Hyperlipidemia in Coronary Heart Disease

II. GENETIC ANALYSIS OF LIPID LEVELS IN 176 FAMILIES AND DELINEATION OF A NEW INHERITED DISORDER, COMBINED HYPERLIPIDEMIA

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ABSTRACT To assess the genetics of hyperlipidemia in coronary heart disease, family studies were carried out in 2520 relatives and spouses of 176 survivors of myocardial infarction, including 149 hyperlipidemic and 27 normolipidemic individuals. The distribution of fasting plasma cholesterol and triglyceride values in relatives, together with segregation analyses, suggested the presence of five distinct lipid disorders. Three of these—familial hypercholesterolemia, familial hypertriglyceridemia, and familial combined hyperlipidemia —appeared to represent dominant expression of three different autosomal genes, occurring in about 20% of survivors below 60 yr of age and 7% of all older survivors. Two other disorders—polygenic hypercholesterolemia and sporadic hypertriglyceridemia—each affected about 6% of survivors in both age groups.

The most common genetic form of hyperlipidemia identified in this study has hitherto been poorly defined and has been designated as familial combined hyperlipidemia. Affected family members characteristically had elevated levels of both cholesterol and triglyceride. However, increased cholesterol or increased triglyceride levels alone were also frequently observed. The combined disorder was shown to be genetically distinct from familial hypercholesterolemia and familial hypertriglyceridemia for the following reasons: (*a*) the distribution pattern of cholesterol and triglyceride levels in relatives of probands was unique; (*b*) children of individuals with combined hyperlipidemia did not express hypercholesterolemia in contrast to the finding of hypercholesterolemic children from families with familial hypercholesterolemia; and (*c*) analysis of informative matings suggested that the different lipid phenotypes owed their origin to variable expression of a single autosomal dominant gene and not to segregation of two separate genes, such as one elevating the level of cholesterol and the other elevating the level of triglyceride.

Heterozygosity for one of the three lipid-elevating genes identified in this study may have a frequency in the general population of about 1%, constituting a major problem in early diagnosis and preventive therapy.

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INTRODUCTION

The hyperlipidemias comprise a heterogeneous group of disorders whose characteristic expression is an eleva-

tion in the plasma concentration of cholesterol and/or triglyceride (2–4). These lipid disorders may occur in familial or nonfamilial form (3). Presumably, each variety of familial hyperlipidemia could arise from the action of a single gene (monogenic or Mendelian inheritance) or could reflect the interaction of several genes at many different loci (polygenic inheritance) (5, 6). Nonfamilial hyperlipidemias are often secondary to such factors as diet, alcohol intake, estrogen therapy, or to diseases such as diabetes mellitus, hypothyroidism, or nephrosis (3). In some cases, neither hereditary nor identifiable environmental factors can be implicated, and such cases are referred to in this paper as sporadic hyperlipidemia.[1]

Current laboratory tests used to define hyperlipidemia and to distinguish among the monogenic, polygenic, sporadic, and secondary disorders include measuring the plasma levels of total cholesterol, low density lipoprotein (LDL)—cholesterol, and total triglyceride (2). However, none of these measurements directly reflects the primary action of genes as do the measurements of proteins and enzymes in such disorders as the hemoglobinopathies. Instead, they are the result of combined genetic and environmental influences.

Monogenic and polygenic causes of hyperlipidemia most likely differ in the underlying biochemical lesion and in the response to diet and drug therapy. Assessing the importance of genetic factors requires a detailed study of cholesterol and triglyceride levels in relatives of hyperlipidemic probands. In hyperlipidemia transmitted as an autosomal dominant trait, lipid levels in first-degree relatives would be composed of two distinct distributions—one reflecting the presence of normal relatives and the other that of affected relatives. This distribution of lipid values would result in a bimodal curve. Bimodality is most easily detected if the mean of the quantitative parameter under study differs significantly between normals and affected, if the spread or variance of the values is not too dissimilar in the two groups, and if the proportion of relatives in each group is nearly equal (7). With dominant inheritance, a characteristic vertical pedigree pattern would be found in both near and distant relatives. In polygenic inheritance the quantitative parameter being studied is continuously distributed, and no distinct separation between normals and affected is evident (8, 9). Thus, the lipid levels in relatives of individuals with polygenic hyperlipidemia would form a single distribution with a mean higher than that of controls. Affected family members are most frequently found among first-degree relatives such as sibs, parents, and children, and would be considerably less frequent in more distant relatives (8, 9). In sporadic hyperlipidemia, lipid levels in relatives of all degrees should be identical with those of controls.

Utilizing as probands for detailed family studies the hyperlipidemic survivors of myocardial infarction identified in the accompanying paper (10), the present investigation was undertaken with the following aims: to delineate the different forms of familial and nonfamilial hyperlipidemia in patients with myocardial infarction, to characterize the mechanisms of inheritance of any familial disorders, and to determine their frequency.

METHODS

Selection of survivors as probands for family studies. Of the 500 survivors of myocardial infarction investigated in the accompanying paper (10), 176 were selected as probands for family studies as follows: (*a*) An attempt was made to investigate the families of all hyperlipidemic survivors whose level of adjusted plasma cholesterol and/or triglyceride equaled or exceeded the 95th percentile value of controls. Of 157 such survivors, family studies were carried out in 131. The remaining 26 hyperlipidemic survivors had either no living first-degree relatives (13 survivors) or fewer than three available first-degree relatives (13 survivors). (*b*) Because 95th percentile cut-off values represented an arbitrary way of distinguishing between normolipidemic and hyperlipidemic individuals, 13 survivors whose plasma cholesterol and/or triglyceride values lay above the 92.5th percentile were included for family study. (*c*) All 11 survivors who were taking clofibrate at the time of examination were considered hyperlipidemic regardless of their lipid values and were included for family study. Of these 11, 5 had lipid values at the time of study below the 95th percentile (10). (*d*) 27 normolipidemic survivors whose plasma cholesterol and triglyceride were both below the 90th percentile were also included as probands and the lipid values of their relatives were used as control family data.

Family studies. Family histories were taken and pedigrees were constructed to include every first-degree relative for each of the 500 survivors of myocardial infarction studied in the accompanying paper (10). Permission to verify causes of death in first-degree relatives was obtained from each of the 500 survivors. From the 176 survivors selected as probands, permission to contact all living first-degree relatives as well as more distant relatives when indicated was requested. A total of 2520 family members were tested, including 1695 blood relatives and 825 spouses. A fasting blood sample and a medical history were obtained from 913 of the 960 living first-degree relatives (age 6 and above) of the 176 probands (95% ascertainment). Similarly tested were 643 second-degree, 135 third-degree, and 4 fourth-degree relatives, as well as 825 spouses of these relatives. Blood specimens from local and out-of-town relatives were collected as described in the accompanying paper (10). The decision to contact the more

[1] The term "polygenic" has been used in the broad sense to imply a multifactorial mechanism, in which observed familial variations could be determined either by multiple genetic factors, by a single genetic locus with one or many alleles interacting with environmental variation, or exclusively by environmental variation. In the strict genetic sense, a sporadic case of hyperlipidemia could be the result of a new autosomal dominant mutation, autosomal recessive inheritance, incomplete penetrance in autosomal dominant inheritance, or a nongenetic entity.

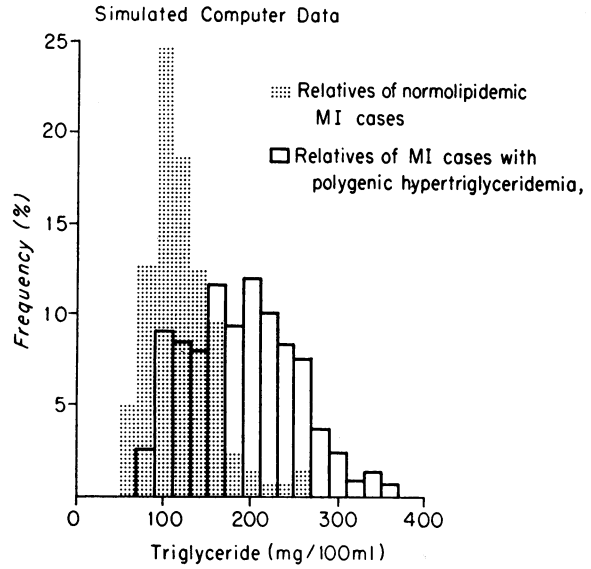

FIGURE 1 Frequency distribution of computer-simulated triglyceride levels in relatives of 60 normolipidemic and 60 hypertriglyceridemic probands. These data were derived from a polygenic model and were generated by a special computer program described in the Methods. The total number of relatives comprising each curve was 300.

distant relatives in a given family was made if one or more first-degree relatives had lipid values equal to or exceeding the 95th percentile cut-off values or if one or more first-degree relatives were said to have died of myocardial infarction. Except for their availability and willingness to cooperate, no other special selection was used in obtaining blood samples from the distant relatives.

Analyses of plasma lipids. Fasting plasma levels of cholesterol and triglyceride were measured as described in the accompanying paper (10). Lipoprotein quantification and phenotyping were performed on repeat plasma samples using methods described in the accompanying paper (11).

Control data. The levels of cholesterol and triglyceride were obtained from 950 spouse controls and adjusted for differences in age and sex as described in the accompanying paper (10).

Classification of hyperlipidemia in families. Classification of the lipid disorders was based on an analysis of the cholesterol and triglyceride levels among relatives of probands. In the absence of knowledge regarding the basic defects in the different hyperlipidemias, no method of sorting data for heterogeneity based on quantitative variation alone can be considered completely unbiased. However, in an attempt to minimize bias, the following approach was developed: Each of the hyperlipidemic families was initially separated into one of two groups depending on whether (group A) or not (group B) the family contained at least one relative besides the proband who would be considered unequivocally hyperlipidemic (i.e., whose lipid level was $\geq$99th percentile for adults of 20 yr of age and older or $\geq$95th percentile for younger individuals).[2] Families in group A were further subdivided depending on whether the predominant lipid elevation in the family occurred in cholesterol alone (group A-1 or familial hypercholesterolemia); in triglyceride alone (group A-2 or familial hypertriglyceridemia); or in both lipids (group A-3 or familial combined hyperlipidemia). For the individual family this assignment to a specific group was determined by inspecting the pedigree and assessing the distribution of percentile values of the adjusted cholesterol and triglyceride levels. Analysis of one 11 member family is given for illustration:

Percentile Distribution of Lipid Levels of Each Family Member (Number)

	10th	20th	30th	40th	50th	60th	70th	80th	90th	95th	99th
Cholesterol		1	2	1	1	1	2			②	①
Triglyceride	1	2		1	1	2		1	①		②

Since this family showed values in the 90th–99th percentile range for both cholesterol and triglyceride, it was assigned to the category of familial combined hyperlipidemia (group A-3). This system was generally useful for classification except for occasional instances of familial hypercholesterolemia and xanthomatosis, where several affected family members had elevations in both plasma lipids (e.g., cholesterol = 410 mg/100 ml and triglyceride = 200 mg/100 ml). However, such affected individuals almost always had a cholesterol/triglyceride ratio greater than 2 (2), so it was easy to make a correct assignment to the category of familial hypercholesterolemia (group A-1). An objective method to confirm our qualitative assessment was sought by calculating a weighted variance[3] for both adjusted cholesterol and triglyceride values of adult relatives in each family. A high variance for cholesterol but not for triglyceride would be expected in familial hypercholesterolemia (group A-1); conversely, a high variance for triglyceride but not for cholesterol would be expected in familial hypertriglyceridemia (group A-2).

Our classification into family groups A-1, A-2, and A-3 on the basis of at least one affected relative could theoretically create artificial bimodality in the lipid distribution of relatives and lead to spurious conclusions regarding monogenic inheritance. To rule out this possibility, we set up a series of simulation experiments using a special computer program developed in collaboration with Dr. Joseph Felsenstein. In these experiments, the inheritance of hyperlipidemia was assumed to be polygenic (heritability = 1), and a lipid value was assigned to each member of a hypothetical family including a mother, a father, and each of their four children, the first child being designated as the proband. Lipid values for father, G_1, and mother, G_2, were chosen from a set of random variates with a distribution having a mean and variance, σ^2, identical with those of our control data. Lipid values for the four offspring were assigned by drawing at random each value from a distribution whose mean was = $(G_1 + G_2)/2$ and variance = $\sigma^2/2$. In this way the values in the offspring

[2] Four families in which the index case was shown to have type III hyperlipidemia were placed in a special group (see accompanying paper [11]).

[3] $$\sigma^2 = \frac{\Sigma(x_1 - \mu)^2 + 0.5\,\Sigma(x_2 - \mu)^2 + 0.25\,\Sigma(x_3 - \mu)^2}{n_1 + 0.5\,n_2 + 0.25\,n_3 - 1}$$

where σ^2 = family variance weighted as to class of relative being considered, in which the weighting factor was proportional to the number of genes in common among relatives, (i.e., first-degree relatives of proband = 1, second-degree relatives = 0.5, and third-degree relatives = 0.25); x_1, x_2, x_3 = adjusted lipid level (cholesterol or triglyceride) in first-, second-, and third-degree relatives, respectively. μ = adjusted mean lipid level of controls; n_1, n_2, n_3 = number of first-, second-, and third-degree relatives, respectively. The proband of each family was excluded from analysis.

reflected polygenic inheritance (9). Thousands of such simulated families were computer generated. The first 60 families fulfilling the criteria of having a hyperlipidemic index case ($\geq$95th percentile) and at least 1 unequivocally hyperlipidemic relative ($\geq$99th percentile) were selected for genetic analysis. When the frequency distributions of lipid values in relatives of these artificially generated data were plotted, the curves for both cholesterol and triglyceride appeared unimodal and significantly shifted to the right of the control curve. An example of such simulated computer data for triglyceride is shown in Fig. 1. Since these simulated family data did not give results resembling monogenic inheritance (i.e., no bimodality), it seemed reasonable to conclude that the approach used in these studies to sort families for heterogeneity did not create artifical bimodality.

Analysis of data. In addition to the methods described in the accompanying paper (10), the following procedures were used in the genetic analysis. It was necessary for testing the single gene hypothesis to decompose each of the frequency distributions of lipid values of affected and normal relatives from the various family groups into two components with the most likely overlapping Gaussian distributions. Initial estimates of the parameters of the respective components were obtained by dissecting the plots of the cumulative frequency distribution on probability paper (probit analysis) by the graphical method of Harding (12). Using these initial values, maximum likelihood estimates of μ_1, μ_2, σ_1, and σ_2 (where μ_1 and σ_1 were the mean and standard deviation of the ith component [$i = 1,2$]) were obtained with the aid of a computer program using a standard iterative method (13, 14). Murphy and Bolling have discussed the applications of this technique to the testing of single locus hypotheses where there is incomplete separation of the phenotypes (15).[4]

Documentation of coronary heart disease in relatives. Copies of 90% of all requested death certificates were obtained for first-degree relatives over 35 yr of age. Death was attributed to myocardial infarction whenever one of the following conditions was listed as a direct cause of death: myocardial infarction, coronary occlusion, coronary atherosclerosis, coronary thrombosis, arteriosclerotic cardiovascular disease, or sudden death. Less specific terms such as organic heart failure, degenerative heart disease, congestive heart failure, and chronic myocarditis were not considered sufficiently indicative of coronary heart disease. Myocardial infarction was accepted in living relatives when their medical history form indicated hospitalization for "myocardial infarction," "coronary thrombosis," or "heart attack."

[4] Although Murphy and Bolling indicated that sometimes convergence of the estimates is not possible when the variances in the two components are not equal (15), this problem did not occur in our data, possibly as the result of the nearly precise initial estimates which greatly reduced the number of iterations (and hence the chance of encountering a point of singularity in the likelihood function) and also because we used less stringent convergence criteria. Occasionally, a small number of outliers with triglyceride values greater than 600 mg/100 ml (<2% of the total sample) severely affected the mean and standard deviation estimates in the second component. For these particular family groups three components were estimated, with the outliers contained in the last component. This proved to be a more effective way of handling the outlying values than winsoring (i.e., making all values greater than 600 mg/100 ml equal to 600), since the winsored values also affected the parameters of the second component.

RESULTS

Lipid levels in relatives of normolipidemic survivors. The mean and SD of age and sex-adjusted cholesterol and triglyceride levels in 113 adult relatives of 27 normolipidemic survivors were 215±39 and 94±54 mg/100 ml, respectively; as compared with the control values of 218±41 and 93±48. Furthermore, the frequency distributions for both cholesterol and triglyceride in these 113 normal relatives appeared unimodal and were identical with those of the controls. This excellent agreement confirmed the validity of the control values and indicated that any differences in the lipid distributions between controls and relatives of hyperlipidemic survivors could not be attributed to obtaining fasting blood samples from many relatives located in diverse geographic areas.

Lipid levels in spouse pairs. To determine whether any familial similarities in lipid levels could result from environmental similarities between family members, plasma lipids in the first 440 consecutively studied husband-wife pairs (including hyperlipidemic and normolipidemic spouses) were compared. Since no significant correlations were found for either cholesterol ($r = -0.023$) or triglyceride ($r = +0.098$), it was concluded that dietary and other environmental factors common to spouse pairs could not account for familial elevations in plasma lipids. Therefore, no corrections were necessary for nongenetic familial effects.

Lipid levels in relatives of hyperlipidemic survivors. Lipid levels in 645 adult first-degree relatives of all 149 hyperlipidemic survivors were compared with those of controls. The age and sex-adjusted values for both cholesterol and triglyceride (mean±SD) were significantly higher ($P < 0.001$) in relatives (235±53 and 126±174, respectively) than in controls (218±41 and 93±48). These differences became more striking when the hyperlipidemic survivors were separated into groups: those with hypercholesterolemia with or without an associated elevation in triglyceride (n = 81) and those with hypertriglyceridemia with or without an associated elevation in cholesterol (n = 107). (36 of these survivors showed an elevation in both lipids and therefore were included in both groups.) The mean±SD value for cholesterol in the adult first-degree relatives (n = 379) of the hypercholesterolemic survivors was 247±56 mg/100 ml (control, 218±41) whereas the mean±SD value for triglyceride in the adult first-degree relatives (n = 434) of the hypertriglyceridemic survivors was 140±200 mg/100 ml (control, 93±48). These data indicated that familial factors play an important role in the etiology of hyperlipidemia in patients with coronary heart disease. The absence of correlations in lipid levels between husband-

TABLE I
Classification of Families

Group	Number of families	Relatives per family* (mean±SEM)	Adjusted cholesterol		Adjusted triglyceride	
			Weighted variance‡	Observed/ Expected§	Weighted variance‡	Observed/ Expected§
A-1 Familial hypercholesterolemia	16	8.4±1.0	11,332	5.40	3,240	0.68
A-2 Familial hypertriglyceridemia	23	10.1±1.2	1,569	0.78	31,803	6.63
A-3 Familial combined hyperlipidemia	47	11.2±1.2	4,972	2.36	45,166	9.42
B Sporadic and polygenic hyperlipidemia	59	8.4±1.1	1,971	0.94	2,595	0.54
C Relatives of normolipidemic survivors	27	8.1±1.2	2,103	1.00	4,794	1.00

* Does not include spouses.
‡ Represents a group mean determined by averaging the individual weighted variances of each family comprising a group. The weighted variance of each family was estimated as described in the Methods.
§ Observed/expected = weighted variance of indicated group/weighted variance of group C.

wife pairs suggested that these familial factors were primarily genetic in origin.

Classification of hyperlipidemic families and development of a genetic hypothesis. In order to search for the presence of major genes contributing to hyperlipidemia, families were sorted for heterogeneity as described in the Methods. The resultant classification is shown in Table I. Group A-1 or familial hypercholesterolemia consisted of 16 families. An abnormally high mean variance was calculated for cholesterol levels in these families as compared with the mean variance for cholesterol of the normolipidemic control families (group C). Group A-2 or familial hypertriglyceridemia consisted of 23 families, with the predominant lipid elevation again reflected in an abnormally high mean variance. Group A-3 or familial combined hyperlipidemia consisted of 47 families in which elevation of both plasma lipids was reflected in high mean variances. Group B consisted of 59 families in which the mean variances in plasma lipids were not significantly higher than those in the control families (group C).

Since the variation in plasma cholesterol and/or triglyceride among relatives in Groups A-1, A-2, and A-3 was 5 to 9 times greater than that of the control families, we tested the genetic hypothesis that the hyperlipidemia in each of these three groups was determined by a different major gene.

Group A-1: familial hypercholesterolemia. The frequency distribution of adjusted lipid levels in 132 relatives of 16 hypercholesterolemic survivors (13 men, 3 women) is shown in Fig. 2. As compared with that of controls, the distribution of cholesterol was suggestive of bimodality—the first mode corresponding to controls and containing the normal relatives and the second mode containing the affected relatives (Fig. 2A). More quantitative evidence for bimodality was obtained by plotting the cumulative frequency distribution on normal probability paper (Fig. 3A). The plot of control values yielded one straight line, indicating a single Gaussian curve (12). The plot of the relatives' values contained two straight line segments, suggesting two Gaussian curves—one for normal relatives and the other for affected relatives (12). The break in the plot (i.e., the nonstraight line segment) reflected the apparent antimode of the two overlapping distributions;

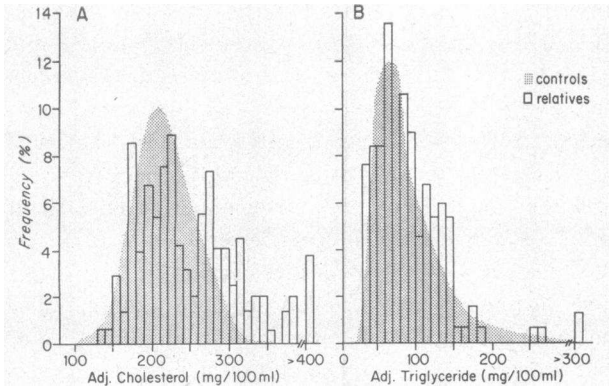

FIGURE 2 Frequency distribution of adjusted lipid levels in 132 near and distant relatives of 16 survivors with familial hypercholesterolemia. Included in this analysis were 68 first-degree, 44 second-degree, 18 third-degree, and 2 fourth-degree relatives. 49 of the 132 relatives were between the ages of 6 and 20. Age and sex-adjustments were carried out as described in the accompanying paper (10). The distribution is divided into increments of 10 mg/100 ml. The smooth stippled curve represents a nonparametric density estimate of the control distribution.

its upper limit corresponded approximately to the 90–95th percentile range of controls. Since these data suggested a mixture of two normal distributions, the best fitting two-distribution mixture was obtained by the maximum likelihood computer procedure described in the Methods. The mean±SD of 214.2±28.5 mg/100 ml for the first component was very close to that of controls (218); the mean of the second component was 299.2±58.3.[5]

The mean±SD for triglyceride of relatives (89.7 ±53.1 mg/100 ml) was not significantly different from that of controls (93±48), and the distributions shown in Figs. 2B and 3B were unimodal.

The pedigrees of each of the 16 families in this group are presented in Fig. 4. Vertical transmission of hypercholesterolemia is evident from the frequent expression in offspring of affected individuals. Although hypertriglyceridemia was observed in some of these hypercholesterolemic individuals, their whole plasma ratio of cholesterol/triglyceride was almost always greater than 2, a finding not observed in individuals with other forms of familial hyperlipidemia (2,3). Finally, at least seven of these families had one or more affected members known to have tendinous xanthomas (viz., families 45, 120, 289, 292, 314, and 497).

In order to determine the pattern of inheritance of the hypercholesterolemia in these 16 families, segregation analyses were carried out. In the absence of a complete separation between the cholesterol distributions of normal and affected phenotypes, relatives were classified as affected or normal depending on whether or not the adjusted cholesterol level equaled or exceeded the 95th percentile of controls (285 mg/100 ml). This value closely reflected the physiologic separation between affected and unaffected relatives as determined both in this study (Figs. 2A and 3A) and in an independent study of familial monogenic hypercholesterolemia (5).[6]

Sib analysis is probably the method of choice for analyzing family lipid data of the type collected in this study. Sibs are usually of a similar age range as probands and will express a given lipid disorder which may not be apparent in children (see below). Furthermore, other methods of segregation analysis, which consider phenotypes of living parents, are more likely to show a deficiency in the number of affected living relatives since many affected hyperlipidemic parents may have already died of myocardial infarction (17).

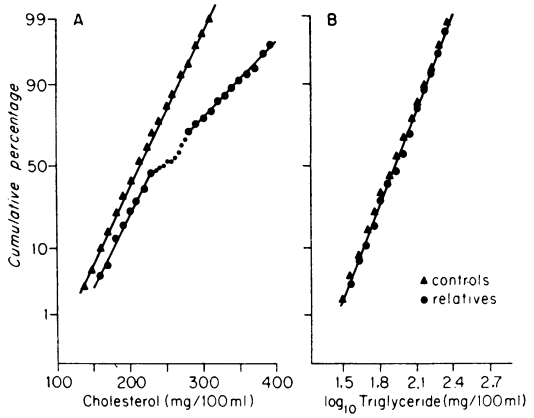

FIGURE 3 Cumulative frequency distribution of adjusted lipid levels in 132 near and distant relatives of 16 survivors with familial hypercholesterolemia. The composition of the relatives group is identical with that described for Fig. 2. The cholesterol and $\log_{10}$ triglyceride values were age and sex-adjusted as described in the accompanying paper (10).

The finding of an apparently bimodal distribution in the levels of cholesterol and not triglyceride in relatives, together with the results of the sib analysis showing that about one-half of living sibs were hypercholesterolemic (Table II), are consistent with autosomal dominant inheritance. On the assumption that affected individuals die prematurely (17), one might expect a deficiency in affected sibs. The lack of such a deficiency in our data presumably reflects the relatively young age (mean = 46 yr) of the probands and their sibs. The analysis of the proportion of affected first- and second-degree relatives (Table III) further confirmed autosomal dominant inheritance and demonstrated nearly complete expression of the hypercholesterolemia gene in affected children. The finding of a higher than expected number of affected second-degree adult relatives is probably a reflection of an ascertainment bias due to the small total number of relatives tested (n = 24) in this category.

Group A-2: familial hypertriglyceridemia. The frequency distribution of adjusted lipid levels in 132 adult relatives of 23 hypertriglyceridemic survivors (18 men, 5 women) is shown in Fig. 5. As compared with that of controls, the distribution of triglyceride was suggestive of bimodality (Fig. 5B). When the cumulative frequencies of these data were examined on log probability paper, the plot of the relatives contained two straight line segments, indicating two Gaussian curves (Fig. 6B). The upper limit of the break in the plot (i.e., the antimode for separation of normal and

[5] The Chi-square goodness of fit was significantly better for a mixture of two overlapping distributions ($P > 0.5$) than for a single distribution ($P < 0.025$).

[6] The cut-off value for separating normal and affected individuals can be derived by at least four methods (reviewed by Murphy [16]), but each of these methods gives approximately the same dividing point when the two component populations are nearly equal size, as is the case in our data. We placed the cut-off point for cholesterol at the 95th percentile of controls, so that the number of normal individuals who are misclassified as affected are minimized and the total number of affected family members is underestimated.

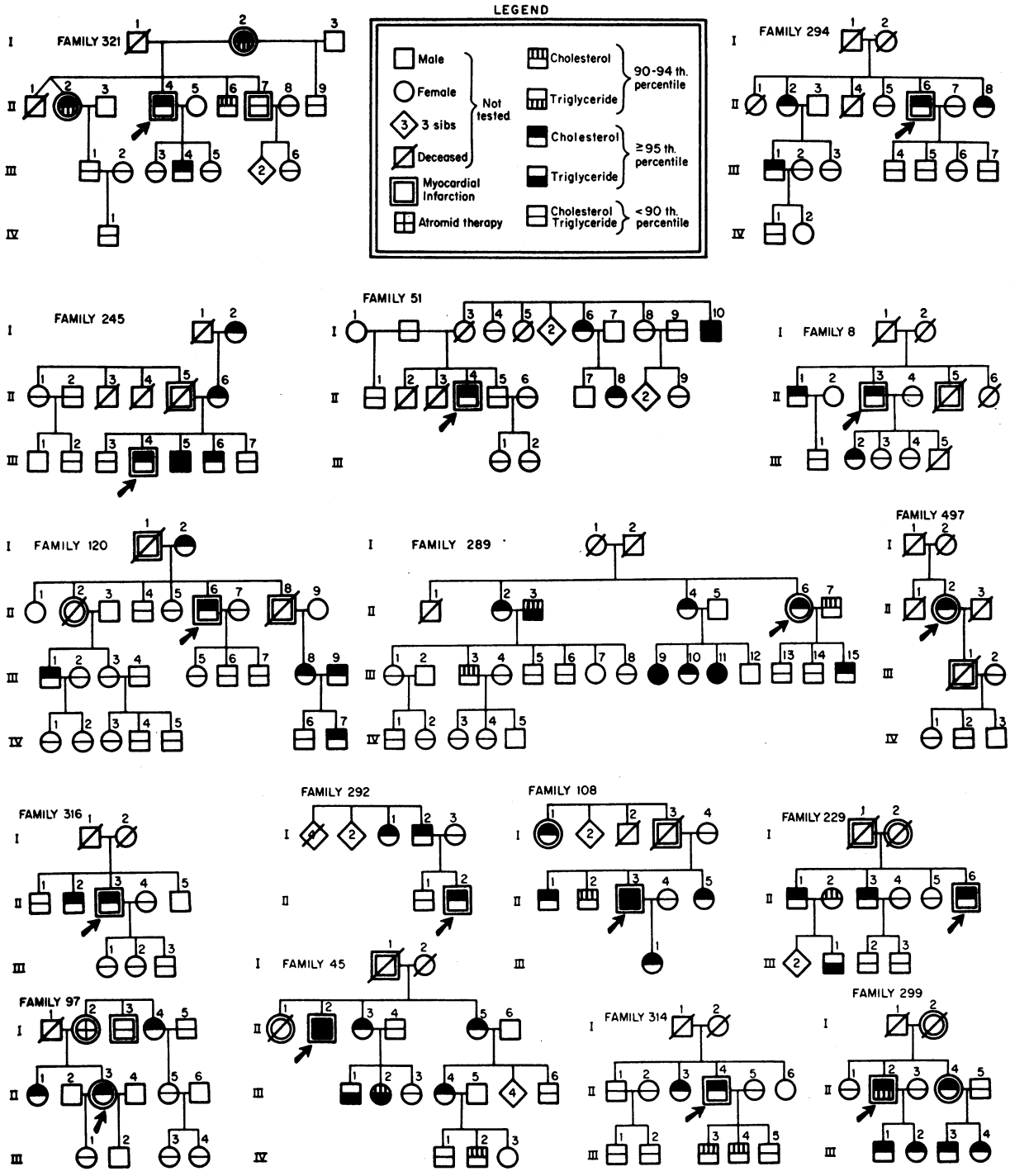

FIGURE 4 Composite showing the pedigrees of 16 families with familial hypercholesterolemia. The legend is shown in the middle of the first row of pedigrees. The proband for each family is indicated by an arrow. Spouses belonging to matings for which there were no data available for the offspring have been omitted from the pedigrees. Although family 497 does not meet the criterion of having at least one relative of the proband with a 99th percentile cholesterol value, it has been included in this group because the proband's son (III, 1) died at age 48 yr of myocardial infarction and was known to have hypercholesterolemic xanthomatosis. Identification and lipid data for each family member are available upon request. These data have been deposited with the National Auxiliary Publications Service (ID no. 02057).

TABLE II

Analysis of Sibships of 16 Survivors with Familial Hypercholesterolemia

	Sibs (no.)					Number affected	
		Dead					
	Total	By all causes	By MI*	Living	Tested	Observed‡	Expected§
Brothers	24	8	2	16	15	5	7.5
Sisters	27	4	2	23	19	14	9.5
Total	51	12‖	4	39	34	19	17.0

* Deaths by myocardial infarction (MI) were documented by death certificates.
‡ Relatives were considered affected if the age and sex-adjusted plasma cholesterol level was equal to or exceeded the 95th percentile of controls.
§ Expected number of affected relatives by the hypothesis of autosomal dominant inheritance, assuming no effect of the gene on mortality at the ages tested.
‖ 5 of these 12 deaths occurred before age 35 yr.

TABLE III

Proportion of Affected Relatives of 16 Survivors with Familial Hypercholesterolemia

Age of relatives and degree of relation	No. tested	Affected relatives		
		Observed		
		90th percentile	95th percentile	Expected‡
		%	%	%
A. ≥ Age 25				
1st degree	44	54.6	50.0	50.0
2nd degree	24	41.7	37.5	25.0
B. < Age 25				
1st degree	22	45.5	38.1	50.0
2nd degree	23	26.1	21.7	25.0

* Relatives were considered affected if the age and sex-adjusted plasma cholesterol level was equal to or exceeded the indicated percentile of controls.
‡ Expected proportion of affected relatives on the hypothesis of autosomal dominant inheritance, assuming no effect of the gene on mortality of the ages tested and assuming no introduction of the gene by those marrying into the family.

TABLE IV

Analysis of Sibships of 23 Survivors with Familial Hypertriglyceridemia

	Sibs (no.)					Number affected	
		Dead					
	Total	By all causes	By MI*	Living	Tested	Observed‡	Expected§
Brothers	47	19	6	28	25	12	12.5
Sisters	38	8	0	30	26	12	13.0
Total	85	27‖	6	58	51	24	25.5

* Deaths by myocardial infarction (MI) were documented by death certificates.
‡ Relatives were considered affected if the age and sex-adjusted plasma triglyceride level was equal to or exceeded the 95th percentile of controls.
§ Expected number of affected relatives by the hypothesis of autosomal dominant inheritance, assuming no effect of the gene on mortality at the ages tested.
‖ 17 of these 27 deaths occurred before age 35 yr.

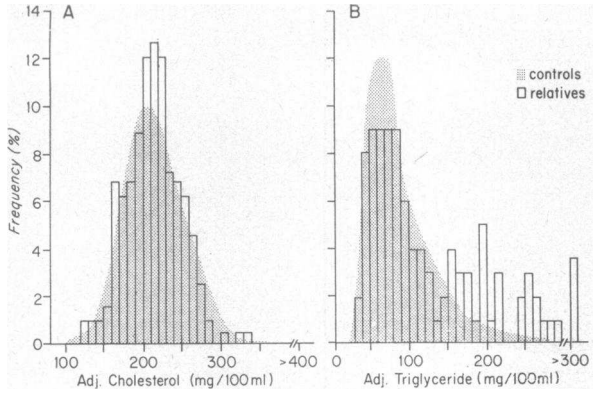

FIGURE 5 Frequency distribution of adjusted lipid levels in 132 adult (≥20 yr of age) relatives of 23 survivors with familial hypertriglyceridemia. Included in this analysis were 90 first-degree relatives, 30 second-degree relatives, and 12 third-degree relatives. Age and sex-adjustments were carried out as described in the accompanying paper (10). The distribution is divided into increments of 10 mg/100 ml. The smooth strippled curve represents a nonparametric density estimate of the control distribution.

affected relatives) corresponded to the 90th to 95th percentile range of controls. The best fitting two-distribution mixture, obtained by the maximum likelihood method, indicated that the mean±SD of normal relatives was 76.2±21.2, while the mean for affected relatives was 180.1±65.3.[7]

Cholesterol levels in these same relatives were distributed as one mode that corresponded to controls (Figs. 5A and 6A). The mean±SD for cholesterol in relatives (217±36 mg/100 ml) was almost identical with that of controls (218±41).

The pedigrees of each of the 23 families in this group are presented in Fig. 7. The inspection of these pedigrees indicates vertical transmission, but with only occasional expression of hypertriglyceridemia in children (about 12% of those at risk), and a high frequency of death by myocardial infarction in presumed carriers of the hypertriglyceridemia gene, such as parents of probands.

All these findings, as well as the results of the sib analysis (Table IV), are consistent with an autosomal dominant mechanism of inheritance. As in familial hypercholesterolemia, no deficiency in affected sibs was noted, suggesting that any effect of the gene on mortality might be delayed until the latter part of the 5th decade of life. Table V analyzes the proportion of affected first-degree relatives (including parents and children as well as sibs) and second-degree relatives. The slight deficiency in the expected number of affected adult first-degree relatives (40% observed; 50% expected) was due to a deficiency in the number of affected living parents.

Group A-3: familial combined hyperlipidemia. A large number of families had affected members who expressed different combinations of elevated cholesterol and elevated triglyceride levels. Thus, affected members of the same family showed either an elevated cholesterol level with a type II*a* lipoprotein pattern, an elevated triglyceride level with a type IV or type V pattern, or they showed both elevated cholesterol and triglyceride levels with a type II*b* pattern (11). The pedigree of a large family with combined hyperlipidemia (family 41) is shown in Fig. 8, and the lipid data for each family member in Table VI. In this family the hyperlipidemia appeared to be transmitted as a simple Mendelian autosomal dominant trait. All offspring of normals were unaffected, and on the average one-half the offspring of affected were hyperlipidemic.

Among 157 hyperlipidemic survivors identified in the accompanying paper (10), 47 (36 men, 11 women) had pedigrees in which the combined disorder appeared to be present. Fig. 9 shows the frequency distribution of adjusted lipid levels in their 234 adult first-degree relatives. As compared with that of controls, the distribution of triglyceride was suggestive of bimodality (Fig. 9B). The cumulative frequency when plotted on log probability paper confirmed the presence of two distributions (Fig. 10B). The best fitting two-distribution mixture, obtained by the maximum likelihood method, indicated that the mean±SD of the normal distribution was 74.0±20.5 mg/100 ml, whereas the

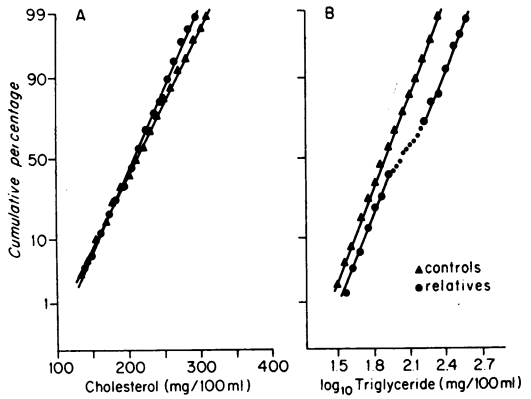

FIGURE 6 Cumulative frequency distribution of adjusted lipid levels in 132 adult (≥20 yr of age) relatives of 23 survivors with familial hypertriglyceridemia. The composition of the relatives group is identical with that described for Fig. 5. The cholesterol and $\log_{10}$ triglyceride values were age and sex-adjusted as described in the accompanying paper (10).

[7] The Chi-square goodness of fit was significantly better for a mixture of two overlapping distributions ($P > 0.5$) than for a single distribution ($P < 0.005$).

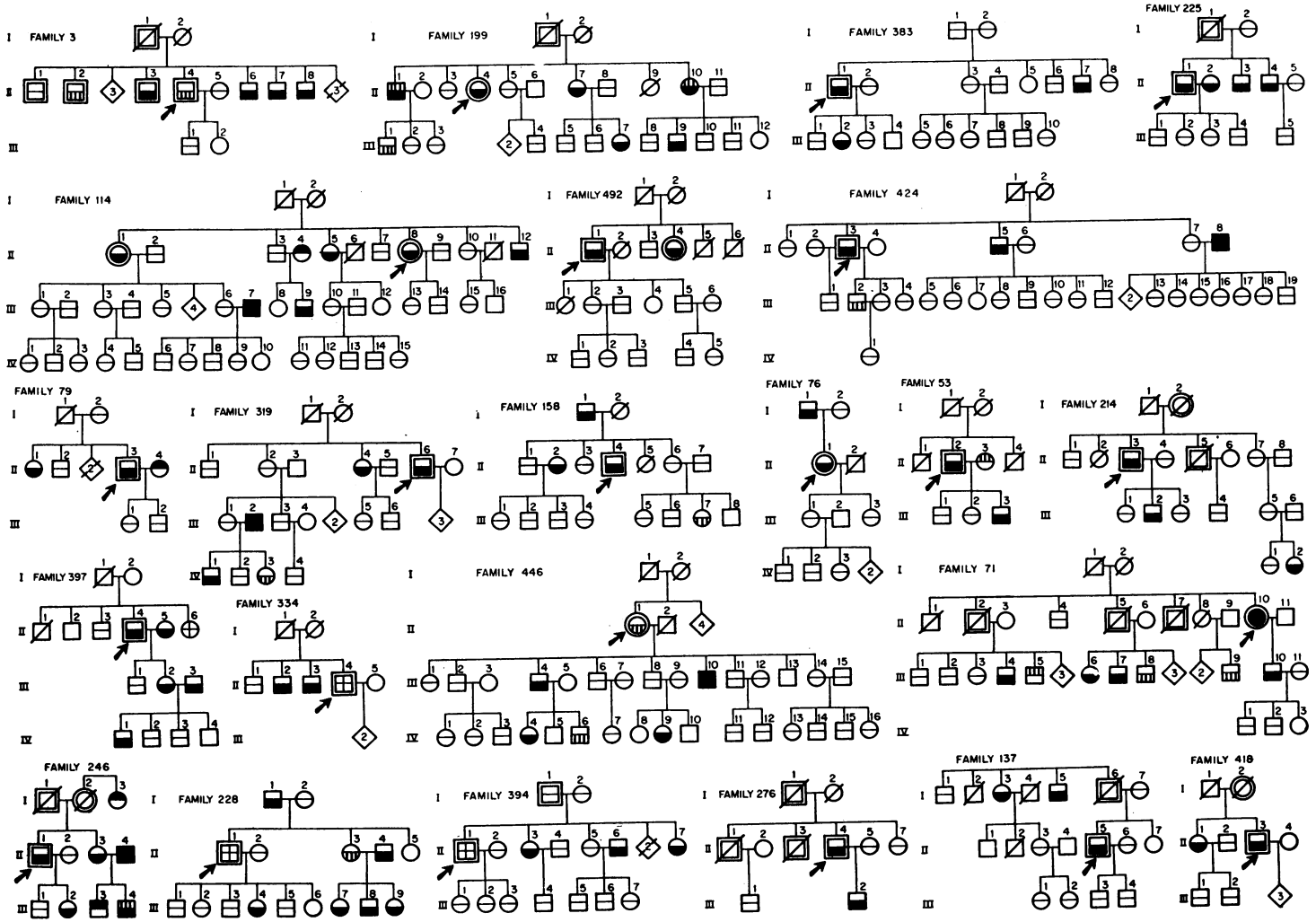

FIGURE 7 Composite showing the pedigrees of 23 families with familial hypertriglyceridemia. The legend for these pedigrees is identical with that shown for Fig. 4. The proband for each family is indicated by ↗. Two individuals (II, 10 in family 71 and III, 10 in family 446) who showed elevations in cholesterol as well as triglyceride had hyperchylomicronemia. Spouses belonging to matings for which there were no data available for the offspring have been omitted from the pedigrees. Identification and lipid data for each family member are available upon request (deposited with the National Auxiliary Publications Service [ID no. 02057]).

TABLE V

Proportion of Affected Relatives of 23 Survivors with Familial Hypertriglyceridemia

Age of relatives and degree of relation	No. tested	Affected relatives: Observed* 90th percentile	Affected relatives: Observed* 95th percentile	Affected relatives: Expected‡
		%	%	%
A. ≥ Age 25				
1st degree	83	42.2	36.2	50.0
2nd degree	31	29.0	20.0	25.0
B. < Age 25				
1st degree	30	16.7	13.3	50.0
2nd degree	68	10.3	10.3	25.0

* Relatives were considered affected if the age and sex-adjusted plasma cholesterol level was equal to or exceeded the indicated percentile of controls.

‡ Expected proportion of affected relatives on the hypothesis of autosomal dominant inheritance, assuming no effect of the gene on mortality at the ages tested and assuming no introduction of the gene by those marrying into the family.

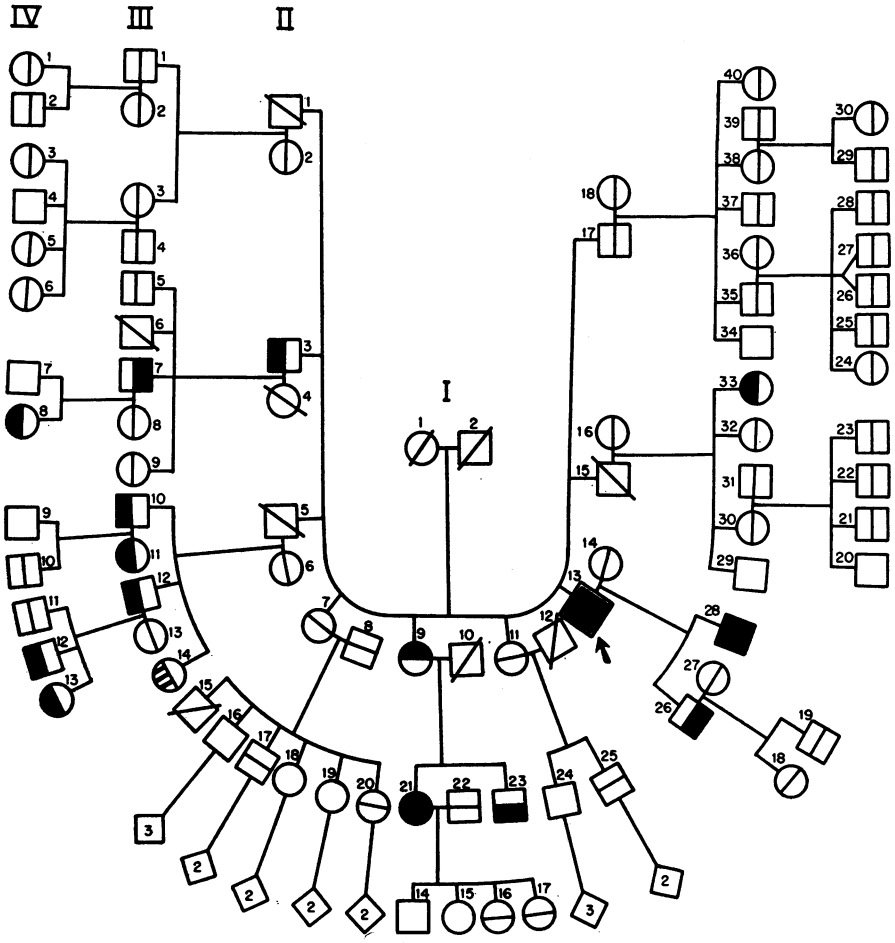

FIGURE 8 Pedigree of a family with familial combined hyperlipidemia. The legend for this pedigree is identical with that shown for Fig. 4. The proband is indicated by an arrow. The kindred is referred to elsewhere as family 41. A summary of the plasma lipid values in relatives and spouses is shown in Table VI.

TABLE VI

Summary of Plasma Lipid Values in Members and Spouses of Family 41

Pedigree position	Sex	Age	Unadjusted lipid levels: Cholesterol	Unadjusted lipid levels: Triglyceride	Lipoprotein type‡
			mg/100 ml		
I 1	F	87	Cause of death unknown		
2	M	86	Cause of death unknown		
II 1	M	50	Cancer*		
2	F	79	226	186	
3	M	84	258	453	IV
5	M	47	Cancer*	—	
6	F	75	252	145	
7	F	79	251	111	
8	M	78	151	84	
9	F	75	364	196	
11	F	67	288	115	
13	M	65	341	268	IIb
14	F	67	268	128	
15	M	47	Trauma*	—	
16	F	59	210	105	
17	M	60	238	81	
18	F	55	197	64	
III 1	M	58	228	135	
2	F	56	201	116	
3	F	56	280	162	
4	M	59	220	36	
5	M	54	231	93	
6	M	40	Smoke inhalation*	—	
7	M	52	293	85	IIa
8	F	50	234	153	
9	F	45	219	56	
10	M	51	239	215	IV
11	F	45	274	171	
12	M	52	259	198	
13	F	51	224	86	
14	F	46	230	152	
15	M	36	Trauma*	—	
17	M	34	251	86	
20	F	27	240	90	
21	F	58	366	300	IIb
22	M	45	219	56	
23	M	55	229	200	
25	M	47	252	152	
26	M	48	248	225	IV
27	F	51	212	104	
28	M	42	328	165	IIb
30	F	37	239	40	
31	M	40	209	59	
32	F	31	229	72	
33	F	26	268	95	
35	M	37	146	104	
36	F	37	163	58	
37	M	29	211	79	
38	F	34	168	46	
39	M	37	232	66	
40	F	15	111	28	
IV 1	F	28	186	72	
2	M	26	195	85	
3	F	33	202	57	
5	F	28	235	60	
6	F	19	162	45	
8	F	23	222	124	
10	M	15	177	72	
11	M	29	179	83	
12	M	24	187	190	
13	F	22	178	166	
16	F	27	180	95	
17	F	25	194	80	
18	F	19	206	104	
19	M	14	151	40	
21	M	17	144	30	
22	M	15	123	25	
23	M	13	189	28	
24	F	14	143	44	
25	M	13	146	38	
26	M	12	145	175	
27	M	12	137	47	
28	M	8	164	60	
29	M	9	147	30	
30	F	8	146	39	

* Cause of death.

‡ Lipoprotein quantification and typing were determined by methods described in the accompanying paper (11).

mean of the abnormal distribution was 172.3±76.6 mg/100 ml.[8]

In these same relatives the distribution of cholesterol was not bimodal but appeared unimodal and shifted to the right of controls (Figs. 9A and 10A). Its mean±SD (250±56 mg/100 ml) was significantly higher than that of the controls (218±41) ($P < 0.001$). Fig. 11 indicates that this apparently unimodal curve was actually composed of two overlapping distributions —a normal cholesterol distribution in relatives whose triglyceride levels were normal (<90th percentile) and a shifted cholesterol distribution in relatives whose triglyceride levels were elevated (≥90th percentile). The difference in mean±SD of these two distributions (238±50 and 277±60 mg/100 ml, respectively), although highly significant ($P < 0.001$), was not great enough to be resolved as a bimodal curve unless the triglyceride level was used as a discriminant (Fig. 9A). It should be noted that while the distribution and median of the cholesterol levels in the normotriglyceridemic relatives resembled those of controls (Fig. 11), the actual mean±SD of the cholesterol levels of the normotriglyceridemic relatives (238±50) was significantly higher than that of the controls (218±41) ($P < 0.01$). This higher mean value in the normotriglyceridemic relatives was due to the cholesterol values of about 6% of relatives who showed hypercholesterolemia without hypertriglyceridemia. These data suggested that familial combined hyperlipidemia

[8] The Chi-square goodness of fit was significantly better for a mixture of two overlapping distributions ($P > 0.5$) than for a single distribution ($P < 0.005$).

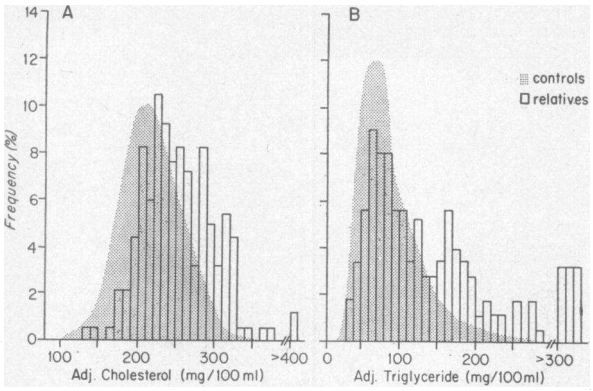

FIGURE 9 Frequency distribution of adjusted lipid levels in 234 first-degree adult (≥20 yr of age) relatives of 47 survivors with familial combined hyperlipidemia. Age and sex-adjustments were carried out as described in the accompanying paper (10). The distribution is divided into increments of 10 mg/100 ml. The smooth strippled curve represents a nonparametric density estimate of the control distribution.

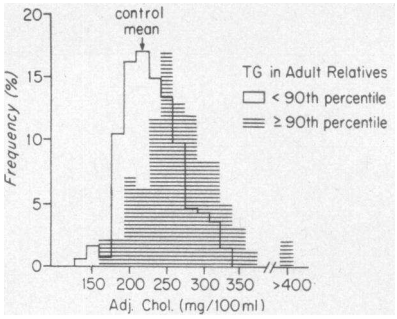

FIGURE 11 Relation between the level of cholesterol and triglyceride in 234 first-degree adult (≥20 yr of age) relatives of 47 survivors with familial combined hyperlipidemia. The 234 relatives were divided into two groups depending on whether their triglyceride level fell above (n = 98) or below (n = 136) the 90th percentile of controls. The mean value for adjusted cholesterol levels in controls (218 mg/100 ml) is indicated by the arrow at the top of the figure.

is determined by a single gene whose primary action is on triglyceride metabolism with secondary effects on cholesterol metabolism. This interpretation comes from the generalization that when several phenotypic effects of a gene are considered, discrimination into distinct genetic classes becomes increasingly evident as one gets closer to the primary effect of the gene (18). As a control, similar analyses of the cholesterol curve of the adult relatives of the familial hypertriglyceridemic disorder were done. These data indicated that although the mean cholesterol of the hypertriglyceridemic relatives (228 mg/100 ml) was slightly higher than that of the unaffected relatives (211 mg/100 ml) ($P < 0.1$, but > 0.05), neither the hypertriglyceridemic nor the unaffected relatives had cholesterol levels that were significantly different from those of controls ($P > 0.05$).

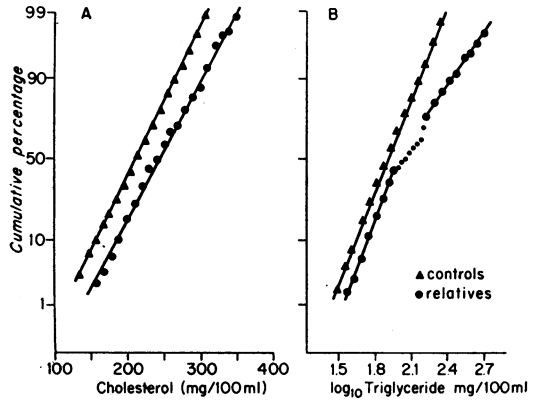

FIGURE 10 Cumulative frequency distribution of adjusted lipid levels in 234 first-degree adult (≥20 yr of age) relatives of 47 survivors with familial combined hyperlipidemia. The cholesterol and $\log_{10}$ triglyceride values were age and sex-adjusted as described in the accompanying paper (10).

The pedigrees of 46 of the 47 families in this group are presented in Figs. 12A and 12B. (The data for the 47th family are shown in Fig. 8 and Table VI). Inspection of these pedigrees demonstrates the following: vertical transmission of hyperlipidemia; the rare occurrence of hypercholesterolemia in children; occurrence of hypertriglyceridemia in about 9% of children at risk; a striking degree of variability in the lipid phenotypes among affected relatives in the same family; and the almost complete absence of hyperlipidemic segregants among the offspring of two normolipidemic parents.

Table VII presents the results of a sib analysis of the 47 survivors with familial combined hyperlipidemia. The proportion affected with hyperlipidemia (nearly 50%) is consistent with an autosomal dominant mechanism of inheritance and excludes the segregation of two genes (one elevating cholesterol and one elevating triglyceride). With segregation of two genes, 75% of sibs would have been expected to manifest hyperlipidemia (i.e., 25% normal, 25% hypercholesterolemic, 25% hypertriglyceridemic, and 25% both). In Table VII, the expected proportion of affected sibs (50% of those tested) assumes that there is no effect of this gene on mortality at the ages tested. However, this assumption is not completely valid, since 10 out of the 24 sib deaths above the age of 35 yr were caused by myocardial infarction (Table VII). Assuming that each of these 10 dead sibs carried the gene for combined hyperlipidemia and that the other 14 sibs were noncarriers, then the ratio of affected to normal would be 61 + 10 = 71 affected as compared with 65 + 14 = 79 normal. It is, of course, possible that some family

TABLE VII

Analysis of Sibships of 47 Survivors with Familial Combined Hyperlipidemia

	Sibs (no.)						
		Dead				Number affected	
	Total	By all causes	By MI*	Living	Tested	Observed‡	Expected§
Brothers	95	24	9	71	60	30 (9, 7, 14)¶	30
Sisters	89	14	1	75	66	31 (7, 11, 13)¶	33
Total	184	38‖	10	146	126	61	63

* Deaths by myocardial infarction (MI) were documented by death certificates.

‡ A relative was considered affected if either his cholesterol or his triglyceride level or both equaled or exceeded the 95th percentile values for cholesterol and triglyceride.

§ Expected number of affected relatives by the hypothesis of autosomal dominant inheritance, assuming no effect of the gene on mortality at the ages tested.

‖ 14 of these 38 deaths occurred before age 35 yr.

¶ Distribution (no.) of phenotypes of affected relatives are indicated in parenthesis in the following order: hypercholesterolemia alone, hypertriglyceridemia alone, and both hypercholesterolemia and hypertriglyceridemia.

members who died of causes other than myocardial infarction also carried this gene. However, this would have no significant effect on the segregation ratio. This distribution of lipid phenotypes among affected adult relatives (Tables VII and VIII) showed about one-third with the characteristic elevation in both cholesterol and triglyceride, one-third with elevated cholesterol alone, and one-third with elevated triglyceride alone. Affected children with hyperlipidemia had hypertriglyceridemia either with or without hypercholesterolemia, but they almost nerver had hypercholesterolemia alone (Table VIII). The sex ratio (male/female) of all affected relatives was 1.06. Similar to the data in familial hypertriglyceridemia, the observed deficiency in first-degree relatives above age 25 reflected a dearth of affected older relatives,

TABLE VIII

Proportion of Affected Relatives of 47 Survivors with Familial Combined Hyperlipidemia

Age of relatives and degree of relation	No. tested	Affected relatives		
		Observed*		
		90th percentile	95th percentile	Expected‡
		%	%	%
A. ≥ Age 25				
1st degree	212	50.0§ (13.5, 16.2, 20.3)	41.7 (10.9, 13.5, 17.3)	50.0
2nd degree	77	37.7 (11.7, 18.2, 7.8)	31.1 (10.4, 16.8, 3.9)	25.0
B. < Age 25				
1st degree	76	15.8 (2.6, 9.2, 4.0)	9.2 (0.0, 7.9, 1.3)	50.0
2nd degree	143	16.1 (3.5, 7.0, 5.6)	13.3 (1.4, 8.4, 3.5)	25.0

* A relative was considered affected if either his cholesterol or his triglyceride level or both equaled or exceeded the indicated percentile value.

‡ Expected proportion of affected relatives on the hypothesis of autosomal dominant inheritance, assuming no effect of the gene on mortality at the ages tested and assuming no introduction of the gene by those marrying into the family.

§ Distribution (percent) of phenotypes of affected relatives are indicated in parenthesis in the following order: hypercholesterolemia alone, hypertriglyceridemia alone, and both hypercholesterolemia and hypertriglyceridemia.

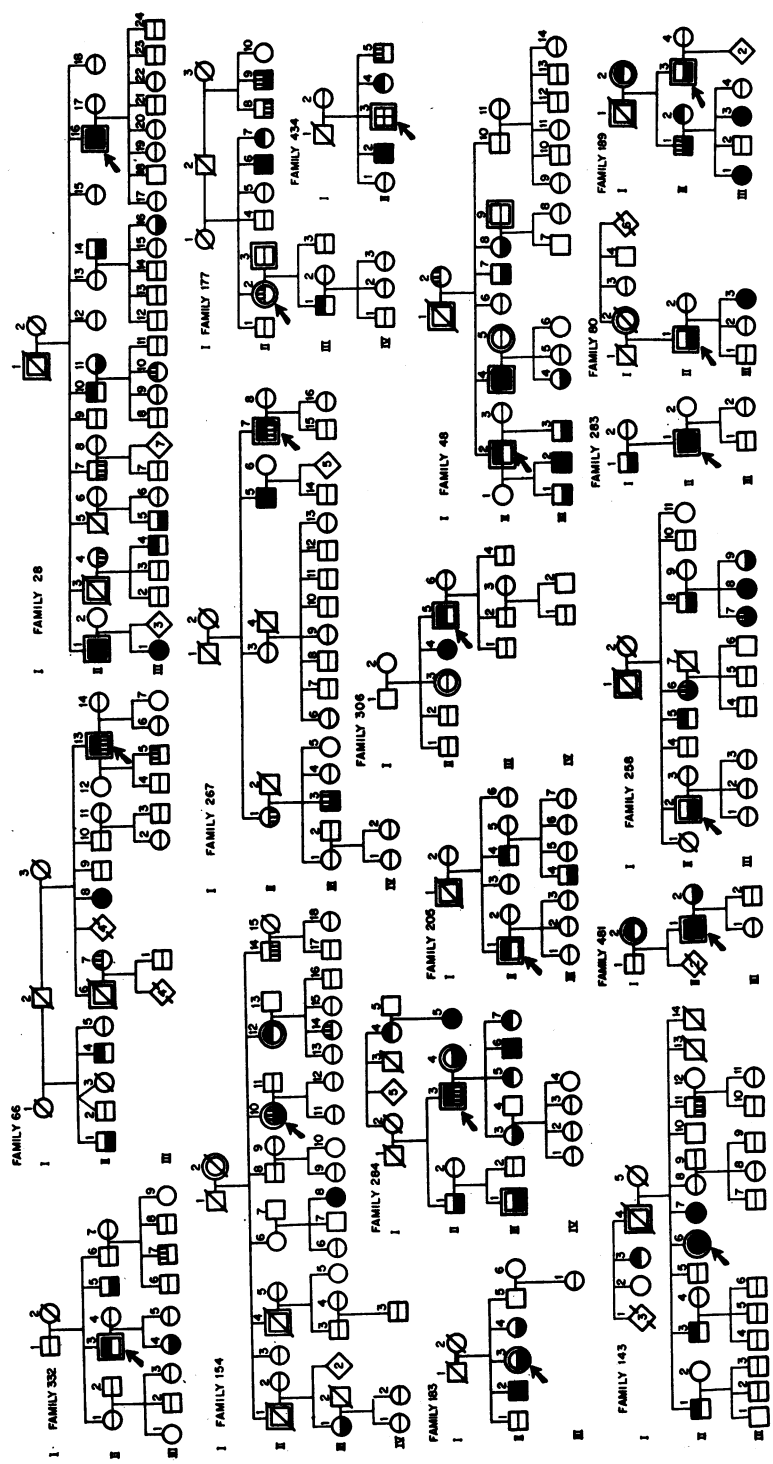

FIGURE 12 A Composite showing 23 of the 47 pedigrees with familial combined hyperlipidemia. The remaining 24 families are shown in Figs. 8 and 12 B. The legend for these pedigrees is identical with that shown for Fig. 4. The proband for each family is indicated by an arrow. Spouses belonging to matings for which there were no data available for the offspring have been omitted from these pedigrees. Identification and lipid data for each family member are available upon request (deposited with the National Auxiliary Publications Service [ID no. 02057]).

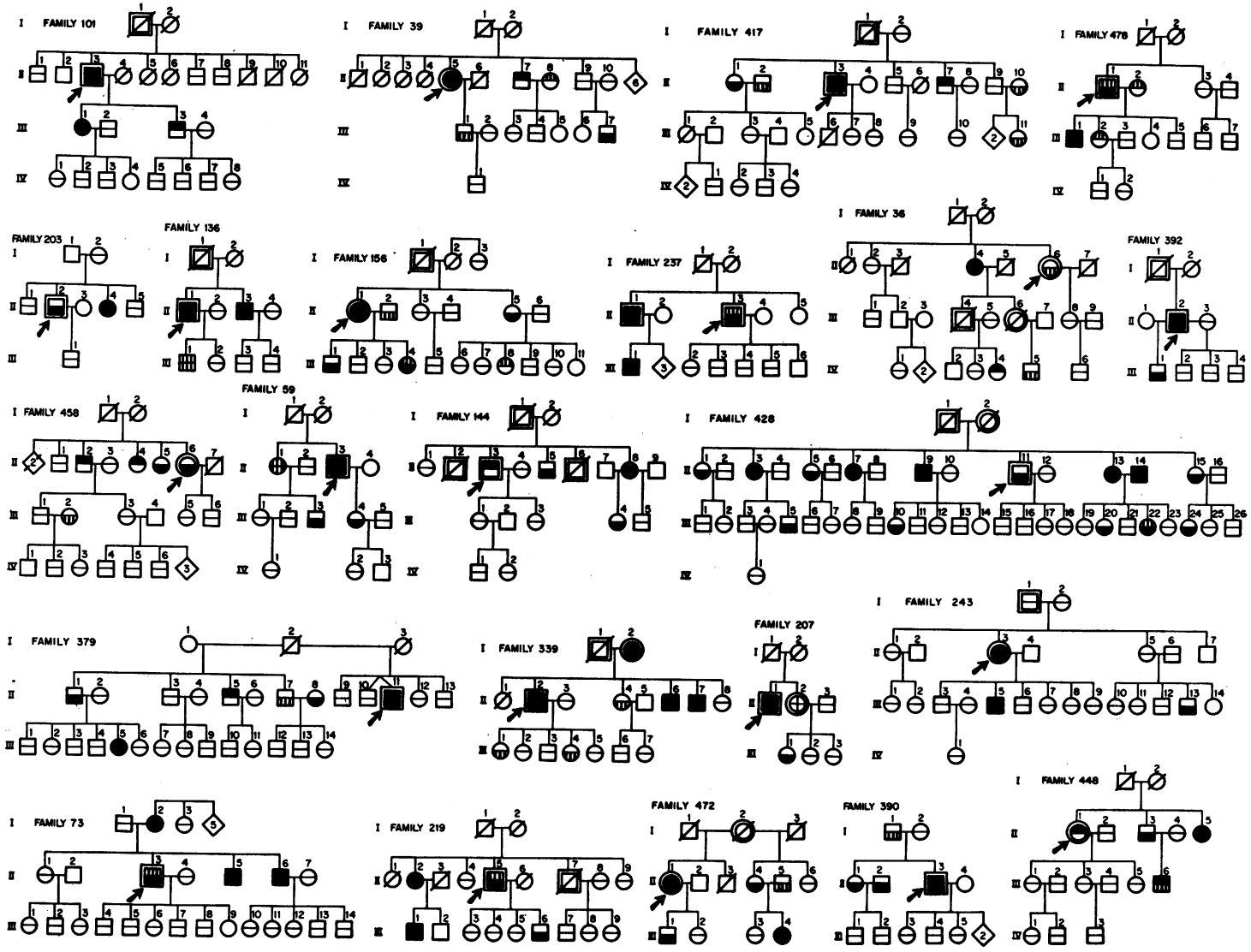

FIGURE 12 B Composite showing 23 of the 47 pedigrees with familial combined hyperlipidemia. The remaining 24 families are shown in Figs. 8 and 12 A. The legend for these pedigrees is identical to that shown for Fig. 4. The proband for each family is indicated by an arrow. Spouses belonging to matings for which there were no data available for the offspring have been omitted from these pedigrees. Identification and lipid data for each family member are available upon request (deposited with the National Auxiliary Publications Service [ID no. 02057]).

TABLE IX

*Analysis of 22 Informative Matings from 20 Families with Familial Combined Hyperlipidemia**

Mating type (no.)	Distribution of phenotypes in offspring (no.)			
	Normal	↑Cholesterol	↑Triglyceride	↑Both
↑Cholesterol × normal (6)	4	1	3	2
↑Triglyceride × normal (9)	10	1	6	5
↑Both × normal (7)	10	0	8	3

* Parents and offspring were considered affected if the indicated lipid level equaled or exceeded the 95th percentile of controls. This analysis includes all matings from families with combined hyperlipidemia which met both of these criteria: (*a*) the parental mating type was affected × normal and (*b*) at least one offspring was affected.

such as parents, whose deaths by myocardial infarction might be ascribed to the effect of the gene.

Further evidence suggesting that familial combined hyperlipidemia is determined by a single gene with variable expression is indicated in Table IX, an analysis of 22 informative matings from 20 of the 47 families. Note the hypertriglyceridemic offspring (elevation in triglyceride alone or in both lipids) from hypercholesterolemic × normal matings and also the hypercholesterolemic offspring (elevation in cholesterol alone or in both lipids) from hypertriglyceridemia × normal matings. In 14 other matings between a hypercholesterolemic and a normal parent, there was a complete absence of hypercholesterolemia among 32 children, a finding quite different from that observed in families with familial hypercholesterolemia (5, 19, 20). In 20 additional matings between two normal individuals. only 2 out of 49 offspring (over half of which were above 20 yr of age) were hyperlipidemic.

Comparison of lipids in relatives, ages 6–20, in familial hypercholesterolemia, familial hypertriglyceridemia, and familial combined hyperlipidemia. Fig. 13 compared the distribution of *unadjusted* plasma lipids in first-, second-, and third-degree relatives, ages 6–20, from families with familial hypercholesterolemia (Fig. 13A), familial hypertriglyceridemia (Fig. 13B), and familial combined hyperlipidemia (Fig. 13C). Unadjusted values were used in this analysis since plasma lipid levels in 110 control individuals (ages 6–20) showed no significant correlation with age (see legend to Fig. 13 and reference 21). The cholesterol distribution was bimodal in the young relatives of probands with familial hypercholesterolemia (Fig. 13A, left). The cholesterol curve in relatives of probands with combined hyperlipidemia was predominantly unimodal, although there was a slight suggestion of a second mode involving about 2% of the total sample (Fig. 13C, left). The triglyceride distribution in familial hypercholesterolemia was unimodal (Fig. 13A, right), but appeared bimodal in familial hypertriglyceridemia (Fig. 13B, right) and in familial combined hyperlipidemia (Fig. 13C, right). These data provide further evidence that familial combined hyperlipidemia is genetically distinct from familial hypercholesterolemia and demonstrate

TABLE X

Analysis of Sibships of 31 Survivors with Sporadic Hypertriglyceridemia

	Sibs (no.)						
		Dead				Number affected	
	Total	By all causes	By MI*	Living	Tested	Observed‡	Expected‡
Brothers	57	28	4	29	23	1	11.5
Sisters	52	15	1	37	34	0	17.0
Total	109	43‖	5	66	57	1	28.5

* Deaths by myocardial infarction (MI) were documented by death certificates.

‡ Relatives were considered affected if the age and sex-adjusted plasma triglyceride level was equal to or exceeded the 95th percentile of controls.

§ Expected number of affected relatives by the hypothesis of autosomal dominant inheritance.

‖ 16 of these 43 deaths occurred before age 35 yr.

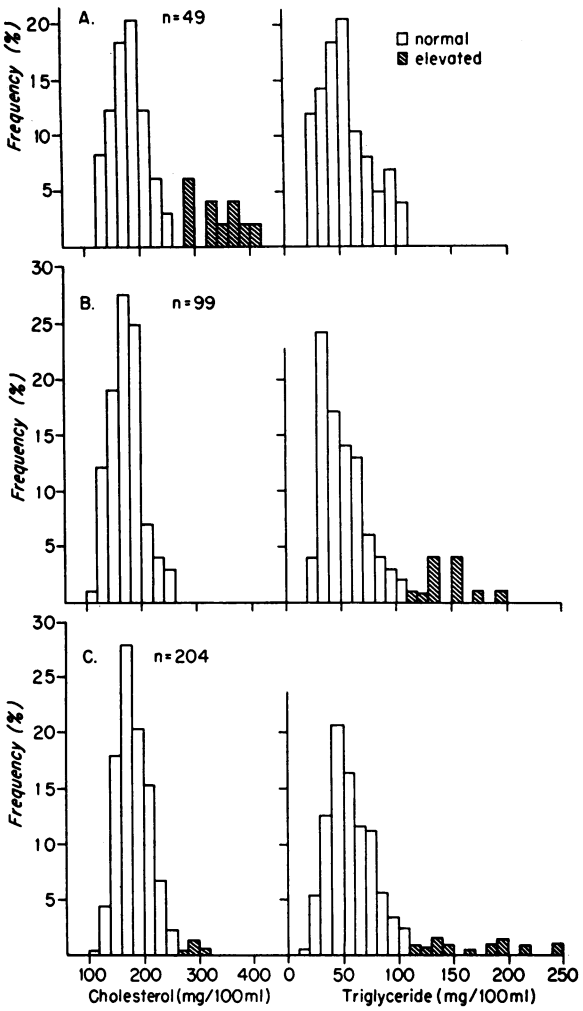

FIGURE 13 Frequency distribution of unadjusted lipid levels in near and distant relatives, age 6–20, of 16 probands with familial hypercholesterolemia (A), 23 probands with familial hypertriglyceridemia (B), and 47 probands with familial combined hyperlipidemia (C). The number of relatives tested for each disorder is indicated in the appropriate panel. No age and sex-adjustments were applied to these data since no significant correlation with age was observed in values from 110 controls, ages 6–20 (see below). Moreover, when these data were reanalyzed using the age and sex-adjustments described in the accompanying paper (10), identical profiles in the lipid distributions were observed for each disorder. The only difference produced by the adjustment to age 45 was that the absolute lipid levels of each individual was higher. The arbitrary designation of normal and elevated was made from a consideration of the lipid levels in the 110 controls, ages 6–20, whose values were collected as part of the family studies of the 27 normolipidemic survivors. Since the highest unadjusted cholesterol and triglyceride values observed in these controls were 250 and 115 mg/100 ml, respectively, these values were arbitrarily considered as maximum upper limits of normal for the age range.

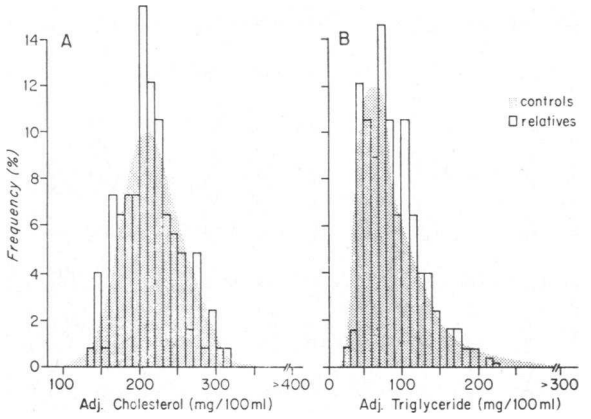

FIGURE 14 Frequency distribution of 158 near and distant adult (≥20 yr of age) relatives of 31 survivors with sporadic hypertriglyceridemia. Included in this analysis were 105 first-degree, 44 second-degree, and 9 third-degree relatives. Age and sex-adjustments were carried out as described in the accompanying paper (10). The distribution is divided into increments of 10 mg/100 ml. The smooth strippled curve represents a nonparametric density estimate of the control distribution.

that the earliest manifestation in affected individuals with combined hyperlipidemia is hypertriglyceridemia alone.

Group B: sporadic hypertriglyceridemia and polygenic hypercholesterolemia. In addition to the 86 survivors of myocardial infarction with monogenic forms of hyperlipidemia (groups A-1, A-2, and A-3,) 59 hyperlipidemic survivors (group B) were identified in whom the family lipid data did not differ significantly from that of the normolipidemic families (Table I). Since these survivors in group B consisted of a mixture of hypertriglyceridemic and hypercholesterolemic individuals, we arbitrarily divided them into two groups: those with pure hypertriglyceridemia (n = 31) and those with hypercholesterolemia with or without hypertriglyceridemia (n = 28).

The distribution of adjusted plasma lipids of 158 adult relatives of these nonfamilial pure hypertriglyceridemic survivors is shown in Fig. 14. Both the cholesterol and triglyceride curves in relatives appeared unimodal, and the mean±SD for cholesterol (211±41 mg/100 ml) and for triglyceride (93±52 mg/100 ml) were almost identical with those of controls. Analysis of the sibs of these 31 survivors (Table X) showed that less than 2% were hypertriglyceridemic. Both lines of evidence suggest that inheritance factors do not play a direct role in the pathogenesis of hyperlipidemia in these survivors. We have therefore designated them as "sporadic" cases in the nongenetic sense.

The distribution of adjusted plasma lipids of 145 adult relatives of the hypercholesterolemic survivors

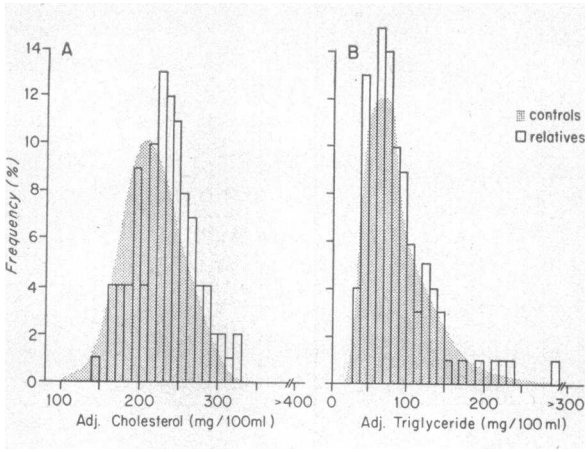

FIGURE 15 Frequency distribution of 145 near and distant adult (≥20 yr of age) relatives of 28 survivors with polygenic hypercholesterolemia. Included in this analysis were 119 first-degree, 20 second-degree, and 6 third-degree relatives. Age and sex-adjustments were carried out as described in the accompanying paper (10). The distribution is divided into increments of 10 mg/100 ml. The smooth strippled curve represents a nonparametric density estimate of the control distribution.

in group B is shown in Fig. 15. The triglyceride curve corresponds to that of controls, while the cholesterol curve is unimodal with a higher mean than that of controls and was shifted to higher values (Fig. 15A). Such a unimodal, displaced curve in relatives of probands is compatible with the hypothesis that multiple cholesterol-elevating genes—possibly in conjunction with environmental factors—are operative in such relatives. Whereas a multifactorial or polygenic mechanism is most likely, such a curve can also be caused entirely by environmental variations, or may be generated by the action of a single genetic locus with one or many alleles. Table XI indicated that about 7% of sibs were hypercholesterolemic by the arbitrary criteria used. This interpretation of multifactorial inheritance of hypercholesterolemia is further substantiated by the finding of significant correlations in cholesterol levels of parent-child ($r = +0.200$) and sib-sib ($r = +0.354$) pairs among normolipidemic families. Such correlations, in the absence of similar husband-wife correlations ($r = -0.023$), fit the concept that one of several mechanisms produce hypercholesterolemia: polygenic, monogenic, and nongenetic.

Summary of genetic analysis of hyperlipidemic survivors of myocardial infarction. A summary of our genetic analysis is presented in Fig. 16. Of the 157 hyperlipidemic survivors of myocardial infarction identified in the accompanying paper (10), 15% could not be classified because of lack of available living relatives (see Methods). 54% had one of the three monogenic disorders, the most frequent being familial combined hyperlipidemia which was observed in about one-third of all hyperlipidemic survivors. Except for four survivors with type III hyperlipidemia (see the accompanying paper [11]), the remaining 31% of hyperlipidemic survivors had either a polygenic or sporadic form of hyperlipidemia.

Frequency of hyperlipidemia in survivors of myocardial infarction and in the general population. Since our study involved virtually all survivors of myocardial infarction under age 60 and a randomly selected group age 60 and above who were admitted to most of the hospitals in a metropolitan area during a period of 11 mo, it was possible to estimate the overall frequencies of the various disorders identified in this study. These data are shown in Table XII. In extrapolating from our observed data in survivors under age 60 to findings in the general population, it was necessary to make several assumptions each of which tended to underestimate the heterozygote frequency of these disorders. (These assumptions are enumerated in the legend of Table XII.) Although the monogenic disorders were about three times more common in the younger (< age 60) survivors than in the older survivors, both polygenic hypercholesterolemia and sporadic hypertriglyceridemia appeared equally common (each with a frequency of about 6%) among young and old survivors. Familial hypercholesterolemia occurred in 4.1% of all consecutively studied survivors under age 60 and in 0.7% of the group of randomly studied older survivors. A rough estimate of its frequency in the general population is 0.1–0.2%. Familial hypertriglyceridemia was slightly more common, occurring in 5.2% of survivors under age 60 and 2.7% of the older group. Its population frequency is estimated at 0.2–0.3%. Familial combined hyperlipidemia occurred in 11.3% of all survivors under 60 and 4.1% of the older survivors. Its population frequency is estimated at

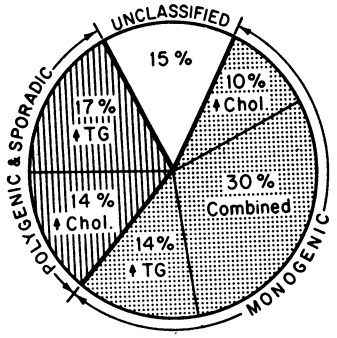

FIGURE 16 Summary of genetic analysis in 157 hyperlipidemic survivors of myocardial infarction. The unclassified category represents those hyperlipidemic survivors in whom family study was not possible because of lack of availability of at least three relatives. Type III hyperlipidemia (not included here) was identified in four, or 2.4%, of these 157 hyperlipidemic survivors (11).

TABLE XI

Analysis of Sibships of 28 Survivors with Polygenic Hypercholesterolemia

	Sibs (no.)						
		Dead				Number affected	
	Total	By all causes	By MI*	Living	Tested	Observed‡	Expected§
Brothers	44	13	1	31	30	1	15
Sisters	58	6	0	52	44	4	22
Total	102	19‖	1	83	74	5	37

* Deaths by myocardial infarction (MI) were documented by death certificates.

‡ Relatives were considered affected if the age and sex-adjusted plasma cholesterol level was equal to or exceeded the 95th percentile of controls.

§ Expected number of affected relatives by the hypothesis of autosomal dominant inheritance.

‖ 7 of these 19 deaths occurred before age 35 yr.

0.3–0.5%. Considered together, these three monogenic disorders were present in about 20% of all survivors of myocardial infarction under age 60. It is estimated that at least 1 in 160 individuals in the general population may carry a major gene for one of these three disorders.

A summary of the major clinical, genetic, and biochemical characteristics of the five disorders identified in this study is provided in Table XIII.

DISCUSSION

The studies reported here present a genetic analysis of the lipid disorders occurring in survivors of myocardial infarction. Perhaps the simplest and least biased way to handle the data is to combine all hyperlipidemic survivors in one group and examine the lipid levels of their relatives. The results of this kind of analysis showed that relatives had significantly higher levels of both cholesterol and triglyceride as compared

TABLE XII

Frequency of Hyperlipidemia

Disorder	Survivors of myocardial infarction			General population*
	< Age 60 (*a*)	≥ Age 60 (*b*)	Ratio *a/b*	
	%	%		%
A. Monogenic hyperlipidemia				
Familial hypercholesterolemia	4.1	0.7	5.9	~0.1–0.2
Familial hypertriglyceridemia	5.2	2.7	1.9	~0.2–0.3
Combined hyperlipidemia	11.3	4.1	2.8	~0.3–0.5
Total	20.6	7.5		~0.6–1.0
B. Polygenic Hypercholesterolemia	5.5	5.5	1.0	—
C. Sporadic Hypertriglyceridemia	5.8	6.9	0.8	—

* These lower limit estimates of heterozygote frequency in the general population are minimal since they were made under the following assumptions: (*a*) that the prevalence rate of coronary heart disease in adults, age 30–59, is 3% (33); (*b*) that the frequency of these disorders as observed among unselected 3-mo survivors of myocardial infarction would be the same among individuals with other manifestations of coronary disease, such as angina pectoris, sudden death, and fatal myocardial infarction; and (*c*) that all heterozygotes for one of these monogenic lipid disorders manifest clinical evidence of coronary disease before age 60 yr. The upper limit estimates were made under the assumption that not all but only one-half of heterozygotes would manifest clinical evidence of coronary disease before age 60 yr.

TABLE XIII

Summary of Clinical, Genetic, and Biochemical Characteristics of Hyperlipidemic Survivors of Myocardial Infarction

Disorder	Typical age	Typical lipid level*		Lipoprotein types‡	Mode of inheritance	Penetrance below age 25§
		Cholesterol	Triglyceride			
		mg/100 ml	*mg/100 ml*			
Monogenic						
Familial hypercholesterolemia	M 45 F 55	353	126	II*a*, II*b*	Autosomal dominant	~0.92
Familial hypertriglyceridemia	M 50 F 55	241	267	IV, V	Autosomal dominant	~0.26
Combined hyperlipidemia	M 50 F 60	300	241	II*a*, *IIb*, IV, V	Autosomal dominant	~0.18
Polygenic						
Hypercholesterolemia	M 55 F 60	308	187	II*a*, II*b*	Polygenic	Not applicable
Sporadic						
Hypertriglyceridemia	M 55 F 60	233	243	IV, V	Nongenetic	Not applicable

* Unadjusted lipid values which represent mean levels of each group.

‡ Lipoprotein quantification and typing was determined by methods described in the accompanying paper (11).

§ Penetrance was estimated by determining the ratio of the proportion of individuals expressing with hyperlipidemia to the expected proportion on the dominant hypothesis.

with those of controls. With this clear establishment of familial factors in the etiology of hyperlipidemia in coronary heart disease, more detailed genetic analysis was undertaken.

Since previous studies had shown the hyperlipidemias to be heterogeneous disorders (2–4), we felt that any genetic analysis should be preceded by sorting the data for obvious heterogeneity. Lacking a specific and diagnostic laboratory test for each of the hyperlipidemias, our first approach was to sort the hyperlipidemias according to the pattern of variation observed in the lipid levels of relatives of affected probands. A comparison of the lipid distributions of relatives with controls should allow a distinction between genetic and nongenetic disorders. Furthermore, among the genetic disorders, autosomal dominant inheritance could be distinguished from polygenic inheritance, provided bimodality in lipid distributions (reflecting the normal and affected relatives) could be detected. Since lipid measurements are far removed from primary gene action, a perfect bimodal curve without overlap in its component distributions would not be expected.

In carrying out the analysis, it was assumed that if there is an inherited disorder in a given family, all affected relatives have the same disorder. In retrospect, such an assumption seemed justified since none of the genetic hyperlipidemic disorders detected has a population frequency of greater than 1 in 100. From the overall results, five different lipid disorders were identified on the basis of different patterns of distribution in cholesterol and triglyceride levels of relatives.

Familial hypercholesterolemia. The disorder easiest to detect, familial hypercholesterolemia, was characterized by the finding in relatives of a normal triglyceride distribution but an apparently bimodal cholesterol distribution. Segregation analysis suggested autosomal dominant inheritance. The criterion which distinguished this disorder from the other familial hyperlipidemias was the nearly complete expression of hypercholesterolemia in affected children. In addition, almost 50% of the 16 families comprising this group were known to have one or more hypercholesterolemic members with tendinous xanthomas. Moreover, nearly all hypercholesterolemic family members manifested ratios of total plasma cholesterol/triglyceride that were greater than 2. These findings indicated that most of our 16 families with familial hypercholesterolemia closely resembled other families previously reported with this disorder (5, 19, 20). However, since all of our hypercholesterolemic families did not have affected members with xanthomatosis and with cholesterol elevations above 400 mg/100 ml, it is possible that this group of 16 families consists of several biochemically and genetically distinct subgroups that can not be further separated by current tests.

Familial hypertriglyceridemia. A second disorder identified in this study, familial hypertriglyceridemia,

was characterized by the occurrence in relatives of a normal cholesterol distribution but an apparently bimodal triglyceride distribution. Segregation analysis in sibs was consistent with autosomal dominant inheritance, but was uninformative when performed on the offspring of affected subjects since hypertriglyceridemia was only expressed in about 13% of young relatives at risk. No detailed genetic analysis of familial "pure" hypertriglyceridemia has been previously published, so it is not possible to make comparisons with other studies. However, on the basis of preliminary analysis of hypertriglyceridemic probands ascertained by a different method, Fredrickson and Levy have also concluded that familial hypertriglyceridemia is determined by a Mendelian autosomal dominant mechanism and that the hypertriglyceridemia is not completely expressed in affected children (19). Moreover, we have recently investigated a large kindred (not part of this study) consisting of 74 members in whom elevated levels of triglyceride but not of cholesterol segregated as an autosomal dominant trait throughout the first-, second-, third-, and fourth-degree relatives of the proband (unpublished observations).

Familial combined hyperlipidemia. The most interesting finding in this study was the delineation of a newly recognized lipid disorder—familial combined hyperlipidemia. It was characterized by variability in expression of lipid levels among affected relatives such that any combination of elevation in LDL cholesterol and very low density lipoprotein (VLDL) triglyceride or both were observed among affected relatives. As described in the accompanying paper (11), affected individuals with this disorder manifested any one of four lipoprotein phenotypes: type II*a*, type II*b*, type IV, or type V patterns. In the individual family with combined hyperlipidemia, the pedigree was often puzzling and confusing because of this variability in phenotypes. But, when the family data were extensive enough and lipid levels were measured in children, the disorder was relatively easy to distinguish from both familial hypercholesterolemia and familial hypertriglyceridemia.

Several lines of genetic evidence indicate that combined hyperlipidemia is genetically distinct from both familial hypercholesterolemia and familial hypertriglyceridemia. First, the pattern of lipid distributions in relatives, showing an apparently bimodal triglyceride curve and a unimodal and shifted cholesterol curve, was unique. Second, the observation that the hypertriglyceridemic relatives with the combined disorder had abnormally elevated levels of cholesterol was not observed in the familial hypertriglyceridemic disorder in which the hypertriglyceridemic relatives had normal levels of cholesterol. Third, in contrast to the findings of familial hypercholesterolemia, elevation in cholesterol alone was not observed in children of adults affected with combined hyperlipidemia. Fourth, in families with this disorder, a hypercholesterolemic × normal mating frequently gave rise to hypertriglyceridemic progeny; conversely, hypertriglyceridemic × normal matings often produced hypercholesterolemic progeny. These observations were consistent with variable expression of a single autosomal dominant gene. It remains to be seen whether all of the 47 families with combined hyperlipidemia actually have the same abnormal gene, since biochemical and genetic heterogeneity cannot be ruled out by the present studies.

Since the earliest manifestation in affected children with combined hyperlipidemia was hypertriglyceridemia alone, the primary biochemical abnormality in this disorder may involve triglyceride metabolism with secondary effects on cholesterol metabolism. Additional evidence supporting this hypothesis is the fact that triglyceride levels in relatives appeared to segregate into two distributions, whereas their cholesterol levels showed a less clear-cut bimodality. Furthermore, the recent demonstration that plasma LDL may arise physiologically from the catabolism of VLDL (22, 23) documents an interrelationship of these two lipoprotein classes and hence of plasma cholesterol and triglyceride, suggesting a possible metabolic site for the lesion in combined hyperlipidemia. In this respect, our postulation that the primary defect in combined hyperlipidemia involves triglyceride transport provides a reasonable explanation for the occurrence of either hypertriglyceridemia alone or both hypertriglyceridemia and hypercholesterolemia in affected individuals. However, the finding of hypercholesterolemia alone in a minority of affected individuals with the combined disorder cannot readily be explained by this hypothesis. Its occurrence therefore provides a challenge for additional work on the pathophysiology and biochemistry of lipoprotein metabolism.

In their recent study of lipid abnormalities in survivors of myocardial infarction, Patterson and Slack did not detect familial combined hyperlipidemia (24). However, only 39 first-degree relatives from families with all types of familial hyperlipidemia were tested, precluding the possibility of picking out this disorder. However, these authors did observe hypertriglyceridemia in relatives of probands with hypercholesterolemia and vice versa.

In keeping with our identification of combined hyperlipidemia as a genetic entity is the recent report of Rose, Kranz, Weinstock, Juliano, and Haft (25). These authors independently concluded that combined hyperlipidemia was different from either familial hypercholesterolemia or familial hypertriglyceridemia. Furthermore, one report in 1968 by Matthews of a kindred in which hypercholesterolemia and hypertriglyceridemia

occurring as different phenotypic expressions of the same mutant gene (26) and another report in 1969 by Schreibman, Wilson, and Arky of a family with familial type IV hyperlipidemia (27) were both, in retrospect, probable examples of familial combined hyperlipidemia.[9] Although widely quoted as an example of familial type IV hypertriglyceridemia (and originally designated as such because of the presence in affected family members of a pre-beta band on lipoprotein electrophoresis of whole plasma), the kindred studied by Schreibman et al. (27) actually contained among the eight affected relatives two with hypercholesterolemia alone, one with hypertriglyceridemia alone, and five with both hypercholesterolemia and hypertriglyceridemia. Like the large pedigree shown in Fig. 10, both Matthews' and Schreibman's pedigrees are consistent with the present evidence for single gene inheritance of familial combined hyperlipidemia.

Problems involved in the detection of major gene effects. Although our analyses suggested that familial hypercholesterolemia, familial hypertriglyceridemia, and familial combined hyperlipidemia are each determined by a different autosomal dominant gene rather than by a polygenic mechanism, it was necessary to use arbitrary cut-off values for cholesterol and triglyceride to classify relatives as normal and affected. Consequently, there must have been misclassification of some individuals, because of the overlapping nature of the lipid distributions. However, the cut-off value chosen (95th percentile) was higher than the apparent antimode of the lipid distributions in relatives (90th percentile). This choice should have minimized the number of misclassified normal relatives, and thus any bias would be against monogenic inheritance.

In addition to the results of the segregation analyses, there are other reasons why a monogenic rather than a polygenic model is more likely for these disorders. The definite absence of unimodality and the highly suggestive presence of bimodality in the appropriate lipid distributions of relatives favored a single-gene mechanism (8). In addition, the proportion of affected relatives was higher than that predicted by a polygenic model. Assuming that the level of cholesterol or triglyceride is entirely determined by multiple genetic factors (i.e., heritability = 1) and designating those with values equal to or exceeding the 95th percentile as affected, the expected number of affected first- and second-degree relatives predicted on a polygenic model would be about 25 and 9%, respectively, (28, 29) rather than the observed 45–55 and 20–30%. With lower heritabilities, these estimates for the proportion of affected first- and second-degree relatives under a polygenic model would be even less (28, 29).[10] Furthermore, whereas the ratio of hyperlipidemic probands having a myocardial infarction was 4:1 (male:female), no deviation from unity in the sex ratio among hyperlipidemic relatives was noted—another argument favoring monogenic inheritance (30). Finally, in contrast to some other polygenically determined disorders where a higher proportion of relatives is affected if the proband is severely affected (28), no such phenomenon was observed in our data. For example, probands whose lipid levels fell in the 95–98th percentile range had as many relatives affected with lipid levels in the 99th percentile as did probands whose lipid levels fell in the 99th percentile (unpublished observations).

Although the above considerations seem most consistent with single gene inheritance, the evidence for major gene effects on quantitative traits such as cholesterol and triglyceride cannot be considered incontrovertible until a specific biochemical defect for each of the three disorders is identified. Alternatively, the discovery of chromosomal linkage to a known marker

[9] Although the family reported by Matthews was originally reported as having several members affected with the type III lipoprotein pattern (26), the presence of the β-VLDL marker which characterizes this phenotype (2, 3) was not demonstrated in those individuals showing an elevation in both cholesterol and triglyceride. By current classification, these individuals would be considered to have a type II*b* lipoprotein pattern.

[10] In studies carried out with Dr. Joseph Felsenstein, the heritability and the components of the total phenotypic variance for both the levels of cholesterol and triglyceride in these monogenic families were estimated by a maximum likelihood computer program. Covariance information between relatives was computed using all degrees of relationship, including near and distant relatives and spouses. The phenotypic variance was partitioned into the following components: additive variance, V*a*; dominance variance, V*d*; and environmental or nongenetic variance, V*e* (9). In this type of analysis, the heritability (i.e., the genetic contribution to the total variance) is defined in the broad sense as,

$$\frac{Va + Vd}{Va + Vd + Ve}.$$

Estimates of heritability in families with the monogenic disorders and in control families were as follows:

Group (Number of family members*)	Heritability	
	Cholesterol	Triglyceride
Normolipidemic families (n = 283)	0.84	0.50
Familial hypercholesterolemia (n = 189)	1.00	—
Familial hypertriglyceridemia (n = 333)	—	0.97
Familial combined hyperlipidemia (n = 734)	0.86	0.87

* Includes probands, relatives, and spouses.

gene, such as to a blood group or polymorphic enzyme, would also provide more definitive evidence for the single gene hypothesis.

In addition to the three presumably monogenic lipid disorders, two other types of hyperlipidemia were identified among survivors of myocardial infarction—polygenic hypercholesterolemia and sporadic hypertriglyceridemia. The finding of a polygenic disorder involving cholesterol but not triglyceride and of a sporadic disorder involving triglyceride but not cholesterol is in keeping with our data on familial correlations of lipid levels. These showed that among normal individuals the variation in plasma cholesterol levels is determined predominantly by genetic factors, while the variation in plasma triglyceride levels is accounted for equally by environmental and genetic factors.[10]

Frequency of hyperlipidemia. Although our calculated figures for the frequency of the three monogenic lipid disorders in the general population are indirect and represent conservative estimates, a heterozygote frequency of 0.1–0.2% for familial hypercholesterolemia agrees with the finding of the Framingham study in which about 1 in 850 individuals in the general population were observed with hypercholesterolemic xanthomatosis (31). Until detailed studies are performed on the families of hyperlipidemic individuals selected at random from the general population, the heterozygote frequencies reported here should be considered only as approximate. Assuming, however, that our estimates are correct and that no more than 1% of the general population carries a gene for one of the monogenic forms of hyperlipidemia, it is predictable that the polygenic and sporadic form of hyperlipidemia affecting 4% of the population will turn out to be the most common forms of these disorders in individuals *without* coronary heart disease. It should be pointed out, however, that the diagnosis of a polygenic disorder depends on quantitative, rather than qualitative data, requiring arbitrary cut-off values. Therefore, making a distinction between normal and affected individuals can be difficult. On the other hand, for a monogenic disorder a clear qualitative difference is ultimately obtainable and the individual diagnosis can be made more confidently.

If the data of this study can be confirmed and if familial hypercholesterolemia, familial hypertriglyceridemia, and familial combined hyperlipidemia can in fact each be proven to be determined by a separate major gene, then these genes affecting lipid metabolism and ultimately the pathogenesis of coronary heart disease are among the most common disease-producing genes in our population. Considering that at least 1 in 160 individuals may carry one of these three genes, the familial hyperlipidemic disorders are major public health problems.

It is tempting to speculate that one reason for the rising frequency of premature coronary heart disease in affluent societies may be the interaction between a genetic factor such as one of these hyperlipidemic genes whose frequency has presumably not changed over many generations and some newly introduced environmental factor(s) such as diet, stress, and/or sedentary habits. Such a mechanism would be analogous to other types of heredity-environment interaction (32) and implies that different approaches may be required in preventing the various hyperlipidemic disorders. The existence of distinct monogenic hyperlipidemias offers defined subpopulations for assessing the effects of various drugs and diets and also emphasizes the need for a vigorous search to identify the underlying metabolic defect and biochemical lesion in each disorder.

Note added in proof. E. A. Nikkila and A. Aro (1973. *Lancet.* 1: 954.) have independently reported that the most common form of familial hyperlipidemia in survivors of myocardial infarction is one in which multiple lipoprotein types are observed among about 50% of the first-degree relatives. These observations are similiar to our data on the familial combined hyperlipidemia disorder.

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