Emerging Genomic Applications in Coronary Artery Disease

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Over the last 4 years, an unprecedented number of studies illuminating the genomic underpinnings of common “polygenic” diseases including coronary artery disease have been published. Notably, these studies have established numerous deoxyribonucleic acid (DNA) variants within or near chromosome 9p21.3, the LPA, CXADR, and APOE genes, to name a few, as key coronary artery disease and sudden cardiac death susceptibility markers. Most importantly, many of these DNA variants confer over a 2-fold increase in risk for coronary artery disease, myocardial infarction, and ventricular fibrillation. Additionally, loss-of-function variants in the hepatic cytochrome 2C19 system have now been found to be the predominant genetic mediators of clopidogrel antiplatelet response, with variant carriers having a >3-fold increase in risk for stent thrombosis. In the near future, many additional rare polymorphisms, structural variants, and tissue-specific epigenetic features of the human genome including DNA methylation, histone modifications, and chromatin state will emerge as significant contributors to disease pathogenesis and drug response. In aggregate, these findings will have the potential to radically change the practice of cardiovascular medicine. However, only the individual clinician can ultimately enable the translation of these important discoveries to systematic implementation in clinical practice. (J Am Coll Cardiol Intv 2011;4:473–82) © 2011 by the American College of Cardiology Foundation

The publication of the initial draft of the human genome sequence in 2000 involved a multinational decade-long effort at a price tag of over $3 billion (1). Now, commercial platforms can sequence whole genomes from several individuals in a day at a tiny fraction of that cost. Instead of $10,000 for sequencing 1 million deoxyribonucleic acid (DNA) bases, the cost is $0.01 (1,2). These technological advances combined with a detailed catalog of common human variation provided by the HapMap Project has spawned the publication of over 550 genome-wide association studies (GWAS) that have strongly linked nearly 800 gene variants to over 150 common polygenic diseases and complex traits (3–5). These studies have also identified numerous highly predictive pharmacogenetic markers of drug response and toxicity (6–9). Together, these findings have the potential to radically change the practice of medicine (4). This review will detail the current discoveries in the field of translational genomics that have potential ramifications in the prevention and treatment of patients with ischemic heart disease (IHD).

Analyzing the Genome

Before commencing with a detailed discussion on the specific applications of genome-based medicine, it is important to first highlight several key concepts regarding the heritability of traits. Monogenic, rare, “simple” Mendelian traits segregate in autosomal dominant, recessive, X-linked, or mitochondrial bases. These relatively infrequent disorders are typically caused by rare, deterministic genetic mutations. Historical examples of such
traits in cardiovascular medicine include hypertrophic cardiomyopathy, long QT syndromes, and familial hypercholesterolemia (10). In contrast, “complex” traits are common and arise from elaborate gene-gene and gene-environmental interactions and confer risk for disease in a probabilistic manner (11). By far, the most diseases in clinical practice today are complex traits and include disorders such as diabetes, myocardial infarction (MI), atherosclerotic coronary artery disease (CAD), and various cancers.

Incremental decoding of the genomic basis of complex traits will require many years of collaborative research efforts by clinicians and scientists and will never be complete (4,12). This is primarily because of the sheer complexity involved in analyzing the 6 billion base pairs, over 15 million single nucleotide polymorphisms (SNPs), and hundreds of thousands of structural variants such as base pair insertions, deletions, inversions, and gene copy number variants present in the human diploid genome. To date, structural variants have not been well characterized because we are only in the early phases of whole genome sequencing. Their understanding is additionally compromised because all sequencing that is done today relies on using the human genome reference template, or resequencing, rather than de novo assembly. Beyond that, there are tissue-specific important epigenetic variations such as patterns of methylation, histone modifications, and chromatin state—all of which are likely contributors to disease susceptibility (12). Other differences, such as protein splicing and folding, differences in the individual’s metabolome, also need to be taken into account. Despite these substantial challenges, over the last 4 years, there has been an unprecedented stream of important genomic discoveries illuminating novel pathways involved in disease biology (13).

These discoveries stem from several recent key advances. First, large-scale efforts have led to the identification of approximately 10 million SNPs that carry at least a 5% minor allele frequency (MAF) and are commonly represented in populations under study (14). In addition, another 10 million or more SNPs are considered rare or low frequency because they fall below the 5% MAF threshold. In aggregate, these SNPs represent only 0.5% of the human genome, but are the most abundant form of human genomic variation (15).

Notably, these SNPs are not inherited independently, but as “bins” or “blocks” that are in linkage disequilibrium. Further, the genotype of 1 SNP may be sufficient to infer the genotype of all other SNPs within a given linkage disequilibrium block (haplotype), thereby “tagging” an entire region of interest (Fig. 1) (15). Thus, by assaying for only 1 million of these tag SNPs, a GWAS is essentially assessing hundreds of thousands of independent haplotype blocks for disease and drug response associations. This hypothesis-free approach in scanning the human genome has yielded hundreds of reproducible disease susceptibility markers in independent cohorts involving tens of thousands of cases and controls (13).

Second, the striking reduction of costs associated with DNA sequencing have enabled targeted resequencing of genomic regions thought to be involved in disease and health (16). This has resulted in the ability to identify rare genetic variants with a MAF of <5%, which complement the common susceptibility SNPs (MAF >5%) established through GWAS (16). Now, these rare SNPs, present in common, incriminated haplotype blocks by GWAS, are being assayed for along with common SNPs in more comprehensive GWAS studies of complex traits with great early success (17). An exemplary case is the recent discovery of 2 apolipoprotein (a) (LPA) polymorphisms—one rare and 1 common variant—that when present together confer at least a 250% increase in risk for CAD (17). This finding was facilitated by data generated from GWAS and resequencing of LPA in thousands of individuals with and without CAD.

Along these lines, Musunuru et al. (18) were recently able to completely sequence more than 16,000 genes in 2 family members affected by familial combined hyperlipidemia—a Mendelian disorder marked by low cholesterol levels and lifelong protection against CAD. Interestingly, individuals affected by the disorder were compound heterozygotes for 2 rare nonsense mutations in the angiopoietin-like 3 protein (ANGPTL3). Further, in a separate cohort, a gene dose effect from the variants was observed with single allele carriers having substantially lower low-density lipoprotein cholesterol levels than noncarriers. Notably, ANGPTL3 is primarily expressed and secreted in the liver with inactivation of the gene leading to lower plasma cholesterol in mice. Thus, ANGPTL3 may serve as a valuable new therapeutic target for low-density lipoprotein reduction and prevention of CAD in the future.

Third, novel approaches in genetic association studies using “Mendelian randomization” principles have assisted in firmly establishing the link between key intermediate phenotypes (inflammation, plasma lipoproteins, glucose levels) and many complex traits (17,19,20). This approach leverages the fact that genes undergo random assortment when forming gametes and are transferred in unbiased fashion
from parent to offspring during the time of conception (20,21). Thus, a Mendelian randomization study that assesses the influence of a gene product on a biologic outcome is, in essence, naturally providing the same principle of a randomized trial but, in some ways, may be considered superior. Potential confounders of these studies include additional potential causative genes that may be nearby and in linkage disequilibrium with gene variants being studied, as well as population admixture from ancestral populations that carry different risks for disease and different genotypes. However, both of these confounders can be easily accounted for by proper design of such studies.

An ideal application of Mendelian randomization principles is well illustrated in a recent publication by Zacho et al. (22) on C-reactive protein (CRP)—a well-known marker of inflammation strongly tied to CAD. Notably, before this study, the question of whether CRP was simply a marker for CAD or actually contributed to disease causation remained unanswered. To answer this question, the investigators assessed 2 independent populations involving over 50,000 subjects and found that 4 defined CRP gene polymorphisms with a known influence on plasma CRP levels did not predict IHD. Conversely, the relationship between elevated CRP and IHD remained strong, thereby indicating that lifelong elevation of CRP levels as indicated by genetic polymorphisms does not confer risk for IHD and that CRP is simply a marker of and not likely a contributor to CAD risk.

These seminal findings recently took on greater meaning with the publication of the high profile JUPITER (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) (23). In JUPITER, individuals with elevated CRP levels treated with rosuvastatin had a 44% reduction in IHD events that was independent of lipid status. Unfortunately, a control group with low CRP and low low-density lipoprotein was not included in the study. Nevertheless, when reconciling JUPITER with genetic data on CRP, it is clear that the benefit from rosuvastatin is most likely secondary to inadvertent decrease in inflammation and not from a direct reduction in plasma CRP levels. Moreover, the genetic data on CRP further validate the previously touted pleiotropic effects of statins with respect to inflammation reduction and potentially support the use of statins to prevent CAD in high-risk patients with elevated CRP levels and normal plasma lipoproteins.
Lipoprotein (a)

The lipoprotein (a) \([Lp (a)]\) molecule is composed of a low-density lipoprotein that is covalently linked to the plasminogen-like glycoprotein, apolipoprotein (a) \((Apo(a))\) \((24)\). Notably, \(Lp (a)\) has been regarded as a putative cardiovascular risk factor for over 4 decades \((24)\). However, the extent of its contribution to IHD has been controversial and unsettled \((25)\).

Now, 3 independently conducted genomic studies have underscored the importance and clearly defined the role of \(Lp (a)\) in CAD and MI \((17,19,26)\).

The initial confirmatory study was an extensive 3-stage GWAS \((26)\). In the first stage, the investigators assayed over 500,000 SNPs in 2,000 cases and controls. Subsequently, the most significant SNPs \((p < 10^{-5})\) were then validated in a second cohort consisting of 875 CAD cases and 1,644 controls. Interestingly, a single haplotype on chromosome 6q26-q27, which encompasses the \(LPA\) gene, remained highly significant and was subsequently validated in a third stage. The final population-adjusted analysis of all 3 stages demonstrated that 2 \(LPA\) haplotypes were highly significant for CAD \((p = 1.0 \times 10^{-13}, p = 1.0 \times 10^{-15})\) with odds ratios (OR) of 1.2 and 1.8, respectively.

In a parallel study that used Mendelian randomization principles, Kamstrup et al. \((19)\) found that common kringle IV type II (KIV-2) copy number polymorphisms in \(LPA\), which are known to influence both \(LPA\) particle size and \(Lp (a)\) plasma levels, correlated with a risk for MI \((OR: 1.2)\) independent of plasma \(Lp (a)\) levels \((19,27)\). Further, in a subgroup analysis of a 9,000-patient prospective cohort with over 16 years of follow-up, they demonstrated that at-risk KIV-2 copy number polymorphism carriers had an impressive 150% increase in risk for coronary events.

In the third and final related study, investigators used a novel cardiac gene chip and assayed for 48,742 common and rare SNPs from several candidate genes in over 7,000 CAD cases and controls \((17)\). Remarkably, the \(LPA\) locus on 6q26-27 that was originally identified in the previously noted GWAS most strongly correlated with CAD \((26)\). Carriers of a single common variant \(rs10455872\) (MAF = 7%) or an independent rare variant \(rs3798220\) (MAF = 2%) had an OR of 1.70 and 1.92, respectively, or a striking 2.5 when carrying both at-risk variants \((Fig. 3)\). Moreover, these variants were shown to tag individual \(LPA\) alleles with fewer KIV-2 repeats, thereby confirming data linking smaller \(LPA\) isoforms to a heightened risk for CAD \((27,28)\).

Most recently, the European Atherosclerosis Society has recommended routine screening for \(Lp (a)\) levels and treatment with niacin for individuals with plasma \(Lp (a)\) levels \(>50 \text{ mg/dl}\) \((20)\). However, the findings from the genomic studies covered here suggest that \(LPA\) screening for susceptibility variants may be a better initial screening tool for many reasons. Current widely used \(Lp (a)\) assays do not fully account for important qualitative features including...
CDKN2A and CDKN2B provided preliminary evidence linking 9p21 SNPs to altered weight gain, a substantially larger number of tumors, and an overall increased death rate in the mutant mice when compared with wild-type mice. Interestingly, there was no evidence for increased atherosclerotic plaque burden in the aortas of the knockout mice.

Although highly informative, these findings raise additional questions regarding the 9p21 risk locus (32). For example, why was there no observed increase of atherosclerotic burden in the mutant mice, when this has clearly been the case in humans? Overall, it appears that the murine model may not be fully adequate for precisely defining the mechanistic influence of 9p21 variants on multiple arterial phenotypes. This theory is supported by the only moderate homology (<50%) present between the human and mouse 9p21 locus. Additionally, the effects from fully deleting a 70-kilobase region, as Visel et al. (41) did in their mouse model, versus carrying a few 9p21 SNPs as humans do, could yield entirely different phenotypes. Most recently, Harismendy et al. (42) demonstrated that the 9p21.3 locus is most likely exerting its effects through key inflammatory pathways triggered by local and long distance gene interactions. In order to determine this, they first examined transcription factor binding and chromatin modification profiles in various human cell lines, which revealed that the 9p21.3 region contains over 30 enhancers — one of the highest densities for predicted enhancers in the human genome. Second, through computational modeling, they found that the 9p21.3 SNP rs10757278, which is the SNP most consistently associated with coronary artery disease in previous GWAS, resides within an enhancer site (ECAD9) and disrupts the binding of STAT1 — a transcription factor critically involved in inflammation and interferon- signaling. These data emphasize the emerging link between inflammation and a variety of discrete vascular phenotypes that were previously thought to be unrelated.

Potential genomic markers of sudden cardiac death are also emerging. Recently, Bezzina et al. (43) assessed over 500,000 SNPs in 515 individuals with ventricular fibrillation and MI and 457 controls with MI alone. Strikingly, the most significant associated SNP, rs2824292 (p = 2.2 × 10^{-10}) was present in over 50% of cases and conferred an impressive 180% increase in risk for ventricular fibrillation. Perhaps most compelling is that the susceptibility allele is located near the gene CXADR, which encodes the Coxsackie virus and adenovirus receptor protein. This transmembrane tight junction protein has been previously implicated in both virus-mediated cardiomyopathies and in sudden cardiac death in candidate gene-based studies. Additional mechanistic studies will need to be performed to precisely define the basis of this variant’s influence on ventricular tachyarrhythmias. However, the genomic underpinnings of ventricular fibrillation illuminated by this study may lead to better predictive algorithms and preventive measures in MI patients at high risk for sudden cardiac death.
Apolipoprotein E (APOE) polymorphisms have been the most widely studied genetic risk factors in humans due to their well-established links to Alzheimer’s dementia, dyslipidemia, and CAD (44,45). Carriers of the E4 allele, which represent approximately 20% of the population of European ancestry, have higher circulating cholesterol levels than their common E2 and E3 counterparts (44,46). Most importantly, these carriers are at increased risk for CAD (44,46).

First discovered in 1977, APOE4 polymorphisms have been firmly linked to dyslipidemia and CAD in over 50 studies (44). Several GWAS have now confirmed this association (47). Further, a decade ago, investigators found that ApoEIV carriers had reduced survival rates after MI, which was abolished by treatment with simvastatin (48). Notably, the derived benefit from statin therapy did not relate to greater lipid lowering, which, once again, provides further evidence for a pleiotropic effect of statin use.

Based on these data, it may be possible to use the APOE4 genotype for CAD risk prediction and institution of statin therapy in patients with moderate risk factors for IHD. Such a strategy would be suitable for a prospective study, or if the data were available from large-scale, placebo-controlled statin trials with multiyear follow-up, validation might be supported. However, the psychosocial concerns of assessing APOE status for CAD and the need for disclosure of risk for Alzheimer’s has, at least in part, limited its use. Importantly, a recent study demonstrated that disclosing APOE genotype data to adult children of an affected Alzheimer’s disease parent did not result in significant anxiety or psychological distress (49). Thus indicating that current concerns related to APOE4 testing and disclosure may be overblown.

In contrast to the APOE4 pharmacogenetic story, recent claims that a KIF6 gene variant predicts statin response have been dubious (50). Surprisingly, over 150,000 KIF6 “Stat-incheck” tests have been ordered based solely on 3 retrospective candidate gene studies that demonstrated a modest increase in CAD risk (OR: 1.1 to 1.5) (51–53) and another study that found statin therapy abrogated this enhanced risk (54). However, several troublesome aspects of KIF6 data exist. First, unlike APOE, KIF6 is not expressed in the vasculature and has no known biologic relevance in dyslipidemia or CAD (55). Second, none of the over 10 GWAS on lipids or CAD have linked KIF6 to either phenotype (26,33–35,47,56–60). Third, a recent well-conducted meta-analysis in over 17,000 individuals found no link between CAD and KIF6 (61). Thus, although many of the examples herein exemplify the promises of genomic medicine, the KIF6 story should serve as a valuable reminder of the pitfalls present when prematurely adopting a genetic test in clinical practice.

Adjunctive aspirin and clopidogrel use in the management of patients with acute coronary syndromes and those receiving coronary stents has substantially reduced the risk for MI, stent thrombosis, and death (62–64). However, in 2006, a variable antiplatelet effect with clopidogrel was observed in hepatic cytochrome (CYP) 2C19 loss-of-function variant carriers (65). Now, several large studies involving thousands of patients have confirmed that genetic resistance to clopidogrel is prevalent even in patients with acute coronary syndromes (66–69). Importantly, the at-risk variants result in reduced clopidogrel active metabolite formation, diminished antiplatelet effect, and a >3-fold increase in risk for stent thrombosis, MI, and death (66–68,70,71). Additionally, recent studies have also identified a common CYP2C19 gain-of-function variant that confers a 2-fold increase in risk for bleeding (Fig. 4) (72). Remarkably, these gain- and loss-of-function variants are highly common with one-third of Europeans and close to one-half of those with African and Asian ancestry harboring the at-risk alleles.

Similar to the APOE and LP4 variants, the CYP2C19 variants were originally identified through hypothesis-driven candidate gene studies. Now, Shuldiner et al. (70), through GWAS, have incontrovertibly confirmed that the predominant mediator of genetic resistance to clopidogrel is the hepatic CYP2C19 locus. To determine this, the investigators first measured platelet aggregation at baseline and within 1 h after the last dose of clopidogrel on day 7. Subsequently, over 400,000 SNPs were simultaneously assessed in a GWAS of platelet reactivity. Not surprisingly, the region most significantly associated with clopidogrel response clustered around the CYP2C19 locus. Moreover, CYP2C19 variant carriers had a striking 345% increase in risk for stent thrombosis, along

![Figure 4. CYP2C19*17 Genotypes and Incidence of TIMI Bleedings](image-url)

Reprinted, with permission, from Sibbing et al. (72). CYP = hepatic cytochrome; TIMI = Thrombolysis In Myocardial Infarction.
with heightened risk MI, and death, which is consistent with previous candidate gene study results.

The clopidogrel story represents the prototypical scenario for individualizing medicine based on key pharmacogenomic information. It is 1 of the most highly prescribed drugs in the world and is used routinely to prevent stent thrombosis, MI, and death in the over 1 million people receiving coronary stents in the United States annually (73). Most importantly, alternatives to standard clopidogrel dosing are readily available for individuals resistant to the drug. These alternatives include the addition of cilostazol, or the use of alternative P2Y12 receptor blocking antiplatelet agents such as prasugrel or ticagrelor (74–77). Further, genotyping for at-risk CYP2C19 variants can be performed in patients undergoing coronary stenting and enables identification of those individuals at greatest risk for thrombotic complications. These high-risk individuals can then be closely monitored during the post-stenting period with platelet function testing to ensure adequate antiplatelet response. This is especially important given that stent thrombosis, although only occurring in <2% of patients, carries a mortality rate of over 40% and occurs most frequently within hours of stenting (78–80). Consistent with this line of reasoning, a recently released U.S. Food and Drug Administration boxed warning has recommended such an individualized approach to antiplatelet therapy in patients receiving coronary stents (74).

Another recent key discovery in antiplatelet pharmacogenomics relates to the identification of a rare SNP in LPA that predicts with high accuracy those individuals most likely to benefit from aspirin therapy (81,82). Chasman et al. (81) reported that the nonsynonymous SNP, rs3798220 (MAF = 3.5%), which encodes for an isoleucine to methionine substitution (Ile4399Met), resulted in an 8-fold increase in Lp (a) plasma levels and a corresponding 2-fold increase in risk for MI and stroke. Interestingly, this heightened risk was completely abrogated in the Ile4399Met carriers by aspirin therapy. This report is especially timely given recent meta-analysis data showing an exceedingly small benefit and substantial risk for bleeding with the nondiscretionary use of aspirin in a primary prevention setting (83). Moreover, as previously mentioned, Clarke et al. (17) recently found that the same rare SNP (rs3798220) conferred a similar relative risk for CAD in a separate study involving over 7,000 CAD cases and controls. Unfortunately, this study did not test the aspirin hypothesis.

Along these lines, in a recent publication of the first clinically annotated whole-genome sequence, over 60 pharmacogenetic variants with an immediate influence on drug efficacy and toxicity were detected in an otherwise healthy 40-year-old male geneticist who had sequenced his own genome (84). Notably, his variants included the at-risk LPA variant (rs3798220), which resulted in a recommendation by the individual’s physician to initiate aspirin therapy for primary prevention of CAD.

Caution in Interpreting Negative SNP Profiling Studies

Earlier this year, Paynter et al. (85) reported on the prognostic capability of 101 SNPs linked to CAD through

### Table 1. Recent Advances in Ischemic Heart Disease Genomics

<table>
<thead>
<tr>
<th>Gene or Locus</th>
<th>Condition</th>
<th>Experimental Methods</th>
<th>Effect Size (OR)</th>
<th>Ref. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>9p21.3 (CDKN2A, CDKN2B)</td>
<td>MI</td>
<td>GWAS</td>
<td>1.2–1.4</td>
<td>32–34,37,39</td>
</tr>
<tr>
<td></td>
<td>AAA</td>
<td></td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intracranial aneurysm</td>
<td></td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PAD</td>
<td></td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>LPA</td>
<td>CAD</td>
<td>GWAS, candidate gene, resequencing</td>
<td>1.7–1.9</td>
<td>17,81</td>
</tr>
<tr>
<td></td>
<td>Enhanced aspirin response</td>
<td></td>
<td>2.2*</td>
<td></td>
</tr>
<tr>
<td>APOE</td>
<td>CAD, dyslipidemia</td>
<td>GWAS, candidate gene, resequencing</td>
<td>1.1–1.4</td>
<td>44,45</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Stent thrombosis (*2 –*5 alleles)</td>
<td>GWAS, candidate gene, resequencing</td>
<td>3.5</td>
<td>70,71</td>
</tr>
<tr>
<td></td>
<td>Bleeding (*17 allele)</td>
<td></td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>21q21 (CXADR)</td>
<td>Ventricular fibrillation</td>
<td>GWAS</td>
<td>1.5–1.8</td>
<td>43</td>
</tr>
<tr>
<td>DAB2IP</td>
<td>Early onset MI</td>
<td>GWAS</td>
<td>1.18</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>AAA</td>
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<td>1.21</td>
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<td></td>
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<td></td>
<td>1.20</td>
<td></td>
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<tr>
<td></td>
<td>PAD</td>
<td></td>
<td>1.14</td>
<td></td>
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</tbody>
</table>

*The odds ratio represents the increased risk for CAD in rs3798220 carriers. The enhanced risk was completely abrogated by aspirin therapy.

AAA = abdominal aortic aneurysm; APOE = apolipoprotein E; CAD = coronary artery disease; CYP = hepatic cytochrome; GWAS = genome-wide association studies; MI = myocardial infarction; OR = odds ratio; PAD = peripheral artery disease; PE = pulmonary embolism.
previous GWAS. An integrated “genetic risk score” for CAD based on the carrier status of these 101 SNPs did predict a heightened risk for CAD, but failed to incrementally add to models based on traditional risk factors. However, there are several important limitations of this study that require addressing. First, all 101 variants received equal weighting in their model, despite clear differential and more potent disease-causing effects from variants such as those in the 9p21 locus, which promotes a washout of any informative potential of the SNPs assessed. Second, many of the SNPs were from GWAS that used variable definitions for CAD cases and healthy controls. Third, some of the most important known CAD SNPs, such as those in LPA, were not included. Fourth, a family history of premature CAD continued to be predictive for the development of CAD even after adjustment for the traditional risk factors, indicating important genetic underpinnings of this disease that have yet to be fully defined. Finally, a recent study has indicated that the simultaneous assessment of all disease-associated SNPs proportionally increases the prediction of disease heritability (86), thus indicating that current genetic risk scores must include all relevant rare and common SNPs (including those yet to be discovered) with adequate weighting to accurately portray the individual’s true genetic risk for disease. To date, no such model has been developed.

**Future Directions**

Over the last 4 years, the breakneck pace of discoveries into the genomic underpinnings of complex traits has largely been enabled by genome-wide assessment of common SNPs (Table 1). Unfortunately, substantial portions of the heritability of many complex traits, including CAD, remain missing. This missing heritability or “dark matter” of the genome will likely be unraveled in incremental fashion in the years ahead through the identification and validation of rare susceptibility SNPs and disease-causing structural variants in properly designed, large-scale, whole-genome sequencing studies (12). Moreover, comprehensive delineation of tissue-specific epigenetic characteristics such as DNA methylation, histone modification, and chromatin state will provide additional insight into biologic mechanisms of disease. However, access to arterial and myocardial tissue for epigenetic studies will be limited, so less-invasive approaches to access these tissues will be needed. Recent advances in rare cell biology that sequester circulating endothelial cells from the peripheral blood are exemplary of such noninvasive approaches (87). Additionally, the concurrent implementation of proteomic and metabolomic technologies in genomic studies will provide vital information on the biologic alterations present in various disease states, while also serving to uncover novel therapeutic targets.

**Conclusions**

In summary, the emerging applications in CAD genomics and pharmacogenomics covered in this review represent a preview of the transformative discoveries that will continue to surface in the months and years ahead. The challenge will be in determining when and how to implement such data into routine clinical practice. The clopidogrel and LPA scenarios represent 2 areas where the evidence threshold for individualizing therapy based on genotype data has been clearly surpassed. However, it will ultimately be the individual clinician that will decide when and how to use these data. Hence, systematic education and training of physicians on the benefits and drawbacks to genomic medicine must be performed for the full potential of the future era of individualized medicine to be realized.

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**REFERENCES**


56. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:616–78.


