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GENETICS (A. MARIAN, SECTION EDITOR)

Genetics—Current and Future Role in the Prevention and Management of Coronary Artery Disease

Robert Roberts[1]

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Abstract
Purpose of Review The purpose of this study is to review genetic risk variants for coronary artery disease (CAD) and how they will change the management and prevention of CAD currently and in the future.
Recent Findings Through the efforts of international consortia, 58 genetic risk variants for CAD of genome-wide significance have been replicated in appropriate independent populations. Only one third of these variants mediate their risk through known conventional risk factors for CAD. Thus, unknown mechanisms contribute to CAD. Secondly, the genetic risk is proportional to the total number of risk variants rather than the intensity of any risk factor. Thirdly, the availability of the genetic risk variants enables one to perform Mendelian randomization (MR) studies since they are randomized at conception, not confounded, fixed for life, and can be used to determine if a risk factor is causative or just a marker. MR can also be used to determine the safety and efficacy of a gene product targeted for drug therapy. Genetic risk variants have been shown to successfully risk stratify for CAD in both primary and secondary preventions.
Summary Contrary to dogma, MR documents that plasma HDL-C is not protective of CAD. The use of genetic risk score (GRS) for CAD is shown to be more effective in risk stratifying for CAD than the Framingham risk score and independent of the conventional risk factors including family history. Furthermore, the GRS predicts the response to statin therapy in primary and secondary preventions. The use of GRS could represent a paradigm shift in the prevention of CAD.

Keywords Genetics · Coronary artery disease · Mendelian randomization · Genetic risk score for CAD · Genetic risk stratifications for CAD

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✉ Robert Roberts
rroberts2@email.arizona.edu

[1] University of Arizona College of Medicine-Phoenix, 550 East Van Buren, Phoenix, AZ 85004, USA

Introduction

Coronary artery disease (CAD) has become a pandemic disease, being the no. 1 killer in both the developing and the developed world [1]. Nevertheless, the application of effective prevention strategies in recent decades has shown a decreased incidence in the Western world. These prevention strategies, which included changes in lifestyle as well as drug therapy, to decrease plasma cholesterol have shown a 40–50 % reduction in cardiac events [1]. The proven success of preventing CAD has led to the postulation by investigators that CAD could be markedly attenuated or eliminated in the twenty-first century [2, 3].

A major gap in our knowledge has been the lack of genetic risk variants, which has been postulated to account for 40–60 % of the predisposition to CAD [4–6]. The twenty-first century addressed this challenge with the discovery of the first genetic risk variant (9p21) for CAD in 2007 [7, 8]. Individual groups pursuing genome-wide association studies (GWAS) discovered several genetic risk variants for CAD and subsequently came together in an international consortium. These efforts have resulted in the discovery of over 50 genetic risk variants associated with increased risk for CAD [9•], all of which have been replicated in independent populations. Over 150 genetic variants have been discovered that regulate plasma lipids [10, 11], which are known risk factors for CAD. The genetic risk variants predisposing to several diseases

associated with increased risk for CAD, such as diabetes and hypertension, are also rapidly being discovered. Mendelian randomization studies indicate that plasma high-density lipoprotein cholesterol (HDL-C) is not protective of CAD [12•], while proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are effective and safe for prevention of CAD [13]. The use of genetic risk variants to predict risk of CAD and the response to therapy appear promising [14•]. This review will discuss how genetic risk variants for CAD will change the management of CAD and continue to do so in the future.

The Era of GWAS

The availability of SNPs spanning the genome annotated to their precise position in the genome led to the first microarrays containing 500,000 to 1,000,000 DNA markers in the form of SNPs [15, 16]. This enabled two independent groups of investigators to discover the first genetic risk variant for CAD, namely, 9p21 in 2007 [7, 8]. The 9p21 risk variant was shown to occur in 75 % of individuals of European ancestry. The increase in relative risk for CAD for one copy was about 25 % and for two copies about 50 % [7, 8]. It was also shown that the 9p21 risk variant was independent of all known risk factors for CAD. Several groups including the Welcome Trust Case-Control Consortium (WTCCC), the German MI Family Study, and the Myocardial Infarction Genetics Consortium (MIGen) identified 11 other genetic risk variants for CAD [17–21]. These studies consistently showed that each genetic risk variant exhibited minimal incremental increase in risk, necessitating large sample sizes to detect such variants. International consortia were formed, including the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) and the Coronary Artery Disease (C4D) Genetics, which confirmed the previous reported genetic risk variants for CAD and uncovered several others [22, 23]. Formation of the CARDIoGRAMplusC4D from the two consortia reported additional variants bringing the total number with genome-wide significance and replication in an independent population to 58 [24, 25].

Conventional Risk Factors for CAD Also Have an Inherent Hereditary Component

Known risk factors for CAD include body mass index, blood lipid levels, blood pressure, diabetes, insulin resistance, family history, and smoking. Risk factors such as blood lipids are under intense genetic regulation. The plasma lipid levels of low-density lipoprotein cholesterol (LDL-C), HDL-C, and triglycerides are known to be 70–80 %, determined by genetic mechanisms [26]. In a collaboration of the CARDIoGRAMplusC4D plus the Global Lipids Genetics Consortium, 95 genetic variants regulating plasma levels were discovered [10], and subsequently, the Global Lipids Genetics Consortium identified an additional 62 genetic variants for plasma lipid levels, resulting in a total of 157 [11]. Other conventional risk factors such as hypertension [27, 28] and diabetes [29, 30] have recently been shown to be associated with multiple genetic risk variants. The role of these genetic risk variants in the management of lipid disorders is emerging as we recognize the variable responses of individuals to various drugs administered to lower plasma lipids.

Genetic Risk Variants for CAD Provide New Insights into the Pathogenesis of Coronary Atherosclerosis

In keeping with the hypothesis, the genetic risk variants are very common with most of them having a frequency in the population of 50 % or greater [9•]. Secondly, the increased relative risk of each variant for CAD is minimal, averaging about 17 % which means an odds ratio of 1.17. It is evident from present studies that the genetic risk for CAD is related to the number of genetic risk variants rather than to the intensity of any variant. It would appear that most people will have several of these variants while those at greater risk are expected to have many more risk variants. Thirdly, most of the genetic risk variants appear to be residing in that part of the genome that does not encode for proteins or are in linkage disequilibrium with the actual risk variant. This would indicate that these variants regulate upstream or downstream sequences that do modify protein-coding genes. Lastly, over two thirds of the genetic risk variants for CAD do not act through known conventional risk factors, implying that other mechanisms contributing to the pathogenesis of coronary atherosclerosis are yet to be discovered. This has implications for development of new therapies in the prevention and treatment of CAD.

PCSK9 Inhibitors—a New Therapy for Prevention of CAD

The finding of a mutation in the LDL receptor in the 1970s by Brown and Goldstein [31] catalyzed the development of statin therapy which today is our main drug for prevention of CAD. PCSK9 [32] inhibits removal of LDL-C from the plasma and discovery of a mutation associated with gain of function [33] induced hypercholesterolemia and increased incidence of CAD. The discovery of a mutation associated with loss of function was associated with a 28 % reduction in plasma LDL-C and an 88 % reduction in risk of CAD [34]. These genetic findings led to the development of antibodies to PCSK9 which is now utilized clinically and shown to be safe and effective in reducing LDL-C and cardiac events [13, 35].

PCSK9 inhibitors decrease plasma LDL-C by increasing the removal of LDL-C from the plasma, while statin therapy decreases the synthesis of cholesterol, thus, PCSK9 inhibitors complement our current armamentarium of statin therapy.

MR: an Approach to Determine Causality of Genetic Risk Factors for CAD and Their Potential Safety and Efficacy as Drug Targets

The FDA requirements to determine the safety and efficacy of drugs are ideally based on the randomized, placebo-controlled clinical trial (RCT). The RCT is usually assessed over a 3–5-year interval with a predetermined adequate sample size. Mendelian randomization is now feasible with the availability of genetic risk variants for CAD. Mendelian randomization enables one to determine whether the product of a particular genetic risk variant is causally related to a disease or is just a marker or surrogate. Utilizing Mendelian randomization (MR) to assess whether the genetic variant is safe and efficacious, one can also determine whether its encoded product is potentially a good drug target. The genetic variants of an individual are randomized at conception and not confounded by other factors. They remain fixed throughout life and thus can be assessed for their effects from birth onward for decades rather than just the few years possible with an RCT. A necessary requirement for a successful MR is the availability of genetic variants that strongly influences the risk factor of interest but is not associated with the clinical outcome other than through their effects on the risk factor. Thus, the recent discovery of multiple genetic risk variants associated with diseases enables the use of MR to assess causation of genetic risk variants and the safety and efficacy of their encoded product as a therapeutic target for several diseases including CAD. If the genetic variant has pleiotropic effects, it might be confounding and not ideal for MR studies.

MR Studies Indicate Plasma Levels of HDL-C Are Not Protective

It is an assumed dogma that elevated plasma levels of HDL-C are protective of CAD. This dogma is in large part based on the results of epidemiological studies and a variety of interventions, namely, niacin, exercise, fibrates, and red wine. All of these interventions are associated with increased plasma HDL-C and decreased cardiac events; however, there is a confounding factor since all of them simultaneously decrease LDL-C [36–38]. MR studies were performed by assessing the effect of the SNP in LIPG p.Asn396Ser and 14 common SNPs associated solely with an increase in plasma HDL-C levels [12•]. The sample size of 50,763 with 4228 myocardial infarctions (MI) was designed with 90 % power to detect a 13 % reduction in risk of MI. Results were replicated in an independent population of 16,685 cases of MI and 48,872 controls, followed by a meta-analysis of both populations (20,913 cases and 95,407 controls). These results show no association between plasma HDL-C levels and protection from MI. In this study, we used as a control 13 SNPs associated solely with increased levels of plasma LDL-C levels. As expected, these SNPs were associated with a twofold increased risk of MI. A recent meta-analysis was performed [39] of 39 trials involving 117, 411 patients randomized to assess the effect of niacin, fibrates, and cholesteryl ester transfer protein (CETP) inhibitors on cardiovascular events (death, MI, and stroke). All interventions increased plasma HDL-C but neither niacin, fibrates, nor CETP inhibitors exhibited any effect on cardiac events, death, or stroke. In patients receiving niacin but not receiving statin therapy, there was a reduction in the incidence of MI. In patients receiving statin therapy, the addition of niacin offered no benefit over that of statin therapy. A similar trend was observed for fibrates. This would suggest that the benefit of niacin and fibrates is due to its minimal effect on the lowering of plasma LDL-C levels. The investigators do not recommend therapy for increasing plasma HDL-C levels. These results are in keeping with results of MR studies showing that an increased plasma HDL-C level is not associated with protection of CAD [12•, 40••]. Recently, RCT assessing CETP shows that despite increased plasma levels of HDL-C, they did not improve clinical outcomes [40••]. The lack of association between plasma HDL-C and CAD has important implications for the management of CAD and also for the direction of future research. MR studies consistently show plasma triglycerides to be a risk factor for CAD [12•, 40••].

MR Proved Several Potentially Beneficial Agents for CAD Were Not Effective

Drug development for CAD has been dormant for the past two decades [41]. The failure rate is 90 % [42], and the cost to market a cardiovascular drug is estimated at $2.5 billion [43]. A potential solution to minimize the failure rate and mitigate the cost would be to determine whether the drug's target is causally related to CAD through MR studies. If genetic variants are available, such is the case for CAD, the MR study is inexpensive and less time-consuming. The role of MR is exemplified by two recent drugs, varespladib and darapladib, both of which were shown in phase III clinical trials to have no effect on cardiac events or stroke [44–46]. However, MR studies performed before the RCT were completed showed no causality between the drug target for varespladib [47–49] and CAD and similarly, no causality between the drug target for darapladib [50, 51] and CAD, predicting a negative response for both drugs. Had the clinical trials waited for the results of the MR studies, the expenditure of millions of dollars could have been avoided.

The drugs varespladib and darapladib are inhibitors of secretory phospholipase A2 (sPLA2) and lipoprotein-associated PL A2 (Lp-PLA2), respectively. Experimental studies [52] had shown that these two enzymes release phospholipid which is shown to be one of the components of atheroma plaques. The MR studies were performed using a functional genetic variant of sPLA2 that is associated with 38 % lower levels with one copy and 60 % lower levels with two copies [47]. In a sample size of 93,000, there was no evidence of a causal effect of sPLA2 for risk of CAD. Further MR studies were conducted [48, 49] in a pooled analysis of 27,230 events and 70,500 controls and confirmed no causal effect of varespladib for increased risk of CAD. In the clinical trial VISTA-16, 5189 patients with acute coronary syndrome received varespladib (500 mg/day) or placebo for a follow-up of 16 weeks [44]. Analysis showed no effect of varespladib on outcomes with a slight increase in the risk of MI. The trial was terminated and any further assessment of varespladib was abandoned.

To assess the causality of Lp-PLA2, an MR study used seven genetic variants of PLA2G7 in a sample size of 10,494 CAD cases and 15,624 controls. Analysis showed that Lp-PLA2 was not associated with any increased risk for CAD [50]. A more recent MR study using a loss-of-function variant for Lp-PLA2 associated with threefold lower levels of Lp-PLA2 showed no difference in risk for CAD [51]. The clinical effect of the Lp-PLA2 inhibitor darapladib was assessed in two phase III RCTs, SOLID-TIMI 52 [45] and STABILITY [46]. SOLID-TIMI 52 randomized 13,026 patients with acute coronary syndrome to darapladib (160 mg/day) or placebo with a follow-up of 2.5 years. In STABILITY, 15,828 patients with stable CAD received 160 mg/day or placebo and were followed for 3.7 years. There was no effect on death, MI, or stroke in either of the studies, and pooling the results in a meta-analysis showed no statistical increased risk of CAD [45, 46]. The concordant results of MR studies and RCT for varespladib and darapladib strongly indicate that MR studies are highly predictive of safety and efficacy and would be helpful if performed prior to phase III clinical trials.

The value of MR studies in evaluating therapeutic agents for CAD is increasing and the results have been very beneficial. Recent MR studies have shown that homocysteine and fibrinogen are not causally related to CAD [40••]. Results of RCT show that folic acid does not improve clinical outcomes [52, 53]. Similarly, MR studies show that hs-CRP, while a marker of inflammation, is not a risk factor for CAD [40••].

MR Studies Confirm the Greater Effect of Early Primary Prevention of CAD

Ference et al. [54] studied nine LDL-C-related genetic variants in a meta-analysis of multiple studies involving over 300,000 individuals. Individuals inheriting genetic variants that decreased plasma LDL-C experienced a marked reduction in cardiac morbidity and mortality over their lifetime [54]. They estimated that a decrease of one unit of LDL-C (38.7 mg/dL) since birth is associated with a 54.5 % reduction in cardiac events, which is threefold greater than observed in clinical trials utilizing statin therapy [54]. These studies emphasize the important role of early primary prevention of CAD, such as is possible with the early administration of statin therapy.

Genetic Risk Score Improves Risk Stratification for CAD—a Paradigm Shift in the Prevention and Treatment

It has been the hope that genetic risk variants would ultimately improve our stratification of risk for CAD, but their clinical application has been slower than expected. First, as expected, it is a multi-gene disorder, so the increased risk of any genetic variant is minimal and would not offer improvement over conventional risk factors such as the Framingham risk score (FRS) or that of the ACC/AHA guidelines as shown by an analysis of 9p21 [55]. It became evident that it would require the accumulative risk of many genetic risk variants to offer any advantage over conventional risk stratification techniques. This required time and analysis of large sample sizes to discover these genetic risk variants for CAD [9•]. Goldstein et al. [56] showed that the genetic risk could be expressed in a summation score utilizing multiple variants which is more discriminatory than the FRS. It is well recognized that attenuation of CAD and its sequel will be primarily through prevention and in particular primary prevention. Secondary prevention following a cardiac event is well proven to be very effective in RCT by modification of risk factors including the lowering of plasma LDL-C by statin therapy. The recent studies utilizing the MR approach (discussed above) clearly support early intervention and show that the effect of prevention is strongly related to the duration of therapy. Primary prevention, while potentially more effective, faces a greater dilemma, namely, how to detect those individuals at increased risk for CAD. Our current approach is to treat such asymptomatic individuals only if they have two or more known conventional risk factors such as diabetes or hypertension. This dilemma is illustrated in the case of a 47-year-old white premenopausal female with an HDL-C of 50 mg/dL, an LDL/C of 175 mg/dL, an untreated blood pressure of 120/80 mmHg, and no other risk factors. According to the clinical guidelines, her estimated risk is only 2 % and treatment with therapy, such as statins to lower LDL-C, is not indicated until she develops other risk factors or has a cardiac event [61]. Decreasing the plasma LDL-C to the range of 70 to 80 mg/dL would be expected

to delay or prevent the development of significant CAD. The identification of multiple genetic risk variants predisposing to CAD has raised the hope that such variants could be used to risk stratify with greater distinguishing power than the FRS or that of the recent ACC guidelines [57]. The latter two methods are both age dependent and can only be applied if conventional risk factors are present. The GRS based on DNA risk variants is independent of age and known risk factors, so it could be applied to stratify risk for CAD at any age. This would be particularly applicable for primary prevention of asymptomatic individuals such as the case previously described. In the premenopausal female, one could take advantage of the minimal coronary atherosclerosis that exists and delay its progression by treating those individuals determined by the GRS to be at greater risk for CAD.

The use of a single genetic risk variant such as 9p21 has been shown to offer no advantage over conventional risk factors [56]. In retrospect, this would be anticipated since there are over 50 genetic risk variants, each associated with minimal increased risk [9•]. A single value for GRS can be obtained by summing the product of the number of high-risk variants inherited by each individual for each susceptibility variant and the log of the odds ratio as determined by previous studies [56, 58]. This provides a single number for genetic risk that is easy to incorporate into determining an individual's 10-year risk. In 2015, Weijmans et al. [59] in the Second Manifestations of ARTerial (SMART) disease study, using a GRS based on 30 SNPs associated with CAD, assessed its predictive value in 5742 patients with symptomatic vascular disease. The predictive value of GRS was comparable to traditional risk factors. However, comparison based on the c-statistic and the categorical net reclassification index (NRI) showed no advantage of GRS over conventional risk factors in risk stratifying for CAD. The authors concluded that GRS might improve risk prediction of the first vascular events but over the 10-year period showed no advantage over conventional risk factors [59]. In a similar study, de Vries et al. [60] utilized a GRS based on 152 SNPs associated with CAD in 5899 subjects followed for 10 years. GRS significantly improved discrimination and reclassification of prevalent cardiac events beyond traditional risk factors and family history with a c-statistic improvement of 0.009. Nevertheless, it did not improve prediction for prevalent disease over that of conventional risk factors. The investigators concluded that this discrepancy could be the result of SNP discovery for prevalent events rather than incident cardiac events. They proposed that GRS could improve prediction of future cardiac events earlier in life when conventional variables used in prediction are not yet available.

In what might be a landmark study, Mega et al. [14•] utilized a GRS based on 27 genetic risk variants for CAD and demonstrated that genetic risk is independent of conventional risk factors. Contrary to the previous studies, the GRS provided an increased discriminating power over that of the FRS and also predicted more accurately the response to statin therapy. In this retrospective study, the investigators genotyped the DNA of individuals enrolled into two primary prevention trials (JUPITER and ASCOT) and two secondary prevention trials (CARE and PROVE IT-TIMI 22), assessing the effect of statin therapy [14•]. The sample size consisted of 48,421 individuals and 3477 events. It statistically stratified individuals into low, intermediate, and high risk in both primary and secondary prevention trials. In addition, it also predicted the intensity of the response to statin therapy for each group in primary and secondary studies. In the primary prevention trials, the number needed to treat to prevent one such event in 10 years was 66 in people at low genetic risk, 42 in those at intermediate genetic risk, and 25 in those at high genetic risk in JUPITER and 57, 47, and 20, respectively, in ASCOT. The risk stratification by GRS was independent of conventional risk factors including family history [14•, 61]. Within months of this study, Tada et al. [61] evaluated the GRS predictive value utilizing the 27 SNPs of Mega et al. (GRS27) and assessed the value of adding another 23 SNPs already proven to be associated with CAD (referred to as GRS50). The study was performed in 23,595 participants from the Malmo Diet and Cancer Study. During a median follow-up of 14.4 years, there were 2213 participants who experienced a first cardiac event. The investigators showed that GRS27 improved significantly the prediction of cardiac events over that of utilizing conventional risk factors. The addition of 23 SNPs, namely, GRS50, further improved discrimination ($p < 0.0001$) and reclassification ($p < 0.0001$). Furthermore, participants below the median age with high GRS50 had a 2.4-fold greater risk than those with low GRS50. The most recent study by Abraham et al. [62], utilizing GRS based on 49,310 SNPs, confirmed the increased predictive value of genetic risk variants over that of conventional risk factors including family history. This study was performed in five prospective population cohorts with a sample size of 12,676 subjects. The GRS provided improved meaningful lifetime cardiac risk stratification over that of conventional risk factors including family history. Furthermore, the combination of GRS with FRS improved the 10-year cardiac risk prediction, particularly in individuals over the age of 60 years. These recent studies show the GRS risk stratification is superior to conventional risk factors, and the addition of more genetic risk variants further enhances the discriminatory power of GRS to stratify for risk of cardiac events.

These results have tremendous implications for the treatment and prevention of CAD, particularly in asymptomatic individuals without other risk factors. Utilization of proven genetic risk variants for CAD as they become

available is expected to further enhance the predictability and accuracy of GRS for cardiac events. The administration of statin therapy early in asymptomatic individuals at high genetic risk is a potential for the future. Such approaches are already supported by MR studies for LDL-C as reviewed above [54]. Adoption of the GRS for routine clinical application will, however, have to be supported in RCTs. Nevertheless, the use of GRS in prospective clinical trials holds great promise for attenuating the current pandemic of CAD.

Conclusion

The discovery of genetic risk variants acting through mechanisms other than conventional risk factors indicates that additional unknown molecular pathways are involved with the pathogenesis of CAD. The availability of genetic risk variants made it possible to pursue MR studies. The result of MR studies indicates that plasma HDL-C is not protective of CAD [12•]. Mendelian randomization studies enabled one to assess whether risk factors are causally related to coronary atherosclerosis and to observe the safety and efficacy of risk variants such as plasma LDL-C over decades rather than the 3–5 years of clinical trial. The genetic risk variants provide new pathways and targets for the development of novel drugs. The discovery of mutations in the PCSK9 gene has spawned a new class of drugs with an entirely different mechanism from that of statin therapy to decrease plasma LDL-C, namely, PCSK9 inhibitors [13]. A major anticipated clinical application for genetic risk variants predisposing to CAD is their utilization to risk stratify so that those at greater risk can be identified for appropriate primary or secondary prevention. Studies document that risk stratification with GRS for cardiac events is superior to conventional risk factors [14•] for both primary and secondary preventions. An exciting new era has dawned for the prevention and management of CAD utilizing genetic risk variants.

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Compliance with Ethical Standards

Conflict of Interest Robert Roberts declares to be a consultant to Cumberland Pharmaceuticals and confirms no conflicts.

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