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HUMAN GENETICS AND CORONARY HEART DISEASE: A Public Health Perspective

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INTRODUCTION

Few scientific disciplines relevant to the study of human coronary heart disease (CHD) have been so fertile in recent years as genetics. New biochemical and microbiologic methods and the widespread application of population genetics have resulted in an explosion of new knowledge and a mushrooming literature. Excellent recent reviews cover several aspects of human genetics and CHD, especially relating to lipid risk factors (13, 14, 75, 90, 101, 146, 220, 223). Also relevant are recent reviews on the genetics of hypertension (162, 228–230, 232).

We review here the current knowledge of familial and genetic contributions to premature CHD with particular emphasis on public health aspects. In an effort to determine the primary contributors to early familial coronary disease, both within specific families and on a population level, we explore the potential familial risk factors of CHD. The importance of this endeavor is emphasized by the fact that 50 to 80% of early CHD in the general population is found within only 5 to 15% of the families or pedigrees (99, 234). Where information is available, we emphasize aspects of diet intervention and reported diet-gene interactions. Recent developments in apolipoprotein molecular biology and associated CHD risks are the subject of another review in this volume (60a).

THE TOOLS

To appreciate the public health impact of familial coronary disease, one first must have some knowledge of population genetics, family studies, and recent biochemical and microbiologic techniques and recognize each method's strengths and liabilities. An initial approach to examining the role of genes in human CHD is to apply standard epidemiologic methods using family history as a risk factor. Such methods include case-control, angiographic, historical prospective, and true prospective studies. These approaches help determine the strength and consistency of risks for early coronary disease associated with belonging to a high-risk family, the prevalence of such families, and their contribution to the overall extent of coronary disease. Such methods cannot be used, however, to uncover specific genetic inheritance, mechanisms of disease, or causality. In fact, very few clearly defined genetic traits have been linked unambiguously to early familial CHD. Currently, the contribution of genetic traits to familial CHD is unknown.

Quantitative population genetics is helpful in determining heritability of coronary disease or specific coronary risk factors, contributions of genes versus environment to quantitative phenotypic traits, modes of transmission, penetrance, and gene frequencies. The final level of analysis of an inherited

trait is to isolate a particular gene product and describe the functional changes and mechanisms of atherosclerosis promotion and define actual associated DNA alterations. This level of understanding is currently available for only two syndromes: familial hypercholesterolemia (28) and type III or familial dyslipoproteinemia (149, 150). But these syndromes account for only a small percentage of all familial premature CHD. With such exciting new methodologies available, losing sight of important practical questions is easy. Such questions include: Which risk factors are most important in high-risk families? Are they amenable to intervention, and, if so, to what extent? Is there important interaction between different inherited traits or between environment and genotype? Are the discovered genetic traits clinically useful? To answer these questions, we must frequently turn to the seemingly more mundane epidemiologic and biometric analyses. In essence, once a particular gene or DNA marker is discovered we must determine its clinical significance.

Two widely used methods in human population genetics are the calculation of heritability, commonly used in twin studies, and pedigree analysis for complex segregation analysis.

The concept of heritability (abbreviated h^2) was developed for use in plant and animal breeding for traits such as muscle mass, milk production, egg laying, or yield per acre. For the most part, these traits are well described by a polygenic model. Other quantitative traits such as cholesterol level and blood pressure are often subjected to the same type of analysis. The value h^2 estimates the fraction of the total variance attributable to genetic factors. Thus, an h^2 value of 0.50 for serum total cholesterol level would imply that 50% of the population variance for serum cholesterol level was due to heritable factors.

In twin studies, h^2 can be estimated by the formula: $h^2 = 2\ (r_{mz} - r_{dz})$, where r_{mz} and r_{dz} are the correlation coefficients between monozygous twin pairs and dizygous twin pairs, respectively. The derivation of this formula and the questionable assumptions required, such as no gene-environment interaction and similar environment between monozygote twin versus dizygote twins, are described in a standard genetics textbook (213a).

Family studies using complex segregation analysis or pedigree analysis allow a more elegant approach to define the role of genes in transmission of quantitative traits. Fewer assumptions need to be made and a variety of genetic models can be tested. Several estimations can be made including the likelihood of a major gene being present, probabilities for dominant versus recessive transmission, gene frequencies with means and standard deviations for specific genotypes, relative fraction of polygenic and major gene inheritance, and the partitioning of environmental variance into various potential sources such as shared household, same-day screening, etc (85, 135, 136).

HOW FAMILIAL IS CORONARY HEART DISEASE?

Many studies have demonstrated that first-degree relatives of early CHD patients are at much higher risk for developing coronary artery disease than the general population. These studies include case control (62, 66, 67, 80, 88, 112, 168, 186), angiographic (5, 15, 35, 36, 65, 72, 189, 210, 216), historical prospective (51, 104, 175, 180–182, 195, 207, 211, 222), and prospective studies (11, 32, 40, 51, 71, 100, 190, 200).

An important early historical prospective study (195) examined CHD among first-degree relatives of 121 male probands in whom onset of CHD occurred prior to age 60 and 96 female probands in whom onset of CHD occurred prior to age 70. Controls consisted of 104 men and 104 women from a London insurance company. Causes of death in first-degree relatives of probands and controls were confirmed by examination of death certificates. The relative risk for death from CHD was highly dependent on age at onset. For example, if probands had onset of CHD between age 35 and 44, only 0.08 cases of CHD were expected among male relatives aged 35 to 44 whereas 2 were observed, yielding a relative risk of 25. With the same age at onset in probands, the relative risk in male relatives aged 55 to 64 was 6.8, and at age 65 to 74 it was 4.9. If the proband had onset of CHD between ages 45 and 49 the relative risk for male relatives aged 35 to 44 was 8.3 and for male relatives aged 55 to 64 was 2.5. This age dependency for relative risk has been repeatedly confirmed (99, 104). Thus age of onset of CHD must be included in a definition for family history, since a family history of two or three CHD deaths at ages 80 to 90 would mean something quite different from the same number of CHD deaths at ages 40 to 50. The use of a nonspecific definition of family history such as "any CHD in a first-degree relative" has yielded relatively unimpressive family history–associated risks in several studies.

Utah Experience

The largest study to date to examine the definition of family history reviewed life table data for 94,292 adult relatives of 8,200 high school students who completed family pedigree health questionnaires as part of their health curriculum (104). Ages at first onset of heart attack or bypass surgery were considered as age of onset of CHD. Reported cases of CHD were validated by questionnaires sent to the individuals reportedly affected or, if deceased, to their close relatives or spouses. To compare family history definitions a historical prospective study design was used. Each family was classified according to its family history of CHD up to the year 1970. Families were then grouped by family history status.

A family history score was calculated that incorporates total number of person years within a family and compares the number of observed versus

expected cases. A family history score greater than two was considered very positive, between one and two mildly positive, and less than 0.5 average or protective. From 1970 until the data were collected between 1983 and 1984, CHD incidence rates were calculated for family members unaffected as of 1970. For families with a family history score prior to 1970 of 2.0 or more, relative risks for men were 9.1, 4.6, 2.5, and 1.8 for age intervals 20 to 39, 40 to 49, 50 to 59, and 60 to 69, respectively. For women, the relative risks were 4.9, 6.8, 3.3, and 1.5 for the same age intervals. Similar relative risks occurred in families with two or more early CHD cases before 1970, where early cases were considered to have CHD onset before age 55. Progressively lower relative risks were seen if the definition for positive family history was two or more affected at any age, one or more affected early, or one or more affected at any age.

Since a family history score of greater than 2.0 occurred in 3.2% of the families in the population, attributable risks for total CHD due to family history scores greater than 2.0 may be calculated as 20.6, 10.3, 4.6, and 2.5% for the age categories specified above. Somewhat lower attributable risks were calculated when the definition for positive family history was two or more early affected, which occurred in 1.7% of the population. Therefore, familial CHD accounts for a sizeable proportion of all *early* CHD deaths.

Another approach to estimating the extent of early CHD that is due to familial proclivity has used a computerized resource of Utah family genealogical records linked to state death certificates. Age-specific CHD mortality rates were compared between different pedigrees. There were 2122 deaths in 327 defined pedigrees that could be used from the file. Eighty percent of CHD deaths between ages 35 and 54 could be attributed to the 16% of pedigrees that displayed a twofold or greater excess in CHD death rates (234). Furthermore, age at CHD death was correlated within families (234). Although these results were considered preliminary, they represent an upper limit to the extent that genetic factors may contribute to the overall population incidence of premature CHD.

Separating Genetic and Environmental Effects

We may appropriately ask how much of the familial risk of premature CHD is due to genetic factors. Both twin studies and other biometric methods can answer this question. Four twin studies have been performed. Using Danish monozygotic (MZ) and dizygotic (DZ) twin pairs, Hauge (89) reported concordance rates for CHD to be 0.39 and 0.26, respectively, in men ($p < .05$) and 0.44 and 0.14 in women ($p < .01$). In a larger Norwegian study of approximately 2000 MZ and 3000 DZ same-sex twin pairs, concordance rates for MZ and DZ CHD death prior to age 65 were 0.65 and 0.25, respectively. When CHD that occurred before age 60 was considered separately, con-

cordance rates were 0.83 and 0.22, respectively (16). Two other twin studies with similar results have been performed on Swedish twin registry data (50, 142).

Although evidence in twin studies strongly suggests that genetic factors play an important role in the familiality of coronary disease, one may question the assumption that MZ and DZ twins share similar environments. Indeed our studies suggest that MZ twins share "environmental traits" including alcohol and coffee consumption, cigarette smoking, as well as type A personality traits such as time urgency more frequently than do DZ twins (103). A particularly enlightening study that fairly successfully separates genetic and environmental influences is a recently reported follow-up study of Danish adoptees (201). Risk of dying from specific and all causes between ages 16 and 58 years in the adoptees was compared on the basis of whether their biologic or adoptive parents had died prior to age 50. If the biologic parent had died prior to age 50 from any cause, the relative risk of death from any cause in the adoptees was 1.71. If the biologic parent died from CHD or stroke, the relative risk for adoptees was 4.52 (95% confidence interval, 1.32–15.4) for cardiovascular causes. If the adoptive parent had died prior to age 50, the relative risk for the adoptees was nearly 1.0 for all causes and 3.02 with a 95% confident interval from 0.72 to 12.8 for vascular causes, while the relative risk was 5.2 for cancer. These results suggest that genetic factors outweigh familial environmental factors in the prediction of premature vascular disease.

FAMILY HISTORY AS AN INDEPENDENT RISK FACTOR FOR PREMATURE CHD

An early attempt to determine the degree to which known risk factors contribute to familial CHD was performed by comparing northern and southern Finnish populations (180, 182). Researchers measured risk factors, including serum cholesterol level, triglyceride level, and blood pressure, and collected medical history information on 1387 first-degree relatives of 203 male probands with myocardial infarction (MI) and in 692 relatives of 106 age-matched healthy controls. Brothers of probands with MI prior to age 45 had an elevenfold increase in risk of CHD by age 55 in North Karelia. This risk compared with a relative risk of sevenfold in south Finland. As in other studies, the risk to the proband's brothers sharply increased as the proband's age of onset of CHD decreased. Hypertension and hyperlipidemia were reported to be more common among relatives of the younger probands, however, a quantitative contribution of these risk factors to early familial coronary disease was not calculated.

Other studies have also documented the aggregation of high serum

cholesterol concentrations, hypertension, diabetes, obesity, and cigarette smoking in some or many high-risk families. Nevertheless, when these risk factors have been controlled for, family history remains a significant independent risk factor in nearly all the studies reported (reviewed in 113). A very recent case-control study further substantiates that family history is an independent risk factor, even after controlling for numerous potential risk factors (171). Researchers compared 366 cases of initial MI with 423 age-, sex-, and neighborhood-matched controls in Boston, Massachusetts. The family history was considered positive if any first-degree relative had a MI prior to age 60. Before adjusting for other risk factors, the odds ratio for MI was 1.81 in those with a positive family history. Adjusting individually for blood levels of apoproteins A-I, A-II, B, and E and for blood levels of total HDL cholesterol, HDL_2 and HDL_3 subfractions, LDL cholesterol, and triglycerides did not change the odds ratio. The odds ratio in logistic regression for positive versus negative family history was still 1.74 (95% confidence interval, 1.12–2.72) after adjusting for age, sex, body mass index, cigarette smoking, type A personality, education level, history of hypertension, diabetes, physical exercise, daily consumption of calories and saturated fat, and blood levels of triglycerides, HDL cholesterol, and LDL cholesterol. This study provides further strong support for the independence of family history as a risk factor.

Prospective Studies

Of particular interest are prospective studies, since the independent role of family history can be accurately assessed by using methods such as the Cox proportional hazards model and since recall of family history is not likely to be biased, as it may be in a retrospective study. The six studies performed to date are reviewed in Table 1.

Only the last two studies used an age-specific definition of family history in deriving the independent risk estimate for family history. This difference may explain the comparatively low relative risks reported in the other studies. In the Rancho Bernardo Study (11), too few events occurred in younger subjects (9 in men under 60, 2 in women under 60) to perform multivariate analysis in that age group.

Khaw & Barrett-Connor have also reported that cigarette smoking interacts strongly with family history in the Rancho Bernardo Study (120). The relative risk for smoking in men and women with a positive family history was 2.5 and 4.0, respectively, whereas men and women with a negative family history had relative risks of 1.1 and 1.7. Finally, a family history of stroke at any age in a first-degree relative was also an independent risk factor for coronary disease incidence in men in this same cohort (121).

Despite the consistent finding of family history as an independent risk

Table 1 Summary of prospective studies of family history as an independent CHD risk factor

Study, year (Reference)	Cohort	Follow-up (years)	#CHD cases	+FHx definition (prevalence with +FHx)	Risk assessment	Other risk factors included in multivariate analysis	Comments.
Western Collaborative Study, 1975 (190)	3,524 men age 39–59	8.5	257	Parental history of CHD at any age (18.4%)	Mantel-Haenzel odds ratio = 1.81 for all new CHD	Total cholesterol[a], beta/alpha lipoprotein ratio[a], schooling, behavior pattern (type A/B)[a]	Odds ratio calculated for men age 39–49. Ages 50–59 not reported. Attributable risk = 13%
Paris Prospective Study, 1980 (32)	7,484 men age 43–54	6.5	1417	Parental history of MI or sudden death at any age (12%)	Cox regression RR = 1.55	Age[a], cigarette smoking[a], total cholesterol[a], diabetes[a], systolic blood pressure	Only paternal history of CHD was significantly related to CHD incidence. Attributable risk = 6.1%.
Framingham Sibling Pairs, 1982 (200)	169 bro-bro sibling pairs	26	114	CHD in older brother of sib pair at any age	Multiple logistic $\beta^* = 0.414$-MI, $\beta^* = 0.440$-CHD death	Systolic blood pressure[a], total cholesterol[a], relative weight, cigarette smoking, age[a]	Oldest and youngest male siblings were selected.

Rancho Bernardo Study, 1984 (11)	4,014 men & women age 40–79	9	183	First-degree relative with heart attack at any age (38%)	Cox regression RR = 1.5 for all CVD in men	Age[a], systolic blood pressure[a], total cholesterol[a], obesity, smoking, diabetes	FHx independent only in men. RR = 5 for FHx in men under 60 (9 cases). Attributable risk = 16%
Nurse's Health Study, 1986 (40)	117,156 women age 30–55	4	275	Parental history of MI age ≤60 (14%)	Cox regression RR = 4.9, 2.4, 3.2 for fatal CHD, nonfatal MI, and AP	History of: hypertension, diabetes, high cholesterol, oral contraceptive, postmenopausal estrogens, obesity, smoking	Based on questionnaire-derived risk factor data. Attributable risk = 35% for fatal CHD
Utah Cardiovascular Genetics, 1988 (100)	1,196 men & women age 20–83	2.5	16	Number of relatives with CHD age ≤55 (50% with 1+)	Cox regression RR = 1.59 for each early CHD case	Age[a], sex[a], total cholesterol[a], hypertension, diabetes, body mass index, triglycerides, HDL cholesterol, interaction terms	Unique, specific, and quantitative definition of family history. Attributable risk = 53%

[a] Statistically significant risk factors in multivariate analysis. Family history was a statistically significant independent risk factor in all the studies.

[b] Abbreviations: +FHx=positive family history, CVD=cardiovascular disease CHD=coronary heart disease, MI=myocardial infarction, AP=angina pectoris, RR=relative risk, β^*=standardized multiple logistic coefficient.

factor, studies 1–5 in Table 1 have been criticized for lack of specific definitions or incomplete evaluation of family history, using questionnaire-determined risk factors, or not evaluating potential risk factor interactions, specifically cholesterol-smoking (166). These objections were largely overcome by the Utah Cardiovascular Genetics Study (100). This report is a 2-½ year follow-up study of 1196 men and women age 20 to 83 who were participants in the Cardiovascular Genetics Research Program in Utah. Approximately half of these subjects were members of families selected for high risk of CHD and the other half for increased risk of hypertension and stroke.

After 2-½ years of follow-up, 16 subjects had had nonfatal, definite CHD including MI, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, angiographic-proven CHD, or definite angina (2 cases). Events ranged from ages 41 to 68 and most were under age 60. Family history was defined as the number of either maternal or paternal (whichever was greater) relatives including grandparents, aunts, uncles, parents, and siblings with CHD prior to age 55. Cox proportional hazard regression was performed correcting for age, sex, cigarette smoking (ever), hypertension history, diabetes history, body mass index, and levels of total cholesterol, total triglycerides, and HDL cholesterol. The Cox regression coefficient for family history was 0.463 ($p < .002$). Family history was second only to age for strength of association with new CHD incidence. Sex and total cholesterol level were also statistically significant risk factors in this population. No interaction pattern including any between the factors family history–total cholesterol, family history–age, family history–smoking, cholesterol–age, and cigarette smoking–cholesterol was significant after entering age, family history of CHD, sex, and serum cholesterol level into the equation.

A Cox regression coefficient of 0.463 implies a relative risk of 1.59 for each additional early CHD case in close relatives as defined above. Approximately 50% of the population screened had no relatives with CHD under age 55, whereas four of the subjects had seven early CHD cases among their close relatives. Such a family history was associated with a *26-fold increase* in risk compared with those with no relatives with early CHD.

The independence of family history as a CHD risk factor in this population was approached in a different way as well (113). The adult participants were divided into three family history groups (low-, intermediate-, and high-risk family history scores). The ability of 60 potential risk variables to discriminate between the family history groups was tested using multiple stepwise discriminant analysis. Significant predictors ($p < 0.01$) included serum cholesterol level, years smoking cigarettes, HDL cholesterol level, triceps skinfold thickness but not obesity, other anthropometric variables, or a variety of blood pressure measurements. In spite of significant differences between

family history score groups, only 39% of the subjects could be correctly classified by discriminant analysis (33% correct classification would be expected by chance alone). The conclusion was that other unknown risk variables must have accounted for the differences between the family history groups.

The total risk for new coronary disease attributable to family history in the Cardiovascular Genetics Study was calculated using the impact fraction, a measure of attributable risk (97). Overall, 52.7% of new coronary disease in this selected population could be attributed to the independent contribution of one or more relatives with early coronary disease (unpublished observation). Attributable risks may be calculated for the other prospective studies cited above. They range from a low of 6.1% in the Paris Prospective Study (which used a nonspecific definition of positive family history as parental CHD at any age) to 35.3% of fatal CHD attributed to parental history of MI under age 60 in the Nurse's Health Study. Therefore, a large fraction of all CHD, especially at earlier ages, can apparently be attributed to a family history of premature CHD, independent of standard risk factors such as levels of total serum cholesterol, triglycerides, and HDL cholesterol, and blood pressure, body weight, cigarette smoking, age, and type A/B behavior pattern.

THE SEARCH FOR FAMILIAL RISK FACTORS

The independence of family history as a CHD risk factor implies the existence of other heritable risk factors that strongly contribute to early CHD in many high-risk families. Inherited risk factors might predispose to CHD in a variety of ways. In prior reviews we have devised a scheme for classifying well over 250 CHD risk factors based on mechanisms of atherogenesis (97, 98).

Figure 1 is a partial list of risk factors classified as initiators, promoters, potentiators, and precipitators that may contribute to early CHD in high-risk families. This scheme helps illustrate that while most familial syndromes of premature coronary disease described thus far are lipid abnormalities, other classes of risk factors may contribute strongly to early CHD in many families. Also, not surprisingly, early familial disease may result from the interaction of two or more risk factors or classes of risk factors.

Inherited Susceptibility

Under each category in Figure 1, inherited susceptibility has been listed as a possible risk factor for some families. This suspicion comes primarily from work with animal models such as White Carneau pigeons, which despite equivalent serum lipid levels, blood pressure, and serum hormone levels are much more susceptible to atherosclerosis than are randomly bred pigeons (214) or very resistant Show Racer pigeons (144). Recent investigation has

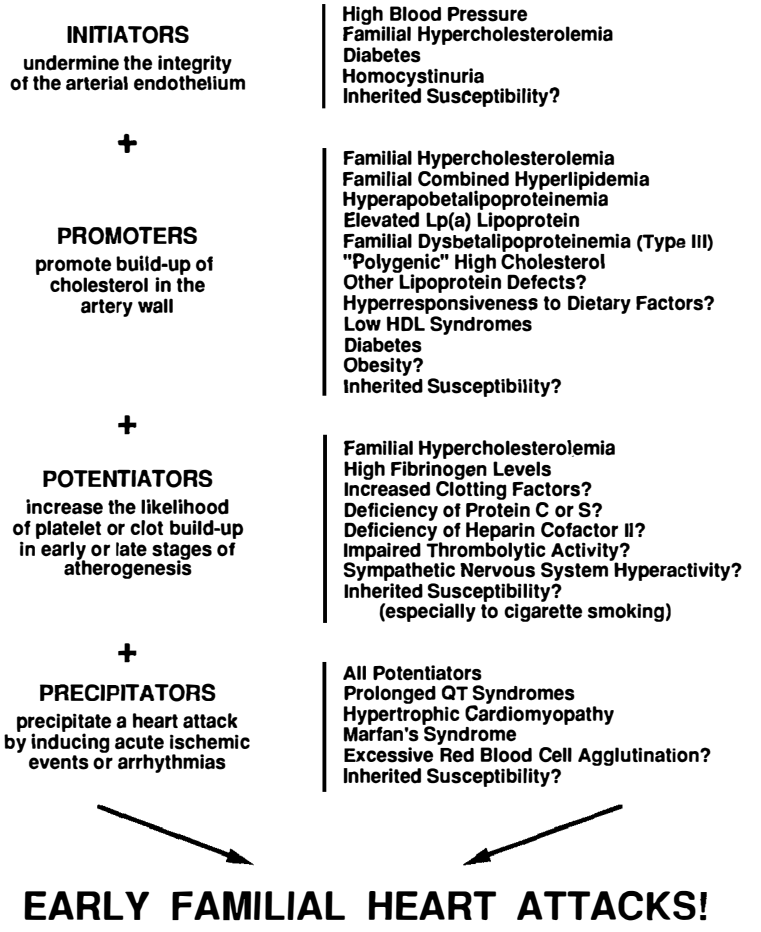

Figure 1 Causes of early heart attacks in high-risk families.

turned to potential arterial wall factors that may enhance atherogenesis. Examples of possible initiating factors that may be under genetic control include increased endothelial permeability, slow endothelial repair rates, and altered endothelial interactions with platelets and lipoproteins.

Levels of circulating immune complexes that act as initiators may contribute to genetic differences. After receiving an atherogenic ration, cynomolgus monkeys exhibited high levels of immune complexes, which gave rise to concentric lesions, in contrast to low levels of immune complexes formed in rhesus monkeys that gave rise to eccentric atherosclerotic lesions more typical of human atherosclerosis (237).

Inherited susceptibility to promoters might include alterations in glycosa-

minoglycans or proteoglycans and other macromolecules in ground substance with altered lipoprotein binding, as found in White Carneau pigeons (215). Another possible promoter independent of serum lipids is deficiency of lysosomal acid lipase, which could impede degradation of imbibed lipoproteins (49, 158, 204). Evidence was presented from a case-control study that a low level of human leukocyte lysosomal acid lipase is an independent CHD risk factor and may be transmitted as a dominant trait (39).

Some families with premature CHD appear to have increased susceptibility to the effects of cigarette smoking. Since cigarette smoking increases platelet adherence and aggregation, increases fibrinogen levels, and decreases fibrinogenic activity, it may be considered a potentiator (97, 98). We compared the effects of cigarette smoking in first-degree relatives of men dying from CHD by age 45 with the relative risk of cigarette smoking in in-law controls. Cigarette smokers experienced a 2.5-fold increased risk among the proband relatives compared with only a 1.7-fold increase among the in-law controls ($p < .05$) (99). Confirmatory results have been reported in the Rancho Bernardo Study as noted above (120). A recent study demonstrated shortened platelet survival primarily among cigarette smokers with a positive family history of CHD, thus suggesting a mechanism for the interaction between family history and cigarette smoking (69).

HERITABLE RISK FACTORS AND MAJOR GENE SYNDROMES LEADING TO EARLY FAMILIAL CHD

A clinical CHD event, even if highly premature and affecting multiple family members, is still a distant phenotype with many intervening steps. Even within a family, multiple genotypes may contribute to final outcome. Nevertheless, several major genes have been identified as important CHD risk factors and most of the "classic" CHD risk factors show considerable heritability. The remainder of this review deals with several cardiovascular risk factors and well-defined genetic syndromes linked to premature CHD. Hypertension is discussed separately as a final topic.

Total Serum Cholesterol

Although the total serum cholesterol level is determined by many ill-defined loci and a few well-described major genes, it is instructive to examine this important CHD risk factor as a single entity. The genetic determinants of total serum cholesterol level can best be described as a combination of polygenic and major gene effects. Some of the major gene effects are described below. Recent National Cholesterol Education Program guidelines recommend that all adults know their serum cholesterol levels and significance (54a). This national effort will uncover many thousands of cases of familial hyperlip-

idemia. Clinicians need to understand the genetic contributions to total cholesterol level to adequately manage such patients.

Heritability estimates (which primarily reflect additive polygene effects in the general population) have been derived from family and twin studies. Correlations between 514 twin pairs participating in the National Heart, Lung, and Blood Institute (NHLBI) twin study suggested a heritability of 0.43 for serum total cholesterol level using standard measures of heritability for twin studies (103, 226). These values compare with 0.34 to 0.68 by Berg (14) and 0.61 in Utah twin studies (103, 226). After investigators of the NHLBI twin study reanalyzed their data with a new method designed to correct bias due to unequal total variances of MZ and DZ twins, total serum cholesterol was no longer significantly heritable (226). This analysis illustrates difficulties in assumptions often used for calculating heritability in twin studies.

By using the more reliable method of components of variance analysis in family studies, several investigators have estimated total heritability of serum cholesterol level due to additive polygenes and environmental contributors to familial correlations. These studies have shown remarkable consistency: three investigators reported polygene heritability of 0.58, 0.49, and 0.53 (193). In Utah family studies, serum cholesterol level was calculated to be 42 to 54% heritable with only 8% of the variance attributable to the effects of a common household (103, 226).

Given the strong heritability of total serum cholesterol level and frequent occurrence of monogenic, dominant traits causing severe hypercholesterolemia, one would expect family screening to be a cost-effective means to identify patients in need of treatment for hypercholesterolemia. An extensive cost-benefit analysis compared a variety of screening and intervention models for mass hypercholesterolemia in the United States (20). In terms of cost per year of lives saved, targeted screening based on family history was second only to mass media or school education as the most cost-effective method. The least cost-effective method was mass screening and medical intervention only in those with serum cholesterol levels above the 90th percentile. These findings may change with the advent of inexpensive cholesterol screening techniques.

GENETIC SUSCEPTIBILITY TO DIET Susceptibility to diet-induced changes in serum cholesterol level has been examined recently. Genetically mediated responsiveness to dietary cholesterol has been documented in several species of nonhuman primates including squirrel monkeys, rhesus monkeys, cynomolgus macaques, and baboons (38). Striking differences in responsiveness to dietary cholesterol were found prior to selective breeding, and these differences apparently require only a few generations of selective breeding to result in consistently hyper- or hyporesponsive offspring.

Studies in humans have suggested differences in responsiveness as well, but the data are much less extensive, and no information on heritability of responsiveness is available (21, 61, 118, 159, 172, 173). Of particular interest are the studies of Mistry et al (159) and McNamara et al (153). In the former study, serum cholesterol responses to dietary cholesterol were inversely related ($r = 0.74$, $p < 0.001$) to the capacity of derepressed blood mononuclear cells to degrade radioiodenated LDL. Both studies showed an inverse correlation between change in mononuclear cell HMG CoA reductase activity and change in serum LDL concentration after dietary cholesterol intake. In neither study, however, were diets rigorously controlled. Nevertheless, both studies demonstrated marked individual variability in responsiveness to added dietary cholesterol and related this variability to cellular compensatory mechanisms that may be relevant to future familial or genetic investigations.

The genetic influence on response to high-fat, high-cholesterol diets was dramatically illustrated (Figure 2) by comparing responses in a number of inbred mouse strains (147). Not only are baseline cholesterol levels different (with about a twofold range), but the change in cholesterol level is unique to each strain and not predictable by initial cholesterol levels. Some strains have very large responses, and two strains in particular (AKR and 129) have virtually no response to a high-fat, high-cholesterol diet. These two strains appeared to have inherited a unique set of DNA markers linked to the apolipoprotein B locus (147). These important studies illustrate the potential fallacy of assuming no gene-environment interaction in human population studies. Further, they graphically demonstrate that while genetic influences may account for a large part of the variability of serum cholesterol levels in populations, they do not dictate the change in serum cholesterol level after dietary intervention.

Serum Triglycerides

While total serum triglyceride levels have not been demonstrated unambiguously in population studies to be an independent cardiovascular risk factor, they are nevertheless a marker for several monogenic familial lipid disorders and are themselves highly heritable. The NHLBI twin study estimated heritability to be between 0.56 and 0.68 for triglycerides (58). This estimate agrees well with Utah twin studies (heritability between 0.75 and 0.81) (103). Utah family studies indicated much lower heritabilities (13–37%) (103).

Although serum triglyceride levels are probably an even more complex phenotype influenced by more factors than serum cholesterol level, they can be an indicator of increased risk for early CHD. Interpreting an individual's serum triglyceride value, however, also requires information on other family

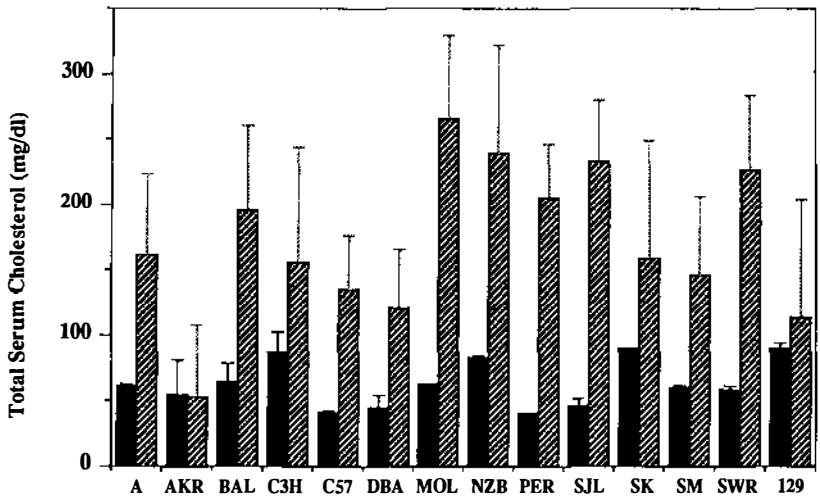

Figure 2 Responses of serum cholesterol level in 14 inbred mouse strains. Solid bars show baseline serum cholesterol levels on regular chow, hatched bars after high-fat, high-cholesterol (added cocoa butter and cholesterol) diet. Standard deviations are shown as error bars. Columns without error bars have only one observation. Abbreviations denote the mouse strains used.

members' lipid levels and CHD history. Familial hypertriglyceridemia, a dominant trait characterized by high triglyceride levels as the sole lipid abnormality in relatives, is apparently not associated with a marked increased risk of CHD, whereas individuals with familial combined hyperlipidemia (see below) may have the same lipid levels as people with familial hypertriglyceridemia but are at much higher CHD risk (30).

HDL cholesterol levels are frequently low in subjects with familial combined hyperlipidemia and high triglyceride level. While this observation is not well documented, it is probably a reflection of the well-known inverse relationship between HDL cholesterol level and total triglyceride level reported in numerous population studies.

HDL Cholesterol

Serum HDL cholesterol level is well established as an important CHD risk factor. Heritability estimates range from 0.14 to 0.46 in the NHLBI twin study (58). These values compare with Utah twin estimates of between 0.51 and 0.74 (103). Utah family studies suggest heritability of 0.45 to 0.57 (86, 103). HDL_2 and HDL_3 were 37% and 28% heritable, respectively, and displayed considerable age-sibling interaction and household environmental effects (86). Although several studies note major genes that determine serum HDL levels and include several syndromes of low HDL with early familial CHD (see below), the predominant genetic influences on HDL in the popula-

tion appear to be polygenic (83, 84, 86). The genetic regulation of HDL is apparently mediated in part through hepatic lipase activity, to which HDL levels are inversely correlated (130).

A unique genetic trait designated Ath-1 in the mouse has been described. Females of an atherosclerosis-prone mouse strain (C57BL/6) had marked (50%) reduction in HDL cholesterol levels when fed a high-cholesterol, high-fat diet. Levels of VLDL and LDL increased markedly with the atherogenic diet, but mouse strains with similar increases in VLDL and LDL did not exhibit lipid-staining aortic lesions when HDL levels remained high. The trait was unambiguously linked to chromosome 1 near the apolipoprotein A-II gene (173a). No similar decreases in HDL on exposure to a high-fat, high-cholesterol diet have been noted in humans, but a gene has been described in humans that affects HDL size and apo A-I/apo A-II ratio and is linked to the same A-II region (173a).

Apolipoproteins and Lipoprotein Subfractions as Familial CHD Risk Indicators

While case-control studies suggest increased predictive power for serum apoproteins B, A-I, and A-II compared with levels of total cholesterol, triglycerides, or HDL cholesterol in discriminating CHD cases from controls, prospective studies have not necessarily demonstrated improved predictive power (31, 109). Nevertheless, one might expect that since apolipoproteins are direct gene products, heritability estimates would be higher. This indeed has been observed: Berg (13) reported heritability estimates for total cholesterol level of 0.34 to 0.68 and total triglyceride level of 0.40 to 0.46, while heritability for apo B level was 0.65, for apo A-I was 0.53, and for apo A-II was 0.68. A study of 97 individuals in 23 high-risk Utah pedigrees demonstrated major gene and polygenic control of apo A-I levels, but no major gene effect for HDL cholesterol levels could be demonstrated in the same families (160). This result suggests additional insights will be gained when apolipoproteins are measured along with other traditional lipid risk factors.

Hyperapobetalipoproteinemia

Another condition in which apolipoprotein determination may provide added predictive power within a family is hyperapobetalipoproteinemia. Elevated levels of apolipoprotein B with or without increased levels of serum LDL cholesterol were a dominantly inherited trait in one large pedigree with early CHD (12, 132). Other observations of suggested increased apo B levels in MI patients (197, 199) led to the designation of familial hyperapobetalipoproteinemia, which appears to be a dominantly inherited trait that conveys increased CHD risk (132, 198). Patients with hypertriglyceridemia and elevated

apo B levels appear to be at higher risk for CHD than hypertriglyceridemia patients with normal apo B levels (129). Hyperapobetalipoproteinemia may be a subset of familial combined hyperlipidemia that is associated with excess apolipoprotein B production (205). A missense mutation that results in tryptophan substitution for arginine at position 4019 in the mature apo B protein has been associated with premature CHD and an increase in LDL apo B levels but normal LDL cholesterol levels in one small, nuclear family (133).

Inheritance of total serum level of apolipoprotein B was studied by using complex segregation analysis among 331 members of 36 Utah pedigrees. A single major locus explained 43% of the variance; the remainder was attributed to random environmental factors. Low homozygotes, heterozygotes, and high homozygotes had mean apo B values of 110.5 ± 2.5, 141.9 ± 4.4, and 208.1 ± 11.5 mg/dl and genotype prevalences of 0.718, 0.259, and 0.023, respectively (87).

Dietary treatment of hyperapobetalipoproteinemia has not been defined. Interestingly, however, subjects with hyperapobetalipoproteinemia defined as LDL apo B levels greater than 120 mg/dl displayed significantly greater and longer-lasting lipemia after oral fat loads than did subjects with normal apo B levels. A pronounced, delayed decrease in HDL_2 levels also occurred in the hyperapobetalipoproteinemia patients (70). These findings suggest possible mechanisms for atherogenesis associated with a high-fat diet independent of fasting lipid levels.

Lipoprotein heterogeneity has been the subject of a recent NHLBI conference (143a). Increased appreciation for such heterogeneity should help uncover new genetically determined traits. A recent report suggested that an LDL subfraction, determined by gradient gel electrophoreses, that has a preponderance of small, dense LDL particles, was associated with a threefold increased risk of MI independent of age, sex, and relative weight. After controlling for levels of total triglyceride, however, researchers found the relationship was no longer significant (8). In the Framingham Offspring Study, 69% of the variance in LDL subfractions was attributable to variations in levels of plasma triglyceride and HDL cholesterol. Smaller, more dense LDL subfractions were more prevalent in subjects with higher triglyceride levels and low HDL levels (154). Similar excess of small, dense LDL has been observed in patients with hyperapobetalipoproteinemia (206) and may be a feature of familial combined hyperlipidemia. In one study (9), an LDL subfraction pattern with more small, dense LDL particles appeared to be a dominant trait. We have been unable to verify this observation in initial attempts at segregation analysis in several Utah pedigrees (unpublished observations). LDL subfractions may be yet another distant phenotypic marker for an inherited syndrome that leads to early familial CHD. Much work remains, however, to disentangle the effects of other potential codeterminants of LDL subfractions.

All the major lipoproteins have been cloned and tested for a variety of restriction fragment length polymorphisms associated with quantitative or qualitative differences between individuals. These studies are reviewed elsewhere in this volume (60a). The finding that some polymorphisms of the apolipoprotein B gene were more common in MI patients than in controls but unassociated with lipid level differences suggests that heritable qualitative differences in lipoproteins as well as quantitative lipid differences may play a role in familial CHD (92).

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is a medium-density lipoprotein (density between LDL and HDL) in which a single apolipoprotein(a) [apo(a)] molecule is covalently bonded by disulfide bridge to the apolipoprotein B of an LDL particle. The LDL component of Lp(a) may be a native LDL (212). The origin(s) and metabolism of Lp(a) are poorly understood. Levels of Lp(a) in blood of fasting subjects appear almost entirely controlled by a single major gene locus. Initially the presence of Lp(a) was detectable only by using immunologic methods in serum and was treated as a qualitative variable (present/not present). More recently developed assays, however, have demonstrated that all persons have some Lp(a) in the blood but that the levels are bimodally distributed; higher levels are present in approximately 20% of the population (161).

Utermann and co-workers (212) have identified six different apo(a) glycoprotein size-isoforms apparently controlled by six different alleles. Lp(a) plasma concentrations were largely determined by the particular apo(a) isoform (212). The apo(a) genome has been fully sequenced and displays remarkable homology to the plasminogen gene (151), to which it is closely linked on the long arm of chromosome 6 (64). We used this close proximity in a family study that demonstrated linkage between codominantly inherited Lp(a) levels and apo(a) size-isoforms linked to restriction fragment length polymorphisms of the plasminogen gene (53).

Increased Lp(a) level is associated with two- to threefold increased risks for CHD and stroke, as documented in case-control and angiographic studies (2, 17, 18, 45, 46, 65, 127, 164, 179). Higher Lp(a) levels were also associated with increased risk of saphenous vein graft stenosis after coronary artery bypass surgery (95). The largest study to date related Lp(a) levels to CHD risk among 308 Hawaiian men of Japanese ancestry and 408 population controls participating in the Honolulu Heart Study (179). The odds ratio for Lp(a) levels above 20 mg/dl was 2.5 for MI under age 60 and diminished with age. The calculated attributable risk of Lp(a) levels in the highest quartile (20.2–71.9 mg/dl) was 28% for a MI under age 60. These results suggest that Lp(a) could be a major genetic determinant of early CHD.

Further substantiating evidence comes from two recent studies. In one (54),

serum levels of total cholesterol, triglycerides, HDL cholesterol, Lp(a), and apoproteins B, A-I, and A-II were measured in 41 patients with MI prior to age 60 and in 78 controls. Discriminant function analysis revealed apo A-I and apo B concentration and family history of early MI best distinguished cases from controls if Lp(a) was not included in the function. When Lp(a) was included, however, it eliminated family history as a significant predictor. Another study (94) measured Lp(a) levels in 1486 men at age 18. Levels of total plasma cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol were also measured. The subjects were divided into probands whose mothers or fathers had suffered a MI at any age (n = 52) and into probands whose parents were free of CHD (n = 1434). If a male child had a Lp(a) concentration greater than 25 mg/dl, parents were 2.5 times more likely to have had a MI. In this study, other lipid fractions were not significantly different in children whose parents had MI versus those without. These studies suggest that much of the risk associated with a positive family history may be attributable to differences in Lp(a) levels.

Recent findings suggest a plausible mechanism for the atherogenicity of Lp(a). LDL particles are thought to bind to proteoglycans, which are a component of the ground substance of arterial intima. Once bound, the LDL are engulfed by macrophages, presumably via the same scavenger receptor that recognizes acetylated LDL rather than the LDL (apo B, E) receptor. Lp(a) that was bound to proteoglycan deposited three to four times as much cholesterol into cultured macrophages than did normal LDL-proteoglycan complexes. The deposition by Lp(a)-proteoglycan was comparable to that by acetylated-LDL (22a).

Diet has reportedly been ineffective in reducing Lp(a) levels (2,128). Cholysteramine is also ineffective, but neomycin (2 g/day) or neomycin (2 g/day) plus niacin (3 g/day) reduced Lp(a) 30 to 45% (78).

Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is a heterogeneous group of genetic disorders, almost all of which affect the LDL receptor. Generally, FH can be recognized when 50% of first-degree relatives have serum cholesterol levels well above the 95th percentile, normal triglyceride levels, and a striking occurrence of hypercholesterolemia in affected children as well as in affected adults. Gene frequency of heterozygotes has been estimated between 1 in 200 and 1 in 500; thus, FH is one of the most common human genetic disorders known. There are nearly as many FH patients in the United States as there are persons infected with the AIDS virus (93), yet FH is often considered an obscure or rare condition both inside and outside the medical profession.

The relative risk for CHD in heterozygotes is very high—up to 20–25 times

normal (98). Given a prevalence of 1/500 to 1/200, between 5 and 10% of early CHD may be attributable to FH. This estimate agrees well with published studies of MI survivors (6, 76). Currently, at least 16 different mutations of the LDL receptor gene have been described (186a). These defects may be classified into at least four groups (28): (*a*) absence of or marked decrease in LDL receptor synthesis, (*b*) defective transport of LDL receptors from endoplasmic reticulum to the golgi apparatus, (*c*) defective binding of a receptor to LDL, and (*d*) defective clustering of LDL receptors into coated pits. These four defect types may account for some of the diversity in binding noted in FH fibroblasts and monocytes (148). Other studies suggest considerable heterogeneity between different FH heterozygotes, both in metabolic studies and in studies of the risk of CHD (23, 42, 127, 139, 174, 192, 202). There is one description of a family with apparent mild-to-moderate FH in which LDL receptors were normal but LDL apoprotein B displayed abnormal binding to the LDL receptor (108). Given the genetic heterogeneity of FH, one might expect to discover heterogeneity in response to treatment. This area remains an important research topic.

In Utah pedigree studies we have accumulated evidence that early CHD may not be inevitable in carriers of the FH gene. In two published pedigrees (235) and one other large pedigree (91), obligate heterozygotes in earlier generations had onset of CHD at later ages. In one large Utah FH pedigree, the founding ancestor of the pedigree, had lived in the 1800s, had had two wives, and lived until age 81. FH was passed to children of both wives. Average age of MI in affected males was approximately 10 years earlier in each succeeding generation. In the most recent generation, every affected male had had a MI by age 42. We hypothesize that living when a high-fat, overly rich diet was scarce probably protected the pioneer progenitors of this pedigree.

The importance of diet in treating FH heterozygotes is often underestimated. A 20 to 30% decrease in serum cholesterol level is not unusual when FH heterozygotes adhere to a low-saturated-fat, low-cholesterol diet. Examples of two responsive FH heterozygotes are presented in Figures 3 and 4.

Cases have been reported (152) of pregnant FH patients with remarkable decreases in serum cholesterol level while on a low-cholesterol diet. Other diet studies of FH are scarce and provide little data, although 10 to 20% reductions in serum cholesterol levels on a diet low in cholesterol and saturated fat are reported (74, 82, 131, 185). Although not all FH heterozygotes will be as responsive to diet, an initial trial of dietary treatment alone is always indicated for at least three to six months in these patients. This trial is an important teaching period in which patients learn the effects of diet on their hypercholesterolemia and develop habits that will allow them to maintain optimal dietary control over a prolonged period.

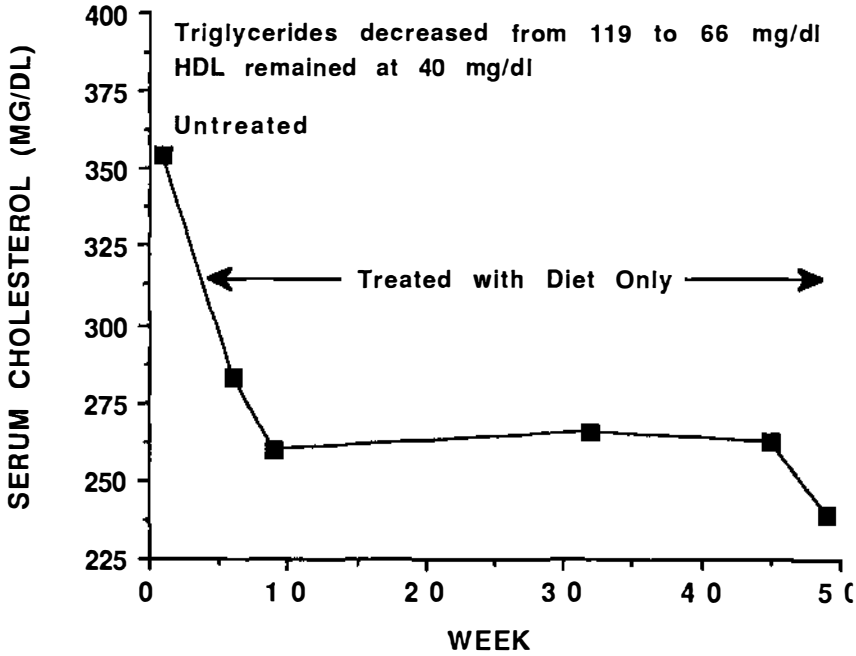

Figure 3 Example of a favorable response to a low-fat, low-cholesterol diet in the treatment of familial hypercholesterolemia (FH). This subject was a 21-year-old woman with FH.

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCHL) is characterized by the presence of hypercholesterolemia, hypertriglyceridemia, or both, with two or more of these patterns present among first-degree relatives. Typically the hyperlipidemia is in the 90th to 95th percentile and thus is not nearly as striking as in familial hypercholesterolemia.

When originally described, FCHL was thought to be a single dominant trait (76). Subsequent segregation analysis on the same families did not yield clear identification of Mendelian patterns, possibly because of inadequate power (235). One important reason that segregation analysis may have been difficult in these families is heterogeneity; three or more genotypes may be involved. In one Utah pedigree with a proband homozygous for lipoprotein lipase deficiency, the mother (an obligate heterozygote) and several of her relatives appeared to display lipid levels similar to FCHL (236). Babirak et al (10) reported similar observations. Some families that have high Lp(a) levels also display lipid patterns similar to those of FCHL patients (J. D. Brunzell, personal communication). Finally, families with heterozygous lysosomal cholesterol ester storage disease also have FCHL patterns (91). It remains

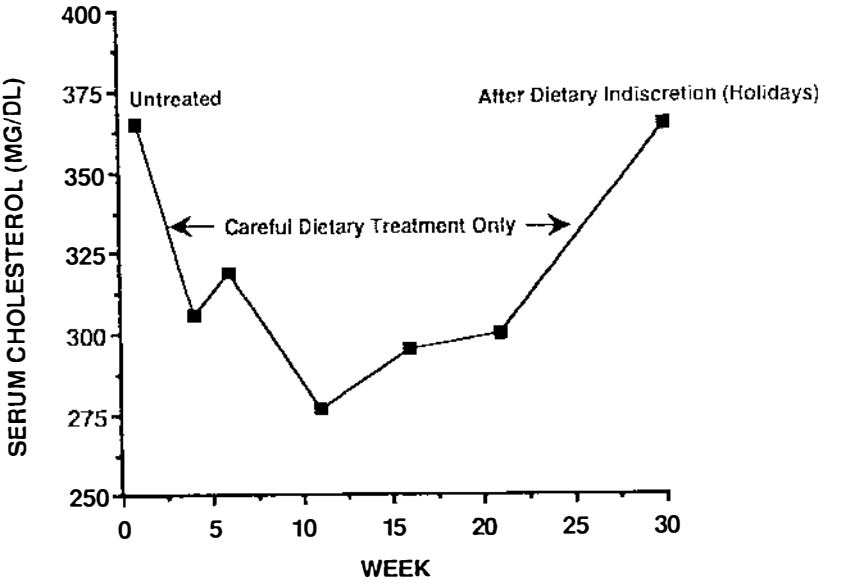

Figure 4 Another example of the response to dietary treatment of FH. This subject was a 32-year-old white man with FH.

unknown why these different subgroups all display similar lipoprotein abnormalities yet some individuals have high cholesterol levels, some high triglyceride levels, others high levels of both, and still other individuals change phenotypes. The explanation may be related to interactions with other genes or environmental factors. Apparently, regardless of the origin of FCHL, subjects display a characteristic increase in apo B synthesis, which may be the underlying pathophysiologic disorder in this syndrome (29, 33, 111, 123, 124). One study has recently challenged these findings with results that suggest decreased VLDL triglyceride removal rates were the primary abnormality in first-degree relatives of FCHL probands (187).

Although no precise genetic description of FCHL exists, this syndrome or set of syndromes is clearly a major contributor to early familial coronary disease. In one study (76), FCHL was the underlying abnormality in 10 to 15% of patients with MI prior to age 60. In families of 101 Finnish probands who had had nonfatal MI by age 50, 24 to 27% had FCHL (6). Individuals with FCHL have been reported to have a three- to four-fold increased risk of early CHD (30). Given a prevalence of approximately 1 in 200 (76), attributable risks for early CHD may be calculated as ranging from only 1.5 to 3%. Such low attributable risks disagree with findings in population studies and suggest errors in available measures of relative risk or perhaps suggest markedly increased risk within some families. We have found that among 32

population-based, coronary-prone Utah kindred, 34 to 53% of the families were affected with FCHL (unpublished observations).

Treatment for FCHL generally includes weight reduction, restriction of dietary saturated fat and cholesterol, and avoidance of alcohol and oral contraceptives. These recommendations appear to be based on anecdotal evidence and clinical impressions. A review of the past 15 years of the medical literature revealed only two articles with actual data for dietary treatment of FCHL. In one (26), guar gum was shown to significantly reduce levels of total cholesterol and triglycerides in 12 FCHL outpatients. Total cholesterol level was reduced by 14% and total triglycerides by 32% after 15 days of receiving guar gum supplements. These decreases persisted for the 60-day duration of the study. There were no significant changes in HDL cholesterol level (26). In the second study, 20 patients with hypertriglyceridemia were treated with diets that included approximately 20 grams of omega-3 fatty acids per day in the form of salmon and/or fish oil (MaxEPA) (176). Of the 20 patients, 8 had FCHL. All subjects appeared to respond similarly. Subjects with type IIB lipid phenotype had a reduction of total cholesterol level from 324 to 236 ($p < 0.001$) and a reduction in triglyceride level from 334 to 118 mg/dl ($p < 0.001$). Interestingly, HDL cholesterol level also decreased significantly from 41 to 34 mg/dl ($p < 0.05$). In a subgroup of patients, fish oil appeared to be more effective in reducing triglyceride and HDL levels than vegetable oil. An important research question is whether subtypes of FCHL display the same response to dietary and other therapies.

Familial Type III Hyperlipidemia and Apolipoprotein E Phenotypes

Familial type III hyperlipidemia, or familial dysbetalipoproteinemia, is a syndrome of VLDL and chylomicron remnant accumulation caused by defective processing. Levels of total cholesterol and triglycerides are elevated and the ratio of measured VLDL cholesterol (mg/dl) to total triglycerides (mg/dl) is greater than 0.3. The defect in processing appears to be caused by amino acid substitutions in the binding regions of the apolipoprotein E molecule. Type III hyperlipidemia appears to confer a significantly increased risk for atherosclerosis, however, the risks are not well defined since this disease is rare (1 in 10,000). Because it is rare, type III hyperlipidemia presumably accounts for only a small portion of early familial CHD. However, we have identified 1 of 13 sibling pairs with CHD prior to age 55 in which both siblings had type III hyperlipidemia (unpublished observations). Influences of apo E phenotypes on serum cholesterol and CHD risk are reviewed elsewhere in this volume (60a).

Another inherited condition that results in a type III hyperlipidemia pattern (presumably 10% of cases or fewer) is familial hepatic lipase deficiency.

Modification of VLDL remnants by hepatic lipase is important for their conversion to LDL and the transfer of apolipoprotein E from VLDL to HDL. Interestingly, in this syndrome, the VLDL cholesterol–to–total triglyceride ratio is normal, but HDL and LDL are enriched in triglyceride and phosphatidyl choline at the expense of cholesterol ester and sphingomyelin (27). While it is not clear whether hepatic lipase deficiency causes accelerated atherosclerosis, cholesterol is deposited into fibroblasts by the beta VLDL in hepatic lipase deficiency (41).

Familial HDL Deficiency

As noted above, most of the variance in HDL cholesterol levels in the population stems from polygenic influences, while a few families may display major gene traits for HDL deficiency. Several families with very low HDL levels and early CHD have been described (188). The most common low-HDL syndrome is familial hypoalphalipoproteinemia (47, 73, 209, 213). Recently, this syndrome has been linked to a DNA polymorphism in the A-I/C-III/A-IV region of chromosome 11 (165, 191).

Rare Lipoprotein Disorders

Several other rare lipoprotein disorders have been associated with early CHD. These recessive disorders include Tangier disease, apolipoprotein E absence, beta-sitosterolemia, cerebrotendinous xanthomatosis, lecithin cholesterol acyl transferase deficiency, and fish eye disease. While these disorders offer interesting insights into lipoprotein metabolism and atherogenesis, they probably contribute little to the overall extent of early familial CHD.

Homocystinuria

Homozygotes for homocystinuria have high levels of homocysteine in the blood stream that causes endothelial damage and severe vascular disease even in childhood. Approximately 60% of homozygotes have thromboembolic events prior to age 40 (163). Boers et al (25) recently determined the prevalence of heterozygotes for homocystinuria in 75 patients with cardiovascular disease, 25 of whom had angiographically documented occlusive peripheral artery disease, 25 who had occlusive cerebral vascular disease, and 25 with previous MI. Abnormally high serum homocysteine concentration after methionine loading occurred in 30% of the patients with occlusive peripheral artery disease and in those with cerebral vascular disease but not in patients with MI. Since the normal population prevalence of heterozygosity for homocystinuria is between 1 in 70 and 1 in 200, this study strongly suggests that heterozygosity for homocystinuria contributes to familial strokes or peripheral vascular disease but apparently not to early familial CHD. Results from other studies have been suggestive but inconsistent in their

findings that heterozygotes for homocystinuria are more common among CHD patients than controls (110, 217). A more sensitive index of abnormal homocysteine metabolism may be the plasma concentration of homocysteine-protein complexes. One report (115) has noted small but statistically significant elevations of homocysteine-protein concentration in 241 patients with angiographically proven CHD compared with 202 controls [5.41 ± 1.62 vs 4.37 ± 1.09 nm/ml in men and 5.66 ± 1.93 vs 4.16 ± 1.62 in women ($p <$ .0005 and .005, respectively)].

Elevated Fibrinogen Levels

Elevated fibrinogen level has been demonstrated to be an independent CHD risk factor in angiographic studies (143, 170) and prospective studies (116, 117, 155, 157, 170, 219). Increased fibrinogen level is associated with hypertension, diabetes, hypercholesterolemia, high hematocrit level, and cigarette smoking, but is still an independent predictor of CHD risk in multivariate assessment (116, 155).

In the Northwick Park Heart Study (156), initial lifetime smoking habits and changes in smoking habits correlated with fibrinogen levels and suggested that much of the increased CHD risk associated with smoking may have been mediated by increased fibrinogen levels. Recent studies, however, have also shown a strong genetic determination. Genetic and cultural heritability of plasma fibrinogen concentration was estimated by path analysis in 85 families with a proband who had had an early MI and in 85 families randomly selected from the general population (81). Genetic heritability was calculated to be 51%, whereas cultural heritability was negligible. Combined effects of smoking and obesity explained only 3% of the variance in plasma fibrinogen levels in this population. Another study (102) used restriction fragment length polymorphisms linked to the fibrinogen gene to designate two different alleles that explained 9% of the observed phenotypic variance ($p < 0.025$). Mean fibrinogen level in the 50 individuals with B1-B1 genotype was 2.74 gm/l, while 37 individuals of genotype B1-B2 had a mean fibrinogen level of 2.98 and finally 4 individuals of B2-B2 genotype had a mean fibrinogen level of 3.69 (102). An increase in fibrinogen level of 0.6 gm/l was associated with a 67% increase in risk independent from other risk factors in the Northwick Park Heart Study (155). Overall risk of CHD attributable to the B2-B2 phenotype in this study would therefore be 14%. Thus high fibrinogen levels may play an important role in early familial CHD.

HYPERTENSION

Numerous twin and family studies have documented the heritability of both systolic and diastolic blood pressures. Heritability estimates range from 0.13

to 0.82 for systolic blood pressure and from < 0.01 to 0.64 for diastolic pressures with average levels for both at about 50% (224). These heritability estimates hold true despite limited or no bimodality in first-degree relatives of hypertensive subjects (79). The lack of clear bimodality is probably due to multiple gene-environment interactions and the heterogeneity of genetic factors that influence blood pressure. Furthermore, blood pressure per se at one age may not entirely reflect the susceptibility to hypertension at another age. Finally, given the multiple mechanisms for control of blood pressure, population variances, not surprisingly, are wide and bimodality difficult to demonstrate.

That inheritance is a major, if not the major, predictive factor for hypertension is amply demonstrated in several animal models, especially rats. Hypertension in all is aggravated by salt intake (227). The locus of susceptibility seems to reside in the kidney in these models, since renal transplant results in acquisition of the trait of the donor animal (22, 44, 119). One hypothesis is that a fundamental inherited deficiency in renal sodium excretion is linked to hypertension and elevated peripheral resistance by a vasoconstricting, natriuretic factor (24, 52).

Salt-Stress-Gene Interaction

While salt intake, stress, and genetic background have each been implicated individually as causative factors in hypertension, recognition of the interaction of all three has been recognized only recently. Salt loading increased renal sympathetic responsiveness to stressful stimuli (such as an air-jet to the head) in several rat models of hypertension. In turn, increased renal sympathetic activity promoted salt retention by the kidneys (34, 77, 114, 125, 126, 145).

Although extrapolating animal results to humans is difficult at best, some parallels have been observed. Falkner (56) reported that young subjects with a positive family history displayed increased resting blood pressure after salt loading, and the increase became more significantly different from controls during mental arithmetic testing. In more detailed studies, long-term moderate salt restriction in young, borderline hypertensives led to diminished blood pressure reactivity in mental arithmetic, hand grip, and bicycle tests and a decreased intralymphocytic sodium concentration (ILSC). ILSC was highly correlated to the change of diastolic blood pressure during these stress tests but not to changes in systolic blood pressure or heart rate (3). Prior studies had revealed a positive correlation between ILSC and blood pressure reactivity in normotensive subjects with a positive family history of hypertension (4).

Other studies showed that after a low-salt diet, borderline hypertensives with a positive family history of hypertension had markedly higher norepinephrine and plasma renin activities while standing than did subjects with a

negative family history or normotensive controls (184). During mental stress, positive-family-history subjects tend to have higher elevations of norepinephrine than do negative-family-history controls (57). Also, urinary sodium excretion is decreased during stress test maneuvers to a greater extent in positive-family-history subjects (96, 141, 194). Hollenberg et al (96) reported marked decreases in renal plasma flow during a mental stress test in borderline hypertensives and in normotensive subjects with a positive family history but not in normotensives with a negative family history.

The studies cited above appear to implicate both increased sympathetic hormones and increased sensitivity to these hormones as mediators of the interactions among salt intake, stress, and family history of hypertension.

Studies at the Cardiovascular Genetics Research Clinic

Studies at the University of Utah Cardiovascular Genetics Research Clinic have provided insights into the role of genetics in hypertension. Heritability for response to special blood pressure tests has recently been reported (103). After correcting for age and environment, estimates of heritability in twins for sitting, standing, mental arithmetic challenge, bicycle, and isometric hand grip test blood pressures were between 19 and 65%; the highest heritabilities were demonstrated for sitting systolic (0.62) and diastolic (0.65) blood pressures. Change in blood pressures before and after mental arithmetic challenge were shown to be approximately 50% heritable in twin pairs (196). These values compare with markedly lower blood pressure heritability estimates in family studies that used components of variance analysis and adjusted for age, sex, and environment. Only sitting diastolic blood pressure ($h^2 = .25$) and bicycle test diastolic blood pressure ($h^2 = .39$) remained heritable after adjustment (103). The effects of a shared or common household accounted for up to 19% of apparent heritability in some blood pressure measurements.

Risks for developing hypertension based on family history of hypertension have been calculated from 96,518 adult relatives of 7,625 Utah students (104). Subjects with two or more first-degree relatives with hypertension at onset prior to age 55 have a 3.8-fold increased risk for developing hypertension between ages 20 and 49 and a 1.4-fold increased risk for developing hypertension between ages 50 and 69. This compares with relative risks of 2.3 and 1.3 for developing hypertension if one or more first-degree relatives had hypertension at any age. Given prevalences of 11 and 53%, respectively, for these two definitions of positive family history of hypertension, at least 40% of all hypertension prior to age 50 can be attributed to positive family history of hypertension.

After findings of elevated red cell sodium-lithium countertransport in hypertensives, tests of electrolyte biochemistry and cellular flux have been

examined with the hope of finding genetic traits that might help determine susceptibility to hypertension. Several of these tests have yielded significantly different means between hypertensive patients and normotensive controls and/or between normotensive persons with and those without a positive family history of hypertension. These tests include red blood cell sodium-lithium countertransport, sodium-potassium cotransport, sodium-potassium ATPase pump activity, ouabain binding sites (sodium-potassium ATPase pump sites), circulating inhibitor of the sodium pump (or digoxinlike factor), intralymphocytic sodium and intraerythrocytic sodium, calcium, and magnesium. Most of these tests have been performed in more than 2500 persons aged 3 to 83 in 98 Utah pedigrees. Briefly, genetic influences appear to dominate in determining levels of sodium-lithium countertransport, sodium- or lithium-potassium cotransport, intraerythrocytic sodium, and the number of sodium-potassium ATPase sites. Environmental influences are most important in sodium-potassium ATPase total activity and the presence of digoxinlike factor (229, 230). Genetic heritabilities of greater than 50% were observed for sodium-lithium countertransport, red cell sodium, and ouabain binding sites (225). Evidence for major gene effects were demonstrated for sodium-lithium countertransport and intraerythrocytic sodium. In contrast, an estimated 89% of the variance of digoxinlike factor measured by a radioimmunoassay for digoxin was determined by the effects of having common households, which strongly suggests environmental factors such as fluid and electrolyte intake determined levels of this variable (107).

Despite strong genetic determination of some red cell electrolyte flux tests, environmental factors are still important. Pregnancy increases the rate of sodium-lithium countertransport, cotransport, ouabain binding sites, and digoxinlike factor (238). Plasma triglyceride concentration and several measures of body fat strongly correlate with sodium-lithium countertransport and somewhat with sodium-potassium cotransport (105). Significant inverse correlations between HDL cholesterol levels and sodium-lithium countertransport were also observed (105). Sodium-potassium cotransport correlated positively with urinary sodium excretion, plasma sodium concentration, and plasma renin activity (233). Finally the sodium-potassium ATPase pump showed strong positive correlation with sodium-lithium countertransport and ouabain binding but showed a strong inverse correlation with red cell sodium concentration and digoxinlike factor (233). Given the diversity of influences on these electrolyte flux tests, the wide overlap of sodium-lithium countertransport or sodium-potassium cotransport between hypertensive and normotensive individuals is not surprising (229).

A recent analysis of daily excretion of urinary kallikrein adjusted for creatinine excretion showed the most striking differences of any variables tested in 116 youths with hypertensive parents compared with 691 youths with

both normotensive parents (19). After adjusting for age, sex, and urinary creatinine excretion, total urinary excretion over 12 hours was 36% lower in youths of hypertensive parents ($p < 0.0001$). A significant major gene effect was detected in complex segregation analysis of 796 persons in 57 Utah pedigrees. A dominant high allele was found in 28% of the population. Because subjects with low urinary excretion of kallikrein were apparently at higher risk of hypertension, this result suggests that 28% of the population have a gene that may be protective. Normotensive adults and youths with the high allele had a family history of hypertension approximately half as often as those who had the low allele.

Modulators Versus Nonmodulators

Williams & Hollenberg (221) have described an apparently discreet bimodal trait in hypertensives. Approximately one half of hypertensives on a low-salt diet had a normal increase in aldosterone in response to angiotensin II infusion or furosemide administration. The other half of the group showed almost no response, and no overlap occurred between the two groups. The nonresponsive group was called "nonmodulators." The authors later demonstrated that nonmodulators, defined as those who showed a blunted adrenal response to angiotensin II on a low-salt diet, also showed a blunted renal response to angiotensin II on a high-salt diet. The normal response to angiotensin II is a decrease in renal plasma flow during angiotensin II infusion. Other important differences between modulators and nonmodulators include inability to increase renal plasma flow upon sodium loading, increased sodium retention, increased time to achieve sodium balance after a change in sodium intake from 10 meq/day to 200 meq/day, greater blood pressure increments during salt loading over a 5-day period, and greater blood pressure dependence on angiotensin II when sodium is restricted. Angiotensin-converting-enzyme inhibitors apparently reverse many of these defects in nonmodulators and produce lower blood pressures in nonmodulators but not modulators (221).

Angiotensin II infusions were performed in 31 hypertensive subjects placed on a high-salt diet (140). The subjects were from 14 Utah pedigrees in which two or more siblings had had hypertension prior to age 60. Blunted renal blood flow response to angiotensin II defined nonmodulation and occurred in 25 of the 31 subjects, a fraction much higher than expected in a random sample of hypertensives ($p = 0.008$). Strong concordance of nonmodulation between sibling pairs was also observed ($p = 0.004$). These studies suggest nonmodulation may be a marker for familial hypertension. If pedigree studies demonstrate clear segregation, modulation status will be the first genetic marker for hypertension that clearly distinguishes susceptible from resistant persons within families.

Familial Dyslipidemic Hypertension

In current studies of genetic and environmental determinants of hypertension, we are screening hypertensive members of sibships in which two or more cases of hypertension occurred prior to age 55. Among the first 131 hypertensive subjects screened, 65% showed one or more lipid abnormalities (levels of LDL cholesterol or serum triglycerides above the 90th percentile or HDL cholesterol level below the 10th percentile). These values were adjusted for expected changes in lipids in hypertensive subjects receiving medication. The expected percentage of such abnormalities was 27% ($p < 0.00001$) (231). Further analyses of these 131 subjects suggested a natural division of the 65% with hyperlipidemia into two subgroups. One subgroup had lipid abnormalities consistent with FCHL, were not obese, and had significantly elevated fasting insulin levels. The second group were more obese, had predominantly high triglyceride and low HDL levels, and had mildly but nonsignificantly elevated fasting insulin levels. Both groups demonstrated a shift of LDL subfractions to the smaller, more dense particles (106). In family pedigrees with presumed familial dyslipidemic hypertension (FDH), lipid abnormalities were more common than hypertension but most patients with hypertension were also hyperlipidemic (231). In 14 pedigrees with early familial CHD, 75% of the hypertensives also displayed a lipid abnormality (unpublished observations).

A potential mechanism for FDH is illustrated in Figure 5. Several studies noted increased insulin levels and insulin resistance in hypertensive subjects (60, 169). One study in nondiabetic hypertensive subjects revealed a marked difference in insulin levels in response to a 75-gram glucose load in hypertensives versus normotensives (115 ± 15 vs 80 ± 112 at 1 hour) (68). Insulin in turn directly promotes sodium retention and may be the causal link between obesity and hypertension (169). Abnormal lipids may play an important causal role by interacting with cells to promote insulin resistance. Infusion of fatty acids in experimental animals resulted in diminished glucose uptake and catabolism and unresponsiveness to insulin in muscle (178, 183). These findings appear to be explained by free fatty acid–induced inhibition of enzymes in the glycolytic pathway and other "postreceptor" mechanisms (43). Adipocytes from rats fed high-fat diets also become much less responsive to insulin-stimulated glucose uptake and metabolism (138). Free fatty acid infusion in human subjects resulted in insulin resistance (122, 167, 208), while phenformin and clofibrate—drugs that decrease free fatty acid release and lower serum triglyceride level—result in improved glucose tolerance (59, 122). Consistent with these observations are findings of elevated insulin responses to a glucose load in subjects with elevated triglyceride turnover (1). Finally, incubation of VLDL with lymphocytes, adipocytes, or aortic en-

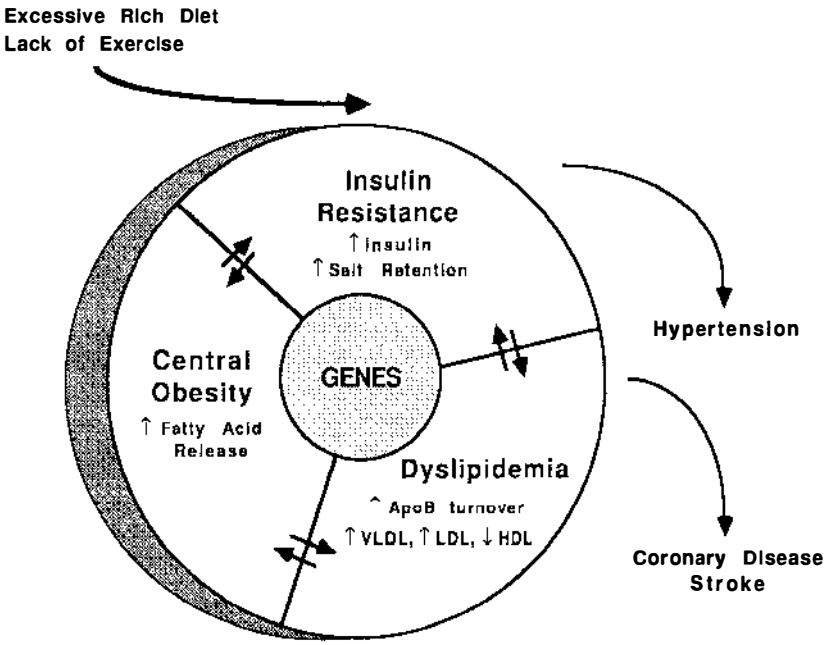

Figure 5 Suggested mechanism(s) in familial dyslipidemic hypertension.

dothelial cells resulted in insulin resistance associated with either decreased insulin receptors or post-binding resistance (203). These findings suggest a causal link between increased triglyceride (or VLDL or apo B) production and insulin resistance.

Not only do high triglyceride levels or increased free fatty acid flux promote insulin resistance, but elevated insulin levels favor production of triglycerides from free fatty acids in the liver (203). This appears to result in a vicious cycle that leads to ever-higher triglyceride levels caused primarily by overproduction. Low HDL levels that frequently occur in hypertriglyceridemia syndromes, familial combined hyperlipidemia, and type II diabetes possibly explain some of the associations between low HDL and FDH. Obesity may be an important contributor to insulin resistance and hyperlipidemia but does not appear to be a prerequisite for FDH, since only about one half of the subjects with hypertension and abnormal lipids described in our study were obese (231).

Approximately 15 to 20% of all hypertensive subjects (~1–2% of the general population) appear to have FDH. A tempting speculation is that much of the CHD risk associated with hypertension (especially mild hypertension) is attributable to FDH and that part of the reason that hypertension-

intervention studies have thus far been disappointing in demonstrating CHD prevention is that inadequate attention has been paid to lipid abnormalities in hypertensive subjects. In fact, many of the drugs used to treat hypertension may further exacerbate this problem.

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