

Genetic Markers for Coronary Artery Disease

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INDEX TERMS: association, atherosclerosis, coronary artery disease, genetics, genome wide association, genomics, linkage, myocardial infarction

LEARNING OBJECTIVES

Upon completion of this activity, participants should be able to:

1. Compare linkage studies with association studies and recognize aspects regarding the utility and limitations of these studies in identifying markers associated with complex disease.
2. Describe genome wide association studies and their advantages and disadvantages for identifying genetic markers.
3. Discuss various aspects regarding the 9p21 risk allele, its association to CAD, and its potential as a clinical marker for CAD.
4. Describe microRNAs and their potential utility as CAD markers.
5. Discuss aspects of genetic markers that would lend to their ability to enter the clinical realm as risk markers for complex disease.

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The hereditary component of coronary artery disease (CAD) is widely recognized. However, identifying clinically useful genetic markers for a complex disease like CAD has been challenging. Linkage-based and association-based genetic studies have pointed to some interesting findings, but many of these studies have lacked reproducibility or statistical significance. Recently, genome-wide association (GWA) and microRNA discoveries have uncovered some potentially promising new markers for CAD. The current status of genetic markers for CAD and their utility in the clinical arena are summarized here.

Coronary artery disease (CAD) is a multifactorial disease that can be influenced by a multitude of environmental and heritable risk factors. While there are many traditional and novel analyte risk markers associated with CAD, a large gap for CAD risk prediction remains. Epidemiological evidence points to an approximate 50% genetic susceptibility to CAD. Many different genetic associations with CAD have been identified through family and population-based analyses, and genetic risk markers may be important for better defining individuals at risk for CAD and CV events. Some potentially promising and interesting markers produced from such studies are highlighted here.

Linkage Analysis and CAD

Linkage analyses are family-based studies investigating genetic variants that segregate with disease in affected vs. non-affected family members. Multiple linkage studies have been performed in CAD cases, investigating the occurrence of myocardial infarction (MI) or subclinical atherosclerosis.¹ While several loci of interest have been identified through linkage studies, the majority of them have not been replicated or directly implicated in CAD.

One gene that was identified through linkage studies is the *MEF2A* gene, encoding the transcription factor myocyte enhancer factor 2A. A seven amino acid sequence deletion from exon 12 of *MEF2A* was identified as the causative mutation in a large autosomal dominant CAD-MI family.² Subsequent studies have variably confirmed or refuted the involvement of *MEF2A* in CAD and/or MI.³⁻⁶ The lack of consistency in findings could point to variable penetrance of the alleles, modifier gene effects, or other genetic variants within the linkage interval.

In a linkage study of Icelandic families, the *ALOX5AP* gene [encoding 5-lipoxygenase activation protein (FLAP)] was identified for its association with MI (OR = 1.8) and stroke (OR = 1.9).⁷ FLAP is involved in the biosynthesis of leukotrienes, which promote chemotaxis and increase vascular permeability. Further fine mapping identified an association with leukotriene A₄ hydrolase (*LTA4H*) in the Icelandic cohort.⁸ It has been, however, challenging to determine which specific *ALOX5AP* SNPs or haplotypes are associated with MI in various ethnic groups and the association between these leukotriene-associated genes and MI has not been consistently replicated.⁹⁻¹² Nonetheless, several studies have highlighted a potentially key role for leukotrienes in atherosclerosis. Importantly, a small molecular FLAP-inhibitor had the ability to reduce both leukotriene production and C-reactive protein (a CAD biomarker) levels in a placebo-controlled, randomized trial.¹³

Candidate Gene Association Analysis and CAD

Candidate gene association analyses are population-based studies investigating variants in candidate genes (identified via linkage studies or based on biological knowledge) that segregate with disease in cases and controls. Many candidate genes have been investigated for association with CAD, including genes coding for apolipoproteins (e.g. *APOE*), matrix metalloproteinases, paraoxonase, cytokines, and other proteins involved in coagulation, blood pressure or lipoprotein regulation (e.g. *PCSK9*), and/or atherosclerotic processes.

Similar to linkage analyses, candidate gene association analyses have been hampered by the general inability to replicate findings in follow-up studies. A genetic analysis of 103 candidate genes in a cohort of 1400 individuals from a founder population demonstrated a lack of association with CAD status.¹⁴ Similar observations were made in a study of 70 candidate genes in 811 acute coronary syndrome patients.⁹ The lack of reproducibility speaks to both the complex nature of CAD and the design flaws of candidate gene studies, which are often single-gene, underpowered studies that may be, in part, afflicted with false positive associations. Because of the limitations of these types of studies, as well technological and genomic haplotype mapping advances, the field has been moving towards large-scale genome-wide (GWA) association studies.

Genome-wide Association (GWA) Studies and CAD

GWA studies are unbiased large-scale population-based studies evaluating the association of hundreds of thousands of markers, generally single nucleotide polymorphisms (SNPs), across the genome with a particular phenotype. Perhaps one of the more interesting loci that has been identified in multiple GWA CAD studies, and confirmed in numerous follow-up case-control analyses is the locus at chromosome 9, band p21.3.¹⁵⁻²² Markers at the 9p21 locus have been shown to give a 15–20% increased risk for CAD in the 50% of Caucasian individuals

heterozygous for the allele, and a 30–40% increased risk of CAD in the 25% of individuals homozygous for the allele.¹⁶ A meta-analysis of case-control studies showed that the odds ratio per copy of the 9p21 risk allele was 1.29 (95% CI 1.22–1.37, $p=0.0079$).²¹ In addition to CAD, the 9p21.3 locus has been associated with stroke, abdominal aortic aneurysms, and intracranial aneurysms, suggesting potential involvement for the 9p21 risk allele with plaque stability and/or vessel wall integrity processes.^{21, 23–28}

While the 9p21 association with CAD has been replicated on multiple occasions, the biological relevance of 9p21 is unclear at this time. The 9p21.3 risk-allele locus spans 50–60 kb and is in linkage disequilibrium (LD) with the 3' end of *CDKN2B*, encoding the cyclin-dependent kinase inhibitor tumor suppressor p15^{INK4B}, with weaker LD extending through *CDKN2B* to *CDKN2A*, which encodes another tumor suppressor p16^{INK4B}. The cyclin dependent kinases are involved in cell cycle regulation and transforming growth factor- β (TGF- β) cell cycle arrest.²⁹ TGF- β has been shown to have impaired signaling and reduced expression in atherosclerotic lesions, overexpression in abdominal aortic aneurysms, and variable expression in different stages of plaque development.^{30–32} A gene encoding a large antisense non-coding RNA (*ANRIL*) spans almost the entire 9p21-CAD association region.³³ A speculated mechanism for the 9p21 risk allele involves antisense regulation of *CDKN2B* (and/or *CDKN2A*), potentially affecting signaling of TGF- β and/or additional cytokine(s) involved in cell cycle arrest/proliferation.^{23, 25, 34} Recently, a SNP in the 9p21 risk allele was shown to be associated with significantly reduced expression of *CDKN2B*, *CDKN2A*, and *ANRIL*.³⁵

Other loci potentially involved in CAD that have been identified through multiple, independent GWA studies and confirmed in follow-up association analyses include 1p13, 1q41, and 10q11.^{15, 17, 19, 20} The 1p13 risk allele is in a 97-kb region of LD containing the *CELSR2*, *PSRC1*, and *SORT1* genes, and has been

found to be strongly associated with low-density lipoprotein (LDL) and total cholesterol concentrations.^{36–42} Of particular interest in this gene cluster is *SORT1*, which encodes sortilin, a pro-neurotrophin receptor involved in adipocyte and muscle glucose metabolism. Sortilin is downregulated in obesity and has been implicated in insulin resistance.⁴³ Examples of other CAD-associated loci identified through GWA studies include 19p13 (*LDLR*), 1p32 (*PCKS9*), and 12q23–24 (*DRIM*, *SH2B3*, *HNF1A-C12orf43*).²² Additionally, a genome-wide haplotype analysis utilizing a sliding-windows approach identified a haplotype of four SNPs in the *SLC22A3-LPAL2-LPA* gene cluster associated with CAD.⁴⁴ *LPA*, encodes apolipoprotein (a), the protein component of lipoprotein (a) [Lp(a)] which is associated with an increased risk for CAD and MI. In addition to the loci described here, other loci associated with atherosclerosis-related phenotypes have been identified via GWA studies and are summarized in Table 1.

MicroRNA

MicroRNAs (miRNAs) are endogenous, small (approximately 22 nucleotides), non-coding RNAs that modulate gene expression and have been shown to play roles in cardiovascular disease pathogenesis, including cardiac hypertrophy, heart failure, and myocardial infarction.^{45, 46} Recently, it was shown that members of the miR-29 family are downregulated in acute MI in mice and humans.⁴⁶ It was further shown that miR-29 downregulation leads to enhanced fibrotic response, indicating a role for miR-29 family members in cardiac fibrosis, an important aspect of post-MI remodeling.

Since miRNAs are expressed in a tissue-specific manner, miRNA-based diagnostics in the clinical setting may be the most immediately available and applicable tests in diseases where tissue is readily available and regularly examined, such as in cancer. However, it is now being recognized that serum-based miRNA biomarker diagnostic tests may have great value in cancer and other disease states.⁴⁷ In the

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Table 1. Genome-Wide Association Studies of Atherosclerosis-related Phenotypes ($p < 5 \times 10^{-5}$)

Phenotype	Chromosomal Region	Reported Gene(s)	SNP	Risk Allele Frequency	P-value	Odds ratio	95% CI	Ref
CAD	1p13.3	<i>CELSR2-PSRC1-SORT1</i>	rs5999839	0.23	4×10^{-19}	1.29	1.18-1.40	17
early-onset myocardial infarction	1p13	<i>CELSR2-PSRC1-SORT1</i>	rs646776	0.81	1.5×10^{-8}	1.17	1.11-1.24	20
early-onset myocardial infarction	1p32	<i>PCSK9</i>	rs11206510	0.81	9.6×10^{-9}	1.15	1.10-1.21	20
early-onset myocardial infarction	1q41	<i>MIA3</i>	rs1746048	0.72	5.9×10^{-7}	1.13	1.08-1.18	20
CAD	1q41	<i>MIA3</i>	rs17465637	0.29	1×10^{-6}	1.2	1.12-1.30	17
CAD	1q43	NA*	rs17672135	0.87	2×10^{-6}	1.43	1.23-1.64	15
early-onset myocardial infarction	2q33	<i>WDR12</i>	rs6725887	0.14	1.3×10^{-8}	1.17	1.11-1.23	20
CAD	2q36.3	pseudogene	rs2943634	0.65	2×10^{-7}	1.21	1.13-1.30	17
CAD	3q22.3	<i>MRAS</i>	rs9818870	NA	7.4×10^{-13}	1.21	1.13-1.30	56
CAD	5q21	NA	rs383830	0.22	1×10^{-5}	1.60	1.16-2.21	15
early-onset myocardial infarction	6p24	<i>PHACTR1</i>	rs12526453	0.65	1.3×10^{-9}	1.12	1.08-1.27	20
CAD	6q25	<i>MTHFD1L</i>	rs6922269	0.25	2×10^{-5}	1.17	1.04-1.32	15
CAD	6q25.1	<i>MTHFD1L</i>	rs6922269	0.25	3×10^{-8}	1.23	1.15-1.33	17
CAD	6q27	<i>SCL22A3-LPAL2-LPA</i>	rs2048327, rs3127599, rs7767084, rs10755578 (CTTG and CCTC haplotype)	NA	1.2×10^{-9} (CTTG), 4.2×10^{-15} (CCTC)	1.20 (CTTG), 1.82 (CCTC)	1.13-1.28 (CTTG), 1.57-2.12 (CCTC)	44**
early-onset myocardial infarction	9p21	<i>CDKN2A, CDKN2B</i>	rs4977574	0.56	1.1×10^{-30}	1.28	1.23-1.33	20
myocardial infarction	9p21.3	<i>CDKN2A, CDKN2B</i>	rs10757278	0.45	1×10^{-20}	1.28	1.22-1.35	18
CAD	9p21.3	intergenic	rs1333049	0.47	3×10^{-19}	1.36	1.27 – 1.46	17
CAD	9p21.3	<i>CDKN2A, CDKN2B</i>	rs1333049	0.47	1×10^{-13}	1.47	1.27–1.70	15
CAD	9p21.3	NA	rs10757274 and rs2383206	NA	NA	NA	NA	16
CAD	10q11.21	<i>CXCL12</i>	rs501120	0.13	9×10^{-8}	1.33	1.20-1.48	17
early-onset myocardial infarction	10q11	<i>CXCL12</i>	rs1746048	0.84	3.4×10^{-5}	1.14	1.08-1.21	20
MI	12q24	<i>SH2B3</i>	rs3184504	0.38	8.6×10^{-8}	1.13	1.08-1.18	57
CAD	12q24.41	<i>HNF1A-C12orf43</i>	rs2259816	NA	5×10^{-7}	1.08	1.05-1.11	56
CAD	15q22.33	<i>SMAD3</i>	rs17228212	0.30	2×10^{-7}	1.21	1.13-1.30	17
CAD	16q23	NA	rs8055236	0.20	6×10^{-6}	1.91	1.33-2.74	15
early-onset myocardial infarction	19p13	<i>LDLR</i>	rs1122608	0.75	1.9×10^{-9}	1.15	1.10-1.20	20
CAD	19q12	NA	rs7250581	0.22	3×10^{-5}	1.06	0.79-1.43	15
early-onset myocardial infarction	21q22	<i>SLC5A3-MRPS6-KCNE2</i>	rs9982601	0.13	6.4×10^{-11}	1.20	1.14-1.27	20
CAD	22q12	NA	rs688034	0.31	4×10^{-6}	1.11	0.99-1.25	15

*NA= not applicable

**Genome-wide haplotype analysis

cardiac arena, much of the focus regarding miRNA is being placed on miRNA-targeted therapeutics. However, miRNA-based diagnostics and prognostics for cardiac disease may become a reality as research uncovers the potential for examining circulating levels of miRNA in cardiac disease, as well as other circulating genetic and non-genetic biomarkers that influence regulation of or are affected by miRNAs.

Clinical Utility of Genetic Markers in CAD

In order for genetic markers to enter the clinical realm, they should demonstrate robust diagnostic or predictive value over current phenotypic risk markers.

Most CAD-associated genetic markers identified to date do not meet these criteria and are not ready for prime time. A great deal of attention has been placed on the 9p21 risk allele and its ability to provide diagnostic and prognostic guidance for CAD and cardiovascular events. Some studies have suggested that the 9p21 genotype may not be useful in stratifying risk in some low-risk populations but may provide discrimination in intermediate-risk individuals.⁴⁸⁻⁵¹ Recent findings have also implied utility for 9p21 in predicting revascularization in early-onset MI cases.⁵² However, concerns regarding genetic testing for 9p21 revolve around the potentially limited information testing for this risk allele in isolation would provide at this stage.

For the most part, newly identified markers confer small relative risks (ORs of 1.1 to 1.5), and most genes involved in complex disease individually contribute to only a small percentage of the overall phenotype.⁵³ And while GWA studies can be useful in identifying risk markers with >5% prevalence, these types of studies are not very useful for identifying less common variants (<1 to 5% prevalence) that may be vital for improved risk stratification. Using computer simulation, one study estimated that over 200 alleles were required to provide a reasonable assessment of CAD risk.⁵⁴ Kathiresan et al. combined nine SNPs to produce a genotype score and demonstrated the increased value of the genotype score over single SNP risk prediction in a cardiovascular cohort.⁵⁵ Thus, it is likely that multiple genetic risk

alleles are needed to accurately assess cardiovascular risk and the future of genetic markers for CAD will likely involve offering a panel of markers and pooling the results for optimal risk prediction.

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