



REVIEW ARTICLE

Pathogenesis of premature coronary artery disease: Focus on risk factors and genetic variants

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Abstract The development of premature coronary artery disease (PCAD) is dependent on both genetic predisposition and traditional risk factors. Strategies for unraveling the genetic basis of PCAD have evolved with the advent of modern technologies. Genome-wide association studies (GWASs) have identified a considerable number of common genetic variants that are associated with PCAD. Most of these genetic variants are attributable to lipid and blood pressure-related single-nucleotide polymorphisms (SNPs). The genetic variants that predispose individuals to developing PCAD may depend on race and ethnicity. Some characteristic genetic variants have been identified in Chinese populations. Although translating this genetic knowledge into clinical applications is still challenging, these genetic variants can be used for CAD phenotype identification, genetic prediction and therapy. In this article we will provide a comprehensive review of genetic variants detected by GWASs that are predicted to contribute to the development of PCAD. We will highlight recent findings regarding CAD-related genetic

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variants in Chinese populations and discuss the potential clinical utility of genetic variants for preventing and managing PCAD.

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Abbreviations

ABCG5 ATP-binding Cassette Sub-family G Member 5
 ABCG8 ATP-binding Cassette Sub-family G Member 8
 ADAMTS7 A Disintegrin-like and Metalloprotease-7
 AIG-1 Androgen-induced Gene 1
 ANGPTL4 Angiopoietin-like Protein 4
 APOA5 Apolipoprotein A5 Gene
 APOB Apolipoprotein B
 APOC3 Apolipoprotein C III
 APOE Apolipoprotein E
 ARHGAP42 Rho GTPase Activating Protein 42
 ASGR1 Asialoglycoprotein Receptor 1
 ATP2B1 ATPase Plasma membrane Calcium Transporting 1
 BTNL2 Butyrophilin-like2
 CAD Coronary Artery Disease
 CDKN2A Cyclin-dependent Kinase Inhibitor 2A
 CDKN2B Cyclin-dependent Kinase Inhibitor 2B
 CETP Cholesteryl Ester Transfer Protein
 CRP C-reactive Protein
 CSF Colony Stimulating Factor
 GTEx Genotype-Tissue Expression
 GUCY1A1 Guanylate Cyclase 1 Soluble Subunit Alpha 1
 GWAS Genome-wide Association Studies
 HDL-C High-density Lipoprotein Cholesterol
 IL Interleukin

LDL-C Low-density Lipoprotein Cholesterol
 LDL-R Low density Lipoprotein Receptor
 LPA Lipase Activator
 LPL Lipoprotein Lipase
 Lp-PLA2 Lipoprotein-associated Phospholipase A2
 NPC1L1 Niemann-Pick C1-Like intracellular cholesterol transporter 1
 NT5C2 5'-Nucleotidase, Cytosolic II
 PCAD Premature Coronary Artery Disease
 PCSK9 Proprotein Convertase Subtilisin/Kexin Type 9
 PDE3A Phosphodiesterase 3A
 PDE5A Phosphodiesterase 5A
 PHACTR1 Phosphatase and Actin Regulator 1
 PMCA1 Plasma Membrane Calcium ATPase 1
 PP1 Protein Phosphatase 1
 RECQL5 Rec Q Protein-like 5
 SH2B3 SH2B Adaptor Protein 3
 SNPs Single-nucleotide Polymorphisms
 SORT1 Sortilin 1
 SPTBN5 Spectrin Beta, Non-Erythrocytic 5
 TRIB1 Tribbles Homolog 1
 TRLs Triglyceride-rich Lipoprotein
 TTC32 Tetratricopeptide Repeat Domain 32
 WDR35 WD Repeat Domain 35
 WES Whole-exome Sequencing
 WGS Whole Genome Sequencing

Introduction

Coronary artery disease (CAD) involves the development of severe coronary atherosclerosis and consequent reduction of blood flow to the heart. Traditional risk factors such as smoking, diabetes, hypercholesterolemia, and systemic hypertension have been implicated in the pathogenesis of CAD. However, 40%–60% of predisposition for CAD cases are inherited, and CAD is therefore generally considered to result from both genetic predisposition and traditional risk factors.¹ In the Framingham Heart Study, a family history of CAD was defined as heart disease in first-degree relatives before the age of 60 in men or the age of 65 in women, and was a powerful predictor of CAD.² Approximately one-third of patients with CAD have a family history of CAD, and individuals with a family history of CAD are approximately 1.5 times more likely to suffer from CAD in their lifespans than those without a family history.^{3,4}

Unraveling the genetic basis of CAD by screening candidate genes that encode proteins with known biological significance in CAD is an important approach to achieving a comprehensive understanding of the heritable risk factors for premature CAD (PCAD). Genome-wide

association studies (GWASs) are used to perform unbiased screens of the genomes of high-risk individuals to identify variant sequences and associations between common genetic variants and disease risk. A single-nucleotide polymorphism (SNP), which is defined as a change in a single nucleotide (A, T, C or G), is the most common type of genetic variation. Genotyping of specific populations can be performed to identify SNPs in coding genes and regulatory sequences that may be associated with variations in disease risk.⁵ GWASs have identified numerous genetic variations associated with CAD in genes such as *APOA5*, *PCSK9*, *GUCY1A1*, *NOS3*, *ANGPTL4*, *LDL-R*, *APOC3*, *LPL* and *LPA*, which are involved in regulation of blood lipid levels, inflammation, vascular endothelial migration, vascular tone, blood pressure and smooth muscle hyperplasia.^{6–9} (Table 1).

In this article we will provide a comprehensive review of genetic variants detected by GWAS that are predicted to contribute to the development of PCAD. We will highlight recent findings regarding CAD-related genetic variants in Chinese populations. Finally, we will discuss how knowledge of these genetic variants could be applied clinically in the prevention and management of PCAD (Fig. 2).

Table 1 Common genetic variants associated with CAD at genome-wide significance.

Genetic Locus	Chromosomal Location	Lead SNP	Risk Allele	Odds Ratio	Potential Mechanism	Reference
<i>PCSK9</i>	1	rs11206510	T	1.08	LDL	2,5,6,20,22,23
<i>LDL-R</i>	19	rs1122608	G	1.14	LDL	2,10,20,24
<i>APOB</i>	2	rs515135	C	1.07	LDL	5,6,24
<i>APOE</i>	19	rs2075650	G	1.14	LDL	6,24
<i>SORT1</i>	1	rs599839	A	1.11	LDL	6,24
<i>ABCG5-ABCG8</i>	2	rs6544713	T	1.06	LDL	6,24
<i>ABO</i>	9	rs579459	C	1.10	LDL, coagulation	6,24,39,40
<i>LPA</i>	6	rs3798220	C	1.51	Lp(a)	6,24
<i>LPL</i>	8	rs264	G	1.11	triglycerides	6,24,32
<i>APOA5</i>	11	rs964184	G	1.13	triglyceride	6,24,28
<i>ANGPTL4</i>	19	rs116843064	G	1.14	triglyceride	6,24,33
<i>APOC3</i>	11	rs964184	G	1.13	triglyceride	6,24,34,35
<i>TRIB1</i>	8	rs2954029	A	1.06	triglyceride	5,6,24
<i>CYP17A1-NT5C2</i>	10	rs12413409	G	1.12	hypertension	6,24,41,42
<i>SH2B3</i>	12	rs3184504	T	1.07	hypertension	6,24,41,42
<i>FURIN</i>	15	rs17514846	A	1.07	hypertension	5,6,24,41,42
<i>ZC3HC1</i>	7	rs11556924	C	1.09	hypertension	5,6,24,41,42
<i>ARHGAP42</i>	11	rs7947761	G	1.04	hypertension	6,24,41,42
<i>NOS3</i>	7	rs3918226	T	1.14	hypertension	5,6,43,44
<i>GUCY1A1</i>	4	rs1842896	T	1.08	hypertension	6,43,44,73
9p21.3	9	rs4977574	G	1.29	Arterial vessel wall	1,5,47,48
<i>ADAMTS7</i>	15	rs3825807	A	1.08	Arterial vessel wall	6,57
<i>C6orf105</i>	6	rs6903956	A	1.65	None	62
<i>C6orf10-BTNL2</i>	6	rs9268402	G	1.16	immunoglobulin	61
<i>TTC32-WDR35</i>	2	rs2123536	T	1.12	gene regulation	61
<i>ATP2B1</i>	12	rs7136259	T	1.11	hypertension	61
<i>PHACTR1</i>	6	rs12526453	C	1.10	inflammatory responses, vasoconstriction	6,71,73,74
<i>EDN1</i>	6	rs12526453	C	1.10	endothelin-1	6,73

Risk factors that predispose individuals to developing PCAD

PCAD is defined as early-onset atherosclerotic disease with 70% or greater stenosis of the coronary arteries or acute myocardial infarction before the age of 45.¹⁰ The pathogenesis of PCAD involves genetic predisposition, and the effects of genetic risk factors are modified by traditional cardiovascular risk factors like smoking, hypertension, diabetes, obesity, and dyslipidemia (Fig. 1). Patients with PCAD have a higher prevalence of hypertension, higher levels of glucose, and greater body mass index (BMI) compared with healthy individuals.¹¹ A meta-analysis found that a family history of CAD, diabetes, dyslipidemia, smoking, and hypertension were significantly and positively associated with CAD in young adults.¹¹ Diabetes, hypertension, and cigarette smoking are critical to the pathogenesis of CAD, and are present in 11.5%, 22.8%, and 17.1% of cases, respectively.¹² Hypercholesterolemia confers a 10- to 20-fold increased risk of developing PCAD,^{13,14} and obesity confers a higher risk for developing CAD.¹⁵ Ethnic origin and persistent smoking were strongly correlated with recurrent episodes of acute or stable obstructive CAD, and had the greatest impact on the prognosis of PCAD compared with other risk factors.¹⁰ A large Mendelian randomization

study that assessed the contribution of the genetic risk of obesity to the risk of developing CAD found that the genetic risk score (GRS) for BMI based on 35 risk alleles better predicted the occurrence of CAD, highlighting the importance of genetic contributions to both obesity and cardiovascular complications.¹⁶ However, paradoxically, a positive family history of PCAD was associated with better long-term survival in patients with angiographic CAD¹⁷ and acute coronary syndrome.¹⁸ A positive family history was also associated with improved overall adverse cardiovascular and cerebrovascular event-free survival.¹⁹ The genetic basis of this apparent paradox remains to be established. Therefore, the genetic risk factors that affect the development of CAD merit further investigation.

Genetic variants affect the risk of developing CAD

Genetic variants related to lipid regulation and CAD

Elevated levels of blood cholesterol, mainly low-density lipoprotein cholesterol (LDL-C), are a well-documented risk factor for CAD. Approximately 20% of all known SNPs associated with CAD are located near gene sequences involved

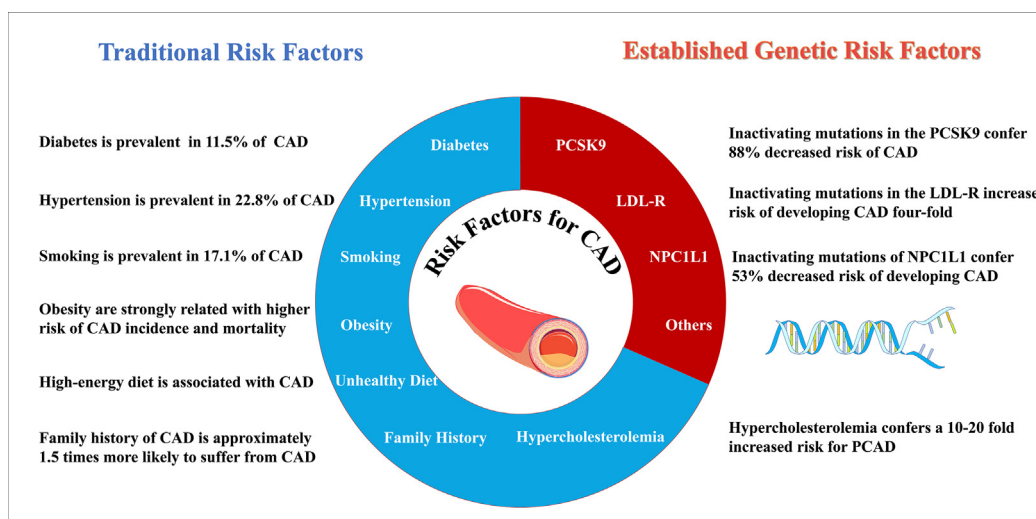


Figure 1 The pathogenesis of premature coronary artery disease (PCAD) involves both traditional and genetic cardiovascular risk factors. The development of PCAD is dependent on both genetic predisposition and traditional risk factors. Traditional risk factors such as diabetes, hypertension, smoking, obesity, a high-energy diet, hypercholesterolemia, and a family history of coronary artery disease are associated with a high incidence of PCAD. Genetic variants in genes such as *PCSK9*, *LDL-R*, and *NPC1L1* contribute to PCAD either directly or via traditional cardiovascular risk factors.

in the regulation of triglyceride-rich lipoprotein (TRLs), LDL-C, HDL-C or lipoprotein(a), indicating the importance of lipid regulation in the development of CAD.² Common variants in nine genes (*PCSK9*, *LDL-R*, *APOB*, *APOE*, *SORT1*, *ABCG5-ABCG8*, *ABO*, *LPA* and *NPC1L1*) associated with LDL-C levels,²⁰ five genes (*LPL*, *APOA5*, *ASGR1*, *ANGPTL4*, *APOC3* and *TRIB1*) associated with triglyceride levels²⁰ and the

gene encoding cholesteryl ester transfer protein (CETP), which is associated with HDL-C levels,²¹ have been linked to CAD.

Under normal physiological conditions, the elimination of LDL-C is primarily dependent on the synergistic effects of LDL-R and PCSK9. Inactivating mutations in *LDL-R* confer an increased risk of CAD,² whereas loss-of-function mutations

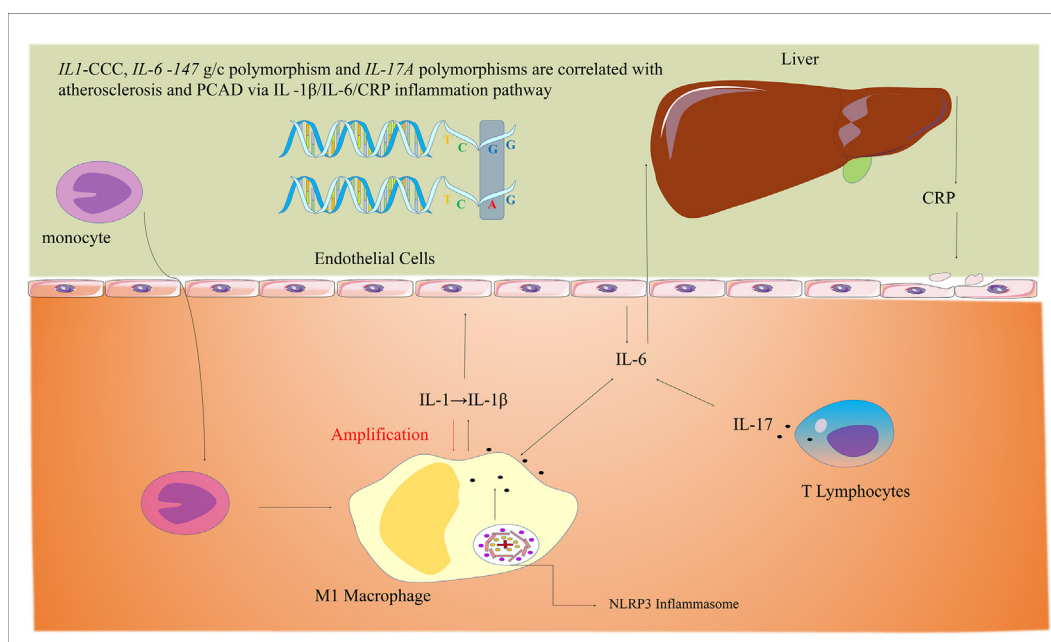


Figure 2 Single-nucleotide polymorphisms in the interleukin (IL)-1β/IL-6 signaling pathway contribute to atherosclerotic premature coronary artery disease (PCAD). The *IL1-CCC*, *IL-6-147* g/c, and *IL-17A* polymorphisms are correlated with atherosclerosis and PCAD via their effects on the IL-1β/IL-6/C-reactive protein (CRP) inflammation pathway. Activation of the NLRP3 inflammasome results in the production of IL-1β and consequent downstream effects on IL-6 and CRP synthesis in the liver. IL-17 produced by activated T cells promotes the production of IL-6, which amplifies local inflammation.

in *PCSK9*, which occur in 2% of individuals of African descent, have the opposite effect.^{2,22,23} Inactivating mutations in *LDL-R* increase the risk of developing CAD 4-fold, and are found in approximately 2% of patients with PCAD.^{2,24} Inactivating mutations in *PCSK9* confer an 88% decreased risk of developing CAD.^{2,23} Disease-related variants in novel genetic loci can indicate unexpected pathways that also lead to dysregulation of lipid metabolism. A striking example is *NPC1L1*, inactivating mutations of which lead to a 53% decreased risk of developing CAD in patients with atherosclerotic disease.²⁵ This observation is consistent with the activity of the protein encoded by *NPC1L1*, which mediates the absorption and transport of cholesterol by intestinal epithelial cells and results in elevated serum cholesterol levels. However, a high proportion of patients with CAD undergoing treatment exhibit average serum LDL-C levels greater than 0.7 g/l, which suggests that currently unrecognized genetic loci contribute to familial hypercholesterolemia or PCAD.^{26,27} LPL also plays a role in hydrolyzing circulating triglycerides, and *LPL* mutations significantly increase triglyceride levels and place individuals at high risk for developing CAD.²⁸ LPL activity is regulated by *APOC3*, *ANGPTL4*, and *APOA5*.^{24,29,30} Mutations in *APOC3*/*ANGPTL4* and *APOA5* attenuate and enhance LPL activity, respectively.^{24,29–31} Individuals harboring loss-of-function mutations in *ASGR1*, which encodes the asialoglycoprotein receptor, have relatively low levels of circulating triglycerides and LDL-C, as well as a low risk of developing CAD.³² However, an attempt to use CETP inhibitors to elevate HDL-C levels failed to decrease the risk of CAD, which suggests that the causal relationship between HDL-C and CAD needs to be reconsidered. Additionally, it has been documented that the individuals with type O blood are less likely to suffer from acute myocardial infarction than those with type A or B blood.³³ Regarding the potential mechanism, the protein encoded by the A and B blood group loci, alpha 1-3N-acetylgalactosaminyltransferase, assists in transferring a carbohydrate molecule to von Willebrand factor (vWF), which promotes coagulation.^{1,34} Mutation of the O blood group locus is associated with loss of vWF activity, which results in a relatively low incidence of myocardial infarction.³⁵ However, a recent study demonstrated a correlation between the *ABO* locus and LDL-C levels, which suggests that activated vWF alone is not enough to predispose to myocardial infarction.³⁶

Blood pressure—related genetic variants and CAD

Several loci that contain variants associated with an elevated risk of developing CAD (*CYP17A1-NT5C2*, *SH2B3*, *FURIN*, *ZC3HC1* and *ARHGAP42*) are related to blood pressure.^{20,37,38} GWAS studies have identified SNPs linked to hypertension. Both nitric oxide synthase 3 (*NOS3*) and guanylate cyclase 1 soluble subunit alpha 1 (*GUCY1A1*), two genetic loci that encode proteins which participate in NO-sGC-cGMP signaling, are important in atherosclerosis development.^{39,40} Loss-of-function mutation of the *NOS3* or *GUCY1A1* gene is associated with an elevated risk of hypertension and CAD,⁴¹ and inactivating mutations in the *GUCY1A1* gene are associated with an increased risk of developing PCAD.⁷ The proteins encoded by both of these

genes play pivotal roles in vascular tone regulation and proatherogenic inhibition by modifying nitric oxide (NO) signaling. SNPs located in the *PDE3A*, *PDE5A* and *MRVI1* genes, which encode proteins in the NO signaling pathway, have also been demonstrated by GWAS to be associated with CAD^{7,20,39,42}; however, the underlying mechanism remains elusive.

Genetic variants in 9p21.3 and CAD

Variants in locus 9p21.3, which is located at band 2.1 on the short arm (p) of chromosome 9, are associated with a risk of developing PCAD.^{5,43} This locus regulates the downstream cyclin-dependent kinase inhibitors CDKN2A and CDKN2B.⁴⁴ Harismendy and associates used innovative unbiased genomic techniques based on chromosome conformation capture to demonstrate a long-distance interaction between a CAD-associated enhancer element and the genes encoding CDKN2A/B.⁴⁵ Other genes may be also regulated by the CAD-associated risk locus on 9p21.3 through this long-distance effect.⁶ Variants in the 9p21.3 locus are also associated with an incrementally increased risk of Alzheimer's disease, vascular dementia, gout, arterial aneurysms, periodontitis and ischemic stroke.^{46–48} This suggests that the CAD-associated susceptibility gene located at 9p21.3 also increases susceptibility to multiple other diseases. Surprisingly, the 9p21 locus is closely related to initial, rather than later, events in CAD development,⁴⁹ which merits further investigation.

Inflammation-related variants and CAD

Recently, the role of immunity and inflammation in the pathogenesis of CAD has received substantial attention, especially after the CANTOS trial showed that anti-inflammatory therapy with canakinumab is effective in treating atherosclerotic disease.⁵⁰ The inflammation signaling cascade from interleukin (IL)-1 β to IL-6 to C-reactive protein (CRP) is one of the major atherogenic pathways (Fig. 2),^{51,52} and inflammation-related genetic variants have been implicated in atherogenesis. Mutation of *IL-1* showed nominal association with PCAD.⁵³ A survey of South African Indian men with PCAD found a strong relationship between SNPs in *IL-6* and CAD.⁵⁴ IL-17 produced by activated T cells promotes the production of cytokines including IL-6, IL-8, and colony stimulating factor (CSF), as well as vascular cell adhesion molecules, which amplify local inflammation. In the Genetics of Atherosclerotic Disease study, which recruited young Mexican individuals with CAD, SNPs in *IL-17* and *IL-35* were associated with increased and decreased risk of developing CAD, respectively.^{55,56}

Other genetic variants and CAD

Other CAD-related SNPs have been identified in genes such as *ADAMTS7*, *SPTBN5* and *NID2*, which encode proteins that regulate cytoskeletal assembly and muscle tissue growth rather than directly affecting CAD.⁵⁷ The protease encoded by the *ADAMTS7* gene is anchored to the extracellular matrix and regulates proteolysis and vascular wall remodeling. Inactivating mutations of the *ADAMTS7* gene lead to

an increased risk of CAD.⁵⁸ The *SPTBN5* gene has been shown to mediate interference of the β subunit of spectrin with the cell membrane and the development of cerebral ischemia in a Japanese population.⁵⁹ Similarly, the *NID2* gene is a vital role in the formation of basement membrane.⁵⁷ While these genes potentially play a pathogenetic role in CAD, the underlying mechanisms remain to be elucidated.

Genetic studies of PCAD-related variants in Chinese populations

Most findings of CAD-related genetic variants are from studies conducted in European populations; however, ethnicity is an important risk factor that may be involved in the pathogenesis of PCAD.⁶⁰ Due to genetic heterogeneity, CAD-related SNPs identified in populations with European ancestry may not be related to CAD in other ancestral populations.⁶¹ American College of Cardiology/American Heart Association guidelines for blood cholesterol management state that ethnicity enhances the risk of developing CAD to the same extent as having a chronic inflammatory disease or a family history of CAD.¹⁰ Thus, evidence of CAD-related genetic variants in Chinese populations is needed.

The first CAD-associated SNP identified in the Chinese Han population was in the *C6orf105* gene at chromosomal locus 6p24.1.⁶² Individuals with this risk locus exhibit lower levels of mRNA transcription from the *C6orf105* gene than those without the risk locus.⁶² Given the long-distance interaction mechanism described above, variants in the *C6orf105* gene may affect the expression of other distant genes, thereby contributing to an increased risk of CAD. *C6orf105* exhibits some homology to the human androgen-induced gene 1 (*AIG-1*), which is regulated by androgen concentrations and, when activated, increases the risk of developing atherosclerosis.⁶³ It is imperative to investigate whether *AIG-1* interferes with *C6orf105* expression and to explore the relationship between CAD and *C6orf105* downregulation in future studies. According to HapMap genotyping data, significant differences in the mutation frequency of *C6orf105* have been observed among different ethnicities, ranging from 5.6% in the Chinese population to 28% in the European population.⁶² However, it is unclear whether *C6orf105* has similar effects on CAD in different populations. One possible reason is that different ethnic groups may be exposed to different environments because of different lifestyles and that the interaction between genes and the environment can modify gene expression, which has different effects.

A mutation in the *RECQL5* gene was identified as being involved in PCAD based on analysis of a family with five affected individuals.⁶⁴ In this family, a TG insertion in the intron 11 receptor splice site led to deletion of exon 12 in the transcribed mRNA. Transcripts containing exon 12 were only rarely expressed in family members homozygous for this mutation, and those who were heterozygous for the mutation expressed about half as many transcripts containing exon 12 as those without any copies of the allele containing this mutation.⁶⁴ The RECQL5 α , β , and γ helicases are encoded by the *RECQL5* gene. RECQL5 α and γ , which

have no known biological effects, reside in the cytoplasm. RECQL5 β is an ATP-dependent DNA helicase that is transported to the nucleoplasm^{65,66} and contributes to the development of genetic diseases by affecting DNA metabolism.⁶⁵ A correlation between *RECQL5* and cancer has been identified⁶⁴; however, it is unclear whether mutation of *RECQL5* confers a highly significant risk of developing PCAD. Knockdown of the *RECQL5* β transcript upregulates LDL-R and β -actin, both of which are associated with CAD.⁶⁶ Therefore, this variant in the *RECQL5* gene is a potential genetic risk factor for PCAD in the Chinese Han population.

Lu and associates found four novel SNPs associated with CAD in the Chinese population located near the *TTC32-WDR35* gene at chromosomal locus 2p24.1, the *GUCY1A1* gene at chromosomal locus 4q32.1, the *C6orf10-BTNL2* gene at chromosomal locus 6p21.32 and the *ATP2B1* gene at chromosomal locus 12q21.33.⁶¹ In European populations, SNPs near *GUCY1A1* and *ATP2B1* are associated with hypertension.^{41,61} SNPs near *TTC32-WDR35* have a negative effect on gene regulation, peptide binding, cell signal transduction and apoptosis,⁶⁷ which may place Chinese individuals at higher risk of developing CAD. *GUCY1A1* encodes the α subunit of soluble guanylate cyclase (sGC), and SNPs near this gene confer a significant risk of hypertension and atherosclerosis.⁶¹ SNPs near *C6orf10-BTNL2* are associated with autoimmune diseases. *C6orf10-BTNL2* encodes immunoglobulins that are involved in T cell functions, and SNPs in this gene lead to the deregulation of the immune response observed in Taiwanese children with Kawasaki disease.⁶⁸ Therefore, it seems reasonable that SNPs located near *C6orf10-BTNL2* confer a risk of developing CAD. *ATP2B1* encodes a plasma membrane calcium ATPase 1 (PMCA1), and mutations in the *ATP2B1* gene show nominal association with essential hypertension in the Chinese Han population.⁶⁹ Intracellular calcium levels are elevated when the *ATP2B1* gene is knocked down in mice,⁷⁰ which may also promote CAD.

SNPs in the phosphatase and actin regulator 1 (*PHACTR1*) gene located at chromosomal locus 6p24.1 exhibit a statistically significant association with PCAD, as determined by the Myocardial Infarction Genetics Consortium.⁷¹ *PHACTR1* mediates actin assembly and regulates cell migration. *PHACTR1* mutation can activate M1 macrophage differentiation and foam cell formation, thereby contributing to local inflammatory responses.⁷² In addition, the *PHACTR1* GG allele increases expression of endothelin-1, an endogenous vasoconstrictor encoded by *EDN1* in endothelial cells, through gene–gene interactions.⁷³ A recent study by Chen et al found that *PHACTR1* mutations were associated with CAD susceptibility, particularly in women,⁷⁴ but whether estrogen mediates this difference is unclear.

Clinical applications of CAD-related genetic variants

Accumulating evidence suggests that genetic risk factors are involved in the pathogenesis of PCAD. Although only a small number of relevant genetic variants have been identified to date, translating knowledge of these variants from bench to bedside nevertheless holds promise (Fig. 3).

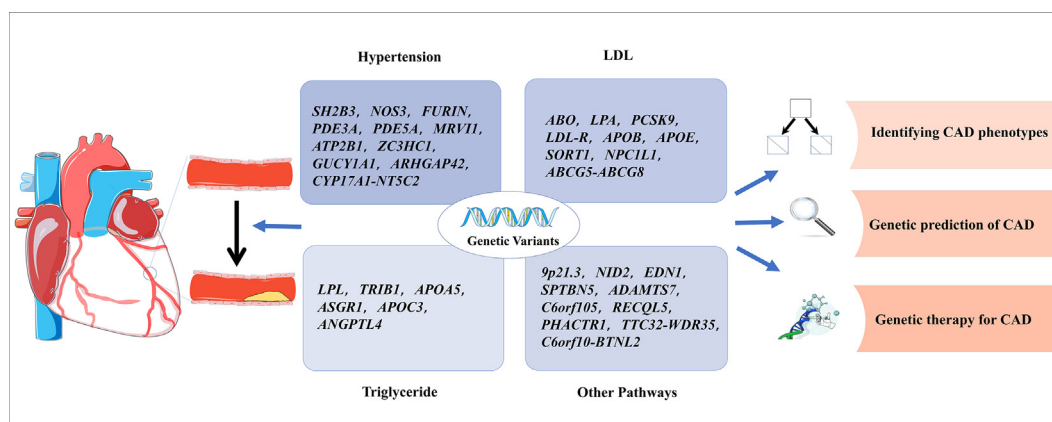


Figure 3 Clinical outcomes and promising applications of genetic variants in premature coronary artery disease (PCAD). Genome-wide association studies have successfully identified genetic variants (in genes related to regulation of hypertension, LDL levels, and triglyceride levels) associated with the development of PCAD. Although translating this knowledge into clinical practice is still challenging, using this information to identify CAD phenotypes, predict the genetic risk of developing CAD, and develop genetic therapies for CAD based on inherited genetic variants is nevertheless a promising approach.

Identifying CAD phenotypes based on inherited genetic variants

GWAS studies benefit in exploring genetic risk factors associated with CAD and its major complications. Known CAD-associated gene loci or SNPs could be leveraged to distinguish atherosclerosis from myocardial infarction.¹ The 9p21.3 and *ADAMTS7* risk loci, which act on the blood vessel walls, lead to atherosclerosis rather than myocardial infarction. Additionally, the 9p21 risk allele exhibits a dose-dependent relationship with the number of atherosclerotic coronary arteries, and 9p21 risk allele frequency is considerably higher in patients with two- or three-vessel disease compared with those with one-vessel disease.⁷⁵ Mutation of the ABO blood group locus is an important risk factor for myocardial infarction, but GWAS studies have not demonstrated any association of this locus with atherosclerosis.¹ One explanation for the relationship between the risk alleles and phenotypes described above is that each locus has a unique potential pathogenetic mechanism. Alternatively, innate predisposition to thrombosis and atheromatous plaque rupture is a possible pathogenetic cause of myocardial infarction.⁷⁶ Therefore, deeper exploration of the molecular mechanisms underlying the risk conferred by these alleles could help identify unexpected pathogenetic phenotypes.

Potential for genetic prediction of CAD

Individuals with LDL-C levels greater than 190 mg/dl are more likely to carry detrimental mutations within the familial hypercholesterolemia gene, and are three times more likely to suffer from CAD than individuals who do not carry these mutations.⁷⁷ In the early 1990s, epidemiologists believed that 40%–50% of CAD cases occurred because of hereditary factors.⁷⁸ However, to date, known genetic variants only account for 20%–25% of CAD cases caused by genetic predisposition, raising the possibility that there are potential pathogenic variants yet to be discovered.³⁹

Alternatively, given the methodologies that have been used to explore genetic risk factors for this disease, many mutations that are true risk factors for CAD may simply have failed to reach the strict *P*-value threshold for statistical significance.²⁰

Given the stable structure of DNA, genes do not mutate easily throughout an individual's life, regardless of age, diet or drug use. Therefore, genetic risk has already been established at birth, which provides a theoretical basis for the utilization of genetic information to predict the risk of developing CAD later in life. Furthermore, coronary atherosclerosis develops over years or decades; hence, it is possible to acquire genetic information and screen asymptomatic individuals to identify those who are at high risk for developing CAD in time to recommend preventive treatment. These individuals should require primary prevention at the preclinical stage, and accept much more intensive therapy in individuals exposed to the traditional CAD risk factors.²⁰ However, high-risk mutations with pronounced effects such as those in *PCSK9* and *LDL-R* are rare in the general population. A more plausible explanation for the development of hereditary PCAD is that patients carry a large number of low-risk mutations that interact to confer high risk of developing the disease. To incorporate known genetic information into an overall risk assessment for CAD, GRSs are proposed to quantify genetic risk in patients with CAD. These include the number of high-risk variants carried and the natural log of the previously determined odds ratio based on a database of known genetic variants.⁷⁸ Thériault and associates selected 182 CAD-related SNPs and found that the GRS was much higher in patients with PCAD in the UK Biobank cohort.⁷⁹ Individuals with PCAD carry more genetic risk variants than those with late-onset CAD.¹ Indeed, the risk of developing PCAD is predominantly determined by inherited factors in patients without traditional risk factors.⁸⁰ LDL-C levels in patients with high-risk GRSs were much lower, which suggests that GRSs can be used to identify these patients earlier than traditional methods of assessing risk.⁸¹ GRS assessment is a promising approach for the proactive identification of disease

recurrence and high-risk relatives.⁸⁰ Individuals of Western European ancestry carry an average of 130–160 risk alleles at 163 identified loci, which suggests that common risk alleles may explain the general susceptibility to CAD in the absence of a positive family history.^{7,82} Is the risk conferred by a positive family history solely attributable to these CAD risk alleles? When higher GRS and family history are both included in a multi-risk CAD prediction model, the correlation between family history and CAD is not weakened, which suggests that GRS-based prediction of a genetic predisposition of CAD does not account for all risk factors.⁸³ Another possible explanation is that family history may also include familial environmental risk factors.⁵ Therefore, the current GRS cannot yet replace the predictive value of family history.

Thus far the strategies based on genetic information have not been used extensively, primarily because the majority of the variants included in the GRS are located in non-coding regions and have unknown pathogenic effects, meaning that the GRS may have only modest predictive power.⁸⁴ The best way to address this problem is to explore the mechanism of gene mutations that occur outside of protein-coding regions and design a new GRS incorporating select genes with large pathogenic effects. However, some difficulties still stand in the way. Genes outside of protein-coding regions do not directly encode proteins but regulate multiple other genetic loci located at adjacent, downstream coding regions or on other chromosomes, through long-distance effects. A very large sample size integrating multiple ongoing projects is also needed to document the complexity of the genome across all human cell types, such as the GTEx (Genotype-Tissue Expression), ENCODE (ENCyclopedia Of DNA Elements) and Roadmap Epigenomics projects.⁸⁵ Additionally, knocking out genes *in vivo* and *in vitro* to verify the causal association between target genes and clinical effects is of paramount importance. Moreover, the high cost of routine genetic screening, the good performance of Framingham and ACC/AHA risk scores and the psychological impact that risk disclosure has on patients restrict the application of GRSs.^{84,86} Because of the reasonable cost and rapidity of whole-exome sequencing (WES), it is recommended as a substitute for the more expensive whole genome sequencing (WGS) approach. Obviously, the main disadvantage of WES is the lack of intron sequencing, and the technique is therefore only appropriate for screening and identifying specific exons of interest. Much work is needed to improve the effectiveness of GRSs in assessing the genetic risk of developing CAD.

The potential of genetic therapy for CAD

Neither WES nor WGS can be used routinely in clinical practice because genetics-based strategies should not only enable the early detection and stratification of high-risk individuals, but also reduce the risk of disease by enabling appropriate medications to be prescribed. Some documented CAD-related SNPs have provided pathophysiological insights into and potential for the development of novel therapies. These targeted therapeutics, which were initially used to treat patients with specific genetic

mutations, have been increasingly recognized as more widely effective treatments.² For example, PCSK9 inhibitors have been used as standard treatment for most CAD patients with or without PCSK9 mutations.⁸⁷ Of note, the pathogenic mechanisms of more than half of all SNPs known to confer a high risk of developing CAD are not modifiable through lifestyle changes, unlike traditional risk factors.⁷⁸ However, there may be other novel protective therapeutic agents like PCSK9 inhibitors waiting to be discovered.

The development of novel, efficient, widely applicable and safe genetic therapies for CAD is rapidly becoming an important public health challenge.² Known genetic pathways function as important predictors of clinical efficacy and can help identify possible adverse outcomes of drugs under development. Novel pharmacological agents should be tested for side effects, which can be predicted based on the relevant risk genes. For instance, mutation of the gene encoding HMG-coenzyme A reductase is inextricably linked to decreased LDL-C levels but an elevated risk of type 2 diabetes.⁸⁸ CETP inhibitors increase HDL-C levels, but genetic variants within CETP are also strongly associated with age-related macular degeneration, a major cause of blindness.⁸⁹ Additionally, variants in the gene encoding lipoprotein-associated phospholipase A2 (Lp-PLA2) that mimic the effects of an Lp-PLA2 inhibitor which has been tested extensively in clinical trials do not decrease the risk of developing CAD.⁹⁰ Therefore, exploring CAD-related risk genes can also help predict clinical trial failure.

It is common to encounter individuals with angiographically normal coronary arteries but multiple cardiovascular risk factors in clinical practice, which raises the possibility that some genes may protect individuals from developing CAD.⁵⁷ Nineteen genetic variants that protect individuals from developing CAD have been identified.⁵⁷ Therefore, CAD protective variants are promising targets for prophylactic strategies in the future, once their functions have been thoroughly investigated.

The challenges still remain in the development of novel CAD-related genetic therapies. In addition to identifying more potential genetic risk factors, exploring currently unknown causative pathways will have important epidemiological, biological and therapeutic implications. It is likely that components of signaling pathways other than the product of the causal gene will be the best druggable targets. Additionally, GWAS only identifies potential pathogenic genes, but provides no information about their biological functions. Bioinformatics approaches could be used to perform full-scale and inexpensive post-GWAS analyses, to help translate GWAS results into clinical applications.⁶

Conclusion

PCAD is an aggressive cardiovascular disorder that has a complex etiology including vascular inflammation, hyperlipidemia, unhealthy lifestyles, ethnicity and inherited genetic risk factors. Analysis of genetic variants has promising implications for the future development of personalized strategies for the prevention, diagnosis and treatment of PCAD.

Conflict of interests

The authors declare that they have no conflict of interest.

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