Genetic Testing for Cardiovascular Disease Susceptibility: A Useful Clinical Management Tool or Possible Misinformation?

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Abstract—Genetic susceptibility tests are already advertised on the Internet to identify individuals at above average risk for cardiovascular disease (CVD), such as deep vein thrombosis, hyperlipidemia, or atherosclerosis, whereas other tests claim to predict response to a particular drug treatment. Some kits are available to the public directly, bypassing a doctor. Their value, however, must be considered carefully, because although a genotype may be strongly and consistently associated with an intermediate trait, and because the intermediate trait is a strong predictor of CVD risk, there may be little or no association of genotype with risk over and above that of the measured trait. This is because multigenic effects and environmental modification (context dependency) of genotype effects determine CVD risk. An individual’s personal characteristics and plasma risk-trait levels (which reflect both genotype and exposure) at present are the best predictors of clinical outcome. Only when genetic tests surpass this, possibly by the inclusion of many functional common variants, in conjunction with their context-dependent effects on risk, might their usefulness in clinical management be realized. Here we review some of the particular issues and concerns raised by CVD-risk genetic testing, and suggest areas of further research to address these issues. (Arterioscler Thromb Vasc Biol. 2004;24:1-9.)

Key Words: XXXXX ■ XXXXX ■ XXXXX ■ XXXXX ■ XXXXX

Genetic Testing for Monogenic and Complex CVD

For “complex” multifactorial disorders, such as cardiovascular disease (CVD) or risk of myocardial infarction (MI), risk prediction from a genetic test is far from straightforward. There are some monogenic causes of early CVD, such as familial hypercholesterolemia (FH), which occurs, in the heterozygous state, in approximately 1 in 500 members of the general population. Although there is locus heterogeneity and a large mutational spectrum (at least three genes and >700 mutations, see http://www.ucl.ac.uk/FH), rapid mutation screening methods have been developed, and FH is amenable to clinical genetic diagnostic approaches\(^1\) and cascade testing\(^1\) to identify presymptomatic relatives, and have been shown to be cost-effective.\(^4\) However, even for FH, the “CVD penetrance” varies enormously, depending partly on the specific mutation present and on modifier genes, but more importantly on the same lifestyle environmental risk factors that determine risk in non-carriers, such as smoking, diet, lack of exercise, alcohol consumption, stress, etc. As a result of the changes in the prevalence of these environmental factors over the past 150 years, the age of death in FH subjects in a single family (ie, all carrying the same mutation) varied by more than 2-fold.\(^5\) Thus genetic testing and mutation identification in an FH proband gives an unequivocal test for carrier relatives who can be offered cholesterol-lowering advice, including statin therapy, but little prognostic information over and above their untreated LDL cholesterol level and general CVD risk profile.

For non-monogenic CVD, research over the last 15 years has examined\(^6\) and identified many “candidate” genes, and functional variants within these genes\(^7\) that could potentially be of value in risk prediction\(^8\) and the commercial tests proposed are based on this knowledge. However, the main problem in their use is that a priori, because of the multifactorial nature of CVD, and as discussed below the context dependency of genotype outcomes, the size of the effect of any single variant in a single gene on the clinical end point of CVD is expected to be relatively modest.

Genetic Prediction Over and Above Accepted Risk Factors

For a genetic test to be useful in clinical management of CVD, it is obviously critical that the test must have additional predictive power over and above those accepted risk factors that can be easily measured, usually inexpensively, and with high reproducibility and replicability. In addition to an individual’s personal characteristics such as age, gender,
degree of obesity, blood pressure, and their lifestyle, “environmental” factors such as smoking habit, alcohol consumption, diet, and exercise, plasma measures of “risk factors” such as total cholesterol, or better still the ratio of total (or LDL) to HDL cholesterol, are well recognized to be useful predictors of risk.10 Recently, measures of the inflammatory state of the subject characterized by levels of C-reactive protein (CRP)10 have also been shown to strongly predict future CVD. Moreover, and critical for its use in clinical settings, CRP evaluation has been shown to add prognostic information at all levels of LDL cholesterol, at all levels of the Framingham Risk Score, and at all levels of the metabolic syndrome.11-13 Variables such as these can be entered into a risk algorithm, such as those based on the PROCAM study14 or used in the Joint British Societies Risk Prediction Chart (JBSRC)15 derived from the Framingham study.16 The accuracy of these scores in risk prediction has been assessed, and with respect to CVD, these classical risk factors gave a positive predictive power and false-negative rate (C-index) of 0.67 for PROCAM and 0.80 for the JBSRC, respectively.17 Similarly, the Framingham score gives a good rank ordering of risk for individuals in NHANES I and II (although prediction of absolute risk was less accurate).18 Genetic tests, therefore, have to add significantly to this predictive power with a good positive predictive value (PPV) and low false-positive rate (FPR) to be useful in identifying at-risk subjects. The question is, “Do any of the currently available genotypes meet this standard?” Any genetic test must also, of course, meet the usual criteria of any useful assay of between-laboratory standardization and must be of high reliability and reproducibility, but a discussion of these aspects is beyond the scope of this review. The usefulness of CVD genetic testing is examined using two examples of well-characterized gene variants.

Apolipoprotein E
The best-studied candidate gene in the lipid field is that coding for apolipoprotein E (apoE),19 in which the most frequent allele is called ε3, and with two less common variants called ε4 and ε2, which have allele frequencies in Europeans of approximately 0.15 and 0.07, respectively. The sequence changes in the gene alter two charged amino acids, which affect plasma clearance of the protein and the cholesterol-rich lipoproteins carrying them, such that the variants explain a large part of the population variance in plasma apoE levels, a smaller part of LDL cholesterol variance, a smaller part of total cholesterol variance, and a very small fraction of CVD risk (estimated to be ≈2%).19

Because APOE variants have the majority of their effects on CVD risk through their effect on plasma lipid levels, including lipid levels, in the risk algorithm they will remove much (if not all) of the risk information that could be obtained from an individual’s APOE genotype. Similar logic applies to other candidate genes proposed in risk panels such as those encoding β-fibrinogen (FIBB) and plasminogen activator inhibitor 1 (PAI-1) involved in coagulation and fibrinolysis, or interleukin 6 (IL-6) in inflammation, or apoCIII (APOC3), lipoprotein lipase (LPL), or cholesteryl ester transfer protein (CETP) in lipid metabolism. If the majority of their risk is mediated through the plasma levels of easily measured lipids (eg, triglycerides), or lipoproteins (eg, HDL), or proteins (eg, IL-6, CRP, fibrinogen or PAI-1), then these genotypes will be poor and incomplete surrogates for the plasma measures. Moreover, because expressed plasma levels reflect genetic and environmental influences, plasma level would be expected a priori, to better-reflect the integrated effect of these exposures on the disease process.20

Receiver-Operator Curves
One way to examine the added diagnostic or predictive value of any new risk factor is to use receiver-operator curves (ROC). ROCs are used to discriminate cases from non-cases and using information from sensitivity and specificity analyses. Risk scores are calculated using the coefficients in the logistic regression model as weights to give the score that discriminates best between cases and controls. ROCs are the true-positive rate (TPR) plotted against the FPR across the range of possible cut points for the risk score. If the FPR rate is equal to the TPR, then the risk score does not discriminate between cases and controls and ROC value equals 0.5. The more TPR that exceeds FPR, the better the discrimination, as indicated by a greater ROC value, with a value of 1 indicating that the score discriminates perfectly, ie, the distributions of the risk score in cases and controls do not overlap. In the PROCAM study, their published risk score algorithm discriminated well with ROC value of 0.82.14

To illustrate this, we present data in Figure 1 from a well-established UK-based prospective study of 3052 middle-aged healthy men, followed-up now for CVD for more than 10 years; the Northwick Park Heart Study II (NPHSII). To date there have been more than 200 CVD events, mainly fatal and non-fatal MIs, and with both classical and novel risk factors being strongly associated with risk.21,22 When we apply the PROCAM score to the NPHSII data, it gives adequate prediction with ROC value of 0.65 and a detection rate (for a 5% FPR) of 11.7% (Figure 1). Adding the APOE genotype to this score increases the ROC value to 0.67 (detection rate=14.0%), but this improvement is not significant (P=0.11). We have reported that several other genotypes are significantly associated with CVD risk in NPHSII, and if we add in two of the commonest, namely IL6,23 and PPARA,24 the ROC value remains unchanged (0.67) with a detection rate of 15.9%, with no significant improvement over the PROCAM score (P=0.17). Thus, by themselves genetic risk factors may give statistically significant effects on risk and shed light on important biological mechanisms of CVD development, but they have limited predictive power when classical risk factors are taken into account. If in the future further independent risk genotypes could be identified, then in combination they may achieve over-and-above risk prediction.

Factor V Leiden
Although the “Leiden” mutation (R506Q) in the factor V (F5) gene is not associated with increased risk of arterial thrombosis,25,26 it is a useful paradigm of susceptibility testing for a less complex disorder, namely venous thrombosis. Also, as discussed later, because of its relatively high prevalence and
penetrance, $F5$ R506Q represents an intermediate situation between genetic tests for monogenic, high-penetrance mutations such as those causing FH and testing in families is useful, and common variants associated with a modest impact on CVD when family testing is not relevant but including carriers as part of a risk screen may be helpful. Most cases of venous thrombosis are not fatal, but death from pulmonary embolism (PE) occurs in 1% to 2% of all patients, and as many as 25% of patients with deep venous thrombosis (DVT) will have the chronic effects of postthrombotic syndrome. Moreover, individuals with an idiopathic DVT or PE have a risk of recurrence approaching 30% over the next 8 to 10 years. Thus, identification of high-risk patients with genetic predisposition to thrombosis is an important clinical goal, particularly among individuals with recurrent DVT. The most common genetic cause is the FVL mutation $F5$ R506Q, which is associated with vascular complications in pregnancy. The mutation is common, with a carrier frequency of 5% in European populations (range 2% to 11%), but this varies greatly across ethnic and racial groups. A high proportion of patients with DVT are FVL carriers (16% to 20%). The prothrombin $F2$ G20210A variant is the second most prevalent prothrombotic mutation but is not associated with significant recurrent DVT or with incident myocardial infarction. However, double heterozygotes for $F5$ R506Q and $F2$ G20210A are at increased risk for DVT, with an OR for recurrence of 5.9 (2.65, 13.20). These data raise the possibility that screening for $F5$ R506Q (alone or in conjunction with $F2$ G20210A) would be of clinical benefit in patients with a DVT to offer carriers earlier or more aggressive treatment. Screening could also be performed in high-risk subgroups, for example in pregnant women or in relatives of $F5$ 506Q carriers who have had a DVT, in which case the identified carriers could then also be offered prophylactic anti-coagulant treatment. As with the other tests considered here, this requires not only consideration of the PPV and the FPR but also consideration of the cost of screening, including not only consumables for the actual tests themselves (estimated to be $\sim$50 to $600 depending on the exact strategy used (Table 1) but also the infrastructure costs, including nurse, consultant, and laboratory time and, most importantly, the cost of treatment monitoring, especially for warfarin. Consideration of the number of person-years of treatment needed to prevent one DVT must also be balanced by the possible morbidity and mortality associated with the increased risk of bleeding.

There presently does not appear to be sufficient advantage to warrant screening for identification and clinical management of $F5$ R506Q carriers. Although DNA screening for $F5$ R506Q has been shown to have a good PPV and low FPR in a theoretical simulation of the general population, it is not cost-effective for men or women with a first DVT, or in pregnant women, or in women with CHD being treated with HRT. Although it has been reported in a simulation study, based on risks in 60-year-old men, that screening and prolonged prophylaxis for $F5$ carriers is cost-effective, and particularly in combination with testing for $F2$ G20210A, these authors conceded that this strategy will not have an acceptable cost-to-benefit ratio in populations in which the prevalence of carriers (or double carriers) is low (ie, combined prevalence $<1.4\%$) or in which the risk of a major bleed is high (rate $>3.2\%$ per year), or in which the risk of recurrent DVT is low (rate $<9\%$ in the first 2 years). It is also relevant to note that the models used in the cost–benefit analysis are based on the assumption that carriers of $F5$ R506Q or the prothrombin mutation, $F2$ G20210A, will benefit from more intense or more prolonged anticoagulation therapy, compared with non-carriers, for the prevention of recurrent venous thromboembolism. However, in three recent randomized, double-blind, placebo-controlled trials, PREVENT (low-intensity warfarin), ELATE (warfarin), and EXULT (ximelagatran) the efficacy of anticoagulation preventing recurrent thromboembolic events was virtually iden-

Figure 1. ROC curves for NPHS men using PROCAM risk score plus APOE genotype. Based on 2451 (of 3012 eligible men) who had complete data for PROCAM and APOE genotyping. Number of CHD events=199 (defined as in21). APOE genotype was fitted as a class variable with 3 categories 33, 22/23, and 34/44. Factors included age, BMI, total cholesterol, triglycerides, systolic blood pressure, and family history. Other factors in PROCAM were not measured in all subjects. For the PROCAM score, the ROC value was 0.65 (0.61 to 0.70), with a detection rate of 11.7% for a FPR of 5.0%. In univariate analysis, genotype CHD risk for APOE was $P=0.01$. The area under the curve increased to 0.67 (0.63 to 0.71) (detection rate=14.0%), but this improvement was not significant ($P=0.11$).

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TABLE 1. Summary of Published Observational and Simulated Cost–Benefit Studies for Screening for F5 506Q Without and With F2 G20210A

<table>
<thead>
<tr>
<th>Group Studied</th>
<th>Type (test)</th>
<th>Testing Strategy</th>
<th>Assumptions</th>
<th>Results and Costs/Carrier Detected</th>
<th>Author Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Men with a first DVT</td>
<td>S* (F5)</td>
<td>Standard anticoagulation therapy for 3 mo vs extend to 1–5 y for F5 carriers</td>
<td>In non-carriers, most VTE occurs in first 2 y ~8% y; in F5, 2 y cumulative rate ~25%; rate of fatal or major bleed ~4.8% y</td>
<td>In non-carriers, by 3 y prevent ~75 VTE but cause ~90 bleeding events; for F5, predict ~300 VTE and cause 80 bleeds</td>
<td>Benefits of prolonged anticoagulation in F5 carriers after 2 y overwhelmed by risk; screening in patients at higher than average risk may be beneficial</td>
<td>40</td>
</tr>
<tr>
<td>Male 60-year-olds</td>
<td>S* (F5)</td>
<td>6 mo standard anticoagulation vs F5 screening plus 2 y therapy for carriers</td>
<td>Based on meta analysis, odds ratio of recurrent DVT in F5 carriers 1.36 vs non-carriers; cost of test $60</td>
<td>Screening — 2 additional QADL/patient @$12 653/QAYL saved</td>
<td>Screening is cost-effective in those with idiopathic DVT and compliant to therapy, but not in those with fatal bleeding expected at rate ~0.34%/y or recurrent DVT rate ~9% in first 2 y</td>
<td>43</td>
</tr>
<tr>
<td>Male 60 year olds†‡§</td>
<td>S* (F5F2)</td>
<td>6 mo standard anticoagulation vs F5F2 screening plus 2 y therapy for carriers</td>
<td>Based on meta-analysis, odds ratio of recurrent DVT in F5F2 carriers 5.9 vs non-carriers; cost of combined test $60</td>
<td>Screening of 1000 VTE patients plus 2 y therapy prevented 6.1 VTEs but induced 0.9 major bleeds; screening — 1 additional QADL/patient @$13 624/QAYL saved</td>
<td>F5+F2 screening is cost-effective in most patients with VTE, because it targets rarer patients at higher risk; not cost-effective when combined prevalence ~1.4% or bleed rate &gt;3.2%/y or recurrent DVT rate ~9% in first 2 y</td>
<td>38</td>
</tr>
<tr>
<td>35-year-old women with VTE</td>
<td>S* (F5)</td>
<td>*No test + 6 mo treat,t testing + 3 y treatment OR 3, testing = life-long treatment for F5</td>
<td>Cost-effective ratio dependant on rate of VTE recurrence, prevalence F5, patient age, and efficacy of anticoagulant therapy</td>
<td>Total cost:$10 393, †$9676, ‡$13 179, which → 0.15 y GA life expectancy</td>
<td>Testing followed by life-long anticoagulation unlikely to be cost-effective in populations with low F5 prevalence/those with low risk for recurrent VTE or for those with high risk of bleeding</td>
<td>41</td>
</tr>
<tr>
<td>Pregnant women N = 937</td>
<td>O (F5)</td>
<td>Screening all women vs only those with high risk (personal or family history of VTE)</td>
<td>Prophylaxis — 50% reduction in vascular complications; APC costs £8; PCR for F5 costs £25</td>
<td>Incremental costs per event prevented = £13 281 universal, £7535 high risk</td>
<td>Universal screening not cost-effective; personal/family history VTE not specific enough; focus on other risk factors (eg smoking) may refine</td>
<td>29</td>
</tr>
<tr>
<td>Women with CHD on HRT (HERS and ERS) (n=3072)</td>
<td>O (F5)</td>
<td>Randomized to HRT or placebo overall VTE ~2%, F5 prevalence 6.4%, HRT increased VTE risk</td>
<td>Rate VTE/1000 y: no F5, placebo ~2.0%, no F5, placebo ~5.8%, F5, HRT = 15.4%, costs of F5 test: $150–$600</td>
<td>1 VTE prevented during 5 y by withholding HRT from the 24 F5 among 376 screened; $50,000 needed for screening to prevent 1 DVT hospitalization</td>
<td>Not cost-effective; this may become cost-effective if screening costs decrease</td>
<td>42</td>
</tr>
</tbody>
</table>

QADL indicates quality-adjusted days of life; QAYL, quality-adjusted years of life.

*Type of study: O, observational; S, simulation.
†Using Markov chain methods.
‡F5 factor V R506Q; F2, prothrombin G20210A.
§Note that calculation did not include loss of life expectancy for fetus if VTE causes miscarriage.

Decision models available from authors for institutional or personal use.

Gene–Environment Interaction and Risk Prediction

Because it is now well-accepted that atherosclerosis and CVD develop as a result of the interplay between the environment adopted by an individual and their genetic predisposition, any genetic test to predict CVD must include such interactions in the algorithm. This interaction has also been termed the “context dependency” of any genetic effect on a risk trait or on CVD risk itself. From a mechanistic viewpoint such interaction suggests that at the molecular level, the effect or byproduct of the environmental insult modifies the molecular function gene product under observation. Genetic predisposition will also be confounded by gene–gene interactions, gender, ethnicity, and pre-existing conditions such as obesity, diabetes, and hypertension as well as factors such as medication, diet, alcohol consumption, exercise, and stress. Taking into account such genotypic modification of environmental factors for CVD risk represents the second hurdle to successfully devising and implementing any genetic test.

Smoking is a major environmental risk factor influencing CVD and there are several examples in which the risk associated with smoking is modified by an individual’s genotype (reviewed in[8]). Smoking is associated with a 2-fold lifetime risk of CVD,[50] but anecdotally disease develops in some smokers earlier whereas some smokers appear relatively protected. Several studies have reported[51,52] that
APOE carriers who were smokers had a particularly high CVD risk compared with APOEe4 never-smokers, while risk was also low in APOEe4 former smokers, supporting the benefit of smoking cessation. A re-analysis of a recent large case-control study showed that compared with APOEe3 smokers, APOEe4 smokers had significantly higher risk of CHD, with a greater than additive interaction between genotype and smoking on risk (relative excess risk of interaction (RERI) of 1.62 (95% CI: 0.4, 2.97). Analysis of the Framingham Offspring data also supports this greater than additive interaction between APOEe4 and smoking on risk (Talmud PJ and Ordovas J unpublished, 2003). These data suggest that carrying the APOEe4 allele does not significantly increase risk of CVD unless the subject is a smoker. Such results indicate that any APOE genetic test result estimating risk in the absence of information about smoking could be misleading.

Another example of the context dependency of a genetic effect on risk of MI is the way in which the beneficial effect of moderate alcohol consumption is modified by the alcohol dehydrogenase 3 (ADH3) γ1/γ2 genotype. While the ADH3 γ1 allele leads to rapid oxidation of ethanol, the γ2 allele results in slow ethanol oxidation.55 Hines et al66 showed that in an all-male nested case-control study (and confirmed in other studies), moderate alcohol consumption of 1 drink per day was associated, as expected, with a decreased risk of MI, which in γ1γ1 men was 0.62 (0.34, 1.13), whereas in γ2γ2 men the decrease was significantly greater, 0.14 (0.04, 0.45). Thus, without taking both genotype and environment into account, an accurate RR would not be obtained. However, in part, the risk reduction was explained by a larger increase in HDL levels in γ2γ2 men (P=0.01 for interaction). The extent to which this genotype effect could be simply estimated by measures of habitual HDL levels is unclear.

Additional Points Relevant to Genetic Tests

There are several more problems that need to be addressed before a genotype-based test is likely to be useful for clinical management.

Size of Risk Effect and Replicability

The use of risk algorithms to estimate an individual's risk is based on epidemiological and clinical (and in some cases interventional) studies that include hundreds of thousands of subjects followed-up for events in a multitude of studies from different laboratories and different countries, for a total of possibly millions of person-years. From these data, the size of the effect associated with a BMI >25kg/m² or a 1 standard deviation increase in plasma cholesterol or SBP or a 1 standard deviation lower HDL, can be estimated with accuracy, and the confidence interval of the estimate will be small.

For the impact of the CVD candidate gene variants identified to date, the situation is markedly different. Because for any gene variant the effect on risk is likely to be relatively small, it requires very large studies to estimate risk with reasonable accuracy and confidence limits. Currently, these genotypes have only been determined in relatively few studies, and the confidence intervals for these risk estimates are still unacceptably large. Thus, basing clinical management on such soft risk estimates is unwise at the least and may be completely inaccurate at worst. The field of CVD genetic research has many examples of an interesting genetic risk association, which has proved unreplicable in later larger studies and, clearly, an honest appraisal of risk is required if genetic tests are to be recognized as valid and to be appropriately used in clinical management.

Genetic data are easily amenable to “meta-analysis,” such as have been used to obtain risk estimates for non-genetic CVD risk factors.57–59 Several such genotype meta-analyses have been published recently and are summarized in Table 2. The problems with these analyses are that they are usually confined to published data, and because studies that fail to find a risk association may be difficult to publish (even if appropriately powered), these meta-analysis estimates may be on the high side of the true effect. Case-control studies also often give higher estimates than prospective studies, which will bias estimates upwards. It is also difficult to adjust appropriately for classical risk factors in such analyses if the full data set is unavailable, so these risk estimates, although statistically significant, may still not be over and above other factors or may not have been able to examine potential environment interactions. There is also the additional difficulty of using multivariate analysis to predict risk from several biological markers, each of small effect, and this represents a limitation of ever using multiple markers of small effect. However, attempts have been made to use approaches other than multiple linear regression, for example neural networking.60

### TABLE 2. Summary Odds Ratios for CHD Risk for CHD Risk-Associated Genes Derived From Meta-Analyses

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant (risk genotype)</th>
<th>Functional Activity Demonstrated (yes/no)</th>
<th>End Point</th>
<th>Risk Estimate (95% CI)</th>
<th>Studies/total cases/controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE</td>
<td>e2, e3, e4 (e4)</td>
<td>Y</td>
<td>CHD</td>
<td>1.44 (1.27–1.62)</td>
<td>9 (2383/3972)</td>
<td>77</td>
</tr>
<tr>
<td>APOB</td>
<td>E415K (K +)</td>
<td>N</td>
<td>MI</td>
<td>1.32 (1.14, 1.54)</td>
<td>15 (2348/2556)</td>
<td>78</td>
</tr>
<tr>
<td>PAI-1</td>
<td>−675 4G/5G (4G4G)</td>
<td>Y</td>
<td>MI</td>
<td>1.15 (1.08, 1.24)</td>
<td>22 (9264/8431)</td>
<td>79, 80</td>
</tr>
<tr>
<td>ACE</td>
<td>Insertion/deletion in intron 16 (DD)</td>
<td>N</td>
<td>MI</td>
<td>1.30 (1.07–1.58)</td>
<td>9 (1521/2120)</td>
<td>79, 80</td>
</tr>
<tr>
<td>MTHFR</td>
<td>A122V (V)</td>
<td>Y</td>
<td>MI</td>
<td>1.2† (1.06, 1.39)</td>
<td>48 (2173/11 854)</td>
<td>82</td>
</tr>
<tr>
<td>ENOS</td>
<td>E289D (DD)</td>
<td>Y</td>
<td>MI</td>
<td>1.31 (1.13, 1.51)</td>
<td>26 (9687/13 161)</td>
<td>83</td>
</tr>
</tbody>
</table>

*This value is well below the initial estimate of more than 3-fold (in some subgroups), which caused such excitement in the field, but well in-line with the estimate of 1.13 from the largest study published to date.72
†Whether this is over and above other classical risk factors (and, in particular, over and above measures of plasma homocysteine) remains to be established.
Extrapolation of Risk Estimates

Currently, risk data for most candidate gene variants likely to be included in gene tests are not available for women or racial/ethnic groups other than whites. This is relevant because the effect of a given variant on trait levels is often influenced by gender. Data are frequently not available for subjects of African, Indian subcontinent, or Asian origin, in whom a different spectrum of risk factors and predisposition may occur. In addition, and potentially more seriously from the point of accurate risk prediction, the size of the impact is often unknown in low-risk compared with high-risk groups, such as those with diabetes or hypertension. Thus, extrapolation to CVD risk prediction used in some genetic test kits would be unwarranted and potentially completely erroneous when applied to populations not studied for that parameter.

Functional Variants or Genetic Markers

While a functional variant (i.e., that alters an amino acid or a transcription factor-binding element in a promoter) is likely to be similarly associated with CVD risk in different ethnic groups, although its allele frequency may vary across groups and their population impact and usefulness in risk prediction will differ. However, many of the variants proposed for CVD tests are not functional, but rather act as genetic markers for functional variants yet to be discovered elsewhere in the gene or even possibly in a nearby gene. Examples of such markers include the insertion allele in intron 16 of the ACE, the SstI site in the 3’ untranslated region of the APOC3, and the CETF intron 1 TaqI variant. All three of these variants have consistently been reported to be associated with increased levels of intermediate plasma risk traits and also with increased CVD risk, but their usefulness in risk prediction is entirely dependent on whether the risk marker is acting as a surrogate for the functional variant in the individual being tested. For all these markers, the degree of linkage disequilibrium (LD) with the actual functional change is likely to be considerably less than 100%. If the association between the two alleles is even as high as 70%, then the marker allele will not be found with the functional allele in 30% of individuals, and the test will therefore be unacceptably inaccurate. While such markers are useful for genetic research, their use to predict an individual’s risk for CVD would be inadvisable. A further complication is that the degree of LD at studied gene loci has been shown to be very different in populations selected from different ethnic groups. Thus, a non-functional polymorphic marker might have a different impact depending on the LD with the functional change, such as FIBB promoter variants. The consequence of this is that such markers will be essentially of no value in risk prediction in individuals of other than white European origin. In individuals of mixed ethnicity, such markers are likely to be particularly misleading. Before inclusion in a genetic test, every variant used should have good in vitro data supporting its functionality in appropriate assays.

Recommendations for Genetic Testing

We would like to propose, as a starting point for discussion in the field, that the minimum criteria for a genetic variant to be included in CVD clinical risk management would be a meta-analysis based on data from a minimum of three different independent studies with at least a total of 1000 cases. This is based on considerations of the frequency of the alleles being examined and the size of the effect on risk. For a relatively common variant (rare allele frequency 0.3 or above), this would have the power to detect a relative risk of 1.25 or more in carriers (at P<0.05 and 80% power). For lower allele frequencies, the total number of cases should be increased to maintain power, for instance a variant with an allele frequency of 0.1 would require 2000 cases. We suggest this level of risk based on the published meta-analysis estimates, which are in the range of 1.20 to 1.70 (Table 2). We believe that it is important that the studies should come from independent laboratories working with samples from different countries to overcome concerns about hidden population stratification. For each study, data must also be available to adjust for at least age, measures of obesity, blood pressure, and smoking habit, as well as plasma cholesterol and HDL-C, because these are the factors used in current risk algorithms, and the genotype must then give predictive value over and above these established risk factors, as assessed by statistically significantly higher ROC values. Finally, this combined data set should show no significant evidence for heterogeneity of risk effect, and the final meta-analysis risk estimate can then be used in genetic test algorithms. As discussed, for acceptable robustness, for each selected gene locus only functional variants (demonstrated in vitro) should be included, and this rules out the ACE and the APOB variants shown in Table 2. When there are several functional variants, a complete test will require haplotype determination for each locus. This is more expensive but technically not difficult, and robust multiplex PCR assays could easily be developed to include multiple sites. We believe that regulatory bodies will be required to oversee the accuracy and validity of such tests, and these could be created by genetic and drug regulatory agencies with help from genetic and epidemiological experts.

Genetic Tests: Future Developments

There are several areas of development that will need to be examined for any future tests to achieve their full potential. Further research using adequately powered studies is urgently required to address each of these issues.

Gene–Environment Interaction

Because CVD is the result of a genetic predisposition in an individual adopting a high-risk environment, if such a combination could be identified, then this might be helpful in clinical management. It might help to motivate the individual to reduce the environmental risk, for example by smoking cessation or weight loss, or by indicating early and more aggressive therapy, such as lipid-lowering or blood pressure-lowering. Before use in any genetic test result, such gene–environment interactions need to meet the criteria of reproducibility proposed, and to date none does, including that for APOe4-smoking.
Gene–Gene Interaction
At the present time, we have little information as to whether the impact of genetic variants on CVD risk are simply the sum of their individual effects, as is the case with most of the classical risk factors, or whether more complex interactive effects are the rule. If an individual has inherited a risk allele at locus 1 and a protective allele at locus 2, then is the net effect risk neutral? Some potentially useful examples of gene–gene interaction effects on risk have already been reported,69,70 and even if such interactive genotype combinations are present in a relatively small proportion of the general population (say 5%), if the CVD risk is large enough to be of clinical relevance (say 2- to 3-fold), testing for such genotype combinations would be useful.

Social and Psychological Impact of Genetic Tests
Although there has been considerable debate about the pros and cons of genetic tests for susceptibility, there is still concern in the general public, and among practitioners, about confidentiality and the unknown future of genetic information, which will not be discussed here because of limited space. These issues are likely to be of particular concern in those countries where health insurance is not universal, and where knowledge of genetic status could conceivably be used to deny coverage by an insurance company. In Europe, there is currently a moratorium on companies using genetic tests or requiring results to be revealed for life-insurance policies below a certain threshold. Although this moratorium is likely to be extended, the widespread availability of CVD or cancer genetic testing may cause commercial pressure for this to change. There are also unresolved concerns about the psychological impact of being given genetic information, because it appears that, in general, “having a gene for heart disease” is viewed fatalistically by a sizeable minority in a recent study.71 A gene test is perceived as being more predictive of future risk than phenotype information, such as high cholesterol,72 even though of course the converse is true. Receiving genetic risk information may therefore have a more detrimental effect than classical risk factor information on the psychological well being of an individual, with the possibility of being viewed by family members and friends, or especially employers, as a “patient.”

Even though some of these concerns could (and should) be addressed in the information provided before and after a genetic test, there is still the potential concern that gene variant data given for CVD risk may subsequently be found to be associated with risk for other diseases that may not be so amenable to treatment. For example, the APOEε4 allele, reported to have a modest risk on CVD, is now known to be associated with earlier onset of Alzheimer disease (AD).73

Conclusion
Although genetic testing would be appropriate in families in which monogenic CVD disorders occur, none of the testing kits currently proposed include such disorders. At present, we believe that predictive genetic testing for CVD is not ready for clinical use. When genetic tests are performed, they should be accompanied by an adequate level of counseling and an infrastructure to deal with frequently asked questions.

This would require the adequate education of health care workers with ongoing provision of resources for dedicated counselors, so that the implications of the tests can be adequately explained. We believe that these tests have been prematurely made available and have the potential to raise unnecessary anxiety and/or provide false reassurance.74,75 If, however, particular environmental subgroups, such as smokers, hypertensives, or obese individuals, could be identified, then when a genotype (or more likely multiple site haplotype or multi-locus genotype) is associated with a high risk, such tests may be useful in clinical management. For this to become reality, further basic research and extensive epidemiological studies are required.76–83

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References


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