Knowledge of genetic factors in the etiology of coronary heart disease has not so far been adequately utilized in attempts to combat premature CHD. The time has now come to utilize genetic information in a setting of family-oriented preventive medicine. This approach would greatly improve the efficiency of preventive efforts, utilizing predictive genetic testing and targeting counseling on those who need it most.1

The optimistic predictions of the impact of genetics on the diagnosis and prevention of coronary heart disease (CHD) have not been realized, and whether DNA testing will be incorporated into clinical cardiology practice in the next decades is unclear. Most of the genetic variations identified thus far either are rare or influence traditional risk factors such as low HDL, elevated LDL, high blood pressure, and diabetes. Several recent advances, however, make a case for renewed optimism (see Part I of this review). Studies in humans and mice have begun to reveal highly penetrant genetic factors that contribute to CHD susceptibility independent of traditional risk factors. As technical advances bring down genotyping costs, we may pass a threshold for the cost–benefit ratio in the near future. One important application in diagnosis is likely to involve the identification of different forms of CHD to allow individualized treatment (pharmacogenetics). The application of multilocus genotyping to assess risk or to screen populations for individuals who are highly susceptible to CHD also is a possibility. Perhaps most important, genetic studies are leading to many insights into disease mechanisms, and these studies, along with biochemical approaches, are pointing to new therapeutic concepts and treatments.

New Therapies

Probably the most significant impact of genetic studies on medicine pertains to the development of therapies. The outstanding example is the impetus that genetic studies of familial hypercholesterolemia (FH) provided in the development of hepatic hydroxymethyl glutaryl coenzyme A reductase inhibitors (statins).2 Several therapeutic approaches based on genetic studies are under development.

The identification of a naturally occurring variant of apolipoprotein AI, known as apoAI Milano, in a village in northern Italy in 19803 led to investigations of the therapeutic potential of HDL and this protein variant as antiatherogenic therapy. Carriers of the apoAI Milano gene are characterized by extremely low levels of HDL cholesterol (10 to 30 mg/dL) and a decreased risk of atherosclerosis relative to the low level of HDL. The apoAI Milano protein differs from native apoAI in that a cysteine is substituted for arginine at position 173. This cysteine confers different properties to this protein as compared with normal apoAI, including the ability to form disulfide-bonded dimers with other apoAI Milano molecules and other HDL proteins such as apoAII. Recombinant apoAI Milano has been formulated in a complex with phospholipids to mimic the properties of nascent HDL (ETC-216, Pfizer).4 Studies in mice and rabbits with experimental atherosclerosis have demonstrated that recombinant apoAI Milano/phospholipid complexes rapidly reduce the lipid and macrophage content of atherosclerotic plaques after a single infusion.5 The effect of short-term intravenous recombinant apoAI Milano/phospholipid complexes on atheroma burden in patients with acute coronary syndromes was studied in a recent clinical trial.5 The intravenous administration of ApoAI Milano for 5 doses at weekly intervals produced statistically significant regression in coronary atheroma volume in the target segment as compared with baseline measurements by intravascular ultrasound. No change was seen with saline placebo control. It remains to be determined whether apoAI Milano has unique properties that result in greater antiatherogenic potential than normal apoAI and whether the exciting results of this first study in humans can be confirmed in large-scale randomized clinical outcome trials.

HDL are involved in reverse cholesterol transport, the process by which cholesterol is transported from peripheral tissues to the liver, where it can be excreted in bile. Studies since the mid-1990s have provided strong evidence that HDL also may protect against atherosclerosis by virtue of its antiinflammatory properties. These studies, based in part on genetic variations that influence the antioxidant properties of HDL, indicate that HDL can selectively remove and destroy proinflammatory oxidized lipids from the vessel wall, thereby
inhibiting the vicious cycle of LDL trapping, LDL oxidation, and inflammation. Navab et al. have demonstrated that short, synthetic amphipathic helices, similar to those in apoAI, exert powerful protective effects against atherosclerosis when administered orally to mice or monkeys.

Another promising therapeutic target is the leukotriene pathway. As discussed in Part I of this review, genetic studies implicated 5-lipoxygenase (5-LO) in atherosclerosis susceptibility in mice and then humans, and recent studies suggest that polymorphisms of other enzymes in leukotriene metabolism also are associated with CHD. 5-LO polymorphisms were implicated originally in asthma, and a variety of leukotriene synthesis inhibitors are widely used to treat asthma. Thus, these inhibitors also may protect against the development of CHD, possibly providing a useful complement to drugs that target risk factors, such as lipids and blood pressure.

**Risk Stratification, Prevention, and Treatment**

Current approaches to the management of asymptomatic individuals and those with documented CHD focus on assessing traditional risk factors, estimating risk, and modifying risk factors through therapeutic lifestyle modification or cardiovascular protective medications or both. National guidelines from the American Heart Association and the National Heart, Lung, and Blood Institute’s National Cholesterol Education Program recommend an approach to initial global risk assessment of the asymptomatic patient to obtain an estimate of absolute cardiovascular risk. On the basis of standard risk factors and related risk correlates, the concept was set forth that asymptomatic patients can be placed into 1 of 3 risk categories: low, intermediate, and high. The techniques used in office assessments include history, physical examination, laboratory testing, and ECG. The focus of the examination is on the detection of risk factors that either can be directly modified or will modify the overall intensity of risk-reduction therapies. The major causal risk factors identified for routine assessment include age, smoking, elevated blood pressure, elevated serum LDL cholesterol, low HDL cholesterol, and diabetes. The approach to therapy is guided by the principle that the intensity of risk-factor management should be adjusted by the severity of risk. Low-risk patients should be encouraged to adhere to healthy lifestyle habits. High-risk patients are advised to directly begin a regimen of aggressive risk reduction through a combination of nondrug and drug regimens. Patients at intermediate risk become candidates for further risk stratification through the measurement of the inflammatory marker high-sensitivity C-reactive protein or noninvasive procedures that test for the presence of myocardial ischemia or coronary atherosclerotic burden, or both techniques.

Although several of the conventional causal risk factors used in global risk assessment clearly have a genetic basis, specific genetic testing has not been recommended for routine clinical practice. Because conventional risk factors account for only ≈50% of the variability in risk, the identification of genetic differences influencing the pathways of measurable atherosclerotic risk factors or novel pathways may allow for the determination of risk that is additive to the measurement of conventional risk factors. Moreover, some genetic differences, such as those in the genes for apoE, lipoprotein lipase (LPL), and interleukin-6, show evidence of specific environmental interactions (eg, with smoking), in which the overall risk is more than additive. Thus, the predictive models used in the Framingham risk score may be greatly enhanced by the addition of pivotal single-nucleotide polymorphisms and haplotypes that have been shown to affect CHD. It can be envisioned that genotypic information could transform the value of the risk score and be integrated in routine clinical practice to guide preventive therapy.

It also seems likely that genetic differences would be useful in predicting specific complications of atherosclerosis. It is interesting that the phosphodiesterase-4D (PDE-4D) gene polymorphisms, examined in Part I, were specifically associated with stroke. The genetic linkage studies of Wang and colleagues suggest that genetic factors may be associated specifically with myocardial infarction rather than with atherosclerotic lesion size. Studies in mice have clearly demonstrated that genetic factors can influence the distribution of lesions in different vessel locations; for example, Teupser and colleagues observed striking differences in the development of lesions in the aortic sinus between strains C57BL/6 and FVB but no differences in the abdominal aorta. Studies in mice also have clearly shown that genetic factors distinct from those for lesion size influence vascular calcification and medial destruction leading to aneurysms.

**Multilocus Testing**

A number of genetic differences identified thus far could potentially serve as useful predictors of prevalent or incident CHD. Such markers, however, may not be useful clinically unless they possess additional characteristics. These characteristics include (1) ability to standardize the assay, (2) independence from established risk factors, (3) association with CHD clinical end points in observational studies and clinical trials, (4) presence of population norms to guide the interpretation of results, (5) ability to improve the overall prediction beyond that of traditional risk factors, (6) generalization of results to various population groups, and (7) acceptable cost of the assays. The use of genetic testing also would be affected by the type of relationship with atherosclerosis and other risk factors.

Many genes have been associated with the increased risk of atherosclerosis, acute myocardial infarction, or both, although a large fraction are likely to be false positives (see Part I of this review). Although most of these variations influence traditional risk factors, they may provide genetic information that is useful in assessing risk to relatives or guiding therapy (eg, LDL receptor, apoAV, apoB, apoE, cholesterol ester transfer protein, LPL, upstream transcription factor-1). Others may provide more reliable and cost-effective assays for risk factors. For example, apo(a) gene variations determine nearly all of the variance of Lp(a) levels, and although Lp(a) levels can be measured using immunoassays, the levels have proven difficult to standardize. DNA-based tests that distinguish the various apo(a) alleles according to haplotype could be developed to reliably predict Lp(a) levels. Several genetic variations clearly influence Lp(a) through mechanisms that are
independent of traditional risk factors, although they may not be independent of markers of inflammation such as C-reactive protein or myeloperoxidase. The recently reported myocyte enhancer factor 2A variation is highly penetrant, although its frequency in the population is probably low. Common variations in the genes for PDE4D, lymphotoxin-α, 5-LO, 5-LO–activating protein, and others discussed in Part I of this review are likely to improve the overall prediction of risk.

The cost of such tests is, of course, an important issue. DNA is stable and simple to isolate, and with multiplexing, many tests (hundreds or thousands) can be performed on the amount of DNA in a few milliliters of blood. Until recently, the cost of typing DNA differences (genotyping) has been $1/genotype, but new technologies are reducing this amount by more than a factor of 10. The number of tests that will be required per gene is another key issue. If, on the one hand, risk is determined largely by genetic differences that are common in human populations, such as the apoE alleles discussed above, then genotyping will be relatively inexpensive. If, on the other hand, susceptibility to CHD results largely from many different rare mutations, such as mutations of the LDL receptor in FH, then genotyping will be much more difficult, expensive, and less reliable. It has proven feasible, even for FH, to design rapid screens that detect a significant fraction of FH mutations. Most of the evidence suggests that common genetic variations will explain a significant fraction of the risk of common diseases, including CHD.

The value of genetic testing in cardiology will become clear only when it has been implemented on a large scale. Although the impact of the testing of individual polymorphisms could be estimated from published odds ratios for risk, these ratios are likely to vary between ethnic groups and to be influenced by genetic and environmental interactions. Also, as discussed above, most of the reported associations require confirmation. Stephens and Humphries have argued that genetic testing may not have much value because the presently known genetic differences may not predict risk over and above that of measured traits, such as plasma lipids. Quite reasonably, they posit that because an individual’s personal characteristics and plasma risk–trait values reflect both genotype and exposure, they may be the superior predictors of clinical outcome. It is our opinion that several potential benefits to genetic testing exist. First, a number of recently identified genes are likely to be independent of measured traits. Second, they may guide therapy (see below). Third, they may reveal highly penetrant disorders that could influence risk substantially for a patient as well as his or her family members. We suggest, therefore, that multilocus testing be evaluated in some large trials to determine its overall value and identify the most important risk factors. The Table lists some of the candidates for multilocus testing.

### Population Screening

Although the prevention of CHD is feasible and scientifically definable by preventing or controlling the major risk factors, the challenge of identifying and treating individuals with

<table>
<thead>
<tr>
<th>Gene</th>
<th>Trait</th>
<th>Risk Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-LO</td>
<td>Increased intima-media thickness</td>
<td>7</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme</td>
<td>CHD</td>
<td>1.3 (1.2–1.6)*</td>
</tr>
<tr>
<td>ApoA</td>
<td>CHD triglyceride levels</td>
<td>20</td>
</tr>
<tr>
<td>ApoE</td>
<td>CHD</td>
<td>1.3 (1.1–1.5)*</td>
</tr>
<tr>
<td>Apo(a)</td>
<td>Lp(a) levels</td>
<td>17</td>
</tr>
<tr>
<td>Cholesterol ester transfer protein</td>
<td>HDL levels</td>
<td>21</td>
</tr>
<tr>
<td>Endothelial nitric oxide synthase</td>
<td>CHD</td>
<td>1.3 (1.1–1.6)*</td>
</tr>
<tr>
<td>5-LO–activating protein</td>
<td>MI, stroke</td>
<td>1.8</td>
</tr>
<tr>
<td>Hepatic lipase</td>
<td>HDL levels</td>
<td>22</td>
</tr>
<tr>
<td>LDL receptor</td>
<td>Cholesterol levels</td>
<td>18, 23</td>
</tr>
<tr>
<td>LPL</td>
<td>Metabolic syndrome</td>
<td>24</td>
</tr>
<tr>
<td>Lymphotixin A</td>
<td>CHD</td>
<td>1.8 (1.4–2.3)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>CHD</td>
<td>1.2 (1.06–1.39)*</td>
</tr>
<tr>
<td>Paraoxonase-1</td>
<td>CHD</td>
<td>21</td>
</tr>
<tr>
<td>PDE4D</td>
<td>Stroke</td>
<td>1.8</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>CHD</td>
<td>1.3 (1.1–1.6)*</td>
</tr>
<tr>
<td>Peroxisome proliferator-activator receptor-γ</td>
<td>Type 2 diabetes</td>
<td>1.2 (1.1–1.4)*</td>
</tr>
<tr>
<td>Upstream transcription factor-1</td>
<td>Familial combined hyperlipidemia</td>
<td>28</td>
</tr>
</tbody>
</table>

*RR and CI for several genes studied in multiple populations and meta-analyzed.

<table>
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<tr>
<th>Trait</th>
<th>Risk Estimate</th>
</tr>
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major CHD risk factors remains great. Nearly the entire US population can be considered at risk because of exposure to ≥1 unfavorable risk factor level or unhealthy lifestyle habits. Because atherosclerosis is clinically manifested later in life, knowledge of propensity for the disease could be available decades before the clinical disease develops, permitting early intervention. DNA tests could assess risk earlier in life, long before phenotype-based tests become useful in adults in assessing risk. A population-wide approach to identifying individuals at higher risk could be justified, allowing for targeted treatment for those found to be at high risk. As it becomes more feasible and less expensive to perform genetic profiling, population screening to identify individuals at risk should be considered.

**Pharmacogenetics**

Pharmacogenetics provides the experimental basis to understand the variability in response to drugs as a function of an individual's genetic makeup. Genetic polymorphisms may influence drug response through a number of mechanisms including pharmacokinetic interactions, pharmacodynamic gene–drug interactions that involve gene products expressed as receptors, and genes that are in the causal pathway of disease and are able to modify the effects of drugs.

Lowering serum lipid levels has been demonstrated to slow progression or even induce regression in atherosclerosis. However, as with any other drug treatment, the magnitude of plasma lipid responses to drug therapies varies considerably among individuals. Several genetic polymorphisms that may play a role in the different responses to lipid-lowering therapy have been identified recently and thus may predict individual differences in plasma lipid responses to drug therapies. Variations in apolipoprotein E (apoE) genotype and plasma lipoprotein lipid changes with statin therapy have been identified recently and thus may predict individual success in hypolipemic drug treatment. Several studies have revealed significant interactions between an individual’s apoE genotype and plasma lipoprotein lipid changes with statin therapy. In these studies, subjects with the E2 allele, and sometimes subjects with the E3 allele, were more likely to respond to statin therapy with a favorable reduction in total cholesterol and LDL cholesterol than were subjects with at least 1 E4 allele. Variation in response to statins on disease progression has also been associated with genetic polymorphisms. A polymorphism for the cholesteryl ester transfer protein gene has been associated with the effectiveness of pravastatin on disease progression. About 16% of the Regression Growth Evaluation Statin Study (REGRESS) trial population (men with angiographically documented atherosclerosis) had the B2B2 genotype, and these subjects did not show any benefit from the use of statins on the progression of atherosclerosis, whereas individuals without this genotype did demonstrate benefit. Another interesting example is the interaction of a common polymorphism in the matrix metalloproteinase stromelysin-1 gene. These effects were independent of the effects of pravastatin on lipid levels.

As previously discussed, the leukotriene pathway may make an important contribution to genetic susceptibility to atherosclerosis as well as to asthma. Genetic variations in 5-LO have been shown to contribute to the variable response to antileukotriene drugs in asthma (Figure 1). Genetic variants in the promoter region are believed to change the binding of these transcription factors and the rate of 5-LO transcriptional activation under inflammatory conditions. Common variations in the GC-rich region of the promoter were associated with the altered transcription of the 5-LO gene as compared with the common allele. In people with asthma, carriers of these genetic variants demonstrated a diminished response to treatment with antileukotriene drugs, indicating a pharmacogenetic effect of the promoter sequences on responses to treatment (Figure 1). If antileukotriene medications are shown to be beneficial in atherosclerosis, then similar pharmacogenetic effects of the 5-LO promoter sequences on responses to treatment would be expected. Genotyping may be used to identify individuals who would derive the greatest benefit of this antiinflammatory directed therapy.

Genetic testing also may reveal useful dietary interactions. In the case of 5-LO, dietary arachidonic acid intake significantly enhanced the proatherogenic effect of 5-LO variants, whereas intake of n-3 polyunsaturated fatty acids attenuated this effect. Because n-3 polyunsaturated fatty acids decrease the formation of leukotrienes by competing with arachidonic acid as substrates for 5-LO, these findings suggest that the antiatherogenic effects of n-3 polyunsaturated fatty acids may be confined to or may be more prominent in individuals with genotypes that favor increased 5-LO activity.

Another interesting example is the interaction of a common polymorphism in the matrix metalloproteinase stromelysin-1 gene. The dependence of homocysteine levels on this interaction is evident when individuals in the study population were divided into those homozygous for the thermolabile allele (genotype TT),
heterozygous for the thermolabile allele (genotype CT), or homozygous for the thermostable allele (genotype CC) and then subdivided according to folate levels (Figure 2A). In individuals with high folate levels, the thermolabile allele had no effect on homocysteine levels. However, in individuals with low folate (first tertile), homozygous individuals had a ∼2-fold increase in homocysteine levels as compared with the individuals carrying the thermostable allele (Figure 2A). The relationship of MTHFR genotypes and folate levels to CHD is, of course, much more complex, and studies with large numbers of individuals are required to observe evidence of this relationship, given the complexity of cardiovascular disease. These investigations have led to some novel therapies that are undergoing testing in clinical trials. A number of genes that are associated with an increased risk for CHD have been identified recently. Several of these genes exhibit common variations that influence CHD risk independently of traditional risk factors (lymphotoxin-α, PDE4D, 5-LO, 5-LO-activating protein). Other genes are rare but are highly penetrant (myocyte enhancer factor 2A); still others are important markers of familial disease (upstream transcription factor-1). Prospective trials to evaluate the effectiveness and cost of genetic testing to risk-stratify individuals, guide therapy, and predict response are needed before these tests are integrated into routine clinical practice. Progress in this area will be driven by studying gene–gene, gene–phenotype, and gene–treatment interactions in large patient populations. Based on the rapid progress being made, an individual’s genotype may well play an important clinical role in the assessment, prevention, and treatment of atherosclerosis.

Conclusions

Investigations into the genetics of atherosclerosis have greatly advanced our knowledge of the mechanisms of this complex multifactorial disease. These investigations have led to some novel therapies that are undergoing testing in clinical trials. A number of genes that are associated with an increased risk for CHD have been identified recently. Several of these genes exhibit common variations that influence CHD risk independently of traditional risk factors (lymphotoxin-α, PDE4D, 5-LO, 5-LO-activating protein). Other genes are rare but are highly penetrant (myocyte enhancer factor 2A); still others are important markers of familial disease (upstream transcription factor-1). Prospective trials to evaluate the effectiveness and cost of genetic testing to risk-stratify individuals, guide therapy, and predict response are needed before these tests are integrated into routine clinical practice. Progress in this area will be driven by studying gene–gene, gene–phenotype, and gene–treatment interactions in large patient populations. Based on the rapid progress being made, an individual’s genotype may well play an important clinical role in the assessment, prevention, and treatment of atherosclerosis.

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Disclosure

Dr Alan M. Fogelman is a principal in Bruin Pharma.

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