherosclerosis in saphenous-vein bypass grafts and the progression of disease in the native circulation. *N Engl J Med* 311:1329–1332, 1984
15. LIPID RESEARCH CLINICS PROGRAM: *Manual of Laboratory Operations*, vol I. DHEW Publication 75–628, Washington, D.C., Dept. of Health, Education and Welfare, 1974
16. SNIDERMAN AD, TENG B, JERRY M: Determination of B protein of low density lipoprotein directly in plasma. *J Lipid Res* 16:465–469, 1975
17. ISHIKAWA TT, MACGEE J, MORRISON JA, GLUECK CJ: Quantitative analysis of cholesterol in 5 to 20 μl of plasma. *J Lipid Res* 15:286–291, 1974
18. HASSTEDT SJ, CARTWRIGHT PE: *PAP: Pedigree Analysis Package*. Technical Report No. 13, Dept. of Medical Biophysics and Computing, Salt Lake City, Univ. of Utah, 1981
19. CANNINGS E, THOMPSON EA, SKOLNICK MH: Probability functions on complex pedigrees. *Adv Appl Prob* 10:26–61, 1978
20. HASSTEDT SJ: A mixed model likelihood approximation on large pedigrees. *Comp Biomed Res* 15:295–307, 1982
21. KHOURY MJ: A genealogic study of inbreeding and prereproductive mortality in the Old Order Amish. Ph.D. thesis, Baltimore, The Johns Hopkins Univ., 1985
22. MELLIES MJ, GLUECK CJ, SWEENEY C, FALLAT RW, TSANG RC, ISHIKAWA TT: Plasma and dietary phytosterols in children. *Pediatrics* 57:60–67, 1976
23. MELLIES MJ, ISHIKAWA TT, GLUECK CJ, BOVE K, MORRISON J: Phytosterols in aortic tissue in adults and infants. *J Lab Clin Med* 88:914–921, 1976
24. GOLDSTEIN JL, BROWN MS: Familial hypercholesterolemia, in *Metabolic Basis of Inherited Disease*, 5th ed, edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, GOLDSTEIN JL, BROWN MS, New York, McGraw-Hill, 1983, pp 672–712
25. FALCONER DS: *Introduction to Quantitative Genetics*. New York, Ronald Press, 1961, p 268
26. KWITEROVICH PO JR, WHITE S, FORTE T, BACHORIK PS, SMITH H, SNIDERMAN A: Phenotypic expression of hyperapobetalipoproteinemia in a kindred with familial combined hyperlipidemia and familial hypercholesterolemia. Submitted for publication