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.

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).
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. 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. Patients with PCAD have a higher prevalence of hypertension, higher levels of glucose, and greater body mass index (BMI) compared with healthy individuals. 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. 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. Hypercholesterolemia confers a 10- to 20-fold increased risk of developing PCAD, and obesity confers a higher risk for developing CAD. 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. 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. However, paradoxically, a positive family history of PCAD was associated with better long-term survival in patients with angiographic CAD and acute coronary syndrome. A positive family history was also associated with improved overall adverse cardiovascular and cerebrovascular event-free survival. 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

| Genetic Locus | Chromosomal Location | Lead SNP | Risk Allele | Odds Ratio | Potential Mechanism | Reference |
|---------------|----------------------|----------|-------------|------------|---------------------|-----------|
| PCSK9         | 1                    | rs11206510 | T           | 1.08       | LDL                 | 2, 5, 6, 20, 22, 23 |
| LDL-R         | 19                   | rs1122608  | G           | 1.14       | LDL                 | 2, 10, 20, 24   |
| APOB          | 2                    | rs515135   | C           | 1.07       | LDL                 | 5, 6, 24       |
| APOE          | 19                   | rs2075650  | G           | 1.14       | LDL                 | 6, 24         |
| SORT1         | 1                    | rs599839   | A           | 1.11       | LDL                 | 6, 24         |
| ABCG5-ABCG8   | 2                    | rs6544713  | T           | 1.06       | LDL                 | 6, 24         |
| ABO           | 9                    | rs579459   | C           | 1.10       | LDL, coagulation    | 6, 24, 39, 40 |
| LPA           | 6                    | rs3798220  | C           | 1.51       | Lp(a)               | 6, 24         |
| LPL           | 8                    | rs264      | G           | 1.11       | triglycerides      | 6, 24, 32     |
| APOA5         | 11                   | rs964184   | G           | 1.13       | triglyceride        | 6, 24, 28     |
| ANGPTL4       | 19                   | rs116843064| G           | 1.14       | triglyceride        | 6, 24, 33     |
| APOC3         | 11                   | rs964184   | G           | 1.13       | triglyceride        | 6, 24, 34, 35 |
| TRIB1         | 8                    | rs2954029  | A           | 1.06       | triglyceride        | 5, 6, 24      |
| CYP17A1-NT5C2 | 10                   | rs12413409 | G           | 1.12       | hypertension       | 6, 24, 41, 42 |
| SH2B3         | 12                   | rs3184504  | T           | 1.07       | hypertension       | 6, 24, 41, 42 |
| FURIN         | 15                   | rs17514846 | A           | 1.07       | hypertension       | 5, 6, 24, 41, 42 |
| ZC3HC1        | 7                    | rs11556924 | C           | 1.09       | hypertension       | 5, 6, 24, 41, 42 |
| ARHGAP42      | 11                   | rs7947761  | G           | 1.04       | hypertension       | 6, 24, 41, 42 |
| NOS3          | 7                    | rs3918226  | T           | 1.14       | hypertension       | 5, 6, 43, 44 |
| GUCY1A1       | 4                    | rs1842896  | T           | 1.08       | hypertension       | 6, 43, 44, 73 |
| 9p21.3        | 9                    | rs4977574  | G           | 1.29       | Arterial vessel wall| 1, 5, 47, 48  |
| ADAMTS7       | 15                   | rs3825807  | A           | 1.08       | Arterial vessel wall| 6, 57        |
| C6orf105      | 6                    | rs603956   | A           | 1.65       | None               | 62           |
| C6orf10-BTNL2 | 6                    | rs9268402  | G           | 1.16       | Immunoglobulin     | 61           |
| TTC32-WDR35   | 2                    | rs2123536  | T           | 1.12       | Gene regulation    | 61           |
| ATP2B1        | 12                   | rs7136259  | T           | 1.11       | Hypertension       | 61           |
| PHACTR1       | 6                    | rs12526453 | C           | 1.10       | Inflammatory responses, vasoconstriction | 6, 71, 73, 74 |
| EDN1          | 6                    | rs12526453 | C           | 1.10       | Endothelin-1       | 6, 73        |
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. 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, whereas loss-of-function mutations...
in PCSK9, which occur in 2% of individuals of African descent, have the opposite effect.\textsuperscript{2,22,23} Inactivating mutations in \textit{LDL-R} increase the risk of developing CAD 4-fold, and are found in approximately 2% of patients with PCAD.\textsuperscript{2,24} Inactivating mutations in \textit{PCSK9} confer an 88% decreased risk of developing CAD.\textsuperscript{2,23} Disease-related variants in novel genetic loci can indicate unexplained 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.\textsuperscript{25} This observation is consistent with the activity of the protein encoded by \textit{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.\textsuperscript{26,27} LPL also plays a role in hydrolyzing circulating triglycerides, and \textit{LPL} mutations significantly increase triglyceride levels and place individuals at high risk for developing CAD.\textsuperscript{28} LPL activity is significantly increased in 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.\textsuperscript{26,27} LPL also plays a role in hydrolyzing circulating triglycerides, and \textit{LPL} mutations significantly increase triglyceride levels and place individuals at high risk for developing CAD.\textsuperscript{28} LPL activity is regulated by \textit{APOC3}, \textit{ANGPTL4}, and \textit{APOA5}.\textsuperscript{24,29,30} Mutations in \textit{APOC3}/\textit{ANGPTL4} and \textit{APOA5} attenuate and enhance LPL activity, respectively,\textsuperscript{24,29,30} Individuals harboring loss-of-function mutations in \textit{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{36}

**Blood pressure–related genetic variants and CAD**

Several loci that contain variants associated with an elevated risk of developing CAD (\textit{CYP17A1}-\textit{NT5C2}, \textit{SHPB3}, \textit{FURIN}, \textit{ZC3HC1} and \textit{ARHGAP42}) are related to blood pressure.\textsuperscript{20,37,38} GWAS studies have identified SNPs linked to hypertension. Both nitric oxide synthase 3 (\textit{NOS3}) and guanylate cyclase 1 soluble subunit alpha 1 (\textit{GUCY1A1}), two genetic loci that encode proteins which participate in NO-cGMP signaling, are important in atherosclerosis development.\textsuperscript{39,40} Loss-of-function mutation of the \textit{NOS3} or \textit{GUCY1A1} gene is associated with an elevated risk of hypertension and CAD,\textsuperscript{11} and inactivating mutations in the \textit{GUCY1A1} gene are associated with an increased risk of developing PCAD.\textsuperscript{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 \textit{PDE3A}, \textit{PDE5A} and \textit{MRVI1} genes, which encode proteins in the NO signaling pathway, have also been demonstrated by GWAS to be associated with CAD\textsuperscript{2,20,39,42}; 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.\textsuperscript{5,43} This locus regulates the downstream cyclin-dependent kinase inhibitors CDKN2A and CDKN2B.\textsuperscript{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.\textsuperscript{5} Other genes may be also regulated by the CAD-associated risk locus on 9p21.3 through this long-distance effect.\textsuperscript{5} Variants in the 9p21.3 locus are also associated with an incrementally increased risk of Alzheimer’s disease, vascular dementia, gut, arterial aneurysms, periodontitis and ischemic stroke.\textsuperscript{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,\textsuperscript{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.\textsuperscript{50} The inflammation signaling cascade from interleukin (IL)-1\beta to IL-6 to C-reactive protein (CRP) is one of the major atherogenic pathways (Fig. 2).\textsuperscript{51,52} Inflammation-related genetic variants have been implicated in atherosclerosis. Mutation of \textit{IL-1} showed nominal association with PCAD.\textsuperscript{53} A survey of South African Indian men with PCAD found a strong relationship between SNPs in \textit{IL-6} and CAD.\textsuperscript{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 \textit{IL-17} and \textit{IL-35} were associated with increased and decreased risk of developing CAD, respectively.\textsuperscript{55,56}

**Other genetic variants and CAD**

Other CAD-related SNPs have been identified in genes such as \textit{ADAMTS7}, \textit{SPTBN5} and \textit{NID2}, which encode proteins that regulate cytoskeletal assembly and muscle tissue growth rather than directly affecting CAD.\textsuperscript{57} The protease encoded by the \textit{ADAMTS7} gene is anchored to the extracellular matrix and regulates proteolysis and vascular wall remodeling. Inactivating mutations of the \textit{ADAMTS7} gene lead to
an increased risk of CAD. 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. Similarly, the NID2 gene is a vital role in the formation of basement membrane. 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. Due to genetic heterogeneity, CAD-related SNPs identified in populations with European ancestry may not be related to CAD in other ancestral populations. 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. 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. Individuals with this risk locus exhibit lower concentrations and, when activated, increases the risk of developing atherosclerosis. 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. 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 RECLQ5 gene was identified as being involved in PCAD based on analysis of a family with five affected individuals. 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. The RECLQ5 α, β, and γ helicases are encoded by the RECLQ5 gene. RECLQ5 α and γ, which have no known biological effects, reside in the cytoplasm. RECLQ5 β is an ATP-dependent DNA helicase that is transported to the nucleoplasm and contributes to the development of genetic diseases by affecting DNA metabolism. A correlation between RECLQ5 and cancer has been identified; however, it is unclear whether mutation of RECLQ5 confers a highly significant risk of developing PCAD. Knockdown of the RECLQ5 β transcript upregulates LDL-R and β-actin, both of which are associated with CAD. Therefore, this variant in the RECLQ5 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. In European populations, SNPs near GUCY1A1 and ATP2B1 are associated with hypertension. SNPs near TTC32-WD35 have a negative effect on gene regulation, peptide binding, cell signal transduction and apoptosis, 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. 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. Therefore, it seems reasonable that SNPs located near C6orf10-BTNL2 confers 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. Intracellular calcium levels are elevated when the ATP2B1 gene is knocked down in mice, 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. 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. In addition, the PHACTR1 GG allele increases expression of endothelin-1, an endogenous vasoconstrictor encoded by EDN1 in endothelial cells, through gene–gene interactions. A recent study by Chen et al found that PHACTR1 mutations were associated with CAD susceptibility, particularly in women, 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.
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. The 9p21 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. 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. 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. Therefore, deeper exploration of the molecular mechanisms underlying the risk conferred by these alleles could help identify unexpected pathogenic 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. In the early 1990s, epidemiologists believed that 40%–50% of CAD cases occurred because of hereditary factors. 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. 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.

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. 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. Individuals with PCAD carry more genetic risk variants than those with late-onset CAD. Indeed, the risk of developing PCAD is predominantly determined by inherited factors in patients without traditional risk factors. 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. GR5 assessment is a promising approach for the proactive identification of disease...
recurrence and high-risk relatives. 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. 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. Another possible explanation is that family history may also include familial environmental risk factors. 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. 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. 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 GRSSs. 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 GRSSs 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. For example, PCSK9 inhibitors have been used as standard treatment for most CAD patients with or without PCSK9 mutations. 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. 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. 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. 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. 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. 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. Nineteen genetic variants that protect individuals from developing CAD have been identified. 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.

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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References

1. Roberts R, Stewart AF. Genes and coronary artery disease: where are we. J Am Coll Cardiol. 2012;60(18):1715–1721.
2. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nat Rev Genet. 2017;18(6):331–344.
3. Kramar NT, Borglykke A, Allin KH, et al. A genetic risk score of 45 coronary artery disease risk variants associates with increased risk of myocardial infarction in 6041 Danish individuals. Atherosclerosis. 2015;240(2):305–310.
4. Hawe E, Tallmadge PJ, Miller GJ, Humphries SE. Second northwick park heart study. Ann Hum Genet. 2003;67(Pt 2):97–106.
5. Assimes TL, Roberts R. Genetics: implications for prevention and management of coronary artery disease. J Am Coll Cardiol. 2016;68(25):2797–2818.
6. Erdmann J, Kessler T, Munoz Venegas L, Schunkert H. A decade of genome-wide association studies for coronary artery disease: the challenges ahead. Cardiovasc Res. 2018;114(9):1241–1257.
7. Erdmann J, Stark K, Esslinger UB, et al. Dysfunctional nitric oxide signalling increases risk of myocardial infarction. Nature. 2013;504(7480):432–436.
8. Brænne I, Reiz B, Medack A, et al. Whole-exome sequencing in an extended family with myocardial infarction unmasks familial hypercholesterolemia. BMC Cardiovasc Disord. 2014;14:108.
9. Brænne I, Kleinecke M, Reiz B, et al. Systematic analysis of variants related to Familial hypercholesterolemia in families with premature myocardial infarction. Eur J Hum Genet. 2016;24(2):191–197.
10. Collet JP, Zeitouni M, Procopi N, et al. Long-term evolution of premature coronary artery disease. J Am Coll Cardiol. 2019;74(15):1868–1878.
11. Poorzand H, Torsouhas K, Hozhabrossadati SA, et al. Risk factors of premature coronary artery disease in Iran: a systematic review and meta-analysis. Eur J Clin Invest. 2019;49(7):e13124.
12. Kumbhalkar SD, Biswak B. Clinical and angiographic profile of young patients with ischemic heart disease: a central India study. J Clin Prev Cardiol. 2019;8:6–12.
13. Watts GF, Lewis B, Sullivan DR. Familial hypercholesterolemia: a missed opportunity in preventive medicine. Nat Clin Pract Cardiovasc Med. 2007;4(8):404–405.
14. Bilan O, Pokharel Y, Ballantyne CM. Genetic testing in hyperlipidemia. Cardiol Clin. 2015;33(2):267–275.
15. Ortega FB, Lavie CJ, Blair SN. Obesity and cardiovascular disease. Circ Res. 2016;118(11):1752–1770.
16. Cole CB, Nikpay M, Stewart AF, McPherson R. Increased genetic risk for obesity in premature coronary artery disease. Eur J Hum Genet. 2016;24(4):587–591.
17. Abdi-All A, Shaheen A, Southern D, et al. Relation between family history of premature coronary artery disease and the risk of death in patients with coronary artery disease. Am J Cardiol. 2016;117(3):353–358.
18. Levi A, Chezar-Azerrad C, Hasdai D, et al. Impact of self-reported family history of premature cardiovascular disease on the outcomes of patients hospitalized for acute coronary syndrome (from the acute coronary syndrome Israel survey [ACSI S] 2000 to 2013). Am J Cardiol. 2018;122(6):917–921.
19. Ruttmann E, Abfalterer H, Dietl M, et al. Positive family history of cardiovascular disease and long-term outcomes after coronary artery bypass grafting: a genetic paradox. Eur J Cardio Thorac Surg. 2020;57(5):986–993.
20. CARDioGRAMplusC4D Consortium, Deloukas P, Kanoni S, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet. 2013;45(1):25–33.
21. Cheng CY, Yamashiro K, Chen LJ, et al. New loci and coding variants confer risk for age-related macular degeneration in East Asians. Nat Commun. 2015;6:6063.
22. Cohen J, Pertsemelidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet. 2005;37(2):161–165.
23. Cohen JC, Boerwinkle E, Mosley Jr TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med. 2006;354(12):1264–1272.
24. Do R, Stitziel NO, Won HH, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. Nature. 2015;518(7537):102–106.
25. Myocardial Infarction Genetics Consortium Investigators, Stitziel NO, Won HH, et al. Inactivating mutations in NPC1L1 and protection from coronary heart disease. N Engl J Med. 2014;371(22):2072–2082.
26. Singh A, Gupta A, Collins BL, et al. Familial hypercholesterolemia among young adults with myocardial infarction. J Am Coll Cardiol. 2019;73(19):2439–2450.
27. Singh A, Collins BL, Gupta A, et al. Cardiovascular risk and statin eligibility of young adults after an MI: partners YOUNG-MI registry. J Am Coll Cardiol. 2018;71(3):292–302.
28. Khera AV, Won HH, Peloso GM, et al. Association of rare and common variation in the lipoprotein Lipase gene with coronary artery disease. JAMA. 2017;317(9):937–946.
29. Myocardial Infarction Genetics and CARDioGRAM Exome Consortium Investigators, Stitziel NO, Stirrupes KE, et al. Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary disease. N Engl J Med. 2016;374(12):1134–1144.
30. Jørgensen AB, Frikke-Schmidt R, Nordergaard GB, Tybjerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med. 2014;371(1):32–41.
31. TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung aBI, Crosby J, et al. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med. 2014;371(11):22–31.
32. Nioi P, Sigurdsson A, Thorleifsson G, et al. Variant ASGR1 associated with a reduced risk of coronary artery disease. N Engl J Med. 2016;374(22):2131–2141.
33. Allan TM, Dawson AA. ABO blood groups and ischaemic heart disease in men. Br Heart J. 1968;30(3):377–382.
34. Gill JC, Endres-Brooks J, Bauer PJ, Marks Jr WJ, Montgomery B. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood. 1987;69(6):1691–1695.
35. Pang H, Zong Z, Hao L, Cao Q. ABO blood group influences risk of venous thromboembolism and myocardial infarction. J Thromb Thrombolysis. 2020;50(2):430–438.
36. Schunckert H, König IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011;43(4):333–338.

37. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;478(7367):103–109.

38. van der Harst P, Verweij N. Identification of 64 novel genetic loci provides an expanded view on the genetic architecture of coronary artery disease. Circ Res. 2018;122(3):433–443.

39. Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet. 2015;47(10):1121–1130.

40. Ehret GB, Ferreira T, Chisman DI, et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. Nat Genet. 2016;48(10):1171–1184.

41. Emdin CA, Khera AV, Klarin D, et al. Phenotypic consequences of a genetic predisposition to enhanced nitric oxide signaling. Circulation. 2018;137(3):222–232.

42. Klärn D, Zhu QM, Emdin CA, et al. Genetic analysis in UK Biobank links insulin resistance and transatlantal migration pathways to coronary artery disease. Nat Genet. 2017;49(9):1392–1397.

43. Palomaki GE, Melillo S, Bradley LA. Association between 9p21 genotypes and heart disease: a meta-analysis. JAMA. 2010;303(7):648–656.

44. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007;316(5830):1489–1491.

45. Harismendy O, Notani D, Song X, et al. 9p21 DNA variants associated with coronary artery disease impair interferon-γ signalling response. Nature. 2011;470(7333):264–268.

46. Helgadottir A, Thorleifsson G, Magnusson KP, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm.

47. Emanuele E, Lista S, Ghidoni R, et al. Chromosome 9p21.3 signalling response.

48. Wang B, Meng D, Wang J, et al. Genetic association of polymorphism rs1333049 with gout. Rheumatol (Oxford). 2011;50(9):1559–1561.

49. Dehghan A, Bis JC, White CC, et al. Genome-wide association study of a genetic predisposition to enhanced nitric oxide signaling. Circulation. 2018;137(3):222–232.

50. Wang H, Liu Z, Shao J, et al. Immune and inflammation in acute myocardial infarction with single nucleotide polymorphisms. Circulation. 2010;122(10):217–224.

51. Idem et al. From CANTOS to CIRT to COLCOT to clinic: will all inflammatory gene polymorphisms in sibships discordant for premature coronary artery disease: the GRACE-IMMUNE study. BMC Med. 2010;8:5.

52. Phulukdaree A, Khan S, Ramkaran P, Govender R, Moodley D, Chuturgoon AA. The interleukin-6 -147 g/c polymorphism is associated with increased risk of coronary artery disease in young South African Indian men. Metab Syndr Relat Disord. 2013;11(3):205–209.

53. Vargas-Alarcón G, Angeles-Martínez J, Villarreal-Molina T, et al. Interleukin-17A gene haplotypes are associated with risk of premature coronary artery disease in Mexican patients from the Genetics of Atherosclerotic Disease (GEA) study. PloS One. 2015;10(1):e0114943.

54. Posadas-Sánchez R, Pérez-Hernández N, Angeles-Martínez J, et al. Interleukin 35 polymorphisms are associated with decreased risk of premature coronary artery disease, metabolic parameters, and IL-35 levels: the genetics of atherosclerotic disease (GEA) study. Mediat Inflamm. 2017;2017:4012795.

55. Abramowitz Y, Roth A, Keren G, et al. Whole-exome sequencing in individuals with multiple cardiovascular risk factors and normal coronary arteries. Coron Artery Dis. 2016;27(4):257–266.

56. Pu X, Xiao Q, Kiači S, et al. ADAMTS7 cleavage and vascular smooth muscle cell migration is affected by a coronary-artery-disease-associated variant. Am J Hum Genet. 2013;92(3):366–374.

57. Yoshida T, Kato K, Yokoi K, et al. Association of genetic variants with myocardial infarction in Japanese individuals with different lipid profiles. Int J Mol Med. 2010;25(4):607–616.

58. Meadows TA, Bhatt DL, Cannon CP, et al. Ethnic differences in cardiovascular risks and mortality in atherothrombotic disease: insights from the Reduction of Atherothrombosis for Continued Health (REACH) registry. Mayo Clin Proc. 2011;86(10):960–967.

59. Lu X, Wang L, Chen S, et al. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. Nat Genet. 2012;44(8):890–894.

60. Wang F, Xu CQ, He Q, et al. Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population. Nat Genet. 2011;43(4):345–349.

61. Seo J, Kim J, Kim M. Cloning of androgen-inducible gene 1 (AIG1) from human dermal papilla cells. Mol Cells. 2001;11(1):35–40.

62. Xie X, Zheng YY, Adi D, et al. Exome sequencing in a family identifies RECSL5 mutation resulting in early myocardial infarction. Medicine (Baltim). 2016;95(5):e2737.

63. Shimamoto A, Nishikawa K, Kitao S, Furuchi Y. Human RecQ5beta, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3alpha and 3beta. Nucleic Acids Res. 2000;28(7):1647–1655.

64. Izumikawa K, Yanagida M, Hayano T, et al. Association of human DNA helicase RecQ5beta with RNA polymerase II and its possible role in transcription. Biochem J. 2008;413(3):505–516.

65. Smith DF. Tetratricopeptide repeat coagagor in steroid receptor complexes. Cell Stress Chaperones. 2004;9(2):109–121.

66. Huseh KC, Lin YJ, Chang JS, Wan L, Tsai FJ. BTNL2 gene polymorphisms may be associated with susceptibility to Kawasaki disease and formation of coronary artery lesions in Taiwanese children. Eur J Pediatr. 2010;169(6):713–719.

67. Xu J, Qian HX, Hu SP, et al. Gender-specific association of ATP2B1 variants with susceptibility to essential hypertension in the Han Chinese population. Biomed Res Int. 2016;2016:1910565.

68. Okuyama Y, Hirawa N, Fujita M, et al. The effects of anti-hypertensive drugs and the mechanism of hypertension in vascular smooth muscle cell-specific ATP2B1 knockout mice. Hypertens Res. 2018;41(2):80–87.

69. Myocardial Infarction Genetics Consortium, Kathiresan S, Voight BF, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009;41(3):334–341.

70. Li T, Ding L, Wang Y, Yang O, Wang S, Kong J. Genetic deficiency of Phacr1 promotes atherosclerosis development via facilitating M1 macrophage polarization and foam cell formation. Clin Sci (Lond). 2020;134(17):2333–2368.

71. Gupta RM, Hadaya J, Trehan A, et al. A genetic variant associated with five vascular diseases is a distal regulator of endothelin-1 gene expression. Cell. 2017;170(3):522–533.
74. Chen L, Qian H, Luo Z, et al. PHACTR1 gene polymorphism with the risk of coronary artery disease in Chinese Han population. Postgrad Med J. 2019;95(1120):67–71.

75. Dandona S, Stewart AF, Chen L, et al. Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. J Am Coll Cardiol. 2010;56(6):479–486.

76. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res. 2014;114(12):1852–1866.

77. Khera AV, Won HH, Peloso GM, et al. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. J Am Coll Cardiol. 2016;67(22):2578–2589.

78. Roberts R. Genetic risk stratification: tipping point for global primary prevention of coronary artery disease. Circulation. 2018;137(24):2554–2556.

79. Assimes TL, Herrington DM. Genetic risk scores in premature coronary artery disease: still only one piece of the prevention puzzle. Circ Genom Precis Med. 2018;11(1):e002006.

80. Thériault S, Lali R, Chong M, Velianou JL, Natarajan MK, Paré G. Polygenic contribution in individuals with early-onset coronary artery disease. Circ Genom Precis Med. 2018;11(1):e001849.

81. Kulko IJ, Jouni H, Austin EE, et al. Incorporating a genetic risk score into coronary heart disease risk estimates: effect on low-density lipoprotein cholesterol levels (the MI-GENES clinical trial). Circulation. 2016;133(12):1181–1188.

82. Schunkert H. Family or SNPs: what counts for hereditary risk of coronary artery disease. Eur Heart J. 2016;37(6):568–571.

83. Tada H, Melander O, Louie JZ, et al. Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history. Eur Heart J. 2016;37(6):561–567.

84. Goldstein BA, Knowles JW, Salfati E, Ioannidis JP, Assimes TL. Simple, standardized incorporation of genetic risk into non-genetic risk prediction tools for complex traits: coronary heart disease as an example. Front Genet. 2014;5:254.

85. Tak YG, Farnham PJ. Making sense of GWAS: using epigenomics and genome engineering to understand the functional relevance of SNPs in non-coding regions of the human genome. Epigenet Chromatin. 2015;8:57.

86. Sandler S, Alfino L, Saleem M. The importance of preventative medicine in conjunction with modern day genetic studies. Genes Dis. 2018;5(2):107–111.

87. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376(18):1713–1722.

88. Swerdlow DI, Preiss D, Kuchenbaecker KB, et al. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. Lancet. 2015;385(9965):351–361.

89. Liutkeviciene R, Vilkeviciute A, Streleckiene G, Kriauciuniene L, Chaleckis R, Deltuva VP. Associations of cholesteryl ester transfer protein (CETP) gene variants with predisposition to age-related macular degeneration. Gene. 2017;636:30–35.

90. Polfus LM, Gibbs RA, Boerwinkle E. Coronary heart disease and genetic variants with low phospholipase A2 activity. N Engl J Med. 2015;372(3):295–296.