Genetic Cardiovascular Risk Prediction: Will We Get There?

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Circulation 2010;122;2323-2334
DOI: 10.1161/CIRCULATIONAHA.109.909309
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

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Major advances in genetics, including the sequencing of the human genome in 2001[1,2] and the publication of the HapMap in 2005,[3] have paved the way for a revolution in our understanding of the genetics of complex diseases, including cardiovascular disease (CVD). After years of inconsistent results and failure to replicate putative candidate gene associations, high-throughput technologies (which genotype more than 500 000 genetic markers known as single-nucleotide polymorphisms [SNPs]) and novel statistical tools have led to a virtual explosion of novel genetic markers associated with complex human diseases. In the context of CVD, these advances have been remarkably successful in uncovering many novel genetic associations with myocardial infarction (MI) and cardiovascular risk factors such as lipids, blood pressure, diabetes, and obesity. A major objective of these studies has always been to provide new insights into the biology of CVD. However, a highly touted additional aim of these discoveries has been to use these genetic markers to usher in a new era of personalized medicine by incorporating genetic information into risk prediction (including for the primary prevention of CVD). In fact, direct-to-consumer testing of recently discovered genetic markers has proliferated despite a lack of evidence for clinical use.[4]

As with all nascent technologies, many fundamental questions remain to be answered: Can genetic markers or gene scores improve CVD risk prediction over and above validated risk algorithms such as the Framingham risk score and a family history of CVD? How many SNPs are responsible for the genetic component of CVD, and how many genetic markers will we need to discover to reliably improve risk prediction? What are the implications of the allelic architecture of CVD and other complex diseases for risk prediction? And, finally, what steps will be needed before this information is brought to patients? In the present review, we will examine each of these questions with regard to risk prediction of coronary artery disease (CAD) and MI in a primary prevention setting.

Cardiovascular Risk Prediction: Is There a Need to Improve Currently Used Algorithms?

For >5 decades, the major cardiovascular risk factors, namely, male sex, hypertension, cholesterol, smoking, and diabetes mellitus, have been well known.[5] On the basis of these factors, a number of risk prediction algorithm scores have been developed, including the Framingham risk score, that provide an estimate of the 10-year risk (and recently, the 30-year risk) of CVD.[6–9] Generally speaking, the metrics used to assess risk scores include an assessment of their performance for risk discrimination, calibration, risk reclassification, and clinical utility (change in management and patient outcomes associated with their implementation; reviewed by Cook and Ridker[10]). Discrimination is summarized by the C statistic, which represents the area under the receiver operating characteristic curve (plotting "sensitivity" in relation to "1−specificity"). The numeric value of the C statistic represents the probability of correctly ranking 2 randomly selected individuals (one likely to develop disease and one not so predisposed) on the basis of their predicted risk from the prediction model under evaluation. Calibration compares the predicted risk with the observed risk in groups of individuals classified by risk level and provides a measure of the overall accuracy of the risk estimates derived by the model. Reclassification, a newer metric, estimates the improvement (or lack thereof) in risk classification of individuals with a novel marker compared with a standard model without the marker of interest. Most currently used risk scores have been validated in many populations and have been shown to have good discriminatory capacity and calibration; however, the risk reclassification and clinical utility of many of these scores have been less well studied and remain an area of active investigation.

One common reason offered for pursuing newer risk factors/markers is that current risk scores explain a modest proportion of CVD incidence in the community. Indeed, a common misconception is that only 50% of the incidence of CVD is explained by the traditional risk factors, and therefore, novel markers of preclinical disease are needed to refine contemporary risk prediction algorithms.[11] In fact, the major risk factors explain a large proportion of the risk of CVD.[12] However, it is estimated that nearly 15% to 20% of MI patients have none of the traditional risk factors and would be considered "low risk" by current risk prediction scores.[13]

Although the importance of traditional risk factors and the utility of current risk prediction algorithms cannot be ignored, efforts to improve risk prediction are needed given that CVD is preventable, the first manifestation may be sudden cardiac

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(*Circulation*. 2010;122:2323-2334.)

Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.109.909309

Table 1. Loci Associated With MI From GWAS

Locus	Nearest Gene	Risk Allele Frequency	*P*	Relative Risk for MI	References
3q22.3	*MRAS*	0.15	7×10^{-13}	1.15 (1.11–1.19)	27
12q24.31	*HNF1A*	0.36	5×10^{-7}	1.08 (1.05–1.11)	27
9p21.3	*CDKN2A, CDKN2B*	0.56	3×10^{-44}	1.29 (1.25–1.34)	22, 23, 26, 28, 29
1p13.3	*CELSR2, PSRC1, SORT1*	0.81	8×10^{-12}	1.19 (1.13–1.26)	28, 29
21q22.11	*SLC5A3, MRPS6, KCNE2*	0.13	6×10^{-11}	1.20 (1.14–1.27)	28
1q41	*MIA3*	0.72	1×10^{-9}	1.14 (1.10–1.19)	28, 29
6p24.1	*PHACTR1*	0.65	1×10^{-9}	1.12 (1.08–1.17)	28
19p13.2	*LDLR*	0.75	2×10^{-9}	1.15 (1.10–1.20)	28
10q11.21	*CXCL12*	0.84	7×10^{-9}	1.17 (1.11–1.24)	28, 29
1p32.3	*PCSK9*	0.81	1×10^{-8}	1.15 (1.10–1.21)	28
2q33.1	*WDR12*	0.14	1×10^{-8}	1.17 (1.11–1.23)	28
6q25.3	*SLC22A3,LPAL2,LPA*	0.02	4×10^{-15}	1.82 (1.57–2.12)	30
6q25.3	*SLC22A3,LPAL2,LPA*	0.16	1×10^{-9}	1.20 (1.13–1.28)	30
12q24.12	*SH2B3*	0.38	9×10^{-8}	1.13 (1.08–1.18)	31

Loci were selected from the GWAS catalog on the basis of a search for the following phenotypes: Coronary disease, CAD, major CVD, MI, and MI (early onset). We also included the 12q24.12 locus, which has been associated with eosinophil levels, asthma, and MI. Reported associations were limited to those with $P<5\times10^{-8}$ and replication in at least 1 independent cohort.

death, the occurrence of an MI is associated with a high early mortality, and survivors may suffer considerable morbidity and a reduction in the quality of their lives. This had led to an intense search for novel biomarkers that can enhance the currently available risk scores. However, the majority of studies that have claimed to identify novel biomarkers that enhance risk prediction beyond the Framingham risk score have been noted to have flaws in their design, analysis, or interpretation.[14] Recently, an expert group has proposed criteria for appropriate assessment of the clinical utility of novel biomarkers for the purpose of enhancing CVD risk prediction.[15]

One category of biomarkers that has evoked extensive study recently is that of genetic variants. The case for risk prediction with genetic polymorphisms must be held to the same standards as used for other biomarkers. Incremental improvement by the addition of a genetic biomarker must be shown over and above well-validated risk scores by use of standard metrics to evaluate their clinical performance. Therefore, useful genetic markers for risk prediction will need to be sufficiently uncorrelated with known CVD risk factors to provide independent information on risk. It can also be argued that any genetic marker should also provide incremental risk information over and above a model that incorporates family history,[8,16,17] given that such information is often readily available.

The interest in incorporating genetic information into risk prediction algorithms stems from the fact that many MI patients have a family history of CVD, which confers a near doubling of the risk of MI among family members, even after adjustment for traditional cardiovascular risk factors.[18,19] Moreover, the heritability of MI, which provides an estimate of the genetic variance in MI risk, has been estimated at 40% to 60%.[20,21] These findings suggest that genetic factors play an important role in CVD and could potentially refine risk prediction for CVD. Indeed, recent genetic studies have confirmed that several genetic factors are associated with MI and CAD, which has led to much excitement around the possibility of their use for risk prediction.

Genome-Wide Association Studies: Initial Successes in Gene Discovery

To date, genome-wide association studies (GWAS) have been remarkably successful in uncovering many novel genetic loci not previously implicated in CVD. Among the first major discoveries for CVD were the simultaneous reports of the association of variants at the 9p21 locus with MI.[22,23] This association represents one of the most consistent and robust SNP-disease associations in the GWAS era, having been replicated in several independent samples in numerous ethnicities. A recent large meta-analysis reported an allele relative risk of 1.27 (95% confidence interval 1.23 to 1.31, $P=1.62\times10^{-12}$) for CAD.[24] Despite the strength and consistency of the associations of 9p21 variants with CVD traits, little is known about the biological role of this locus and how it may confer increased CVD risk. This SNP is located in a gene desert, with the nearest gene, *ANRIL*, being several thousand kilobases away. Despite significant effort, the function of *ANRIL*, a noncoding RNA, has not yet been elucidated but continues to be an area of active investigation. In a recent study, the mouse ortholog of the 9p21 locus (CDKN2A/2B) associated with CAD was successfully knocked out. These mice had reduced survival, a more rapid increase in body weight, and a hyperproliferative smooth muscle cell phenotype that suggested altered vascular cell dynamics.[25] If these findings are confirmed, this would represent a novel mechanism for MI that is unrelated to conventional risk factors.

Several additional GWAS for MI have also been completed that identify a number of novel loci (Table 1).[26–30] In addition, GWAS for blood pressure[32–34] and lipid traits[35–37] have also discovered additional loci that are associated with these traits. To date, more than 100 new genetic variants have

Table 2. Studies Using Genetic Information in CVD Risk Prediction

Authors	Markers Used	Source of Genetic Markers	Outcome	Covariates: Conventional Risk Factors	Covariates: Family History	Metric to Assess Incremental Utility	Evidence for Clinical Utility	Comments
Paynter et al[42]	Multiple	GWAS	Incident CVD	Yes	Yes	AUC, NRI	No	Women only
Paynter et al[43]	Single (9p21)	GWAS	Incident CVD	Yes	Yes	AUC, NRI	No	Women only
Brautbar et al[44]	Single (9p21)	GWAS	Incident CVD	Yes	No	AUC, NRI	Minimal	. . .
Kathiresan et al[45]	Multiple	GWAS	Incident CVD	Yes	Yes	AUC, NRI	Minimal	Lipid SNPs only
Kathiresan et al[28]	Multiple	GWAS	Prevalent CVD	No	No	None	No	. . .
Talmud et al[46]	Single (9p21)	GWAS	Incident CVD	Yes	No	AUC	Minimal	Diabetes not in model
Morrison et al[47]	Multiple	Candidate genes	Incident CVD	Yes	No	AUC	Minimal (in blacks only)	. . .
Humphries et al[48]	Multiple	Candidate genes	Incident CVD	Yes	No	AUC	Minimal	G×E interactions considered; diabetes not in model
Junyent et al[49]	Multiple	Candidate genes and GWAS	Prevalent CHD	Yes	No	OR	No	Gene score associated with CHD case status
Trichopoulou et al[50]	Multiple	Candidate genes	Incident CHD	Yes	No	OR	No	Gene score associated with CHD case status; G×E interactions considered
Yamada et al[51]	Multiple	Candidate genes	Prevalent CVD	Yes	No	OR	No	Gene score associated with CHD case status

AUC indicates area under the curve; NRI, net reclassification index; CHD, coronary heart disease; G×E, gene-environment interactions; and OR, odds ratio.

Studies were identified by use of a PubMed search that used the following key words: Cardiovascular disease, coronary disease, genetics, risk prediction. We also reviewed the references of relevant articles to find additional articles of interest. From these studies, we included only those studies that evaluated the utility (or made positive or negative claims regarding utility) of genetic information in cardiovascular risk prediction.

been discovered with GWAS that relate to MI or MI risk factors[38] (13 SNPs have been replicated for MI; 26 for high-density lipoprotein levels, 16 for low-density lipoprotein levels, and 26 for triglyceride levels; 42 for diabetes and fasting glucose; 10 for hypertension; 6 for C-reactive protein levels; and 16 for body mass index). These SNPs have been rigorously replicated in 1 or multiple additional independent studies that confirmed that they represent genuine associations with CAD. After years of inconsistent results,[39,40] these studies have provided an early glimpse into the underlying genetic risk of CAD. However, these initial studies represent the first steps toward understanding the complete allelic architecture of CAD, and it is likely that many more genetic variants remain to be discovered.[41] Despite our limited understanding of genetic risk for CAD, a number of studies have attempted to incorporate these newly discovered genetic risk variants into CVD risk prediction tools, with limited initial success (as reviewed below; Table 2).

Use of Genetic Information for Cardiovascular Risk Prediction: Overview of Initial Experience With Single Genetic Variants and Genetic Risk Scores

Several studies have evaluated the predictive power of the addition of single SNPs and combinations of risk SNPs to genetic risk scores for MI risk based on pre- and post-GWAS results (Table 2). Additions of single SNPs at 9p21 to the Framingham risk score have not been found to consistently improve risk prediction.[43,46] A genetic risk score that incorporates 9 CAD-associated SNPs resulted in a >2-fold higher odds ratio for MI in subjects in the highest quintile of the risk score than in those in the lowest quintile but did not evaluate the incremental value of the addition of such a score to traditional risk factors.[28] In a separate report, a genetic risk score that used SNPs that were strongly associated with lipid levels conferred a 15% increase in CAD risk per lipid-associated SNP allele.[45] Despite the increased CAD risk per allele, the genetic score did not improve discriminative ability over and above traditional risk factors and showed only modest improvement in risk reclassification. The limited success of these initial studies has led to the development of more elaborate genetic risk scores comprising many SNPs that encompass both MI risk alleles and SNPs associated with other cardiovascular risk factors in an effort to increase the genetic risk explained and to improve the predictive performance of genetic risk scores. A genetic risk score comprising 101 validated SNPs from large-scale GWAS of MI and other cardiovascular risk factors was evaluated for cardiovascular risk prediction in more than 18 000 women. After adjustment for traditional risk factors, the genetic risk score was not associated with CVD events, and the addition of the genetic risk score to a standard risk prediction model did not significantly improve discrimination or reclassification.

Despite the incorporation of multiple CAD-associated SNPs, genetic risk scores to date have explained less than 5%

of the interindividual variance in risk[52] and have not led to clinically meaningful improvements in risk prediction. However, the modest improvements in risk reclassification seen in some of these studies[44,45] highlight the future potential for the use of genetic markers for risk prediction as additional genetic variants are discovered.

Although the results of these initial studies of genetic risk prediction have been underwhelming, several important insights into the future of cardiovascular genetic risk scores can be gleaned from these studies. First, the incremental predictive utility of genetic risk scores that explain a small fraction of the heritability will likely be marginal. To significantly improve risk prediction, genetic risk assessment will need to be markedly refined. It has been suggested that approximately 20% of the heritability needs to be explained to provide similar discrimination as obtained from standard risk prediction models.[53] Second, the addition of predictors with relative risks <10 will have a limited effect on risk discrimination,[54] although their contribution to risk reclassification warrants further study. Therefore, it is not surprising that the addition of recently discovered genetic markers for MI, with relative risks ranging from 1.1 to 1.3, has led to limited improvements in risk prediction. For useful risk prediction, genetic risk scores with many additional markers will likely be needed to improve contemporary CVD risk stratification algorithms. It must also be emphasized that relative risks across extreme comparisons (ie, top quintile of genetic risk to bottom quintile of genetic risk), which are often reported for genetic risk scores, are not relevant for risk prediction. For translation to risk prediction, the reported risks should be compared with "average" risks found in the general population in which risk prediction will likely be used in a primary prevention setting.

"Effect" Estimates From Initial Discovery GWAS: Caveats and Implications for Risk Prediction

An important additional consideration that explains the limited success of genetic risk prediction is that effect estimates for associations from discovery GWAS may be biased and of limited utility in risk prediction. First, genetic effect estimates from GWAS are likely inflated owing to the "winner's curse," whereby early reports of relatively large effect sizes become attenuated with further replication in studies of increasing sample sizes.[55,56] Second, estimates from meta-analysis in the genetic literature frequently assume "fixed effects" despite important between-study heterogeneity. It has been demonstrated that some markers become statistically nonsignificant at a genome-wide level when "random-effects" models are used and therefore may have poor generalizability across populations.[52,57] Third, several GWAS have used extreme subjects to identify genetic associations by sampling high-genetic-risk cases (ie, "hypercases" who are frequently younger with fewer risk factors and a positive family history) and low-genetic-risk control subjects (ie, "hypercontrols" who lack such factors), which further inflates effect estimates.[58] Moreover, the odds ratios generated from case-control GWAS to date (which have used prevalent cases and controls) likely overestimate the true risk ratio, because MI is not a "rare" disease, and control subjects have not been sampled by an appropriate sampling strategy (eg, incidence-density sampling) to provide odds ratio estimates that approximate risk ratios.[59,60] Lastly, most GWAS to date may also suffer from major potential survival biases, because enrollment into the study is conditioned on survival after MI. Given that 30% to 70% of MI patients die before admission to the hospital,[61–63] analyses of prevalent MI cases are poorly representative of most incident MIs.

Although these practices are acceptable for gene discovery, the relative risk estimates associated with putative genetic variants are unlikely to be applicable to the general population in which risk prediction is applied for a future time horizon. Accordingly, it is likely that such estimates will perform poorly in prospective assessments of CVD prediction in a primary care setting. These observations highlight an important point: If risk prediction is the objective, then GWAS of incident CVD are needed in large prospective cohort studies of representative populations to complement currently available studies. To date, and to the best of our knowledge, there have been no published GWAS for incident CVD that have used a prospective cohort design despite calls stressing the importance of such a study design in genetic epidemiology.[64]

How Many SNPs Do We Need for CVD Prediction? Theoretical Predictions With the C Statistic

Despite criticisms that the C statistic is insensitive to most biomarkers studied in the "-omics era,"[65] it still represents an important, yet perhaps overly conservative, starting point for evaluating the possibility of using genetic variants or risk scores in CVD risk assessment. A number of simulation studies have provided important information on the feasibility of genetic risk prediction in CVD using the C statistic as the metric for discrimination. We review these investigations, acknowledging that similar simulations are warranted that use additional newer metrics, such as risk reclassification.

For genetic studies, the C statistic is a function of the heritability, the genetic variance explained by the genetic variants, the prevalence of the disease condition, and the minor allele frequency in the population.[66,67] For CVD, it can be estimated that the upper bounds of the C statistic are $\approx$0.90 for populations with a 10% prevalence of disease and $\approx$0.85 for populations with a higher disease prevalence.[66,68] Although achieving the maximum C statistics would provide excellent discrimination, this would require identifying all the genetic variants associated with CVD. A more reasonable goal would be to achieve C statistics of $\approx$0.80 to 0.85, which would still represent an improvement over current risk prediction models. It has been estimated that to achieve this level of discrimination, $\approx$100 uncorrelated genetic variants (ie, in linkage equilibrium) with relative risks of $\approx$1.5 and minor allele frequencies of 10% that explain $\approx$20% of the heritability of CVD would be needed.[66] However, few CVD genetic variants were identified in the GWAS or pre-GWAS eras with relative risks in this range. If we assume that CVD genetic variants will have mean relative risks of 1.1 to 1.2, then even 100 genetic variants would only explain 1.0% to 9.1% of the variance of CVD and provide C statistics

≈0.75,[67] which would be similar to but not much better than current prediction models. To achieve a higher level of discrimination based on the genetic relative risks observed most frequently to date (ie, relative risk 1.1 to 1.25), it can be estimated that 150 to >400 genetic variants would be needed, depending on the frequency of these genetic variants (ie, >5% minor allele frequency).[66]

Although the large number of genetic variants required for a useful genetic risk score may seem daunting, it can be argued that these simulations may artificially inflate the estimated number of genetic variants needed for reliable CVD risk prediction because of reliance on the C statistic, which may be insensitive to the small effects of genetic variants. Using a method that relied on the accuracy of genetic risk prediction (ie, genetic variance explained by the genetic score) instead of the C statistic, it has been reported that many fewer genetic variants may be needed for useful CVD risk prediction.[69] Under the assumption that a reasonable number of genetic variants (ie, <1000) with weak effects explain the heritability of CVD, the authors estimated that a large case-control study with 20 000 subjects and a 1:1 case-to-control ratio could identify 80 to 120 genetic variants that explain >50% of the genetic variance of the disease.[69] They also demonstrated that this set of genetic variants would likely have good to excellent calibration in a validation data set and would have relative risks of ≈4.0 for the top 5% of subjects based on the genetic risk score compared with the average risk in the population. However, this inference is based on simulation modeling with an assumed risk of disease (prevalence of 5% to 10%, with a sibling relative risk of 1.45 to 2.90). In addition, published empirical studies have not reported on the proportion of variance explained as a potential metric for risk prediction, thereby underscoring the need for additional research. Although there is some debate about the optimal metric for assessing the utility of genetic markers in simulation studies (and the appropriate genetic models for these simulations), these studies provide valuable insight into the future feasibility of genetic risk prediction. Similar evaluations of novel metrics, such as the net reclassification index, have not been reported as yet and would provide important new information for the assessment of genetic risk prediction.

Although these simulations are apparently encouraging for genetic risk prediction, they depend on assumptions relative to the total number of genetic variants needed to explain the totality of genetic variation in CVD. If the heritability of CVD can be explained by 100 to 1000 SNPs, then risk prediction may be possible with a relatively small number of SNPs (≈100) in the near future. However, the total number of SNPs that explain the heritability of CVD is currently unknown. Recent studies examining the allelic architecture of CVD using simulated data have shed some light on this problem and have suggested that the genetic component of CVD (and other complex diseases) may be influenced by many more genetic variants (or other factors) than anticipated,[41,70] which could have profound implications for risk prediction.

GWAS and the Allelic Architecture of CVD: How Many SNPs Explain the Genetic Risk of CVD?

GWAS were designed on the basis of the theory that the genetic architecture of complex diseases would follow the "common disease–common variant" hypothesis that predicts that common diseases, such as CAD, are caused by many common genetic variants, each of which explains a small portion of the variance in the risk of disease.[71] Most detected genetic variants have allele frequencies >5% and have small to very small effect sizes (ie, relative risks of 1.1 to 1.3), and each explains <1% of the variance in risk of disease. Although theory posits low effect sizes for complex disease, the very weak effect sizes and the low variance explained by recently uncovered SNPs have been somewhat unexpected. These findings have profound implications for risk prediction, because the total number of genetic variants needed to explain the heritability of a disease is proportional to the proportion of variance explained by each genetic variant. It has been argued that if current GWAS have indeed detected the common SNPs that explain the highest fraction of the genetic variance, which may be quite likely, the remaining variants to be found will explain exponentially smaller proportions of the remaining genetic variance.[41] GWAS data for complex traits (such as diabetes and height) have been shown to follow this pattern quite convincingly.[41,72] Many prior simulation studies estimating the total number of SNPs required to explain heritability or the number of genetic variants required for accurate risk prediction have not considered these recent insights into the genetic architecture of CVD (and related traits) and have frequently oversimplified the models by assuming fixed genetic relative risks for each genetic variant that remains to be identified. Such simplifications would markedly underestimate the number of SNPs that explain the heritability of CVD.

Given that the strongest common SNP associated with CAD has a relative risk of 1.3 and other recently identified variants have relative risks of 1.1 to 1.2, the remaining undiscovered variants are expected to have small to very small effect sizes, and therefore, it is possible that hundreds to thousands of genetic variants are needed to explain the relatively high heritability of CVD. This may pose a challenge for risk prediction for a number of reasons. First, the demonstration of robust disease associations with thousands of genetic variants with weak effects would require studies with >100 000 individuals, which would be larger than even the largest genetic consortia currently in place.[73] Second, as the number of genetic variants implicated in CVD increases, the possible combinations of risk alleles become unmanageable, such that every individual would have a unique genetic signature, with little overlap between individuals, making genetic risk prediction very challenging if not nearly impossible. Higher-risk genetic profiles requiring hundreds of genetic variants would also be exceedingly rare.[74] Third, if thousands of SNPs are required to explain a substantial proportion of the variance of CVD, it has been argued that most individuals would have many of these "risk variants," which could seriously hamper their usefulness for

risk prediction. As one author has said presciently, "In pointing at everything, genetics would point at nothing."[41] To date, such dire pronouncements have rung true, because genetic risk prediction has not been successful across the spectrum of complex disease. However, a recent study using a novel approach to genetic risk scores has provided some evidence that these initial concerns may be unfounded and that genetic risk prediction could still be possible even if thousands of genetic variants are needed.[75,76]

Using GWAS data from the Wellcome Trust Case Control Consortium, Evans et al[75] constructed a genetic risk score by including the top SNPs ranked by statistical significance. Instead of limiting the genetic risk score to genome-wide significant SNPs, they lowered the statistical threshold to include many more SNPs, acknowledging that some would likely represent false-positives. In doing so, they also captured many unidentified true-positives that increased the genetic variance explained. Using this genetic risk score on a validation sample, they demonstrated that as the probability value threshold for inclusion into the risk score was lowered, the C statistic increased for many (but not all) common diseases, including CVD. Despite low C statistics (≈0.70) and low levels of genetic variance explained (≈3%) for risk scores that included thousands of SNPs, they showed by simulation that as the sample size of the discovery data set increased, the top SNPs would be enriched for true-positives compared with false-positives. With a discovery set that included 10 000 case subjects and 10 000 control subjects, the top 5% of associated SNPs would be expected to explain up to 20% of the genetic variance. This analysis is noteworthy for a number of reasons. First, it provides empirical evidence that there may be many SNPs with weak effects that are beyond the detection limit of current GWAS (using contemporary statistical thresholds for genome-wide significance). In particular, for CVD, even when the threshold was relaxed to as low as $P<0.80$, the C statistic continued to increase, which suggests that the genetic architecture of CVD may consist of numerous SNPs with very weak effects that are currently undetectable. Second, the study by Evans et al[75] implies that it may be possible to construct useful genetic risk scores without actually identifying most of the true-positive associations at a genome-wide significance level. Whether empirical evidence will confirm these findings and prove the utility of this method remains to be seen, which emphasizes the need for additional research.

The Potential Causes of "Missing Heritability" in Initial GWAS

Although GWAS has uncovered many novel genetic associations in several complex diseases, the overall genetic variance explained by these associations has been lower than expected. For CVD, the very low variance explained by recently discovered SNPs, despite large studies with substantial statistical power, implies that either hundreds to thousands of genetic variants with very low effects are needed to explain most of the heritability of CVD, as discussed above, or that other factors explain the so-called missing heritability (which has been dubbed "the dark matter" of human genetics).[70] Explanations for the missing heritability in CVD and other complex diseases include overestimation of heritability by conventional methods, measurement imprecision of phenotypes, gene-gene and gene-environment interactions, linkage disequilibrium of associated SNPs with "true" causal variants, existence of low-penetrant variants, and the potential contributions of structural variation, epigenetic modifications, and rare genetic variation to disease risk.[70]

A primary concern before missing heritability can be evaluated is the accuracy of current estimates of heritability. Heritability represents the proportion of the total variance in the phenotype explained by genetic factors. Given that familial clustering of disease is due to both a shared environment and shared genes and that genetic factors are subject to much less misclassification than environmental exposures, heritability estimates (made on the basis of current family-based methods) can be confounded by poorly measured shared environmental factors.[70,77] Recent studies of quantitative traits that used novel methods to estimate heritability suggest that current methods are likely unbiased,[78] and therefore, incorrect heritability estimates are unlikely to explain a major component of the missing heritability in complex disease.

A large component of the missing heritability in complex disease may be due to interactions, both gene-gene and gene-environment. Interactions can be viewed simplistically as combinations of risk predictors in which the combined presence of 2 predictors leads to larger (or smaller) effects than expected for either predictor alone. Failure to incorporate interactions into GWAS has likely led to an underestimation of the true genetic effects and reduced the statistical power to identify novel genetic variants. Given that populations may be made up of genetically "susceptible" and "null" subpopulations based on their environmental coexposures (so-called context-specific genetic effects) and that genetic risk estimates are weighted averages of the risks in both subpopulations, ignoring interactions will often bias genetic exposures to the null when they are present.[79] Interactions, however, are difficult to study, because they can occur at many levels and add to the already large number of statistical tests performed in GWAS. However, larger sample sizes, novel analytic methods, and a "systems biology" approach may soon uncover important interactions that could explain a significant portion of the heritability.[80,81] Improvements in our understanding of interactions would also be expected to improve genetic risk prediction models.

Linkage disequilibrium of associated SNPs with true causal variants and the existence of poorly penetrant genetic variants may also explain some additional genetic variance. Deep resequencing efforts are ongoing in an effort to identify these causal variants.[82] Copy number variants, a form of structural genetic variation that includes small deletions, insertions, or inversions in the genome, have also been proposed as an additional source of missing heritability. Fortunately, most common copy number variants are relatively well "tagged" by HapMap SNPs and are well represented in GWAS.[83] In the Myocardial Infarction Genetics Consortium (MIGen), no copy number variants were found to be associated with MI despite good coverage of common copy number variants.[28] Rare copy number variants could

potentially represent an important source of genomic variation that would not be detected by GWAS, a possibility that has been demonstrated recently for obesity.[84] Although this may also be true for CVD, this possibility invokes an important consideration, ie, that most genetic variations in the genome, whether they represent SNPs or copy number variants, may be rare. If most of the genetic variation resides in rare variants not captured by HapMap SNPs, this variation would not be detected by GWAS. Indeed, studies conducted on lipid traits have demonstrated that a significant portion of the genomic variability was attributable to rare variants detectable only by sequencing.[85] Current efforts to sequence the genome in large cohorts of individuals are under way.[86,87] It is very likely that these strategies will uncover many new genetic variants that are biologically linked with disease; however, unless they identify rare variants that explain relatively large proportions of genetic variance (ie, with strong effects), their value for risk prediction will likely be limited. Furthermore, because rare variants may be specific to certain groups (ie, founder populations or families), their impact on risk prediction in the general population may be very modest.

Although the current trend of pooling samples in increasingly larger consortia to maximize statistical power for GWAS has led to some additional SNP discovery, it is likely that this approach will soon be exhausted, and more refined approaches will need to be prioritized in future efforts (Table 2). Several additional strategies to identify the missing heritability have been suggested recently.[70] These include assessment of analytic pooling strategies for rare variants, targeting recent African American samples with narrow linkage disequilibrium for resequencing efforts (to uncover rare variants), and conducting studies of family-based cohorts (in which susceptibility alleles/risk variants are likely enriched).[70]

A final point must be made about the missing heritability of CVD and the phenotypes that are currently being evaluated, which may represent "crude" representations of disease. Disease classifications have evolved in clinical medicine by the fitting of similar patterns of symptoms into categories, often organized by organ system, to reduce complexity and simplify diagnosis. Although this practice has been extremely helpful for the management and treatment of disease, it is increasingly apparent that these crude divisions of diseases may be suboptimal for identifying causal genetic factors. In CVD, it is very conceivable that MIs, like many other diseases, could be classified by primary pathophysiological process, for example, by an increased propensity for endothelial dysfunction, accelerated atherosclerosis, or thrombosis. MIs may represent the culmination of multiple different causal pathways, with each pathway having its own set of genetic associations. Using new tools from the "–omics" toolbox, disease phenotypes based on causal pathways could be extended to cellular and molecular profiles. This concept of refining disease phenotypes to produce distinct phenotypes of increasing homogeneity has been described as "deep phenotyping."[88] Deep phenotyping of disease could lead to improvements in the resolution of genetic signals and provide increasingly specific genetic insights that may enhance future genetic research.

Family History of CVD as a Marker of Genetic Risk for CVD

A parental history of premature CVD is a well-established risk factor for incident CVD.[18,19] Although a portion of the familial aggregation of CVD is mediated by nongenetic factors, a positive family history of premature CAD is thought to represent a good surrogate for increased genetic risk. Although individual genetic variants or genetic risk scores have not yet led to significant improvements in risk prediction, the addition of family history improves risk reclassification and has been formally added to risk prediction models.[8,16,19] In a recent study that incorporated >100 CVD-risk SNPs into a prediction model that included family history, the genetic risk score was not associated with incident CVD, but the association with family history remained strong.[42] In fact, the magnitude of the association between family history and CVD was not attenuated when the genetic variants were added, which suggests that current genetic variants explain only a very small fraction of the familial risk. It is possible that as additional common variants associated with CVD are uncovered, a larger proportion of the familial risk will be explained; however, an alternative possibility is that a significant proportion of the familial genetic risk is related to other shared environmental factors (including behavioral and lifestyle factors) or to rare familial genetic variants. Indeed, it is possible that a significant proportion of the familial genetic risk may in fact be specific to a given family (ie, "private genetic epidemiology"[89]), which could significantly hamper genetic risk prediction in the general population unless a few rare genetic variants (with large effects) explained a large portion of the familial risk (as seen with BRCA1 and BRCA2 in breast cancer).

Although family history may currently represent the best marker for the genetic risk of complex disease, a number of important limitations exist. A major limitation stems from the fact that family history predicts the same risk for all members of the immediate family despite the fact that 50% of the genetic variation occurs within families. Furthermore, even under ideal conditions of complete ascertainment of family history over 3 generations, up to 55% of CVD cases are expected to have no family history.[90] In a recent simulation study, the maximal value of the area under the curve for family history of CVD under such idealized conditions was only 0.71 (compared with >0.90 for a genetic risk score that explained 100% of the genetic variance of CVD).[68] However, a complete multigenerational family history would still explain 16% of the genetic variance of CVD, which is significantly better than any currently reported genetic risk score for CVD. This underscores the importance and clinical utility of ascertaining family history of CVD as a marker of genetic risk at the present time.

Translating Genetic Risk Prediction to Clinical Use

With a combination of the approaches outlined above, it is plausible that a greater portion of the genetic variance of

Table 3. Summary of Challenges Facing Genetic CVD Risk Prediction, Their Implications, and Potential Solutions

Challenges for Risk Prediction	Possible Issues and Implications	Potential Solutions
General considerations for CVD prediction	Conventional risk factors explain a large proportion of the risk for CVD Family history information is predictive, easily obtained, and free Determining predictive performance of genetic information	Genetic risk must be incremental to standard factors and family history . . . Use of a combination of c-statistic and reclassification measures
Biases in genetic effect sizes from GWAS	Use of extreme cases and extreme controls Incidence-prevalence bias Survivor bias	GWAS for incident CVD in population-based cohorts
Allelic architecture of CVD	Small to very small effect sizes Hundreds to thousands of genes may underlie CVD risk	Larger sample sizes
Missing heritability	Inaccurate estimates of heritability Gene-gene and gene-environment studies Poorly penetrant SNPs Identification of causal variants Structural variants (ie, CNVs) Rare variants Imprecise phenotypes	Heritability by identity-by-descent methods Case-only and family-based studies Larger sample sizes Sequencing, studies in populations with narrow LD (eg, African-Americans) Exome and whole-genome sequencing, studies in populations with narrow LD, family-based studies, founder populations Deep phenotyping by use of "-omics" methods
Large number of genes explain genetic risk	Unique genetic signature for each individual High genetic risk will be rare	Larger sample sizes
Translation of genetic risk prediction to clinical practice	External validation Generalizability across ethnicities Optimize false-positive and false-negative rates using appropriate cutoffs Assessment of predictive values and likelihood ratios in populations with differing baseline risks Efficacy and effectiveness (ie, need for screening RCTs) Cost-effectiveness Clinical utility over other "-omic" approaches	Cohort studies in appropriate populations Cohort studies in diverse ethnicities, recalibration Evaluation of prediction in individuals of varying baseline risk Randomized screening trials Cost-effectiveness studies Evaluation of genomic predictors vs other "–omics" predictors

LD indicates linkage disequilibrium; CNVs, copy number variants; and RCTs, randomized controlled trials.

CVD will be explained in the near future. Whether this will lead to genetic risk prediction that can be useful in clinical practice remains to be determined. Although high levels of prognostic performance for genetic risk prediction (ie, discrimination, reclassification, and calibration) are important, many additional considerations exist (Table 3).

First, any new predictive model will require validation in independent cohorts. It is currently unclear whether genetic risk scores will be transportable across ethnicities and races with varying allele frequencies and environmental exposures.[91] Independent validation and recalibration may be required for use in such populations. Second, appropriate cutoffs for risk scores are needed to optimize false-positive and false-negative rates depending on their relative importance.[52,58,92] Furthermore, similar to many other cardiovascular tests, genetic risk scores will also be subject to Bayes' theorem, which implies that inappropriate use of these tests will lead to poor predictive accuracy. Reports of predictive values and likelihood ratios for populations of varying cardiovascular risks would be needed to provide guidance as to which patients would benefit most from screening. Whether genetic risk scores should target only individuals with a family history of CVD or those with intermediate CVD risk (in whom the prevalence of CVD and the likelihood of true-positives may be higher) rather than the general population warrants further study.[93,94] Cost will also represent an important consideration in deciding which segments of the population should undergo such testing.[92,95] Third, the level of evidence required for genetic risk scores before clinical use will need to be robust. Clinically significant improvements in predictive performance (that are also cost-effective) should represent the threshold for clinical utility; marginal improvements that meet an arbitrary threshold for statistical significance will not suffice for translation to clinical use. Fourth, the type of evidence required for clinical use will also need to be clarified. Whether genetic risk scores will require "biomarker trials" in which a management strategy using a risk score is compared with a strategy without the use of the risk score[96] or whether a well-validated risk score with good prognostic performance in prospective cohorts will be sufficient evidence for clinical use will need to be determined. Fifth, education of patients and clinicians on the use and interpretation of such risk scores will also be needed to limit genetic determinism. The advantage of genetic risk scores is that they remain stable throughout life and can predict the genetic risk of disease at any age; however, their constancy may also be a liability, because it may be challenging to use them to assess efficacy of treatment or other risk-reduction strategies. In fact, it could be argued that for any genetic variant that has a measurable product, either in blood or other accessible tissue,

it would be much more useful to track the product than to obtain a genotype, which would obviate the need for genetic risk scores altogether. Whether genomic risk scores will eventually become a reality or will be complemented or superseded by proteomic or other "-omic" risk models remains to be seen.

Conclusions

At this early stage in the GWAS era, many questions remain about the feasibility and utility of genetic risk prediction for CVD. Although genetic information is far from ready for clinical use in CVD prediction, genetics has made important clinical inroads in other areas, such as pharmacogenomics for predicting efficacy and adverse events of common cardiovascular drugs (reviewed in Marín et al[97]). Whether "we will get there" for genetic CVD prediction, as we have asked in the title of our review, remains an open question. CVD may represent a particularly difficult phenotype for genetic risk prediction. Nonetheless, we hope that the many challenges faced for genetic CVD risk prediction will not be insurmountable and that novel strategies will lead to a greater understanding of the heritability of CVD. However, we must also appreciate the complexity of the human genome and the challenges inherent in achieving the goal of personalized medicine in CVD risk prediction. At this stage, clinicians should continue to inquire about family history for risk prediction, because this continues to represent a simple, cheap, and clinically useful risk factor for CVD that likely represents the net effect of hundreds of genetic risk variants that have yet to be discovered. Regardless of whether genetic information will be used clinically in CVD risk prediction, GWAS have been a resounding success for cardiovascular medicine through their identification of many genetic variants not previously linked with CVD that will undoubtedly provide novel mechanistic insights in the years to come.

Sources of Funding

Dr Thanassoulis is supported by a Research Fellowship from the Canadian Institute of Health Research and the Fonds de Recherche en Santé du Québec. Dr Vasan is supported in part by National Institutes of Health contract NO1-HC-25195 and RO1-HL93328.

Disclosures

None.

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KEY WORDS: cardiovascular disease ■ epidemiology ■ genetics ■ risk prediction ■ risk stratification