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STATE-OF-THE-ART PAPERS

Genes and Coronary Artery Disease

Where Are We?

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Susceptibility to coronary artery disease (CAD) is claimed to be 40% to 60% inherited, but until recently genetic risk factors predisposing to CAD have been elusive. Comprehensive prevention of CAD requires manipulation of genetic risk. The availability of microarrays of single-nucleotide polymorphisms enabling genome-wide association studies (GWAS) led to the discovery of 33 genetic risk variants for CAD. Surprisingly, 23 risk variants mediate their risk through unknown mechanisms, with only 10 associating with hypertension or lipids. Thus, there are several mechanisms contributing to the pathogenesis of CAD yet to be elucidated. The first risk variant discovered by GWAS was 9p21.3, which occurs in 75% of all populations except African, with a mean increased risk of 25% per copy. Of the 33 variants for CAD, the increased risk varies from 6% to 92% with a mean increased risk of 18%, occurring on average in 47% of the population. The maximum number of risk alleles per individual would be 66. In the CARDIoGRAM (Coronary Artery Disease Genome-wide Replication and Meta Analysis) study of 23 variants, the average per individual was 17, the minimum 7, and the maximum 37. The top 10th percentile has an odds ratio of 1.88 and the lowest percentile an odds ratio of 0.55. Routine genetic screening is unlikely until management is improved by genetic testing. Risk variants should provide pathophysiological insights and targets for novel therapy. While risk variants are less potent predictors of CAD, compared with biomarkers, they have the advantage of not changing in one's lifetime and are unaffected by diet, sex, age, or medication.
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Coronary artery disease (CAD) is the number 1 killer in the world and is felt to be largely preventable. Reduction of known risk factors for CAD such as hypercholesterolemia, hypertension, and smoking have been assessed in multiple randomized placebo-controlled clinical trials and is associated with 30% to 40% less clinical events such as death and myocardial infarction (1). Epidemiological and family studies have repeatedly shown that genetic predisposition accounts for 40% to 60% of the risk for CAD (2). Prevention and treatment of CAD to be comprehensive would be expected to also include modification of the effects of these genetic risk factors, analogous to the current treatment of hypercholesterolemia, which is partly genetic and partly environmental. The barrier (3) until 2005 was the lack of technology. The first microarray having 500,000 single-nucleotide polymorphisms (SNPs) as DNA markers became available and within 5 years the results have been nothing short of remarkable. In total, over 1,319 genetic variants have been identified to be associated with increased risk for 160 diseases.

Genome-Wide Association Studies and the Common Allele Hypothesis

Genome-wide association studies (GWAS) have used as many as 1,000,000 SNPs, variants in DNA sequence that occur at a frequency ≥1% in a population, to genotype large samples of unrelated individuals with common diseases and compare the frequencies of genetic variants to that in equally large samples of control individuals. Genetic variants occurring at a higher frequency in cases than controls are considered to associate with disease risk. The limitation of the GWAS approach is the exceedingly high threshold required for an association due to potential false positives from the multiple testing of 1,000,000 SNPs. The threshold of significance is a p value $<0.05/1{,}000{,}000$ or $<5 \times 10^{-8}$. To further avoid false positives, it has become the standard to require replication of these markers showing genome-wide significance in an independent and appropriate population of adequate sample size. This stringency demands large sample sizes. To detect a risk variant that increases the risk by 10%, occurring in 15% of the population, requires a discovery sample size of 20,000 to provide 80% power and a similar replication sample size. All 33 risk loci for CAD discussed in this review satisfy this rigid requirement (4–11).

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Abbreviations and Acronyms

CAD = coronary artery disease

GWAS = genome-wide association studies

LDLC = low-density lipoprotein cholesterol

lncRNA = long noncoding RNA

SNP = single-nucleotide polymorphisms

GWAS Identifies 33 Genetic Variants Associated With Increased Risk for CAD

Table 1 lists the 33 genetic variants that exhibited genome-wide significance and have been replicated in appropriate independent populations. The first CAD risk variant, 9p21, was published in 2007, and within 2 years, 11 more novel genetic variants were mapped showing increased risk for CAD (12–16). It was evident from the results of these studies that most genetic variants would have modest to minimal risk effect. The sample size to detect more of these variants would have to be greater than initially expected, which enticed most of the investigators involved in performing GWAS for CAD to pool their resources. This led to the formation of an international consortium designed CARDIoGRAM (Coronary Artery Disease Genome-wide Replication and Meta Analysis) study and involved the collaboration of 14 GWAS, each of which on its own had previously been successful (17). This brought together many disciplines and considerable expertise with a total sample size of 86,995 individuals (22,233 cases vs. 64,762 controls) of European ancestry. This involved investigators from the United Kingdom, Germany, the United States, and Canada. This consortium combined the resources of over $200 million to pursue the genetic risk responsible for CAD. Genotyping was performed with the 1 million chip array followed by imputation of over 2 million SNPs from the

Table 1 Risk Loci for Coronary Artery Disease/Myocardial Infarction Discovered by Genome-Wide Association Studies

Chromosomal Location	SNP	Nearby Genes	Risk Allele Frequency (Allele)	Odds Ratio (95% CI)	Ref. #
Loci that associate with cholesterol					
1p32.3	rs11206510	*PCSK9*	0.82 (T)	1.15 (1.10–1.21)	(22)
1p13.3	rs599839	*SORT1*	0.78 (A)	1.29 (1.18–1.40)	(11)
2p21	Rs4299376	*ABCG8*	0.33 (G)	1.08 (1.04–1.13)	(19)
6q25.3	rs3798220	*LPA*	0.02 (C)	1.92 (1.48–2.49)	(4,13)
8q24.13	rs17321515	*TRIB1*	0.54 (A)	1.10 (1.06–1.15)	(19)
9q34.2*	rs579459	*ABO*	0.21 (C)	1.10 (1.07–1.13)	(6)
11q23.3	rs964184	*ZNF259, APOA5-A4-C3-A1*	0.13 (G)	1.13 (1.10–1.16)	(6)
19p13.2	rs1122608	*LDLR*	0.77 (G)	1.14 (1.09–1.19)	(22)
Loci that associate with hypertension					
10q24.32	rs12413409	*CYP17A1, CNNM2, NT5C2*	0.89 (G)	1.12 (1.08–1.16)	(6)
12q24.12	rs3184504	*SH2B3*	0.44 (T)	1.13 (1.08–1.18)	(14,15)
Loci that do not associate with known risk factors					
1p32.2	rs17114036	*PPAP2B*	0.91 (A)	1.17 (1.13–1.22)	(6)
1q41	rs17465637	*MIA3*	0.74 (C)	1.20 (1.12–1.30)	(11)
2q33.1	rs6725887	*WDR12*	0.15 (C)	1.16 (1.10–1.22)	(22)
3q22.3	rs2306374	*MRAS*	0.18 (C)	1.15 (1.11–1.19)	(8)
5q31.1	Rs2706399	*IL5*	0.52 (G)	1.07 (1.03–1.11)	(19)
6p24.1	rs6903956	*C6orf105*	0.07 (A)	1.65 (1.44–1.90)	(7)
6p24.1	rs12526453	*PHACTR1*	0.67 (C)	1.13 (1.09–1.17)	(22)
6p21.31	rs17609940	*ANKS1A*	0.75 (G)	1.07 (1.05–1.10)	(6)
6q23.2	rs12190287	*TCF21*	0.62 (C)	1.08 (1.06–1.10)	(6)
7q22.3	rs10953541	*BCAP29*	0.75 (C)	1.08 (1.05–1.11)	(5)
7q32.2	rs11556924	*ZC3HC1*	0.62 (C)	1.09 (1.07–1.12)	(6)
9p21.3	rs4977574	*CDKN2A, CDKN2B, ANRIL*	0.46 (G)	1.25 (1.18–1.31) 1.37 (1.26–1.48)	(5,9,10)
10p11.23	rs2505083	*KIAA1462*	0.42 (C)	1.07 (1.04–1.09)	(5,16)
10q11.21	rs1746048	*CXCL12*	0.87 (C)	1.33 (1.20–1.48)	(11)
10q23.31	rs1412444	*LIPA*	0.34 (T)	1.09 (1.07–1.12)	(5,19)
11q22.3	rs974819	*PDGF*	0.29 (T)	1.07 (1.04–1.09)	(5)
13q34	rs4773144	*COL4A1, COL4A2*	0.44 (G)	1.07 (1.05–1.09)	(6)
14q32.2	rs2895811	*HHIPL1*	0.43 (C)	1.07 (1.05–1.10)	(6)
15q25.1	rs3825807	*ADAMTS7*	0.57 (A)	1.08 (1.06–1.10)	(5,6)
17p13.3	rs216172	*SMG6, SRR*	0.37 (C)	1.07 (1.05–1.09)	(6)
17p11.2	rs12936587	*RASD1, SMCR3, PEMT*	0.56 (G)	1.07 (1.05–1.09)	(6)
17q21.32	rs46522	*UBE2Z, GIP, ATP5G1, SNF8*	0.53 (T)	1.06 (1.04–1.08)	(6)
21q22.11	rs9982601	*MRPS6*	0.15 (T)	1.19 (1.13–1.27)	(22)

*The locus at 9q34.2 is associated with myocardial infarction, but not with coronary atherosclerosis.
CI = confidence interval; SNP = single-nucleotide polymorphism.

HapMap Project. SNPs showing a significant positive association in the Discovery population were analyzed for replication in an independent population with a sample size of 56,682. Thirteen new genetic risk variants for CAD were identified with confirmation of 10 previously identified risk variants (6). In another study involving CARDIoGRAM investigators, Reilly et al. (18) identified 2 novel variants, 1 for CAD ADAMTS7 and 1 for myocardial infarction, the ABO blood group locus. This was followed by the results from the Coronary Artery Disease (C4D) Genetics Consortium (5), which identified 4 additional risk loci related to CAD. This study also involved replication in an independent population of Caucasians and East Asians. In the same issue of *Nature Genetics*, Wang et al. (7) reported on the identification of a genetic variant at 6p21, which increases the risk for CAD in the Chinese population, but has no risk effect in the Caucasian population. The IBC 50K CAD Consortium, utilizing a 50K SNP array covering approximately 2,100 candidate genes, identified 3 additional novel CAD loci (19).

Common Features of CAD Genetic Risk Variants

There are several common features that characterize genetic risk factors.

- Eight of the variants mediate their risk through lipids and another 2 through hypertension, with 23 loci acting independently of known risk factors (5–7,9,10,18–22).
- The increased relative risk of each variant for CAD varies from 6% to 92% with a mean increased risk of 18%.
- The frequency of these variants in the population varies from 2% to 91% with a mean frequency of 47%.
- In CARDIoGRAM with the analysis of 23 loci, the number of alleles per individual could vary from 0 to 46. We observed the average to be 17, with a maximum of 37 and minimum of 7.
- Most genetic risk variants exhibit higher risk in individuals with early onset than for those with late onset of CAD.
- The top 10th percentile is associated with odds ratio for CAD of 1.88 and the lowest percentile is associated with an odds ratio of 0.55.
- Most of the genetic risk variants for CAD are located in DNA sequences that do not code for protein.

Biological and Therapeutic Implication of Loci That Do Not Associate With Known Risk Factors

Perhaps the most exciting result of the GWAS approach is the observation that 23 of the 33 loci do not associate with any known risk factor for CAD. The corollary being there are other mechanisms contributing to the pathogenesis of CAD, which have heretofore not been considered. While the mechanisms through which these genetic factors mediate their risk remains to be determined the results of the GWAS have robustly indicated they do exist. Secondly, it provides the impetus to search for clues that will ultimately identify the molecular pathways through which the risk is manifested. It is highly likely the 23 loci associated with unknown mechanisms will act through a few common molecular pathways. This is speculative but would be in keeping with the observation that 8 of the risk variants act through just 2 pathways: cholesterol and hypertension. The utilization of these pathways to identify new targets for drug therapy should be a worthy future pursuit. These findings also emphasize the necessity of identifying genetic risk factors to provide comprehensive prevention and treatment of CAD.

Refining the Phenotype: Distinguishing CAD From Myocardial Infarction

In all of the GWAS for CAD the phenotype has been either CAD defined as ≥50% obstruction in 1 or more coronary vessels as determined by coronary angiography or documented myocardial infarction. The phenotype of CAD has consistently been used interchangeable with that of myocardial infarction. This is because myocardial infarction is almost always induced by the superimposition of a thrombus on existing CAD (atherosclerosis). In the initial discovery of 9p21, the phenotype by the Ottawa Group (10) was the presence of CAD on the basis of coronary angiography, while the phenotype of the DeCode Group was myocardial infarction (9). Because myocardial infarction seldom occurs without atherosclerosis it is not surprising several studies have since linked 9p21 to the phenotype of myocardial infarction (23–26). Despite this commonality of CAD and myocardial infarction, it is intuitive that some of the genetic risk factors specifically predispose to CAD and others to myocardial infarction. In an attempt to separate the 2 phenotypes, Dandona et al. (27) analyzed the genotypes of 950 patients with early onset CAD documented by angiography with and without myocardial infarction. The association of the 9p21 risk allele in patients having CAD without myocardial infarction was compared with patients having CAD and myocardial infarction. There was a strong association between the 9p21 risk allele and CAD with no association of the risk allele with myocardial infarction. The association of 9p21 with CAD and not with myocardial infarction was also observed by Horne et al. (28), Reilly et al. (18), and more recently by Ardissino et al. (29). In support of 9p21 acting at the vessel wall is the finding that 9p21 is a significant risk factor for abdominal aortic aneurysm (30) and atherosclerotic stroke (31,32).

In the Dandona et al. study (27) it was also observed that 9p21 in a dose-dependent relationship was strongly associated with the severity of CAD as determined by the number of coronary vessels involved. Left main disease also correlated with the dosage of 9p21 risk allele. The frequency of the 9p21 risk allele was significantly less in individuals with 1-vessel disease compared to 2- or 3-vessel disease. Similarly, results were observed in an independent population of

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764 cases with late onset disease (27). Several investigators have confirmed 9p21 as a predictor of the severity of CAD (20,29,33). In contrast, Hinohara et al. (23) in a study involving Japanese and Korean populations did not observe any relationship between the dose of 9p21 and the number of coronary vessels involved. However, the sample size consisted of 77 cases with 1-vessel disease, 54 with 2-vessel disease, and 46 with 3-vessel disease, which is inadequate to determine whether 9p21 is or is not associated with severity of CAD. In a study by Chen et al. (34) no relationship between 9p21 and the severity of CAD was observed. The sample of 212 cases was insufficient as indicated by the authors. In a study by Anderson et al. (35) with a sample size of 1,011 cases, the authors observed a strong relationship between 9p21 and the presence of CAD but no relationship between gene dose and the number of vessels.

All of the studies in which coronary angiography is available consistently show that 9p21 mediates its risk by acting at the vessel wall to influence atherosclerosis and is not associated with myocardial infarction. In contrast, there is not consistent agreement on whether 9p21 relates to the progression and severity of CAD. All of the studies have been cross sectional. A definitive solution to this problem may require a prospective longitudinal follow up study with adequate sample size including serial coronary angiograms. Similar data indicate the ADAMTS7 risk allele acts at the vessel wall influencing coronary atherosclerosis.

The recent identification of the ABO blood group locus as a risk factor for myocardial infarction has important epidemiological, historical, biological, and therapeutic implications. In this CARDIoGRAM study by Reilly et al. (18), a GWAS was performed in 4,372 patients with documented CAD by angiography and confirmed myocardial infarction and 2,739 patients with documented CAD without myocardial infarction. Several markers at 9q34.2 showed a strong association with myocardial infarction but no association with CAD. This association with myocardial infarction was replicated in an independent population and a meta-analysis of the combined sample showed markers at 9q34.2 to be associated with myocardial infarction with genome-wide significance.

It has been claimed for decades that blood group O offers protection from myocardial infarction compared with blood groups A or B (36). The locus for these blood groups at 9q34.2 is responsible for a single gene. The A, B, and O are different forms (alleles) of the same gene. The A and B genes encode for a protein (alpha 1-3N-acetylgalactosaminyltransferase), which transfers a carbohydrate molecule onto von Willebrand's factor. This modification slows the normal process of proteolysis and prolongs the life of von Willebrand's factor. This predisposes to coronary thrombosis, which is presumably the cause of the myocardial infarction. In contrast, the O gene encodes for a transferase protein, which is mutated and as a result lacks the activity to transfer the carbohydrate onto von Willebrand's factor, so individuals with blood group O do not have increased risk for myocardial infarction. This has important therapeutic implications for patients undergoing bypass surgery, angioplasty, and other such procedures.

Functional Analysis of Genetic Risk Variants

A major goal for the future will be to elucidate the mechanisms whereby genetic variants mediate their risk. This will require great ingenuity as most of them are in regulatory regions rather than protein coding genes. Regulatory regions are known to act on nearby and far-removed genes on the same chromosome or even to act in *trans* on genes on other chromosomes (37). It is also bothersome that a regulatory region not only acts at great distances, but also on many targets. The genetic risk factor of which the most is known is 9p21.3. Discussion of the functional studies on 9p21.3 illustrates both the problems and a reason for the lack of success.

Frequency and Risk Analysis of the 9p21.3 Risk Locus

McPherson et al. (10) and Helgadottir et al. (9) independently and simultaneously discovered the first common CAD risk variant located on chromosome 9p21.3, using the GWAS approach that was confirmed by many others (11,38). The 9p21.3 risk allele is carried by 75% of the European population (50% heterozygous and 25% homozygous risk) and confers risk for coronary atherosclerosis by an unknown mechanism that is independent of known risk factors (9,10,38). The risk for CAD is increased by 25% with 1 copy and 50% by 2 copies of the 9p21.3 risk allele. In premature CAD, 9p21.3 increases the risk 2-fold (10). A recent meta-analysis concluded that the 9p21.3 risk locus was significantly associated with early age of onset of heart disease (21). The association of the 9p21.3 locus with CAD risk has also been documented in other ethnic groups including Chinese (39), Korean (24), Japanese (23), Indian (40), and Pakistani (41), but surprisingly not among Africans (42), suggesting that selective pressure has maintained this allele in non-African populations.

Functional Analysis of 9p21.3

The 9p21.3 locus also contributes to the risk of intracranial and abdominal aortic aneurysms (30), vascular dementia and late onset Alzheimer's disease (43). It is also noteworthy that the 9p21.3 locus has been also associated with aggressive periodontitis (44) and gout (45), diseases with a marked inflammatory component. However, it should be noted that the 9p21.3 risk allele did not associate with general markers of inflammation, such as C-reactive protein among cases with CAD (46,47).

The 9p21.3 CAD risk allele is in a region of DNA that has no sequences that encode for protein. The risk allele is contained in a long noncoding RNA (lncRNA) of 126,000 bps, referred to as ANRIL and officially known as

CDKN2BAS lncRNA, which is adjacent to genes encoding the cyclin-dependent kinase inhibitors *CDKN2A* and *CDKN2B* (48–50). The lncRNA is transcribed into several alternate transcripts and expression of the 9p21.3 risk allele is consistently associated with higher expression of the CDKN2BAS lncRNA yet lower mRNA expression of the nearby genes *CDKN2A* and *CDKN2B* (48–50). We have shown that several conserved sequences in this region contain enhancer elements (48), recently confirmed by others (51). Targeted deletion of the 9p21.3 homologous region in the mouse was associated with reduced expression of *CDKN2A*, confirming this region contains regulatory enhancers (52). However, these mice did not exhibit atherosclerosis. Because there is only 50% homology of the DNA sequences between man and mouse, it is possible the 9p21.3 risk allele only developed in higher primates and involves a primate-specific pathway, or these results could indicate the mechanism whereby 9p21.3 confers risk is not confined to reduced *CDKN2A* expression. A recent report used the technique of chromatin conformation capture to identify a long-range interaction between conserved enhancer sequences at the 9p21.3 locus that remodel chromatin in the vicinity of the genes encoding *CDKN2A* and *CDKN2B*, methylthioadenosine phosphorylase (*MTAP*), and affect the genes much further downstream of interferon-ω1 (*IFNW1*) and interferon α-21 (*IFNA21*) (51). Interferon α-21, similar to 9p21.3 risk allele, is a gene unique to higher primates. If its expression were associated with the 9p21.3 risk genotype, it would be the first evidence of an inflammatory cytokine specifically associated with increased risk of CAD and could account for the primate specificity of the risk locus.

What Is the Clinical Utility of GWAS CAD Risk Loci?

The recent excitement of identifying 33 genetic variants associated with increased risk for CAD is yet to be realized in managing patients. The mean increased relative risk of each variant is only 15%. Thus, any 1 variant is not likely to add much over that of available markers, particularly if including the family history. Because most individuals have at least 15 to 20 of these risk variants, the total risk may be the sum of their interactions. The other possibility is some synergistic gene-to-gene interactions may occur that are not accounted for in current estimates. The expected clinical application is early detection, and stratification of those at higher risk together with new and appropriate therapies for treatment. The fact that 23 of the risk variants for CAD do not act through any of the known risk factors is very promising and exciting. It indicates the pathogenesis of CAD is due to mechanisms yet to be elucidated. The best class of drugs currently to prevent CAD is the statins, which decrease the synthesis of cholesterol. This pathway came into the limelight with the recognition that the gene that encodes the receptor for cholesterol was abnormal in familial hypercholesterolemia, a rare disorder (1 in 5,000 individuals) (53). It would be a decade before a drug would be marketed, however, progress is likely to be more rapid today. Given there are 23 variants acting through unknown mechanisms, it is highly likely another pathway equivalent to that of the cholesterol is waiting to be identified. The issue of genetics for early detection and prevention is more complex. On the basis of the known 33 genetic risk variants, the maximum number an individual could have if homozygous for all alleles is 66. In the CARDIoGRAM study (6), we analyzed 23 variants (46 alleles) and observed an average of 17 per individual, with a minimum of 7 and a maximum of 37. There are some advantages of genetic DNA variants over that of conventional biomarkers such as cholesterol. While cholesterol varies with sex, age, diet, and preferably detected during fasting, DNA variants do not change in the lifetime of the individual and does not vary with meals or drugs, thus 1 blood sample at birth is all that is needed. However, cholesterol is a much stronger predictor of CAD than any of the genetic variants.

Are these genetic variants ready for routine testing? The short answer is no and likely to remain as such until it is proven that their application in some way improves patient management. If we have to wait until specific treatment is available it will require several years. The time from discovery of the defective gene encoding the cholesterol receptor until the first statin was available was almost 20 years. We would expect today that once a mechanism is discovered, it would not take so long to develop appropriate therapy. However, the increased risk of these variants is significant and because most are acting through unknown mechanisms, there is increased excitement and enthusiasm to adopt genetic testing. Fifty percent of the population has blood group A or B, which is associated with increased risk of MI, with implications for increased thrombosis after bypass surgery or angioplasty. Would it be prudent for individuals of group A or B to receive aspirin daily? One approach to utilizing 9p21 to alter management could be on the basis of the ATP III (Adult Treatment Panel III) recommendations for treatment of low-density lipoprotein cholesterol (LDLC). The recommendations are to treat LDLC exceeding 190 mg/dl if the individual has only 1 risk factor and to treat LDLC of 160 mg/dl with 2 risk factors. There is consistent agreement that 9p21 is an independent risk factor and thus would change the levels of LDLC required for statin therapy. This could be considered for approval by the ATP Committee before development of new therapy.

The risk associated with 9p21.3 for individuals likely to get CAD before the age of 55 years (males) and 60 for females is twofold greater. This is equivalent to many risk factors such as smoking. Several investigators have questioned the clinical utility of GWAS loci as a standalone approach to predict risk in the context of CAD (54,55). However, among patients after coronary artery bypass grafting, the ability to predict mortality was significantly improved by genotyping for the 9p21.3 risk locus (56).

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Furthermore, using 12 GWAS variants, we found that these significantly improved prediction of CAD over traditional risk factors (57). With the accrual of additional risk loci, perhaps in combination with the detection of serum biomarkers that might be discovered to associate with these risk loci, GWAS risk loci are likely to have even greater power to predict disease severity and age at onset. What is the major barrier to genetic testing for CAD? It is not a technological problem. One can put 30 or 200 risk variants on a chip and have the result within an hour with today's technology. The interpretation can be produced immediately and such algorithms are already available. The real barrier is that no specific treatment exists as of yet. Until the mechanisms are discovered and management is improved, genetic testing is unlikely to become routine. The discovery of 33 genetic risk variants for CAD and the increased awareness of prevention will no doubt catalyze the search for improved treatments and the elucidation of their biology and pathophysiology.

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