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Invited Editorial: Dissecting the Genetic Contribution to Coronary Heart Disease

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Introduction

Coronary heart disease (CHD) is a significant contributor to morbidity and mortality in many countries and is the leading cause of death in the United States. There is ample evidence that the development of CHD is mediated by many genetic loci. However, identification of the specific genes that affect CHD risk has been a major challenge.

The underlying cause of most CHD is atherosclerosis, a disease characterized by accumulation of lipids in the intima of large- and medium-sized arteries. The development of atherosclerotic disease spans 30–50 years; fatty streaks begin to appear in childhood, and fibrous plaques are apparent in young adults. The lipids that are contained in streaks and plaques are derived from circulating lipoproteins. In the past 50 years, a variety of risk factors for atherosclerosis have been identified, including heredity (positive family history of CHD), increased serum lipid level, male gender, cigarette smoking, sedentary life-style, a diet high in fat and cholesterol, and various diseases such as hypertension, diabetes, and obesity. Serum cholesterol level is one of the most important risk factors for atherosclerosis. Two of the five major classes of particles that transport cholesterol and lipids in serum are associated with risk of atherosclerotic disease: serum levels of low-density lipoprotein cholesterol (LDL-C) are positively correlated with disease risk, and high-density lipoprotein cholesterol (HDL-C) levels are negatively correlated (Gofman et al. 1966; Gordon et al. 1977).

Because of the importance of lipoproteins as intervening variables for atherogenesis, identifying the genes that affect lipoprotein metabolism can contribute substantially to understanding the etiology of atherosclerosis. Among the scores of genes that are known to influence lipoproteins and related phenotypes are genes encoding apolipoproteins (the protein constituents of lipoproteins), lipid-processing enzymes, lipid-transfer proteins, and receptors involved in regulation of lipoproteins (see reviews by Zannis and Breslow 1985; Humphries 1988; Lusis 1988; Berg 1989; Chan and Dresel 1990). The genes encoding these apolipoproteins, enzymes, and receptors are candidate loci for atherosclerosis.

Rare variants of some of these candidate loci produce extreme lipoprotein phenotypes that contribute to development of premature atherosclerosis (reviewed by Lusis [1988]). These genetic defects account for only a small proportion of individuals who develop atherosclerotic disease. One of the most common and best understood of these variants causes defects in the LDL receptor (LDLR) and results in familial hypercholesterolemia (FH) (reviewed by Brown and Goldstein [1986]). However, individuals with FH, who generally have total serum cholesterol (TSC) values greater than 300 mg/dl, account for only 5% of individuals with atherosclerotic disease seen in cardiac-care units. As shown in the Framingham study, the majority of individuals who develop CHD have TSC levels of 200–240 mg/dl (Castelli et al. 1990). Much research remains to be done to identify the specific genes that contribute to increased risk of disease among individuals who are *not* in the upper extreme of the distribution of TSC levels and to understand the interaction of these genes with each other and with environmental factors.

The strategy of three papers in this issue of the *Journal* (i.e., Boerwinkle et al. 1991; Kamboh et al. 1991; Reilly et al. 1991) is to investigate effects that allelic variation in apolipoprotein genes have on lipoproteins and related phenotypes in the general population. Al-

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though such genes do not produce the extreme phenotypes associated with rare disorders such as FH, they typically account for a much higher proportion of the phenotypic variance in lipoproteins and other intervening variables for atherosclerosis. Thus they may be of substantial public-health importance.

Among the most widely used methods for detecting effects of individual loci on atherosclerosis are analyses of population-level associations. There are two general types of association studies. The first is the case-control study, in which one determines, for a trait related to atherosclerosis, whether individuals of contrasting phenotypes differ in the frequencies of alleles at the candidate locus. Cases may be individuals with defined dyslipoproteinemias, angiographically determined heart disease, or previous myocardial infarction, or they may be individuals from the extremes of the distribution for some quantitative precursor. In the second type of study, one determines whether individuals with specific candidate genotypes differ with respect to a particular risk factor (e.g., HDL-C levels). The underlying assumption of all association studies is that these allelic differences mark an altered protein that causes a change in lipoprotein structure or regulation. The alleles may themselves result in differences in the function or regulation of the protein, as in the case of the allelic variants at the apolipoprotein E (*APOE*) locus, or they may be in strong linkage disequilibrium with an allele that causes the difference.

Sing and Orr (1976) were the first to investigate the effects of polymorphic loci (serum protein and red cell markers) on a risk factor for atherosclerosis (i.e., TSC). At the time of their study, these loci, which were not known candidate loci for atherosclerosis, were the only ones for which useful polymorphisms had been found. Since then, many studies have indicated population-level associations between either atherosclerosis endpoints or risk factors and allelic variation at candidate loci (see reviews by Cooper and Clayton 1988; Humphries 1988; Berg 1989). Although such studies can be very informative, their results have sometimes been contradictory, probably because of small sample sizes, the failure to include effects of covariates such as sex, diet, ethnicity, and socioeconomic status, and the methods used to classify cases and controls. For example, a classification scheme in which individuals with 50% blockage of arteries are classified as cases, whereas those with less blockage are classified as controls, could result in substantial genetic heterogeneity among cases. The conflicting results of some association studies also reflect the fact that the genes and environmental factors that affect atherosclerosis may vary between populations.

Much progress has been made both in identifying polymorphisms in candidate loci and in demonstrating their effects on risk of (or risk factors for) atherosclerosis. Of particular interest are the effects of allelic variation in loci encoding the apolipoproteins. Apolipoproteins are involved in maintaining the structural integrity of lipoproteins, are ligands for lipoprotein receptors, and also act as cofactors for several lipid-processing enzymes. Each size and density class of lipoproteins has characteristic apolipoproteins. Many apolipoproteins have been described, including apolipoproteins (a), AI, AII, AIV, B, CI, CII, CIII, D, E, G, H, J, and S (see Breslow 1988; Kamboh and Ferrell 1990).

Beginning with reports of the effect of lipoprotein (a) on CHD (Berg et al. 1974), many investigators have found protein and DNA polymorphisms of apolipoproteins that affect variation in serum lipid levels in the general population (see reviews by Humphries 1988; Lusis 1988; Berg 1989; Chan and Dresel 1990). The best-characterized association is that between apolipoprotein E (apoE) isoforms and serum levels of apoE, total cholesterol, and apoB-containing lipoproteins (reviewed by Davignon et al. 1988). ApoE and apoB are the major apolipoprotein constituents of very-low-density lipoproteins (VLDL) (Reichl and Miller 1989) and also are ligands for the LDL receptor; thus, these apolipoproteins are important in the catabolism of cholesterol (reviewed by Brown and Goldstein 1986). Boerwinkle and Sing (1987) demonstrated that apoE isoforms account for approximately 8% of the variation in total serum cholesterol levels in 102 families from Nancy, France. Furthermore, individuals with the ε4 allele may be at increased risk of developing atherosclerosis (reviewed by Davignon et al. 1988). This hypothesis is supported by the recent results of Hixson et al. (1991), who reported that apoE genotype is associated with the extent and severity of arterial lesions in young males who died of accidental causes.

Identifying New Candidate Gene Polymorphisms

An important first step in implicating specific genes in the development of atherosclerosis is the demonstration that allelic variation at a candidate locus is associated with differences in a phenotype related to the disease process. The paper by Kamboh et al. in this issue of the *Journal* reports an allelic variant for

apoJ, a recently described and poorly understood apolipoprotein associated with subclasses of HDL-C that also contain CETP (cholesteryl ester transfer protein) and apoAI. This allele is present in Nigerian and American black populations only. These investigators found no significant differences among individuals with different apoJ genotypes for serum levels of total cholesterol, HDL-C, two subclasses of HDL (HDL_2-C and HDL_3-C), LDL-C, VLDL-C, and triglycerides, indicating that the effects of this allele, if present, are very small.

Black populations also have unique variants at the *APOC2* and *APOD* loci (Sepehrnia et al. 1988; Kamboh et al. 1989). The existence of unique allelic variants for candidate genes in specific populations is useful in sorting out genetic effects on CHD. Different populations, whether they are different human ethnic groups or different species, have distinctive patterns of polymorphic genes and risk factors that contribute to the development of atherosclerosis. Comparisons of results from different populations can reveal hitherto unrecognized effects of single genes or of a suite of genes and, as a result, can define new elements in the genetic control of lipoprotein metabolism.

Interactions with Environmental Factors and with Other Loci

Genetic effects on atherogenesis can be clarified by investigating the effects that possible interactions between loci—as well as between genes and environmental factors—have on intervening variables. The presence of such interactions has been hypothesized on the basis of reports that risk of disease varies greatly both within and between individuals of different genders (e.g., see Higgins and Thom 1989), ethnic backgrounds (e.g., see Mitchell et al. 1990), dietary habits (e.g., see McGill 1979), and *LDLR* genotypes (Hobbs et al. 1989). Furthermore, not all individuals respond similarly to dietary intervention (e.g., see Katan et al. 1986). Studies of genotype × genotype and genotype × environment interactions in humans are just beginning. For example, Pedersen and Berg (1989) have reported that an *LDLR* variant interacts with the apoE variants to affect LDL-C levels, thus demonstrating genotype × genotype interaction. Tikkanen et al. (1990) have reported that the effect of apoE genotype on serum cholesterol values is moderated by dietary fat and cholesterol intake, although other investigators have not found significant effects (Clifton et al. 1990; Xu et al. 1990).

Boerwinkle et al. (1991), in this issue of the *Journal,* report on the response of serum lipid levels to a high-cholesterol diet in 71 normolipidemic men with different apoE and apoB genotypes. As expected, the investigators found significant effects of the high-cholesterol diet and apoE and apoB genotypes on serum lipid levels and also report considerable individual variation in response to the high-cholesterol diet. However, there was no significant interaction between an individual's apoE or apoB genotype and his response to dietary cholesterol. Therefore, although APOE and APOB are associated with variation in lipid levels, these loci probably are not responsible for a significant proportion of individual variation in response to dietary change in normolipidemic Caucasian men.

The results of this investigation illustrate both the power and some of the problems with research on genotype × environment interactions. Because intervention studies (using diet, exercise, etc.) are difficult to perform on large groups of human subjects, individual studies permit only limited comparisons, e.g., the effects of a high- versus a low-cholesterol diet in males who are primarily of a single ethnic group. Many studies of other population groups and other environmental covariates are needed. Once lipid data and DNA have been obtained for such studies, DNA markers for additional candidate loci can be developed, and the effects of these loci on response to intervention can be determined. However, a comparison of results across these studies may be difficult and will depend on the development of additional analytical methodologies, such as metaanalysis (Berlin and Colditz 1990).

Gender-specific Effects on Means and Variances

In 1954, Lerner (1954) proposed that genes influence not only the mean levels of quantitative traits but also the variation around the mean. He suggested that differences in intragenotypic variances may be due to an inability by individuals of a specific genotype to "buffer" their phenotypes against other genetic and environmental factors. Murphy (1979) extended these concepts, with particular reference to human populations. More recently, Berg (1988, 1990; also see Berg et al. 1989) has investigated the effects of "variability genes" on CHD risk factors. For such genes, the mean value of two genotypes may be similar, but the intragenotypic variances differ. Increased phenotypic variance within a genotype may be due either to genotype

× environment or genotype × genotype interaction or to linkage disequilibrium.

In this issue of the *Journal,* Reilly et al. (1991) report gender-specific effects of apoE genotypes on both means and variances in lipid and lipoprotein traits. They examined the effects of the three most common apoE genotypes (ε2/ε3, ε3/ε3, and ε3/ε4) in a group of 587 Caucasian adults in Rochester, MN. They found significant associations between apoE genotype and mean serum levels of apoB, apoCII, and apoCIII, in both males and females. Associations between apoE genotypes and mean serum levels of total cholesterol, triglyceride, and HDL-C were gender specific. In general, these mean effects are consistent with those reported by other groups, although many of the previous analyses were not done separately for males and females.

Reilly et al. are among the first to report genotype-specific effects on variances of quantitative CHD risk factors. With the exception of apoAI levels, the variances of all lipid and lipoprotein traits differed between genders and among apoE genotypes. To investigate further the relationships among genotypes and phenotypes, Reilly et al. present results of a multivariate graphical analysis. Their novel representation of the standardized, multivariate means (i.e., centroids) and variances of each genotype revealed that the centroids of the three apoE genotypes differed among genotypes but not between males and females; the variances were gender specific and genotype specific; and the contribution of each trait to the multivariate mean and variance was gender specific and genotype specific.

Knowledge of gender- and genotype-specific means and variances could be important in decisions concerning the need for health care. This point is illustrated by Reilly et al.'s estimate of the proportion of individuals of different apoE genotypes who have TSC levels greater than 240 mg/dl (assumed to be an arbitrary threshold above which risk of CHD increases). In the population studied, Caucasian males of genotype ε2/ε3 have lower mean TSC but higher SD than do ε3/ε3 males (187.3 ± 41.7 vs. 193.6 ± 31.8, respectively). As a consequence, even though mean TSC is lower in ε2/ε3 males, a greater proportion of them (10.4% vs. 6.4%) are at increased risk of CHD.

Our understanding of the effects that allelic differences at candidate loci have on the means and variances in levels of quantitative risk factors for CHD is at a preliminary stage. Additional research to determine the differential effects of candidate loci on means and variances of quantitative traits is required. Especially important will be the development of new analytical methods to estimate the significance of such effects.

Conclusion

The genetics of atherosclerosis has been very difficult to unravel, except for those rare situations in which a mutation produces an extreme effect. Fortunately, there is great potential to sort out the effects of specific genes on the development of atherosclerosis: there are very few complex diseases about which so much information is available from metabolic and epidemiological studies and for which so many candidate genes and quantitative risk factors already have been identified.

Association studies can provide the first statistical evidence that a specific candidate gene is involved in the development of atherosclerosis. After specific candidate loci have been implicated, additional metabolic and molecular genetic studies can be performed to identify the mode of action and regulation of these genes. The identification of candidate genes that affect atherosclerosis in the general population also provides a powerful tool with which to investigate how interactions between genes and gender, age, environmental factors, and other loci affect risk of disease. These studies can be performed either with data on unrelated individuals, as done in the three articles in this issue of the *Journal,* or with family data. Hypotheses about the effects of interactions among loci and between candidate loci and environmental factors will always be difficult to evaluate in human populations, but they can be tested in animal models, in which dietary and other environmental factors are more easily controlled (e.g., see Blangero et al. 1990).

All known candidate genes, however, account for only a small proportion of the variance in TSC levels. Among Caucasians, apoE polymorphisms (which give the most consistent results across groups) account for approximately 4%–8% of the variation in TSC (Ordovas et al. 1987; Davignon et al. 1988), and polymorphisms at genes associated with the three most common single-gene disorders (familial hyperlipidemia, familial defective apoB100, and familial combined hyperlipidemia) may each account for an additional 1% of the variation (Davignon et al. 1988; Soria et al. 1989; Wojciechowski et al. 1991). Since the heritability of TSC may be as high as .60, many of the genes affecting TSC (and other intervening variables)

remain to be identified. Complex segregation analysis of family data has suggested the existence of other, still unidentified genes that have a major effect on either a lipoprotein or other intervening variable for CHD (e.g., see Hasstedt et al. 1987; Austin et al. 1988; Moll et al. 1989). Linkage analyses using a panel of candidate loci and other markers can establish the chromosomal locations of these major genes, and further research by genetic epidemiologists, biochemists, molecular biologists, and physiologists can lead to their identification and characterization.

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