Are Genetic Tests for Atherosclerosis Ready for Routine Clinical Use?

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Abstract: In this review, we lay out 3 areas currently being evaluated for incorporation of genetic information into clinical practice related to atherosclerosis. The first, familial hypercholesterolemia, is the clearest case for utility of genetic testing in diagnosis and potentially guiding treatment. Already in use for confirmatory testing of familial hypercholesterolemia and for cascade screening of relatives, genetic testing is likely to expand to help establish diagnoses and facilitate research related to most effective therapies, including new agents, such as PCSK9 inhibitors. The second area, adding genetic information to cardiovascular risk prediction for primary prevention, is not currently recommended. Although identification of additional variants may add substantially to prediction in the future, combining known variants has not yet demonstrated sufficient improvement in prediction for incorporation into commonly used risk scores. The third area, pharmacogenetics, has utility for some therapies today. Future utility for pharmacogenetics will wax or wane depending on the nature of available drugs and therapeutic strategies. (Circ Res. 2016;118:607-619. DOI: 10.1161/CIRCRESAHA.115.306360.)

Key Words: atherosclerosis ■ familial hypercholesterolemia ■ genetic testing ■ pharmacogenetics ■ risk score
Diagnostic Testing for Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) presents a strong case for the utility of incorporating genetic testing into clinical practice. FH is caused by genetic defects in low-density lipoprotein (LDL) cholesterol (LDLC) metabolism that cause unusually elevated levels of LDL. The classic defects are autosomal dominant forms found in a few genes that are inherited in heterozygous, combined heterozygous, and homozygous patterns. Left untreated, FH is associated with extremely elevated risk of coronary heart disease (CHD) and death, estimated as ≈8.5-fold over a lifetime with the excess risk concentrated among younger individuals, even <40.6,8

FH is defined clinically via one of 3 criteria, which uses genetic testing to varying degrees. The US Medical Pedigrees With FH to Make Early Diagnoses and Prevent Early Death (MEDPED) criteria are based solely on measured LDLC levels, with cutoffs varying by age and type of relative with FH.9 The Simon–Broome criteria require measured elevated LDLC in combination with either tendon xanthomas in the patient or a first- or second-degree relative or a functional genetic mutation.7 The Dutch Clinical Lipid Network criteria assign points based on family history, measured LDLC, clinical history, and genetic testing, with genetic testing suggested for all probable diagnoses, as well as for all first-degree relatives of diagnosed cases.10 The largest population-based study, which used the Dutch Clinical Lipid Network criteria, estimated the prevalence at ≈1 in 250 people with substantial under diagnosis.11

The most common genetic defects resulting in FH are in the gene encoding the LDL receptor, LDLR, with well over 1200 defective alleles identified to date.12,13 Somewhat less common mutations affect the apolipoprotein B gene (APOB) or PCSK9, which encodes proprotein convertase subtilisin/kexin type 9 enzyme involved in LDL receptor processing. The elevated LDLC levels in FH can typically be traced through a pedigree to the patient, though the observed LDLC levels can vary widely in people with the same mutation,10,14,15 and all 3 diagnostic criteria incorporate familial inheritance patterns together with elevated LDLC. However, a detailed knowledge of familial health is often not available, and the additional FH diagnostic criteria may be absent. In these cases, unless cholesterol is measured and found to be extremely high or a genetic test is done, FH is often not detected. Consequently, FH is believed to be substantially underdiagnosed, with cases often recognized either in adulthood by cholesterol screening after subclinical disease may have developed and exposure to high cholesterol levels has already occurred or at the time of premature CHD.16,17 Clinically diagnosed FH can also be found with none of the known defects in LDLR, APOB, or PCSK9, which could be a monogenic defect not yet identified or could be polygenic in origin. A recent study in Slovenia, which has the only current country-wide screening program of children, suggested that multifactorial hypercholesterolemia accounts for a substantial percentage (43%) of the identified FH cases and is less likely to have an associated positive family history.18 Some of these polygenic FH cases can also be captured using genetic testing. Risk scores using 6 and 12 single nucleotide polymorphisms (SNPs) associated with LDLC levels have been developed and shown to have good discrimination in separating FH cases with no known mutation from healthy controls, though the clinical utility is less clear.19,20

Although genetic testing is incorporated into many diagnostic guidelines, the utility as a screening tool is less clear. Universal screening using genetics in adults has not been adopted but cascade screening using genetics and lipid profile after identification of an index case is currently recommended in the Netherlands, Norway, and the United Kingdom, among others, though it is not the recommended practice in the United States. However, efforts to identify and test people with FH include the Cascade Screening for Awareness and Detection of Familial Hypercholesterolemia (CASCADE FH) Registry20 and a related Flag, Identify, Network, Deliver FH initiative to identify possible FH cases from health record data. Similarly, assessing the landscape of FH in the United States, a recent statement from the American Heart Association, encourages genetic testing in diagnosis as complementary to family history and traditionally recognized clinical FH criteria. Although genetic analysis will fail to resolve some FH cases, especially in the absence of a known monogenic mutation, and the impact on insurance coverage for treatment is unclear, this recommendation notes that genetic tests could provide certainty of diagnosis in many cases, diminish the burden of undiagnosed FH, facilitate research gains, and improve health outcomes all in a way that lowers costs associated with FH.21 Universal
phenotypic screening of children to identify carriers of FH mutations for lifelong observation and management through diet and medication was recommended for consideration in the European Atherosclerosis Society Consensus Panel along with the inclusion of children in cascade screening, though the ethics and acceptability of genetic testing in children is a source of concern. Wider use of genetic testing, either with targeted sequencing or broader genotyping, could also enable future identification of additional causal mutations in those who tested negative for known mutations.

With or without a genetic diagnosis, most patients with heterozygous FH have until recently been treated with high-intensity statin monotherapy or a combination of statins plus ezetimibe. Although these options work moderately well in this setting, these same therapies are relatively ineffective in cases of homozygous FH when there is little or no residual LDL receptor activity. Although such patients are rare, the traditional approach to reducing very high lipid levels has been apheresis, an expensive and complicated intervention that is often unavailable even in large population centers. However, in 2013, the US Food and Drug Administration (FDA) approved 2 new treatments for homozygous FH, lomitipide (a microsomal triglyceride transfer protein inhibitor) and mipomersen (an antisense oligonucleotide that binds to apo B coding RNA). Further, in 2015, the FDA approved the use of 2 different PCSK9 inhibitors for those with either heterozygous or homozygous FH. These advances signal a major therapeutic shift and strongly support increased diagnosis of FH through both clinical and genetic surveillance. This is particularly important for PCSK9 inhibitors that have proven highly effective at reducing LDLC in heterozygous FH with reduced LDL receptor activity and in those cases of homozygous FH with residual LDL receptor activity. Although currently it may be clinical expedient to simply start the therapy and see if it works, genetic testing and identification of the precise causal mutation may eventually allow tailored care with more predictable clinical outcomes.

**Prevention and Risk of Future CVD Events**

Widely used CVD risk prediction models typically capture a 10-year time frame and share a core of strong primary risk factors. The Systematic Coronary Risk Evaluation (SCORE) model used in Europe is based on sex, age, systolic blood pressure, total cholesterol, and smoking status, whereas the US-based Pooled Cohort equation additionally incorporates whether blood pressure is treated, high-density lipoprotein cholesterol, race, and diagnosed type 2 diabetes mellitus. The Reynolds Risk Score includes most of the pooled cohort risk factors and adds a family history of heart attack in a parent under 60 and C-reactive protein levels and hemoglobin A1c levels among those with diabetes mellitus. The QRISK®2 model includes chronic kidney disease, atrial fibrillation, rheumatoid arthritis, socioeconomic status based on postal code, and body mass index as well as age, sex, ethnicity, smoking status, the ratio of total to high-density lipoprotein cholesterol, type 1 and 2 diabetes mellitus, systolic blood pressure, and a family history of angina or heart attack in a first-degree relative under 60. These models target a range of related CVD outcomes, and all have strong baseline predictive ability reflected in the area under the receiver-operator characteristic curve value, a measure of discrimination between predicted events and nonevents, in the range of 0.7 to 0.8.

**Evaluating New Predictors**

New markers of risk, including genetic factors, are evaluated in the context of the above models for population risk prediction. Guidelines have been developed for evaluation of whether new markers add to current risk prediction models both for biomarkers in general and for genetics in particular. There is consensus that the primary statistical test should be the association of the new marker with the outcome after adjustment for the components of the existing comparison model (eg, SCORE, Pooled Cohort, or QRISK®2). Once a significant association has been established, additional measures of improvement in prediction can be presented. These measures include the change in the area under the receiver-operator characteristic curve and in calibration or consistency between the observed and predicted rate of events as measured by the Hosmer–Lemeshow test. Clinical risk thresholds for action can also be incorporated into evaluation measures, such as the net reclassification improvement, which summarizes movement across threshold-defined categories, and the reclassification calibration, which allows for comparison of calibration across categories. In addition to these statistical measures, the guidelines also point out that a new marker should substantially change risk assessment in a way that will affect clinical practice, ideally tested in a randomized trial, and be cost-effective.

**Genetic Markers of Risk**

The substantial heritable component of both CHD and stroke suggest that genetic variants, if they can be identified, may be strong risk predictors with lifelong value in preventive management. Twin studies suggest heritability for CHD between 40% and 60%, with higher heritability for premature CHD for which nongenetic exposures may have less effect. In total, the loci from genome-wide association studies reaching genome-wide significance explain 13.3% of the CHD heritability. The strong effect of genetics is further supported by a 50% increase in risk of CHD for a sibling or one parent with CHD after 50 years of age to a 6-fold increase in risk with CHD before age 50 in both parents. Over the past decade, 58 independent loci associated with CHD have been identified by genome-wide association studies, with the most recent analysis including 60,801 cases. The majority of the independent loci have a minor allele frequency >5%, with less common alleles unlikely to have substantial utility in prediction. Some of these loci suggest biological functions related to lipid risk factors, particularly LDL and inflammatory processes represented by the IL6 receptor locus, but there are also loci whose role in CHD remains uncertain. The most notable pathophysiological uncertainty is around the locus on chromosome 9p21, despite being the first identified CHD common marker, as well as the strongest. In total, the loci from genome-wide association studies reaching genome-wide significance explain 13.3% of the overall heritability of CHD, whereas a larger set including loci meeting a more relaxed false discovery rate significance threshold explain 22.6% of the CHD heritability.
genetics of stroke has shown similar patterns, with heritability estimates of 17% to 32% and population attributable risks of only 4.5% to 7.2% for identified loci. Additional loci for which the strength of association varies as a consequence of ancestry are more likely to be identified by expanding the reach of genome-wide association studies into new populations. The most recent genome-wide association studies primarily includes participants from populations with European ancestry but demonstrates similar genetic associations for non-European ancestry groups, specifically South Asian, East Asian, African American, and Hispanic populations. Current funding is increasing emphasis on inclusion of non-European populations in ongoing genetic analysis, including the Human Heredity and Health in Africa Initiative, jointly funded by National Institutes of Health and the Wellcome Trust.

Once genetic markers have been identified, thought must be given to the optimal strategy for incorporating multiple loci into CVD risk prediction because currently identified SNPs have small effect sizes (odds ratios in the range of ≤1.2). Using a single SNP for prediction is ineffective, as demonstrated by analysis of the 9p21.3 locus. Despite having the strongest single SNP association with CHD and remaining significantly associated with CVD events after adjustment for current risk factors, no substantial improvement in prediction was observed. Subsequent efforts have followed 2 main strategies in combining multiple SNPs in a simple additive model to generate genetic risk scores. The first strategy is focused on SNPs with direct associations with overall CHD or stroke, which limits the number of variants included but captures pathways unrelated to the standard risk factors. Initial risk scores based on 13 SNPs showed significant association with CVD but no substantial improvement in risk prediction. More recent studies based on ≤50 SNPs showed similar significant relationships for incident disease and suggest that the prediction improves as more SNPs are incorporated. Two studies examining 28 SNPs and 50 SNPs showed improvements in prediction which were independent of family history (Figure 1).

The second strategy includes additional SNPs that affect standard CVD risk factors. This strategy would be expected to more closely approximate the full genetic effect on CVD by incorporating the indirect effects on intermediate risk factors. However, these scores have shown weaker association with CVD in fully adjusted models and smaller improvements in prediction than scores derived from SNPs with direct CVD. Scores derived from SNPs associated with a single CVD risk factor have also been examined. A blood pressure score and a lipid score were both found to be associated with incident CVD after adjustment for standard risk factors, but neither showed an improvement in prediction.

In theory, larger sample sizes and the identification of additional SNPs with small effects should increase the predictive ability of genetic risk scores with an upper bound determined by the overall heritability. The theoretical maximum area under the receiver-operator characteristic curve for genetic markers alone would be in the range of 0.7 to 0.8, which is the same range as models with traditional risk factors (Figure 2). Although important for understanding pathophysiology, gene–gene interactions, and gene–environment interactions are unlikely to substantially improve overall performance. What remains uncertain, however, is the degree to which the full set of SNP-based predictors will be independent of other traditional risk factors for CHD, such as lipid levels and blood pressure. Additionally, as smaller effect sizes are incorporated and larger populations are used for discovery, careful consideration is required for selection, weighting, and evaluation of SNPs, an area of active research. Lengthening the time scale of prediction from 10 to 30 years, or even lifetime risk, may show a stronger effect for genetic markers compared with measured risk factors and the question of when to test for increased risk remains open. Integration of genetic and epigenetic information, the latter reflecting some age effects, is another promising possibility.
Risk Scores in Practice

Despite association with outcomes, the impact of genetic risk scores on clinical decision-making is unclear. Trials of risk prediction in general have demonstrated increased accuracy of classification, but only small effects on subsequent cardiovascular care.\(^6\) Additionally, these effects occur when risk information is included with related counseling and followed by repeated presentations and feedback. Similarly, observational studies of genetic risk assessments provided directly to consumers showed no association with increased anxiety or changes to health behaviors.\(^6\)

However, the incorporation of genetic risk may have a different effect on physician and patient behavior than other markers. Promising results have been shown by the Myocardial Infarction Genes (MI-GENES) trial, in which patients with a high or low genetic risk score for CHD were randomized to receive conventional risk score information or a conventional risk score plus genetic risk score at baseline.\(^6\)

Participants who were informed that they had a high genetic risk were more likely than participants with a lower genetic risk score or those who were not informed of their genetic risk to initiate and remain on statin therapy, leading to a lower LDLC at 6 months.\(^7\) However, no effect was seen for lifestyle changes, such as diet and exercise. Additionally, it is not clear whether this result is due only to the change in risk after incorporation of the genetic information or represents an additional effect of genetic information on behavior.

Additional trials incorporating genetic risk prediction in CVD are in progress. The Personal Genomics for Preventive Cardiology trial planned to enroll 100 patients at intermediate or high cardiovascular risk based on traditional models.\(^7\)

Longer trials, incorporating the results from current trials on strategies for conveying genetic risk to patients and physicians, will be critical in establishing best practices on conveying genetic information and could be incorporated into larger pragmatic trials, such as the Million Hearts Study.

Pharmacogenetics in CVD Management

The third area of potential utility is the stratification of patients to improve treatment efficacy and safety. Pharmacogenetic effects are typically constrained to pathways closely related to the primary effects on the drug target (pharmacodynamics) and aspects of drug absorption, distribution, metabolism, and elimination (pharmacokinetics). The number of variants causing these effects is typically smaller than the number of variants contributing to CVD overall.\(^8\) However, the magnitude of pharmacogenetic effects are typically larger than individual variant effects on overall CVD, perhaps in part because of evolutionary pressures on genetic variation for efficient absorption of nutrients and strong defenses against xenobiotic toxins.\(^9\) The clinical utility of these effects depends both on their magnitude.
and the constellation of available therapies. We present here the major pharmacogenetic interactions with CVD treatments in order of increasing potential for clinical utility.

Blood Pressure Treatment

No genetic tests are currently suggested for managing blood pressure treatment. Elevated blood pressure is treated by several classes of drugs, including β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II blockers, diuretics (sodium channel inhibitors), and calcium channel blockers. Analyses focusing on the genes encoding each drug target have not been successful at identifying variants with large effects.7 Similarly, an approach testing 39 SNPs associated with resting blood pressure for modification of atenolol (β-blocker) or hydrochlorothiazide (diuretic) response found no significant effects after adjustment for multiple comparisons.77 Combining the 4 atenolol-response SNPs or 3 hydrochlorothiazide SNPs into risk scores revealed potential for stratifying response but uncertain translational value. In a genome-wide meta-analysis among 844 individuals from 3 studies, the locus (rs2273359) with the strongest association with response to hydrochlorothiazide did not reach genome-wide significance (P=5.5×10^{-8}) nor were candidate SNPs significantly associated in these data.78

Associations with treatment response have been identified using race, a possible measure of shared genetics. Blood pressure reduction with a combination of hydralazine and isosorbide was found in the African American Heart Failure Trial (A-HEFT) to reduce mortality by 43% and heart failure hospitalization by 39% among individuals who self-identified as black.79 This is a potentially important observation as black patients with congestive heart failure may respond less well than white patients to standard treatments, such as angiotensin-converting enzyme inhibitors. However, although the A-HEFT trial led to the first FDA approval for a race-based pharmacological intervention, uptake of this therapy has been slow, and there has been criticism of the concept of self-identification as a proxy for genetic testing.80,81

Aspirin Treatment in CVD Prevention

The candidate gene approach for response to aspirin in CVD prevention has not identified variants that would prompt pharmacogenetic testing.4 Antiplatelet therapy with aspirin inhibits the cyclooxygenase-1 enzyme (COX-1) by irreversible acetylation, causing decreased production of thromboxane A2 that otherwise would stimulate clotting and vasoconstriction. Genetic variation in PTGS1, the gene encoding COX-2, was shown to be associated with aspirin treatment and CVD outcomes82 but has not been consistently replicated. By contrast, significant genetic interactions were observed with the response to randomized allocation to aspirin (100 mg/alternate days) or placebo for genes encoding a component of lipoprotein(a) (LPA), a cardiovascular risk factor,83 and catechol-O-methyltransferase (COMT), an enzyme that is involved in metabolism of catechols, such as epinephrine.84 For LPA, a single copy of the rare variant allele at rs3798220 increased CVD risk by ≥2-fold in the placebo group but had no effect in the group receiving aspirin. For the common genetic variation at rs4680 in COMT encoding methionine or valine at amino acid 148, compared with the heterozygotes, both the ≥34% reduction in CVD events for the homozygous methionine-encoding alleles and ≥50% increase in events for the homozygous valine-encoding alleles were eliminated with preventive aspirin treatment. These effects imply that genotype influences whether preventive aspirin is either beneficial or harmful. However, effects on aspirin response at LPA and COMT have not yet been confirmed in an independent population and thus are not considered for testing.

Statin Therapy

Genetic influences on statin-induced LDLC lowering have been robustly validated, but only explain a small proportion of the variance in response and have not been incorporated into clinical practice. Initial candidate analysis focusing on genes proximal to the known pharmacodynamic and pharmacokinetic pathways of statin mechanism identified associations with LDLC lowering in the HMGR gene, encoding the target of statin therapy,86–88 APOE, LDLR, and genes encoding statin transporters.89–91 Subsequent genome-wide genetic analysis confirmed many of these associations for treatment response with rosuvastatin,92 simvastatin,93 and atorvastatin.94 For rosuvastatin, the median LDLC response increased proportionally with the inherited number of identified alleles. The total combined genetic effect explains only a few percent of the variation in LDLC lowering, although the odds of achieving an LDLC reduction >50% roughly doubled with each additional allele.95 A meta-analysis for LDLC response to statin, which included the largest total sample to date (N>40,000) and multiple different statins, identified genome-wide significant associations with LDLC lowering at APOE, LPA, SORT1, and SLC10A1.98

Another potential setting for genetic testing in statin efficacy was suggested by the relationship between risk reduction and overall CHD genetic risk. Individuals with elevated genetic risk, based on a 27 SNP score, experienced significantly greater relative reduction in CHD events with statin therapy in meta-analysis across several studies.75 The interaction between cardiovascular risk and statin therapy was not because of greater LDLC response among individuals with greater genetic risk or other potential clinical characteristics, as demonstrated in multivariate analysis.

Statin pharmacogenetic effects on safety have also been investigated. SLC10A1 encodes a statin transporter that has confirmed genetic effects on simvastatin toxicity among
estimated adoption of testing for alternative to simvastatin at 80 mg/d has resulted in only limited reduction in exposure to the active metabolite of clopidogrel compared with noncarriers. The low metabolizing carriers also experienced a 53% increase in the primary composite cardiovascular outcome during treatment with clopidogrel compared with clopidogrel-treated noncarriers. This pharmacogenetic interaction has been questioned but ultimately supported by large meta-analysis. Genetic variation in a second gene ABCB1, encoding an efflux transporter of clopidogrel, has also been associated with response to clopidogrel but more weakly than for CYP2C19 variants. In 2009, on the basis of the data for adverse outcomes, the FDA imposed black box warning regarding CYP2C19 pharmacogenetics of clopidogrel, though the American College of Cardiology Foundation/American Heart Association guidelines recommend selective rather than routine genetic testing. Two point-of-care testing systems are currently FDA-approved and reimbursable through Medicare. Crucially, although there is retrospective and other observational evidence that genetic information may improve outcomes, there is a lack of completed prospective evidence examining the benefit of testing for CYP2C19 genotype in the context of the newer, more potent alternative P2Y₁₂ inhibitors, prasugrel or ticagrelor. The pharmacology of these new drugs is not influenced by CYP2C19 variation but they carry higher bleeding risk and greater cost because of patent protection. Trials evaluating the effect of CYP2C19-informed clinical choices among P2Y₁₂ inhibitors on cardiovascular outcomes as well as safety and cost effectiveness are ongoing. The Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response After Percutaneous Coronary Intervention (TAILOR-PCI) trial, which compares treating all patients with clopidogrel to assigning the patients with any CYP2C19*2 and *3 alleles to ticagrelor and the others to clopidogrel, will examine both major cardiovascular and bleeding risks. The POPular Genetics trial is examining the opposite comparison, with the control group receiving either prasugrel or ticagrelor as local standard care, whereas CYP2C19*1 homozygous participants in the intervention group are randomized to clopidogrel instead. Positive results for either trial would support the use of genotyping to guide treatment decisions, whereas the different designs will add information on how best to use the genotype results for all patients.

**Anticoagulation Therapy With Warfarin**

Pharmacogenetic effects are most pronounced for warfarin and its derivatives, and these effects have engendered a vigorous appraisal of their translational potential. Warfarin’s

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**AntiplATELET Therapy With P2Y₁₂ Inhibitors**

By contrast to the settings of blood pressure medications, aspirin treatment, and statin therapy, genetic effects in the pharmacokinetic pathway of clopidogrel, a thienopyridine class P2Y₁₂ inhibitor of platelet function, have justifiably received serious consideration for genetic testing. Clopidogrel is activated from a prodrug through metabolism by CYP2C19. Genetic variation in CYP2C19 includes the reference CYP2C19*1 form, the low metabolizer CYP2C19*2 and CYP2C19*3 forms, and the high metabolizer CYP2C19*17 form, as well as other, less common, forms. At the standard dose of 75 mg/d, low metabolizer forms of CYP2C19 are associated with reduced platelet inhibition and adverse cardiovascular outcomes, whereas the high metabolizer form carries increased bleeding risk. In Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction (TRITON–TIMI 38), carriers of low metabolizing CYP2C19 alleles had a 32% reduction in exposure to the active metabolite of clopidogrel compared with noncarriers. The low metabolizing carriers also experienced a 53% increase in the primary composite cardiovascular outcome during treatment with clopidogrel compared with clopidogrel-treated noncarriers. This pharmacogenetic interaction has been questioned but ultimately supported by large meta-analysis. Genetic variation in a second gene ABCB1, encoding an efflux transporter of clopidogrel, has also been associated with response to clopidogrel but more weakly than for CYP2C19 variants. In 2009, on the basis of the data for adverse outcomes, the FDA imposed black box warning regarding CYP2C19 pharmacogenetics of clopidogrel, though the American College of Cardiology Foundation/American Heart Association guidelines recommend selective rather than routine genetic testing. Two point-of-care testing systems are currently FDA-approved and reimbursable through Medicare. Crucially, although there is retrospective and other observational evidence that genetic information may improve outcomes, there is a lack of completed prospective evidence examining the benefit of testing for CYP2C19 genotype in the context of the newer, more potent alternative P2Y₁₂ inhibitors, prasugrel or ticagrelor. The pharmacology of these new drugs is not influenced by CYP2C19 variation but they carry higher bleeding risk and greater cost because of patent protection. Trials evaluating the effect of CYP2C19-informed clinical choices among P2Y₁₂ inhibitors on cardiovascular outcomes as well as safety and cost effectiveness are ongoing. The Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response After Percutaneous Coronary Intervention (TAILOR-PCI) trial, which compares treating all patients with clopidogrel to assigning the patients with any CYP2C19*2 and *3 alleles to ticagrelor and the others to clopidogrel, will examine both major cardiovascular and bleeding risks. The POPular Genetics trial is examining the opposite comparison, with the control group receiving either prasugrel or ticagrelor as local standard care, whereas CYP2C19*1 homozygous participants in the intervention group are randomized to clopidogrel instead. Positive results for either trial would support the use of genotyping to guide treatment decisions, whereas the different designs will add information on how best to use the genotype results for all patients.

**Anticoagulation Therapy With Warfarin**

Pharmacogenetic effects are most pronounced for warfarin and its derivatives, and these effects have engendered a vigorous appraisal of their translational potential. Warfarin’s
anticoagulation properties are caused by its inhibition of a component of the vitamin K epoxide reductase complex (VKORC1), thus limiting vitamin K levels for use in the clotting cascade.\textsuperscript{124,125} Warfarin-class drugs have a relatively narrow therapeutic window requiring frequent monitoring of the international normalized ratio (INR) and adjustment of dose to balance bleeding risk with efficacy. Common genetic variants that affect the expression or activity of VKORC1 strongly influence the maintenance dose of warfarin. Variation in the gene for a second enzyme that metabolizes warfarin, CYP2C9, also has strong effects on maintenance dose. The combination of 1 \textit{VKORC1} variant and 2 \textit{CYP2C9} variants (\textsuperscript{*2}, \textsuperscript{*3}) together with clinical information explain 43\% of the variation in maintenance dose, compared with 26\% of variation explained by clinical information alone.\textsuperscript{126} A third gene, \textit{CYP4F2}, has a smaller association with warfarin response and is not typically considered for pharmacogenetic applications.\textsuperscript{127}

After some small trials and observational studies validating the contribution of genetics to warfarin dose,\textsuperscript{128,129} 2 large trials examined whether accounting for genetics reduces the time to establish INR maintenance dose or an increase the proportion of time patients experienced INR measures within the therapeutic range over 4 or 12 weeks.\textsuperscript{130,131} Dosing algorithms using both genetics and clinical characteristics were only slightly better or not statistically different from algorithms using clinical characteristics alone. However, the integration of the repeated, interim INR measurements in both dosing algorithms likely attenuated potential differential effects of genetics.\textsuperscript{132} A third trial was conducted to compare dosing according to an algorithm combining genetics and clinical characteristics to dosing in standard care, rather than an optimized clinical algorithm.\textsuperscript{133} Over the 12-week trial, the genetic algorithm resulted in superior metrics of warfarin dosing for time to first reach therapeutic or stable INR levels and fraction of time within range of therapeutic INR.

More recently, a fourth trial examined the issue of genetic guidance in the choice between warfarin and a recently approved alternative anticoagulant therapy, edoxaban that inhibits clotting factor Xa.\textsuperscript{134} Edoxaban and other new non-vitamin K oral anticoagulants have been found to have at least noninferior efficacy to warfarin in clinical trials for management of atrial fibrillation and superior safety profile for some adverse events, but greater risk of gastrointestinal bleeding.\textsuperscript{135,136} Participants with nonvalvular atrial fibrillation were randomly allocated to warfarin treatment or either higher (60 mg/d) or lower (30 mg/d) dose edoxaban. Investigators were instructed to dose warfarin-allocated participants using standard care guidelines or a nongenetic dosing algorithm. The trial assessed risk of adverse events within strata defined by prespecified combinations of \textit{VKORC1} and \textit{CYP2C9} alleles as normal, sensitive, or highly sensitive responders to warfarin. Over the first 90 days, individuals in the sensitive and highly sensitive strata have fewer overt bleeding events on edoxaban compared with warfarin (Figure 4). These differences were no longer significant beyond 90 days, though an increased risk of major or life-threatening bleeding remained in the highly sensitive responders.

Warfarin remains a widely used treatment option, however, and although guidelines issued by the Clinical Pharmacogenetics Implementation Consortium suggest genetic testing for guiding warfarin dosing, testing is not in routine clinical use nor is testing covered by Medicaid and Medicare.\textsuperscript{137} Without trial data addressing genetic testing for hard clinical outcomes with warfarin rather than INR, establishing the prospective utility of genetic testing for warfarin remains elusive. Ongoing trials will address this gap.

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\begin{tabular}{|l|c|c|c|}
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 & Edoxaban & Warfarin & HR (95\% CI) \tabularnewline
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\textbf{Any overt bleed} & & & \tabularnewline
Normal responder & 6.8\% & 5.1\% & 6.2\% & 1.13 (0.92-1.39) \tabularnewline
Sensitive & 6.3\% & 4.6\% & 8.0\% & 0.77 (0.59-1.00) \tabularnewline
Highly sensitive & 7.2\% & 3.5\% & 15.6\% & 0.45 (0.22-0.90) \tabularnewline
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\textbf{Major or clinically relevant non-major bleed} & & & \tabularnewline
Normal responder & 5.1\% & 3.8\% & 4.6\% & 1.15 (0.91-1.45) \tabularnewline
Sensitive & 4.5\% & 2.8\% & 5.8\% & 0.78 (0.58-1.07) \tabularnewline
Highly sensitive & 3.3\% & 2.4\% & 14.1\% & 0.22 (0.09-0.57) \tabularnewline
\hline
\textbf{Major bleed} & & & \tabularnewline
Normal responder & 3.1\% & 0.6\% & 1.1\% & 1.08 (0.66-1.77) \tabularnewline
Sensitive & 0.8\% & 0.4\% & 1.4\% & 0.60 (0.30-1.38) \tabularnewline
Highly sensitive & 0\% & 0\% & 2.3\% & \tabularnewline
\hline
\textbf{Clinically relevant non-major bleed} & & & \tabularnewline
Normal responder & 4.1\% & 3.3\% & 3.8\% & 1.12 (0.86-1.45) \tabularnewline
Sensitive & 3.7\% & 2.4\% & 4.7\% & 0.80 (0.57-1.13) \tabularnewline
Highly sensitive & 3.3\% & 2.4\% & 13.4\% & 0.24 (0.09-0.60) \tabularnewline
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\caption{Response to edoxaban vs warfarin in first 90 days of therapy in participants classified by combinations of \textit{VKORC1} and \textit{CYP2C9} alleles as normal, sensitive, or highly sensitive responders to warfarin. Reproduced from Mega et al\textsuperscript{134} with permission of the publisher. Copyright ©2015, Elsevier.}
\end{table}
and include the Genetics Informatics Trial (GIFT), which examines genetics-guided dosing in orthopedic patients to prevent subsequent deep vein thrombosis, as well as INR control, and is enrolling 1600 participants to be followed from the time of surgery until the completion of warfarin treatment in 4 to 6 weeks.138

**Impact of Changing Pharmacopeia**

The preceding examples illustrate how commercial, structural, and practical aspects of clinical care influence the potential for utility of pharmacogenetics in CVD. Even when comparatively large genetic effects are observed, changes in clinical care because of costs or development of new and typically better treatments may obviate the utility of genetic testing. For example, although the low cost of generic formulations of simvastatin make it attractive, the myopathy risk associated with 80 mg/d dosing need not arise now that patent protection for more potent statins with less toxicity risk have expired (atorvastatin) or will soon (rosuvastatin). Moreover, the newly approved PCSK9 inhibitors potentially represent a novel alternative LDLC lowering treatment, although at substantially higher treatment cost. Similarly, potential replacement of clopidogrel with alternative P2Y1 inhibitors, such as prasugrel and ticagrelor, as well as potential replacement of warfarin with non-vitamin K oral anticoagulants, will change the relevance of the pharmacogenetic effects that have been concerns to date. These new treatments may have their own genetic interactions, and ongoing postapproval pharmacogenetic surveillance will be important.

In theory, pharmacogenetics may also have the ability to save failed drugs. One recent example, which has yet to be successfully replicated, is a genetic reanalysis of neutral trial data for dalteparbin, which claimed a significant effect interaction with treatment by genotype. Until such effects are tested in further prospective trials, it is unclear whether such post hoc hypothesis-free stratification of trial results on the basis of genome-wide scanning will represent a wild goose chase or evolve into a useful new tool for drug development.140

**Conclusions**

Genetic testing for prediction and management of atherosclerosis is a work in progress. The urgency to add genetics to clinical practice may increase with the general availability and reduced cost of genetic information, whether from a test indicated by a different condition, childhood screening, or direct-to-consumer testing. As this information becomes increasingly available, the question changes from whether to incorporate genetic markers to how best to manage the genetic information already available. Currently, clinicians have concerns about standards for interpretation of genome sequence information reflected in the American College of Medical Genetics guidelines, where difficulties in communication among findings related to complex diseases are noted and classification of these results as other findings or risk alleles with qualifications based on the level of evidence is recommended.142 Nonetheless, methods of incorporation of genetic information into clinical practice are being studied in multiple targeted trials mentioned above, as well as more general initiatives, such as the Vanderbilt Pharmacogenomic Resource for Enhanced Decisions in Care & Treatment (PREDICT) program.143

Another challenge for providers of genetics tests as well as clinicians is the constantly moving target of establishing validity and utility of associations.144 To address the need for rapid and consistent synthesis of available research on the expanding number of known relationships, ClinGen, the Clinical Genome Resource program funded by National Institutes of Health, was created. The aim is to build and maintain a central clinical genomic knowledge base to provide consistency in the presentation and interpretation of genetic results.145 It has been suggested that instead of considering a genome sequence as a test, it may be more useful to frame it as a resource to revisit over a lifetime.146 Although this resource is not yet part of routine clinical practice for CVD, it will likely be an important component of increasingly tailored clinical care in the future.

**Disclosures**

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