BACKGROUND: It has long been recognized that 50% of the susceptibility for coronary artery disease (CAD) is due to predisposing genetic factors. Comprehensive prevention is likely to require knowledge of these genetic factors.

CONTENT: Using a genome-wide association study (GWAS), the Ottawa Heart Genomic Study and the deCODE group simultaneously identified the first genetic risk variant, at chromosome 9p21. The 9p21 variant became the first risk factor to be identified since 1964. 9p21 occurs in 75% of the population except for African Americans and is associated with a 25% increased risk for CAD with 1 copy and a 50% increased risk with 2 copies. Perhaps the most remarkable finding is that 9p21 is independent of all known risk factors, indicating there are factors contributing to the pathogenesis of CAD that are yet unknown. 9p21 in individuals with premature CAD is associated with a 1-fold increase in risk, similar to that of smoking and cholesterol. Routine genetic testing will probably remain controversial until a specific treatment is developed. Over a period of 5 years, however, GWASs have identified 30 genetic variants for CAD risk, of which only 6 act through the known risk factors.

SUMMARY: The 9p21 variant has now been established as an independent risk factor for CAD and, along with the additional 29 risk genetic variants recently identified, is likely to provide the thrust for genetic testing and personalized medicine in the near future.

Prevention of Heart Disease

The number one killer in the world remains coronary artery disease (CAD), despite the tremendous advances that have been made in treating this disease.

Several investigators have postulated that CAD can be prevented if treated early (1, 2). The 21st century is expected to be known for the prevention of this disease rather than just treatment of the crisis. CAD is believed to be largely preventable on the basis of evidence from randomized placebo-controlled clinical trials. Modifying such known risk factors as cholesterol, hypertension, and smoking has repeatedly shown that 30% to 40% of deaths from CAD can be prevented (3–5), and the fact that a major proportion of the susceptibility to CAD is due to genetic risk factors has been recognized for more than 5 decades. Thus, if this disease is to be markedly reduced or eliminated, comprehensive prevention will require knowledge of the genetic risk factors. Epidemiology, family, and other studies suggest that approximately 50% of the susceptibility to CAD is due to genetic risk (6).

The power of a family history is illustrated in a recent study from the state of Utah (7). Fourteen percent of this population has a family history of CAD, which accounts for 72% of the cases of early CAD (<50 years) and 48% of all CAD events occurring at all ages. Similarly, 11% of the Utah population has a family history of stroke, and 86% of all early strokes occur in this cohort (7). In the Framingham Study, men with a family history of CAD had a 2.6-fold increased risk for CAD, and women had a 2.3-fold increased risk (8). A similar increased risk for CAD was observed in the recent INTERHEART (1) and PROCAM (9) studies for the participants with a family history of CAD.

Rare Genes vs Genes Predisposing to Common Diseases

In contrast to rare single-gene disorders, 80% of deaths worldwide are due to 20 diseases (10). CAD, cancer, and infections account for more than two thirds of these deaths (10). These diseases are common, and genetic predisposition is thought to be due to common polymorphisms. Unlike rare single-gene disorders, genetic susceptibility to such diseases is imparted by multiple genes. In single-gene disorders, the gene is both necessary and sufficient for disease expression; in common diseases, genetic predisposition is thought to be due to common polymorphisms. Unlike rare single-gene disorders, genetic susceptibility to such diseases is imparted by multiple genes. In single-gene disorders, the gene is both

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2 Nonstandard abbreviations: CAD, coronary artery disease; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; pRb, retinoblastoma protein; p53, protein 53; LINE, long interspersed element; MIR, mammalian-wide interspersed repeat; SINE, short interspersed element; CARDioGRAM, Coronary Artery Disease Genome-wide Replication and Meta-analysis.

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necessary and sufficient to induce the phenotype. In fact, once the mutation for a single-gene disorder has been identified, one can inject the human gene into the egg of a mouse (transgenic), and the phenotype of the offspring will confirm that this mutation induces the phenotype (11). In contrast, for such polygenic disorders as CAD, no one gene is sufficient or necessary to induce the disease (12). It was also appreciated in the 1990s that the genetic susceptibility for common diseases is carried by multiple genes, with each gene having minimal effect. It is the sum of the genes that predisposes an individual to the disease, rather than any single gene. The detection and mapping of the locations of the genes predisposing to a polygenic disease would require hundreds of thousands of DNA markers. Computerized studies have estimated that at least 1 marker would be required every 6000 bp (13). Moreover, linkage analysis based on genotyping pedigrees lacks the analytical sensitivity to map the locations of these predisposing genes (14, 15). It would require what is now known as a case–control association study (15).

The Basis for the Genome-Wide Association Study

The fundamental principle involved in case–control association studies is quite simple and depends on comparing the frequencies of a DNA marker in cases and controls. If a particular DNA marker is more frequent in cases than controls, then that marker is in close proximity to a DNA sequence associated with an increased risk for the disease. In the 1990s, the approach taken to case–control association studies was the candidate gene approach. In this approach, a particular gene known to be a good candidate for the disease is selected and genotyped in a few hundred cases and a few hundred controls. Many candidate genes were claimed to be associated with an increased susceptibility to CAD, as well as to many other diseases, such as diabetes and hypertension. This approach was recognized to be biased, however, and to require replication in an independent population. Subsequent attempts to replicate these results in independent populations have generally been unsuccessful (16, 17).

The appropriate technology for pursuing an unbiased approach to predisposing genes for common disorders, which has been referred to as “genome-wide association studies” (GWASs), was not feasible until 2005 (12), when a microarray was developed with 500,000 single-nucleotide polymorphisms (SNPs) that were selected as DNA markers to span the human genome at an interval of every 6000 bp (on average). Microarrays now have up to $1 \times 10^6$ markers, so that the difference between the frequencies of a DNA marker in cases and controls has to be significant at a $P$ value of $5.0 \times 10^{-8}$. This level of significance is referred to as “genome-wide significance” (18). Although it is overly stringent, this level of significance became accepted as the norm. For reducing false positives further, replicating the findings in an independent sample with the significance level determined by a Bonferroni correction was also recommended. The success to follow this technological breakthrough would be fast and extensive.

Discovery of 9p21: The First Genetic Risk Variant Predisposing to CAD

In just the 4 years since the identification of the initial risk variant on chromosome 9p21 (hereafter referred to as “9p21”), a total of 30 risk variants for CAD have been discovered and replicated in appropriate independent populations. Because the results of the studies on most of these variants were not published until 2011, there is very little functional information on the individual variants besides the observation that most of them reside in non–protein-coding regions. That fact suggests that these variants have a regulatory role in the expression of protein-coding genes. Elucidation of the risk target of these variants and their mechanisms is further complicated by the observation that most of them do not act through the known risk factors for CAD. The 9p21 variant, which is estimated to occur in $>4 \times 10^9$ individuals, occurs in a non–protein-coding region and mediates its risk independently of all known risk factors. Because this variant was the first to be identified, there is more information for it, which we discuss in detail below. Furthermore, the 9p21 variant exemplifies several of the features that the genetic variants have in common as risk variants for CAD, including the exciting potential of its novel risk-mediating pathway to provide new drug targets, as well as the barriers to overcome before routine clinical use of any such drugs.

9p21: A Risk Factor in All Ethnic Groups except African Americans

Within a month after the initial discovery of the 9p21 variant (19, 20), the Wellcome Trust Study (20) confirmed the 9p21 variant as a risk factor for CAD, and numerous other studies throughout Europe and North America subsequently confirmed 9p21 as a CAD risk factor in Caucasians (21–23). These studies established that 9p21 is a risk factor for CAD, its risk is independent of known risk factors, and it has a frequency of 75% in Caucasians. These conclusions were well summarized in a metaanalysis of 22 studies of 9p21 by Palomaki et al. (24). GWASs of populations of different ethnic origins followed. 9p21 was shown to be a risk
factor for CAD in the Chinese population (25), the Korean population (26), and the Italian population (27). 9p21 was confirmed as a risk factor in the Korean (28), Japanese (28), and Chinese populations (29). In a study involving a large number of Southeast Asians, Assimes et al. (30) showed that 9p21 was a CAD risk factor with features similar to those shown in the Caucasian studies; however, African Americans did not exhibit 9p21 as a risk factor in this study (30). In a more recent study, the Coronary Artery Disease Genetics Consortium (31) performed a GWAS of a discovery population of 30,482 individuals that included 6996 cases of South Asian ancestry from Pakistan and India, with replication in a sample size of 40,593 individuals that included 6187 South Asians. 9p21 was confirmed to be a risk factor for CAD with a frequency and risk odds ratio similar to those of the Caucasian population (26). Although the studies of African Americans have been limited, 9p21 appears to be a risk factor in all ethnic groups that migrated out of Africa. These groups appear not to have had the time to intermingle and break up the DNA haplotype that carries the risk. In contrast, in Africans, who have had a much longer history, the 9p21 risk region (haplotype) has been broken into smaller haplotypes that produce minimal or no risk for CAD (30).

9p21: An Independent and Novel CAD Risk Factor

The last risk factor announced for CAD was in 1964, when smoking was added to the risk factors already known from the Framingham Study (32). Given the prevalence of the 9p21 variant, 1 or 2 copies are estimated to be present in >4 × 10^6 individuals. 9p21 has also been shown to increase the risk by 2-fold in premature CAD (21, 28, 33). Because 9p21 will be one of many genetic risk factors discovered and there are surely more to come, “9p21” will probably become a label for a class of genetic risk factors. However, as we elucidate the risk-mediating mechanisms of the genetic variants and as they become part of a panel for genetic testing, they may remain as separate factors relating to their specific functions or risk-mediating pathways. Two concerns that have been expressed are that the 9p21 haplotype, despite its prevalence, may not contribute greatly to the predictability of cardiovascular events and that there is no specific treatment currently. Although both of these concerns are appropriate, they should not deny 9p21 of its recognition as a risk factor for CAD or as part of the genetic risk group. Collins has pointed out repeatedly that neither the frequency nor the effect of a genetic variant correlates with the therapeutic impact of a drug discovered on the basis of a target in the variant’s risk-mediating molecular pathway (34). An example is the hydroxymethylglutaryl-CoA reductase inhibitors known as statins. The identification of a genetic defect in the gene encoding the cholesterol receptor responsible for familial hypercholesterolemia, a rare disorder (1 in 5000), led to the development of the current number one drug for the prevention of CAD (35). Had we not already known cholesterol to be a risk factor for CAD and ignored this observation, we might not have had statin therapies today.

9p21: A Risk Factor for Intracranial Aneurysms and Abdominal Aortic Aneurysms

Studies by the deCODE group based on a GWAS have implicated 9p21 as a risk factor for abdominal aortic aneurysms, intracranial aneurysms, and stroke (36). The risk for heterozygous and homozygous carriers of the risk allele was estimated at 1.36 and 1.74, respectively, for abdominal aortic aneurysms, and 1.38 and 1.72 for intracranial aneurysms. A subsequent study by Bilguvar et al. (37) that included 21,000 cases of stroke caused by intracranial aneurysms and 8000 controls showed an increased risk of 24% to 36% for stroke in patients with the 9p21 risk allele. A GWAS by Wahlstrand et al. in Sweden showed that 9p21 was associated with an increased risk for stroke independent of hypertension (38). Subsequent studies have confirmed the association of the 9p21 allele with intracranial aneurysms. Yasuno et al. (39) used arrays of 832,000 SNPs to perform a GWAS of 5891 cases of intracranial aneurysms and 14,181 controls and found the 9p21 risk variant to be a major risk factor for stroke and intracranial aneurysms. Matarin et al. (40) showed 9p21 to be a risk factor for stroke, a finding in accordance with the results of previous GWASs.

Genomics of the 9p21 Risk Variant

The 9p21 genome region is complex and requires some consideration. The region consists of CDKN2B-AS [CDKN2B antisense RNA (non-protein coding), also known as ANRIL (antisense RNA in the INK4 locus)], which encodes an antisense noncoding RNA spanning 126,000 bp (41). CDKN2B-AS is adjacent to the CDKN2A [cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)] and CDKN2B [cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)] genes, which encode

3 Human genes: CDKN2B-AS, CDKN2B antisense RNA (non-protein coding), also known as ANRIL (antisense RNA in the INK4 locus); CDKN2A, cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4); CDKN2B, cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4); ADAMTS7, ADAM metallopeptidase with thrombospondin type 1 motif, 7; ABG, ABG blood group (transf erase A, alpha 1–3-N-acetylgalactosaminyltransferase; transf erase B, alpha 1–3-galactosyltransferase).
the cyclin-dependent kinase inhibitors. In fact, the sequence of CDKN2B-AS overlaps that of CDKN2B. This region has been the target of cancer research for some time because of genes that regulate cell growth, namely CDKN2A and CDKN2B. Two pivotal pathways are involved with cell growth (42). One is regulated by the retinoblastoma protein (pRb), which arrests cell growth in the G1 phase, and the other is regulated by protein 53 (p53), which arrests cell growth in the G1 and G2 phases. CDKN2A or CDKN2B can inhibit the pRb pathway and arrest cells in the G1 phase, whereas an alternative transcript of CDKN2A inhibits the p53 and pRb pathways. Numerous studies have shown that knock-out of CDKN2A or CDKN2B in mice is associated with the appearance of tumors within months (43). The potential connection between 9p21 and atherosclerosis may be related to the antiproliferative action of the cyclin-dependent kinase inhibitors, which are known to be depressed in individuals with the 9p21 risk allele.

Phylogenetic studies have shown that CDKN2B-AS (i.e., ANRIL) is highly conserved in primates but is not present in orthologous sequences from dog, mouse, rat, and opossum (44). The CDKN2B-AS region is complex, with many repetitive elements. Exons 7 and 12 consist entirely of Alu repeats, exons 8 and 16 consist of long terminal repeats, exon 14 contains long interspersed elements (LINEs) of the LINE2 family, and exons 4, 17, and 20 contain multiple MIR (mammalian-wide interspersed repeat) short interspersed elements (SINE) repeats. Within this CDKN2B-AS region is a 58 000-bp region that contains the SNPs associated with increased CAD risk. SNPs outside of this region, even those in CDKN2A or CDKN2B, show only a weak association with CAD. Thus, there is confirmatory evidence that the 58 000-bp haplotype contains the polymorphism(s) responsible for the increased risk for CAD. Notable, however, is that expression of the 2 adjacent genes, CDKN2A and CDKN2B, inhibits proliferation in the vascular endothelium, particularly smooth muscle proliferation. Smooth muscle proliferation is a good culprit for atherosclerosis. Another possible link with atherosclerosis is the presence of multiple conserved enhancers in the CDKN2B-AS region, which have been shown to be associated with the upregulation of several genes that induce proliferation (44). This phenomenon was studied in greater detail by Harismendy et al. (45), who identified 33 enhancers in the region, several of which are in the 58 000-bp risk region.

The Site of Action for the 9p21 Variant Is the Vessel Wall

Although the mechanism of action by which the 9p21 variant mediates its increased risk for CAD is unknown, evidence suggests it is at the vessel wall. Several studies indicate 9p21 is involved with the initiation and possibly the progression of atherosclerosis without having an effect on plaque rupture or thrombosis, events known to precipitate myocardial infarction. Several independent investigators have shown that 9p21 is a risk factor not only for CAD but also for abdominal aortic aneurysms and intracranial aneurysms. Neither abdominal aortic aneurysms nor intracranial aneurysms are associated with atherosclerosis. The pathology in aneurysms involves thinning and loss of tissue from the wall, leading to rupture rather than atherosclerosis or fatty plaque. In the Ottawa Heart Genomic Study, 950 cases of early-onset CAD were analyzed for the number of coronary vessels showing ≥50% obstruction in coronary angiograms. A similar analysis with coronary angiography was also performed with 517 patients with late-onset CAD. Approximately 50% of the patients had myocardial infarction superimposed on the atherosclerosis (46). The analysis of the 9p21 variant as a risk factor indicated a strong association with coronary atherosclerosis, and 9p21 was a predictor of severity, as assessed by the number of vessels involved (46). Individuals with single-vessel disease had the lowest dose of 9p21 risk, whereas individuals with 3-vessel disease had the highest frequency of 2 copies of the 9p21 risk allele. Interestingly, there was only a minimal association between 9p21 risk and myocardial infarction. Several other studies have since confirmed the close association of the 9p21 risk allele with atheroma without any association with myocardial infarction (47–51). Some studies have suggested that 9p21 initiates the process of atherosclerosis rather than being related to its progression or severity (47). All of the studies suggest that 9p21 exhibits greater risk in individuals who develop premature CAD (<55 years in men and <60 years in women). Thus, 9p21 mediates its risk at the vessel wall, which leads to the onset of atherosclerosis in coronary vessels and abnormal growth or other abnormalities in the abdominal aorta and intracranial vessels.

Molecular Mechanisms for 9p21

Several investigators have confirmed that the polymorphism(s) that increase the risk for CAD at the 9p21 locus are confined to a 58 000-bp region that is part of the DNA region from which the CDKN2B-AS transcript originates. CDKN2B-AS encodes a long noncoding antisense RNA of 126 000 bp and is transcribed into several alternative forms. It is also well established that CDKN2B-AS is adjacent to CDKN2A and CDKN2B, which are involved with cell cycle regulation and encode cyclin-dependent kinase inhibitors that inhibit cell growth and proliferation. It appears that the CDKN2B-AS transcript regulates the expression of CDKN2A and CDKN2B; however, the mechanism has...
yet to be elucidated. One postulate is that increased expression of CDKN2B-AS increases the expression of the genes encoding the cyclin-dependent kinase inhibitors, preventing cell proliferation. Owing to some form of feedback or cell stimulus, however, the increasing concentrations of the CDKN2B-AS transcript attract the suppressor polycomple complex. The CDKN2B-AS transcript binds to transcription factor chromobox 7, which trimethylates lysine 27 on histone H3, thereby silencing the CDKN2A and CDKN2B genes. This mechanism was elucidated by Yap et al. (52). This mechanism is also compatible with the findings that increased expression of the long transcript of CDKN2B-AS is associated with increased expression of CDKN2A and CDKN2B in the presence of the 9p21 nonrisk allele and that cells with the 9p21 risk allele show a decrease in the long transcript with a corresponding increase in the short CDKN2B-AS transcript, which is associated with decreased expression of CDKN2A and CDKN2B. These findings are also compatible with those of a study by Visel et al. (53), in which knock-out of the CDKN2B-AS region in the mouse produces marked suppression of CDKN2A and CDKN2B and a phenotype of cell proliferation. One potential mechanism is that the polymorphism in the 9p21 risk allele (the 58 000-bp CDKN2B-AS region) may induce higher concentrations of the CDKN2B-AS transcript, further suppressing CDKN2A and CDKN2B expression.

GWAS Has Identified 30 Genetic Variants for CAD Risk

Even though the technological advances occurred only about 6 years ago, the success of GWAS in mapping genetic variants for disease risk has been nothing short of remarkable. The first description of a CAD risk variant, the 9p21 risk allele, was published in 2007, and within 2 years, 11 more novel genetic variants showing an increased risk for CAD were mapped (54). The results of these studies indicated that most of the genetic variants have a modest to minimal effect on risk, with relative risk ratios varying from 10% to 30%. It was evident from these studies that to detect most of the variants predisposing to CAD would require larger sample sizes than previously expected. In response to this observation, 12 previously successful GWASs pursuing genes related to CAD formed CARDIoGRAM (Coronary Artery Disease Genome-wide Replication and Meta-analysis) (55). This consortium involved several universities across Europe and North America. This cooperation yielded a discovery population with a sample size of 86 995 (22 233 cases and 64 762 controls), all of European ancestry. Genotyping was performed with the 1 × 10^6 chip array, followed by importation of >2 × 10^6 SNPs from the HapMap Project. SNPs that showed a significant positive correlation and reached genome-wide significance (i.e., 5.0 × 10^-8) in the metaanalysis were replicated in an independent population with a sample size of 56 682. This analysis led to the identification of 13 new genetic variants for CAD risk and confirmation of 10 previously identified risk variants (56). Another study involving the CARDIoGRAM investigators (57) identified 2 novel variants for CAD: ADAMTS7 (ADAM metallo-protease with thrombospondin type 1 motif, 7) and the locus encoding the ABO blood group. The ADAMTS7 locus, like the 9p21 locus, increases the risk for coronary atherosclerosis but has no effect on the incidence of myocardial infarction. In contrast, the ABO [ABO blood group (transferase A, alpha 1–3-N-acetylglactosaminyltransferase; transferase B, alpha 1–3-galactosyltransferase)] locus is the first locus with genetic variants identified to increase the risk for myocardial infarction but to impart no increased risk for the underlying atherosclerosis. The genes for the A and B proteins encode a transferase protein that adds a carbohydrate moiety to von Willebrand factor, thereby prolonging its half-life and predisposing patients to thrombosis and myocardial infarction. The gene encoding the O protein does not possess this function, and thus the von Willebrand factor half-life is not prolonged.

The Coronary Artery Disease Genetics Consortium recently identified 4 additional loci related to CAD (31). This study involved a genome-wide search with replication in an independent population of Caucasians and East Asians. There was no difference between Asians and Caucasians in the frequency or risk effect of these 4 loci. Wang et al. identified a genetic variant at 6p21 that increases the risk for CAD in the Chinese population but has no effect in the Caucasian population (58). In just 5 years, 30 genetic variants have been identified to be associated with an increased risk for CAD (Table 1).

Relative Insights Gained from the CAD Genetic Variants

Although 30 genetic variants for CAD risk have been identified, very little information is known about them except for the 9p21 variant. Several observations, however, could be very insightful regarding the future utility of these risk factors:

1. Six of the variants mediate their risk through known risk factors for CAD. The corollary of this observation is that several mechanisms contributing to the pathogenesis of CAD have yet to be elucidated. Thus, several potential targets remain for the devel-
Table 1. CAD and myocardial infarction risk loci discovered by GWASs.

<table>
<thead>
<tr>
<th>Band</th>
<th>SNP</th>
<th>Nearby genes*</th>
<th>Risk allele frequency (allele)</th>
<th>Odds ratio (95% CI)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p32.3</td>
<td>rs11206510</td>
<td>PCSK9</td>
<td>0.82 (T)</td>
<td>1.15 (1.10–1.21)</td>
<td>MIGC(b) (65)</td>
</tr>
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<td>1p32.3</td>
<td>rs599839</td>
<td>SORT1</td>
<td>0.78 (A)</td>
<td>1.29 (1.18–1.40)</td>
<td>Samani et al. (66)</td>
</tr>
<tr>
<td>1q41</td>
<td>rs17465637</td>
<td>MIA3</td>
<td>0.74 (C)</td>
<td>1.20 (1.12–1.30)</td>
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</tr>
<tr>
<td>2q33.1</td>
<td>rs6725887</td>
<td>WDR12</td>
<td>0.15 (C)</td>
<td>1.16 (1.10–1.22)</td>
<td>MIGC (65)</td>
</tr>
<tr>
<td>3q22.3</td>
<td>rs2306374</td>
<td>MRAS</td>
<td>0.18 (C)</td>
<td>1.15 (1.11–1.19)</td>
<td>Erdmann et al. (67)</td>
</tr>
<tr>
<td>6p24.1</td>
<td>rs12526453</td>
<td>PHACTR1</td>
<td>0.67 (C)</td>
<td>1.13 (1.09–1.17)</td>
<td>MIGC (65)</td>
</tr>
<tr>
<td>6q5.3</td>
<td>rs3798220</td>
<td>LPA</td>
<td>0.02 (C)</td>
<td>1.92 (1.48–2.49)</td>
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</tr>
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<td>9p21.3</td>
<td>rs4977574</td>
<td>CDKN2A, CDKN2B</td>
<td>0.46 (G)</td>
<td>1.25 (1.18–1.31)</td>
<td>McPherson et al. (19), Helgadottir et al. (20)</td>
</tr>
<tr>
<td>10q11.2</td>
<td>rs1746048</td>
<td>CXCL12</td>
<td>0.87 (C)</td>
<td>1.33 (1.20–1.48)</td>
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<td>10q11.2</td>
<td>rs1222608</td>
<td>LDLR</td>
<td>0.77 (G)</td>
<td>1.14 (1.09–1.19)</td>
<td>MIGC (65)</td>
</tr>
<tr>
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<td>rs9982601</td>
<td>MRPS6</td>
<td>0.15 (T)</td>
<td>1.19 (1.13–1.27)</td>
<td>MIGC (65)</td>
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<td>rs17114036</td>
<td>PPA2B</td>
<td>0.91 (A)</td>
<td>1.17 (1.13–1.22)</td>
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<td>1.10 (1.07–1.13)</td>
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<td>rs12413409</td>
<td>CYP17A1, CNNM2, NT5C2</td>
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<td>ZNF259, APOA5, APOA4, APOC3, APOA1</td>
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<td>1.13 (1.10–1.16)</td>
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<td>0.44 (G)</td>
<td>1.07 (1.05–1.09)</td>
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<td>rs216172</td>
<td>SMG6, SRR</td>
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<td>1.07 (1.05–1.09)</td>
<td>Schunkert et al. (56)</td>
</tr>
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<td>rs1293687</td>
<td>RASD1, SMCR3, PEMT</td>
<td>0.56 (G)</td>
<td>1.07 (1.05–1.09)</td>
<td>Schunkert et al. (56)</td>
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<td>17q21.32</td>
<td>rs46522</td>
<td>UBE2Z, GIP, ATP5G1, SNF8</td>
<td>0.53 (T)</td>
<td>1.06 (1.04–1.08)</td>
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<td>rs974819</td>
<td>PDGF</td>
<td>0.29 (T)</td>
<td>1.07 (1.04–1.09)</td>
<td>CADGC (31)</td>
</tr>
<tr>
<td>7q23.3</td>
<td>rs1093541</td>
<td>BCAp29</td>
<td>0.75 (C)</td>
<td>1.08 (1.05–1.11)</td>
<td>CADGC (31)</td>
</tr>
<tr>
<td>10p11.23</td>
<td>rs2505083</td>
<td>KIAA1462</td>
<td>0.42 (C)</td>
<td>1.07 (1.04–1.09)</td>
<td>CADGC (31)</td>
</tr>
<tr>
<td>6p24.1</td>
<td>rs6903956</td>
<td>C6orf105</td>
<td>0.07 (A)</td>
<td>1.65 (1.44–1.90)</td>
<td>CADGC (31)</td>
</tr>
<tr>
<td>15q25.1</td>
<td>rs1994016</td>
<td>ADAMTS5</td>
<td>0.60 (C)</td>
<td>1.19 (1.13–1.24)</td>
<td>Reilly et al. (57)</td>
</tr>
</tbody>
</table>

*Genes not mentioned in the text: PCSK9, proprotein convertase subtilisin/kexin type 9; SORT1, sortilin 1; MIA3, melanoma inhibitory activity family, member 3; WDR12, WD repeat domain 12; MRAS, muscle RAS oncogene homolog; PHACTR1, phosphatase and actin regulator 1; LPA, lipoprotein, Lp(a); CXCL12, chemokine (C-X-C motif) ligand 12; SH2B3, SH2B3 adaptor protein 3; LDLR, low density lipoprotein receptor; MRPS6, mitochondrial ribosomal protein S6; PPA2B, phosphatidic acid phosphatase type 2B; ANKS1A, ankryin repeat and sterile alpha motif domain containing 1A; TCF21, transcription factor 21; ZC3H1, zinc finger, C3HC-type containing 1; CYP17A1, cytochrome P450, family 17, subfamily A, polypeptide 1; CNNM2, cyclin M2; NT5C2, 5'-nucleotidase, cytosolic II; ZNF259, zinc finger protein 259; APOA5, apolipoprotein A-V; APOA4, apolipoprotein A-IV; APOC3, apolipoprotein C-III; APOA1, apolipoprotein A-I; COL4A1, collagen, type IV, alpha 1; COL4A2, collagen, type IV, alpha 2; HHIPL1, HHIPL-like 1; SMG6, smg-6 homolog, nonsense mediated mRNA decay factor (C. elegans); SRR, serine racemase; RASD1, RAS, dexamethasone-induced 1; SMCR3, Smith-Magenis syndrome chromosome region, candidate 3; PEMT, phosphatidylethanolamine-N-methyltransferase; UBE2Z, ubiquitin-conjugating enzyme E2Z; GIP, gastric inhibitory polypeptide; ATP5G1, ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9); SNF8, SNF8, ESCR2-II complex subunit, homolog (S. cerevisiae); LIPA, lipase A, lysosomal acid, cholesterol esterase; PDGF, platelet-derived growth factor; BCAp29, B-cell receptor-associated protein 29; KIAA1462, KIAA1462; C6orf105, chromosome 6 open reading frame 105.

b MIGC, Myocardial Infarction Genetics Consortium; CADGC, Coronary Artery Disease Genetics Consortium.
opment of novel therapies, which would probably be essential for the comprehensive prevention and treatment of CAD.

2. The increased relative risk of CAD for each variant is minimal, varying from 6% to 17%.

3. The number of risk variants for CAD per individual varies from 15 to 37.

4. Most genetic variants exhibit a higher risk for early-onset CAD than for late-onset CAD.

5. The top 10th percentile and the lowest 10th percentile are associated with odds ratios for CAD of 1.88 and 0.55, respectively.

6. More than 70% of the genetic variants for CAD risk are located in DNA sequences that do not code for protein, implying that most of these polymorphisms exert their effects through the regulation of protein-coding sequences.

The Advantages of DNA Risk Variants over Conventional Biomarkers

Sampling for CAD risk factors such as cholesterol requires the patient to fast, and the results may be altered by various drugs. In contrast, DNA variants do not change during an individual’s lifetime and can be sampled at birth, with no reason to repeat such sampling during the individual’s life. The DNA variant has several advantages over conventional risk markers: (a) It does not vary over time; (b) it does not vary with meals or drugs; (c) it does not vary with an individual’s sex; (d) it can be determined with a single blood test; and (e) a single variant may exert multiple influences.

Should Genetic Risk Variants Be a Part of a Routine Clinical Assessment?

The simple answer to this question is not based on our current information. The first concern is that we do not have any specific treatments for any of the genetic risk variants. Many would argue (and appropriately so) that until we have evidence that genetic risk variants will alter the management of CAD, there is no reason to screen routinely. The current information is insufficient to determine the impact of these risk factors on the management of CAD. Multiple genetic risk factors have now been confirmed, however, and most do not act through known cardiovascular risk factors. The corollary of this observation is that several factors yet to be elucidated are contributing to the pathogenesis of CAD. Second, comprehensive prevention of CAD is unlikely to be successful without treatment that reduces genetic risk.

Despite the lack of specific treatments to manage the genetic risk factors, the compelling evidence of their importance has undoubtedly catalyzed a major research thrust. This effort will both pursue the role of genetic screening for early prevention and explore the risk-mediating pathways as targets to develop more-appropriate therapy. The information on 9p21 indicates its relevance as a risk factor. That the 9p21 variant is a predictor of the severity of CAD is reflected by the number of coronary vessels involved (46). This role was assessed in a population of cases with early-onset CAD and confirmed in an independent population with late-onset CAD. The role of 9p21 as a predictor of CAD severity has since been confirmed by multiple investigators (47–51). The 9p21 risk factor in individuals with early-onset CAD carries a 50% increased risk in heterozygotes and a 2-fold increased risk in homozygotes (19), comparable to the 2-fold increased risk of a current smoker (59), the 40% increased risk of a 10-mm increase in blood pressure (60), and the 30% increased risk associated with a standard deviation increase in LDL cholesterol or a standard deviation decrease in HDL cholesterol (61).

Despite the increased relative risk associated with the 9p21 variant allele, Paynter et al. (62) found that adding 9p21 to conventional risk factors with the technique of risk allele counting did not improve risk prediction. In contrast, Davies et al. (63) used logistic regression to construct a weighted genetic risk score for assessing the effect of combining 9p21 with 11 other genetic variants. They demonstrated a significant improvement in risk prediction; however, the absolute increase was modest. A longitudinal study of a population in the UK by Talmud et al. (64) showed that the 9p21 variant had modest effects but did reclassify a significant number of individuals into different risk categories. The role of genetic screening will ultimately depend on whether the results will alter the management of a patient with CAD. The technological barrier has been removed, because it is currently possible to genotype the DNA from a blood or saliva sample for hundreds of genotypes on a single microarray within 60 min and to produce an interpretable display in the process. We are currently performing, on an experimental basis, point-of-care genotyping for 26 genotypes for anti-platelet therapy; results are available in 45 min.

The importance of the genetic risk variants may ultimately lie in their contribution to the development of new therapies. Twenty-two of the 30 genetic risk variants are mediating their risk through pathways yet to be elucidated. The pathogenesis of coronary atherosclerosis has been dominated by the cholesterol hypothesis, and, not surprisingly, therapy has been directed toward reducing the synthesis of cholesterol. It is highly likely that one or more of these novel pathways...
will be amenable as targets for the development of new therapies. Specific treatment for cholesterol required decades to develop. With 22 genetic risk variants acting through unknown mechanisms, it is expected that the impetus to elucidate them will be greater and that the time to success will be shorter.

**References**


9p21 and Coronary Artery Disease


