Title

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Journal

Current Atherosclerosis Reports, 19(2)

ISSN

1523-3804

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Publication Date

2017-02-01

DOI

10.1007/s11883-017-0641-6

Peer reviewed

HHS Public Access

Author manuscript

Curr Atheroscler Rep. Author manuscript; available in PMC 2018 January 24.

Published in final edited form as:

Curr Atheroscler Rep. 2017 February ; 19(2): 6. doi:10.1007/s11883-017-0641-6.

The Genetic Architecture of Coronary Artery Disease: Current Knowledge and Future Opportunities

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Abstract

Purpose of review—We provide an overview of our current understanding of the genetic architecture of coronary artery disease (CAD)and discuss areas of research that provide excellent opportunities for further exploration.

Recent findings—Large-scale studies in human populations, coupled with rapid advances in genetic technologies over the last decade, have clearly established the association of common genetic variation with risk of CAD. However, the effect sizes of the susceptibility alleles are for the most part modest and collectively explain only a small fraction of the overall heritability. By comparison, evidence that rare variants make a substantial contribution to risk of CAD has been somewhat disappointing thus far, suggesting that other biological mechanisms have yet to be discovered. Emerging data suggests that novel pathways involved in the development of CAD can be identified through complementary and integrative systems genetics strategies in mice or humans. There is also convincing evidence that gut bacteria play a previously unrecognized role in the development of CAD, particularly through metabolism of certain dietary nutrients that lead to proatherogenic metabolites in the circulation.

Summary—A major effort is now underway to functionally understand the newly discovered genetic and biological associations for CAD, which could lead to the development of potentially

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Conflicts of Interest

None.

novel therapeutic strategies. Other important areas of investigation for understanding the pathophysiology of CAD, including epistatic interactions between genes or with either sex and environmental factors, have not been studied on a broad scope and represent additional opportunities for future studies.

Keywords

coronary artery disease; genome-wide association study; rare variants; microbiome; metabolomics; gene-environment interactions

Introduction

Coronary artery disease (CAD) and other forms of cardiovascular disease remain the number one cause of death for both men and women in Western societies[1], even in the contemporary era of high-potency statin therapy[2]. Individuals with CAD are typically asymptomatic, with the first manifestations of this pathogenic condition being clinically significant endpoints such as myocardial infarction (MI). It is generally accepted that CAD is characterized by lifetime exposure to an atherogenic environment in the context of genetic susceptibility factors. In this regard, large-scale studies in human populations and rapid advances in genetic technologies over the last several years have revolutionized our understanding of the genetic basis of CAD. However, the susceptibility alleles identified to date, most of which are common in the population, still only explain a small fraction of the overall heritability for CAD. This observation implies either the existence of additional variants with smaller effect sizes, higher order interactions between genes and environmental factors, rare susceptibility alleles, and/or other unknown biological/genetic mechanisms. In this review, we highlight recent discoveries that provide a framework for our current understanding of the genetic etiology of CAD. We also discuss areas of investigation that provide opportunities for further expansion of our knowledge and/or translation to improved clinical care.

Contribution of Common Genetic Variants to CAD and Clinically Associated Risk Factors

Beginning with the first genome-wide association studies (GWAS) [3–6], gene finding efforts over the last decade have transformed our understanding of the genetic architecture of CAD. The majority of these GWAS have been in individuals of European descent and have investigated various blood biomarkers that are associated with clinical disease as well discrete CAD phenotypes. For example, the most recent meta-analysis carried out by the CARDIoGRAMplusC4D Consortium, which included ~185,000 cases and controls, revealed ~50 distinct loci that were associated with CAD at the genome-wide significance threshold (Table 1)[7••]. Another 10 regions were identified that exhibited nominally significant associations as well [7••]. Meta-analysis of GWAS data in large numbers of subjects have also been carried out for numerous quantitative phenotypes clinically associated with CAD, including conventional risk factors, such as lipid levels and blood pressure, as well as less traditional biomarkers related to other biological mechanisms, such as inflammation, oxidation, coagulation, and amino acid metabolism. Depending on the sample size used,

dozens to hundreds of genes have been identified for each respective biomarker[8]. Thus, there is wealth of data already generated and, to the credit of the genetic research community and various consortia involved, many of the datasets are freely accessibly for other investigators to mine and use in their own analyses.

Whether for CAD phenotypes or related biomarkers, several important insights can be gleaned from the results of these genetic studies. First, the evidence clearly confirms the hypothesis that common variants contribute to common diseases/complex traits. However, the CAD loci with the strongest genetic effects, such as *SLC22A3-LPAL2-LPA* and chromosome 9p21, still only confer a 20–37% increased risk and the vast majority of the loci modulate risk by 10% or less (Table 1). Furthermore, the ~60 loci for CAD collectively explain <20% of the heritability, raising the question of where to search for the remaining genetic risk. Second, with the exception of the dozen or so regions that harbor genes known to be involved in CAD-related pathways(i.e. lipids or blood pressure), the biological mechanisms through which most of these loci affect atherosclerosis or its clinical biomarkers are not evident and remain a challenge to decipher. This is compounded by the fact that the identified loci often exhibit extensive linkage disequilibrium (LD) between variants and harbor multiple genes, which make it difficult to identify the underlying causal genes/variants simply from the GWAS results alone. One approach to overcome this difficulty has been to use trans-ethnic analyses. This was based on the assumption that causal variants would be shared among different populations, but the shorter LD blocks in subjects of African ancestry, for example, would improve mapping resolution and possibly even identify additional ethnicity-specific risk loci. CAD-associated regions were generally found to be concordant between subjects of European or Asian ancestry, but most loci failed to replicate or showed considerably reduced effect sizes in African ancestry populations [9, 10]. While the smaller sample sizes used for non-European ancestry subjects could have decreased statistical power, these observations also raise the possibility that the genetic mechanisms leading to CAD are different in subjects of African ancestry compared to Eurasian populations.

Computational and Bioinformatics Approaches for Identifying Causal Biological Pathways and Positional Candidate Genes

Beyond just GWAS analyses, investigators have developed and applied various computational and bioinformatics methods to identify causal biological pathways and prioritize positional candidate genes. Although the gold standard for determining causality with biomarkers has relied on randomized clinical trials, Mendelian randomization with GWAS data has also emerged as highly useful tool for determining the causal relationship between clinical biomarkers and CAD [reviewed extensively in ref.[11]]. The central tenet behind this approach is that a genetic variant leads to natural randomization of individuals to high or low biomarker levels. In the absence of pleiotropic effects of the variant, it follows that if a biomarker is a causal driver of disease, the genetic determinants of the biomarker will also be associated with disease risk.

Based on this premise, Mendelian randomization and genetic studies have confirmed the causal relationship between CAD and certain classic risk factors, including blood pressure [12, 13], low-density lipoprotein (LDL) cholesterol [14, 15], triglycerides [16], obesity [17], and type 2 diabetes [18–20], and pointed to novel causal associations with interesting traits, such as telomere length [21] and height [22]. However, these analyses also highlight several notable exceptions. For example, chronic kidney disease [23], fibrinogen [24], high-density lipoprotein (HDL) cholesterol [14], homocysteine [25], or uric acid [26] do not appear to be casually related to CAD. With the exception of IL-6 receptor signaling [27, 28], genetic analyses do not support causal roles for inflammatory biomarkers either, including C-reactive protein (CRP) [29, 30], ceruloplasmin [31], myeloperoxidase [32], and paraoxonase [33].

Other bioinformatics analyses have also been informative for interrogating GWAS data and providing additional biological insight in the pathogenesis of CAD. One such widely used approach has been the integration of transcriptomics in so called expression quantitative trait locus (eQTL) analysis. An eQTL is defined as a SNP that affects transcript levels of a nearby (*cis*) or distant (*trans*) gene, and its coincident mapping with an association signal for a clinical phenotype provides strong evidence that the given SNP has a functional effect. eQTL analyses are now easily facilitated by publicly available multi-tissue datasets [34] and, in their simplest application, can be used to narrow down the number of positional candidates at GWAS loci by focusing on those genes for which an eQTL is observed. More sophisticated network analyses have also been carried out with eQTL data. As demonstrated recently, SNPs associated with cardiometabolic risk yielded more *cis* and *trans* eQTLs in vascular and metabolic tissues from CAD patients compared to eQTLs from tissues of healthy subjects, with some of the eQTLs even being tissue specific [35].

Other computational approaches have involved pathway analyses with GWAS data. For example, a gene-set enrichment-based analysis revealed several core biological processes that were associated with CAD, including those related to extracellular matrix integrity, innate immunity, and growth factor signaling [36]. Another recent study incorporated multiple bioinformatics approaches by intersecting all transcript-coding genes at CAD-associated loci identified in GWAS with lead variants and proxy SNPs at these loci that were either deleterious amino acid changes, yielded eQTLs, or were located in putative regulatory regions [37]. The results of these integrative analyses led to the conclusion that the great majority of causal variation at loci for risk of CAD occur in noncoding regions and that genetic variants at these loci affect 98 genes that were not previously linked to CAD. A similar integrative analysis revealed that a transcriptional network linking multiple independent CAD loci is regulated by TCF21 [38]. However, it should be noted that while such studies have provided additional insight into the biology of CAD, they are still hypothesis generating and require experimental follow up to validate the inferred associations.

Evidence that Rare Variants Influence Risk of CAD

Rare variants, typically defined as those with <1% frequency in the population, have been postulated to be one piece of the puzzle for the so-called “missing heritability” of CAD.

Since rare variants are generally not captured on GWAS chips or easy to impute, investigators have turned to other approaches for determining whether they are associated with risk of CAD. Utilizing family-based studies, targeted re-sequencing of candidate genes, whole exome or genome sequence analyses, or a chip-based array containing pre-selected exonic variants, several genes have been found to harbor rare variants that modulate risk of CAD (Table 2).

In one study, analysis of an extended German pedigree revealed a digenic pattern of inheritance where family members with early-onset MI (≤60 years of age) were more likely to be heterozygous for protein altering mutations in *GUCY1A3* and *CCT7* [39]. The biological mechanism for how *GUCY1A3* and *CCT7* increase risk of MI appears to involve nitric oxide signaling and platelet function (Table 2). Targeted re-sequencing of candidate genes have also revealed rare variants that are associated with risk of CAD through lipid metabolism pathways. One of the first successful examples of this approach was the identification of rare loss-of-function variants in *PCSK9* that lowered LDL cholesterol levels and conferred protection against CAD [40]. Notably, *PCSK9* was originally identified in a family-based genetics study where gain-of-function mutations were shown to be the cause of autosomal dominant hypercholesterolemia [41]. More recent targeted re-sequencing studies demonstrated that loss-of-function mutations of *APOC3* and *ANGPTL4* decreased risk of CAD through lowering of plasma triglyceride levels [42, 43]. By contrast, a loss-of-function mutation in *SCARB1*, which mediates selective hepatic uptake of HDL particles, led to elevated plasma HDL cholesterol levels and, surprisingly, increased risk of CAD [44•] (Table 2).

A more agnostic approach to identifying rare variants has been to comprehensively interrogate the coding portion of the genome through whole-exome sequencing. These efforts identified rare variants in *APOC3* as well as *APOA5*, *LDLR*, and *NPC1L1* that modulated risk of CAD or MI through their effects on triglyceride or cholesterol metabolism [45–47] (Table 2). Extending this unbiased strategy to whole-genome sequencing also revealed that subjects heterozygous for a 12bp deletion in *ASGR1,* which encodes a subunit of a lectin that plays a role in the homeostasis of circulating glycoproteins, had lower non-HDL cholesterol levels and reduced risk of CAD [48]. Lastly, another complementary approach has been to evaluate the contribution of low-frequency coding variation to CAD through the use of a customized exome array. While this strategy is limited to pre-selected variants, it is more cost-effective compared to exome or whole-genome sequencing and thus provides the advantage of including large sample sizes. Using the exome array, previously reported associations of low-frequency missense variants in *PCSK9* and *LPA* with CAD were confirmed [40, 49, 50]. Significant associations with either increased or decreased risk of CAD, depending on the biological consequence of the variant, were also observed with *ANGPTL4*, *LPL*, and *SVEP1* [50] (Table 2). Interestingly, the missense substitution in *SVEP1*, which encodes a cell adhesion molecule, was associated with a very modest increase in systolic and diastolic blood pressure. Since this effect alone cannot explain the magnitude of the association with increased risk of CAD, it suggests the existence of additional unknown and potentially novel genetic mechanisms leading to atherosclerosis [50].

Taken together, the results of rare variant studies illustrated several interesting concepts. As might be expected, the effect sizes of the identified variants on intermediate lipid traits and risk of CAD were, for the most part, larger compared to those for common variants identified in GWAS. However, it also became apparent that low frequency polymorphisms collectively still only explain a very small proportion of the heritability for CAD, suggesting that a significant fraction of genetic susceptibility to CAD is unlikely to reside in rare variation. Lastly, most of the identified genes were in pathways already known to be involved in the pathogenesis of CAD, such as lipid metabolism. However, the results of these rare variant studies did support the notion that triglycerides are causally associated with the development of CAD, which corroborate the results of Mendelian randomization studies.

Insight from Genetic Studies in Mice

Given the difficulties in human genetic studies, mouse models provide another strategy for gene discovery that can be extrapolated to humans as well as a model system for understanding the mechanisms underlying CAD-related phenotypes. Most atherosclerosis studies in mice have focused on perturbing specific candidate genes on genetically hyperlipidemic backgrounds [51], with hundreds of genes being reported in the literature as modulating aortic lesion formation. However, a systematic review of results from these mouse knockout or transgenic studies and human GWAS revealed surprisingly little evidence that atherosclerosis-causing genes in mice are associated with risk of CAD in humans [52]. It is worth noting that the human GWAS results used for this comparison were from a prior meta-analysis [53] that included approximately half the number of subjects as the most recent study from the CARDIoGRAMplusC4D Consortium discussed above [7••]. Furthermore, overexpression or complete deficiency of single genes in mice may not represent the subtle genetic perturbations that are likely to underlie common forms of CAD in humans, thus rendering such comparative genomics analyses difficult.

An alternative approach with mouse models is to exploit naturally occurring genetic variation among inbred strains, analogous to studies in human populations. In this regard, a genetics platform was recently developed for GWAS in mice [54], termed the Hybrid Mouse diversity panel (HMDP). Since each strain is renewable, diverse molecular, proteomic, metabolomic, and clinical phenotypic data can be collected *ad infinitum* in multiple mice of the same strain/genotype, thus facilitating systems genetics analyses. Studies with the HMDP have been applied to a variety of traits relevant to human CAD and consistently identified positional candidate genes for follow-up functional experiments [55–61].

To specifically examine atherosclerosis in the HMDP, an F1 hybrid strategy was used where the dominant-acting and atherosclerosis-promoting transgenes, human apolipoprotein E-Leiden (APOE-Leiden) and human cholesteryl ester transfer protein (CETP), were bred from strain C57BL/6J onto over 100 hundred different strains in the HMDP [62]. In addition to aortic lesion formation, global gene expression profiling was carried out and levels of clinically relevant biomarkers were measured in plasma. Systems genetics analyses led to several interesting conclusions. For example, the relationships between atherosclerosis and CAD-related risk factors in mice resembled those found in humans [62], and the combined

variations in plasma levels of LDL/VLDL-cholesterol, glucose, insulin, and various other metabolites accounted for approximately 30 to 40% of the variation in aortic lesion formation. Additionally, broad sense heritability for atherosclerosis was much larger than narrow sense heritability and the ratio of lesion size between male and female mice varied extensively among the strains [62]. These observations indicate the existence of both gene-gene and gene-sex interactions. Lastly, some of the loci mapped by GWAS in the HMDP overlapped with those previously identified in human and mouse studies [62]. The advantage of having gene expression data in several atherosclerosis-relevant tissues, such as aorta, liver, and adipose, also enabled enrichment analysis of pathways contributing to atherosclerosis and prioritization of candidate genes at associated loci in both mice and humans. Collectively, these data provide a rich resource that can be further leveraged for understanding the complex interactions underlying atherosclerosis and provide new directions that can be explored in future human genetics studies.

Role of Intestinal Microbiota in the Development of CAD

It has become widely appreciated that our gut symbionts play integral roles in human health since perturbations of this bacterial community or the products they can produce have been associated with increased susceptibility to a variety of diseases. The first indications of these associations were for colitis and inflammatory bowel disease, but altered gut microbial composition or function has now been shown to affect various cardiometabolic phenotypes that could subsequently affect risk of CAD, such as lipid metabolism and obesity-related metabolic abnormalities [63–69].

A more direct role for intestinal microbes in the development of atherosclerosis was recently demonstrated by the identification of a novel meta-organismal pathway involving multiple interactions between gut bacteria, dietary nutrients, and host metabolism [70–75]. These studies revealed a mechanism whereby bacterial metabolism of dietary choline and L-carnitine in the intestine leads to the formation of an intermediate, trimethylamine (TMA), which is absorbed from the gut and subsequently oxidized by one or more hepatic flavin monooxygenases (FMOs) to generate trimethylamine *N*-oxide (TMAO) [71, 76]. Plasma TMAO levels were positively associated with aortic lesion formation in mice and increased risk of prevalent/incident CAD in humans, the effects of which could be mediated, at least in part, through foam cell formation, reverse cholesterol transport, bile acid metabolism, and platelet hyperresponsiveness [70, 72, 73, 77–79].

One important question raised by these studies is whether intrinsic host genetic variants are associated with plasma TMAO levels and, by extension, risk of CAD. While GWAS analyses with the HMDP identified three loci [62, 80], two GWAS in humans failed to identify loci significantly associated with TMAO levels [80, 81], thus precluding the determination of its causal nature with CAD with the use of a genetic instrument in Mendelian randomization analyses. Taken together, these studies suggest that the contribution of host genetic factors to plasma TMAO levels are relatively weak and/or complex, at least in humans, and that variation in diet or the repertoire of gut microbiota may be more important determinants. Interestingly, studies in humans have uncovered a surprising number of associations between genetic variants and the abundance of various

bacterial taxa [82–86]. This concept is supported by a separate HMDP obesity study, which identified seven host loci that were associated with common bacterial genera [87•]. Thus, it is possible that host genetic factors could control TMAO levels indirectly by regulating the abundance of TMA-generating bacteria. However, the existence of such loci in mice or humans remains to be determined.

Integrative Metabolomics and Genetics Reveals Novel Sex-specific Association with CAD

Metabolomics is an emerging field of biomedical research that is based on characterizing the repertoire of small molecules in biological samples. This has led to the identification of metabolites in several pathways that may serve as novel clinical biomarkers for CAD [88], such as those discussed above for TMAO. Furthermore, the availability of metabolic profiles with genomic, transcriptomic, proteomic, and clinical data have yielded comprehensive datasets that can potentially be leveraged in systems genetics analyses, similar to those implemented in the HMDP, to identify underlying biological networks that drive disease susceptibility [89]. However, such studies have generally been carried out in an unbiased fashion, both from a genetics and metabolomics perspective, and efforts are underway to intersect these high dimensional data to identify pathways causally related to CAD and other diseases.

Another complementary metabolomics strategy is to focus on targeted pathways that have been directly implicated in the development of CAD. This approach was exemplified by a recent study seeking to understand the relationship between CAD and betaine, another choline-derived metabolite that has also been associated with atherosclerosis in mouse models and increased risk of CAD in humans [70]. AGWAS for plasma betaine levels identified two significantly associated loci, one of which mapped to carbamoyl-phosphate synthase 1 (*CPS1*) on chromosome 2q34 [90•]. *CPS1* encodes a mitochondrial enzyme that catalyzes the first committed reaction and rate-limiting step in the urea cycle, raising the interesting question of how CPS1 would therefore be associated with betaine levels. This is best explained by a series of known stepwise demethylation reactions that metabolize betaine (also known as trimethylglycine) to glycine. The major catabolic route for glycine is through the glycine cleavage complex, which forms ammonia and metabolized by CPS1 to carbamoyl phosphate for entry into the urea cycle.

Based on previously reported effects of the *CPS1* locus [91], follow-up targeted metabolomics and genetic analyses revealed a distinctive sexually dimorphic pattern of pleiotropic associations with decreased levels of the most proximal precursors (i.e. choline, TMAO, and betaine) and the most distal urea cycle metabolites, such as citrulline, arginine, and ornithine [90•]. By comparison, the strongest and most significant metabolite association was observed with increased plasma glycine levels. Importantly, the *CPS1* locus also yielded a strikingly significant and protective association with risk of CAD in women but not men [90•]. This association represents one of the first female-specific genetic risk factors for CAD reported in the literature and its magnitude (~12% decreased risk) was equivalent to the most significantly associated loci identified for CAD to date [7••].

Taken together, the results of this study illustrate several important concepts that could be informative future studies. For example, given that the known loci for CAD still only explain <20% of the genetic variation in risk [7••], the association of *CPS1* with CAD in only women suggests that a portion of the "missing heritability" may also reside in sex-specific associations. This observation highlights the need for genetics studies of CAD and related biomarkers to be of sufficient size in order to permit adequately powered analyses in men and women separately. In addition, the *CPS1* locus had previously been associated with various other CAD-related traits [92, 93, 25, 94–99, 24], but the direction of the associations with these biomarkers was opposite to what would be expected for a variant that decreases risk of CAD [90•]. Thus, it is possible that the protective association with CAD is mediated through lower TMAO levels or increased glycine levels. In this regard, prior *in vitro* studies demonstrated that glycine has anti-inflammatory properties in endothelial cells, activated macrophages, and other leukocytes [100–104]. A recent clinical study also reported an inverse relationship between plasma glycine levels and risk of MI [105], consistent with the glycine-raising effects of *CPS1* and its cardioprotective association with CAD. Lastly, the association of *CPS1* with TMAO levels was modest compared to glycine levels, suggesting that TMAO may not be the likely causal metabolite [90•]. However, additional follow up studies will still be needed to replicate these findings and to determine whether the underlying causal mechanism(s) by which *CPS1* decreases risk of CAD is directly related to glycine metabolism or whether it involves TMAO, the urea cycle, or other unknown pathways/intermediates. More broadly, this study also suggests that integration of metabolomics in future genetic studies can potentially reveal novel pathways that are relevant to the development of CAD.

Translational and Clinical Implications of Genetic Studies

An important question from genetic studies of CAD is whether the findings can be leveraged towards development of novel therapeutic strategies. Notably, genetic studies can provide valuable insight into whether a drug target modulates a pathway that is causally related to the atherosclerosis. For example, although statins or ezetimibe were initially developed without the knowledge provided by genetic studies, their cardiovascular benefits were further validated when lipid-lowering variants of genes targeted by these drugs (*HMGCR* and *NPC1L1*) were shown to be associated with reduced risk of CAD [46, 15]. The initial identification of *PCSK9* as cause of autosomal dominant hypercholesterolemia [41], followed by the discovery of additional *PCSK9* variants that were associated with both LDL cholesterol levels and CAD [7••, 40, 50], has also advanced the development of several different classes of therapeutic agents that inhibit PCSK9 [106]. Two of these drugs (evolocumab and alirocumab) have already been approved for use in patients with familial hypercholesterolemia, statin intolerance, or insufficient LDL cholesterol control with statins [107, 108]. Furthermore, a recently published clinical trial with CAD patients demonstrated that the addition of evolocumab to statin therapy resulted insignificant regression of coronary plaque volume compared to patients receiving only on statins [109]. Similarly, and based on the cardioprotection conferred by loss-of-function variants of *APOC3* [42, 45], two phase 2 studies with an anti-sense inhibitor of *APOC3* demonstrated significant reductions of plasma triglycerides in patients with hypertriglyceridemia or type 2 diabetes without any

notable safety issues [110, 111]. By contrast, attempts to reduce risk of CAD by raising HDL cholesterol levels through inhibition of CETP has not been successful thus far [112, 113]. These null clinical effects are consistent with the results of genetic analyses that raised questions regarding the causal role of HDL cholesterol levels in the development of atherosclerosis [14, 44•]. However, it should be noted that inferring causality from Mendelian randomization studies alone can be difficult. For example, since the biological function of HDL cholesterol-associated variants are mostly unknown, they may have varying effects on CAD risk. In addition, circulating levels of HDL cholesterol per se may not be a good surrogate for the atheroprotective effects of HDL, leading to the hypothesis that HDL function and its cholesterol efflux capacity are better indicators. This notion is consistent with recent studies demonstrating inverse associations between cholesterol efflux and prevalent CAD and incident cardiovascular events [114, 115], although a separate study reported paradoxically opposite associations with prospective risk of MI, stroke, and death [116]. Thus, the nature of relationship between HDL levels/function and atherosclerosis has yet to be fully determined. Lastly, inflammatory pathways, and particularly those mediated by *IL6R*, have also been causally associated with atherosclerosis and provide additional therapeutic opportunities. For example, the interleukin 6 receptor (IL6R) antagonist tocilizumab is under consideration for treatment of CAD and is currently being evaluated for reducing cardiovascular risk in patients with rheumatoid arthritis [117, 118].

Another promising therapeutic avenue for CAD may involve selective manipulation of the gut microbial ecosystem by either altering bacterial composition or targeting biological processes specific to intestinal bacteria. Studies in mice have demonstrated that transplantation of cecal microbes from an atherosclerosis-prone strain with high TMA/TMAO levels to a low TMAO-producing and atherosclerosis-resistant strain can enhance production of these pro-atherogenic metabolites with a corresponding increase in aortic lesion formation [75]. These observations raise the intriguing question of whether using the converse strategy with bacterial transplantation from individuals with low TMA/TMAO-producing capacity, such as vegans [72], can decrease risk of CAD. Although such approaches have yet to be implemented in humans with respect to CAD, fecal transplantation studies have shown that transfer of gut microbes from lean donors through a duodenal infusion into patients with metabolic syndrome can improve insulin sensitivity [119]. In another strategy, a small molecule choline analog, 3, 3-dimethyl-1-butanol (DMB), was designed to competitively inhibit diverse and phylogenetically distant classes of microbial TMA lyases, which were previously identified as enzymes that catalyze the conversion of choline to TMA [120, 121]. Chronic feeding of DMB to mice in the context of a high-choline diet led to shifts in the proportions of some bacterial taxa and substantial reductions in plasma TMAO levels, macrophage cholesterol accumulation, foam cell formation, and atherosclerotic lesions, without any evidence of toxicity or adverse cardiometabolic effects in the animals [122•]. Taken together, these results suggest that targeting gut microbial composition or production of bacterial-derived metabolites through transplantation or specific pharmacological manipulation may serve as potentially novel therapeutic approaches for treating CAD.

Opportunities and Areas of Investigation for Future Studies

Despite the advances made over the last several years, there is still much that we do not know about the genetic basis of CAD. As noted above, one looming question is the large fraction of the heritability that cannot be explained by the genetic discoveries thus far. It is possible that heritability of CAD is over-estimated but it is also likely that epistatic interactions between genes (GxG) or gene-environment(GxE) and gene-sex interactions play important roles in determining risk of CAD as well. However, GxG and GxE analyses have proven difficult, particularly on a genome-wide level, because of the requirements for both genotype and exposure data, and, with respect to GxE interactions, imprecise exposure assessment. In this regard, diet and smoking are well known environmental exposures relevant to risk of CAD, and there is a large body of epidemiological data showing consistent adverse associations with ambient air pollution as well [123]. However, obtaining accurate measures of dietary intake or air pollution exposure in large numbers of subjects are difficult and have hindered progress in this area. Nonetheless, GxG and GxE interactions for CAD represent excellent opportunities for future studies if methodological and statistical challenges can be overcome.

Elucidating the molecular mechanisms for the association of variants with CAD represents another area that is being actively pursued but is laborious and often requires extensive experimental follow up. For example, despite being identified nearly 10 years ago, it is only recently and partially being understood how the 9p21 locus influences risk of CAD [124]. This novel atherogenic mechanism appears to involve the regulation of ribosome biogenesis in vascular smooth muscle cells and macrophages by a circular non-coding RNA encoded by *CDKN2B-AS* that is transcribed from the 9p21 locus [124]. Since most loci for CAD or causally associated biomarkers fall within non-coding regions, the availability of HAPMAP, ENCODE, and multi-tissue eQTL data should facilitate the identification candidate genes that could be studied further with mouse models or evaluated as therapeutic targets.

Conclusions

In summary, recent efforts to elucidate the genetic architecture of CAD have revealed multiple mechanisms that contribute to the pathogenesis of atherosclerosis (Figure 1). There is ample evidence that common genetic variants influence risk of CAD via perturbations of lipid metabolism, blood pressure, inflammation, platelet function, as well as other unknown biological pathways that have yet to delineated. However, evidence from studies evaluating the contribution of rare variants is less compelling, although it should be noted that studies determining the effects of rare variant can be hampered by inadequate power. While some novel genes have been identified, mostly related to lipid metabolism, it does not appear that low frequency variation explain a significant portion of the “missing heritability” that has thus far remained elusive. In addition, the realization that metabolic interactions between the microbiome and dietary nutrients can promote the development of CAD provides excellent opportunities for furthering our understanding of the atherosclerotic process. By comparison, the cross talk between genes and with environmental factors or sex have not been explored to the same degree as the search for main effects even though these interactions are also likely to be important components in determining the overall risk for

CAD. Taken together, genetic discoveries made over the last several years offer new avenues for further research and therapeutic development that will have a fundamental impact on our understanding of the atherosclerosis and the treatment of patients with CAD.

Acknowledgments

Work in the author's laboratories is supported, in part, by NIH grants R01ES021801, R01ES021801-S3, R01ES025786, P01ES022845, R01MD010358, R01HL133169, and R01HL128572; U.S. EPA Grant RD83544101; a grant from the Whittier Foundation; and a Transatlantic Networks of Excellence Award from Foundation Leducq. The funders had no role in preparation, review, or approval of the manuscript.

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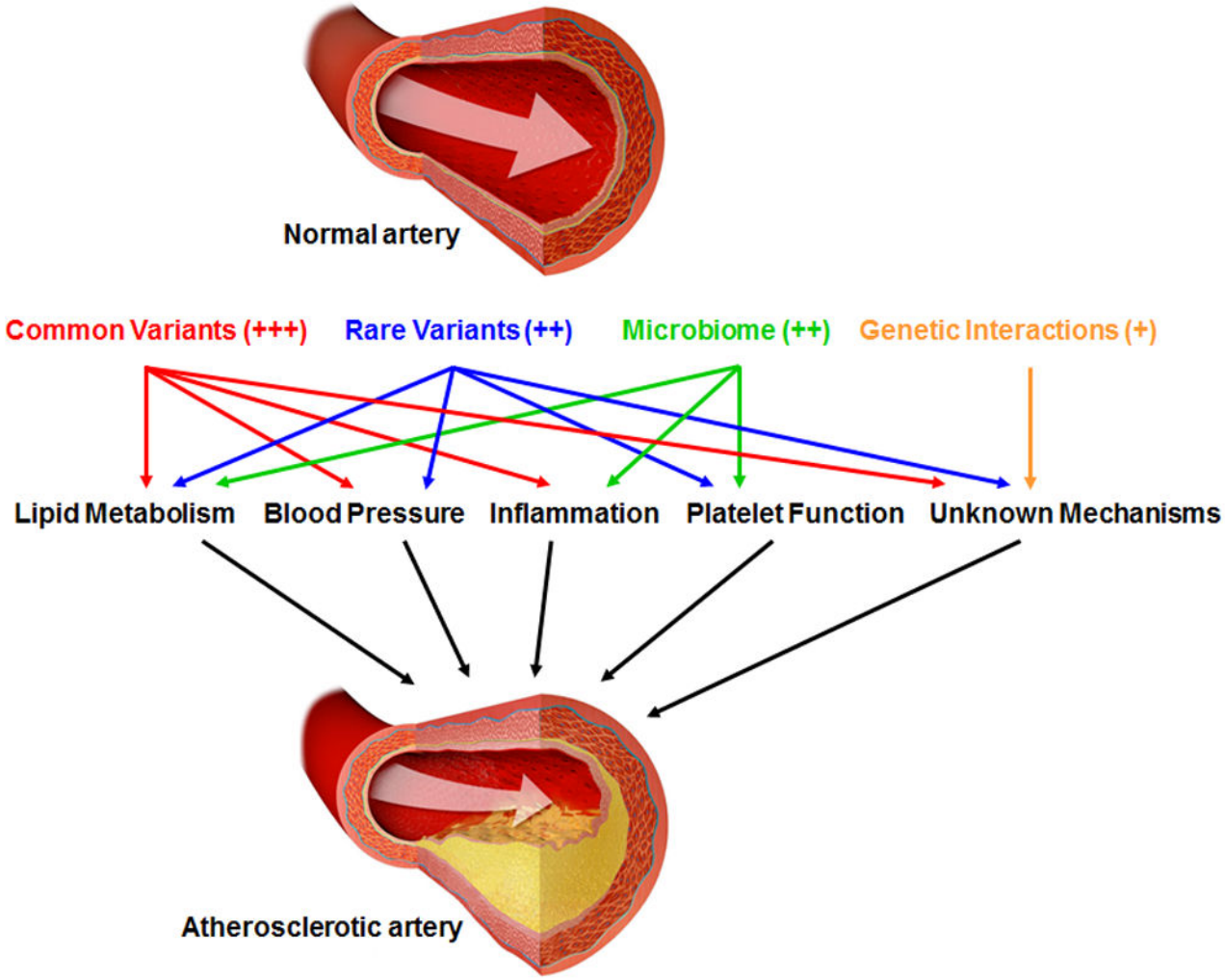

Figure 1. Genetic mechanisms that promote the development of atherosclerosis

There is strong evidence that common variants are associated with risk of CAD, particularly through pathways related to lipid metabolism, blood pressure, and inflammation. However, these variants collectively still only explain a small fraction of the heritability of CAD. Rare variants have also been identified in the population that affect risk of CAD, mostly through effects on lipids or blood pressure, but evidence that these types of alleles make a substantial contribution the genetic variability in CAD is only moderate. Similarly, emerging data provides evidence for a direct role of intestinal microbes in atherosclerosis and thus far has been shown to involve the effects of gut bacteria-derived metabolites of choline and L-carnitine on lipid and inflammatory pathways. It is also widely hypothesized that gene-gene, gene-sex, and gene-environment interactions contribute to risk of CAD but these are areas that have yet to be explored on a large scale, presumably due, in part, to the challenges posed by the required statistical methodology and imprecise measures of exposure. Positive signs in parentheses indicate strong (+++), moderate (++), or weak (+) evidence for involvement of the indicated genetic mechanism in the pathogenesis of CAD.

Table 1

Loci Identified for CAD through Large-scale Genetic Studies.

Chr	Locus/Nearest gene(s)	Lead SNP	Risk Allele/Other Allele	EAF	OR (95% CI)*	P-value
1	*PPAP2B*	rs9970807	C/T	0.92	1.13 (1.10 – 1.17)	5.0×10^{-14}
1	*PCSK9*	rs11206510	T/C	0.85	1.08 (1.05 – 1.11)	2.3×10^{-8}
1	*SORT1*	rs7528419	A/G	0.79	1.12 (1.10 – 1.15)	2.0×10^{-23}
1	*IL6R*	rs6689306	A/G	0.45	1.06 (1.04 – 1.08)	2.6×10^{-9}
1	*MIA3*	rs67180937	G/T	0.66	1.08 (1.06 – 1.11)	1.0×10^{-12}
2	*LINC00954*	rs16986953	A/G	0.11	1.09 (1.06 – 1.12)	1.5×10^{-8}
2	*APOB*	rs515135	C/T	0.79	1.07 (1.04 – 1.10)	3.1×10^{-8}
2	*ABCG5/ABCG8*	chr2:44074126:Del	Del/Ins	0.75	1.06 (1.04 – 1.09)	2.6×10^{-8}
2	*VAMP5/8-GGCx*	rs7568458	A/T	0.45	1.06 (1.04 – 1.08)	3.6×10^{-10}
2	*ZEB2-AC074093.1*	rs17678683	G/T	0.09	1.10 (1.07 – 1.14)	3.0×10^{-9}
2	*WDR12*	chr2:203828796:I	Ins/Del	0.11	1.15 (1.11 – 1.18)	2.2×10^{-18}
3	*MRAS*	chr3:138099161:I	Ins/Del	0.16	1.08 (1.05 – 1.10)	2.9×10^{-9}
4	*EDNRA*	rs4593108	C/G	0.80	1.07 (1.05 – 1.10)	8.8×10^{-10}
4	*GUCY1A3*	rs72689147	G/T	0.82	1.07 (1.05 – 1.10)	6.1×10^{-9}
4	*REST-NOA1*	rs17087335	T/G	0.21	1.06 (1.04 – 1.09)	4.6×10^{-8}
6	*PHACTR1*	rs9349379	G/A	0.43	1.14 (1.12 – 1.16)	1.8×10^{-42}
6	*KCNK5*	rs56336142	T/C	0.81	1.07 (1.04 – 1.09)	1.9×10^{-8}
6	*TCF21*	rs12202017	A/G	0.70	1.07 (1.05 – 1.09)	2.0×10^{-11}
6	*SLC22A3-LPAL2-LPA*	rs55730499	T/C	0.06	1.37 (1.31 – 1.44)	5.4×10^{-39}
6	*PLG*	rs4252185	C/T	0.06	1.34 (1.28 – 1.41)	1.6×10^{-32}
7	*NOS3*	rs3918226	T/C	0.06	1.14 (1.09 – 1.29)	1.7×10^{-9}
7	*HDAC9*	rs2107595	A/G	0.20	1.08 (1.05 – 1.10)	8.1×10^{-11}
7	*ZC3HC1*	rs11556924	C/T	0.69	1.08 (1.05 – 1.10)	5.3×10^{-11}
9	*9p21/CDKN2B-CDKN2A*	rs2891168	G/A	0.49	1.21 (1.19 – 1.24)	2.3×10^{-98}
9	*ABO*	rs2519093	T/C	0.19	1.08 (1.06 – 1.11)	1.2×10^{-11}
10	*KIAA1462*	rs2487928	A/G	0.42	1.06 (1.04 – 1.08)	4.4×10^{-11}
10	*CxCL12*	rs1870634	G/T	0.64	1.08 (1.06 – 1.10)	5.6×10^{-15}

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Chr	Locus/Nearest gene(s)	Lead SNP	Risk Allele/Other Allele	EAF	OR (95% CI)*	P-value
10	*LIPA*	rs1412444	T/C	0.37	1.07 (1.05 – 1.09)	5.2×10^{-12}
10	*CYP17A1-CNNM2-NT5C2*	rs11191416	T/G	0.87	1.08 (1.05 – 1.11)	4.7×10^{-9}
11	*PDGFD*	rs2128739	A/C	0.32	1.07 (1.05 – 1.09)	7.1×10^{-11}
11	*SWAP70*	rs10840293	A/G	0.55	1.06 (1.04 – 1.08)	1.3×10^{-8}
12	*ATP2B1*	rs2681472	G/A	0.20	1.08 (1.05 – 1.10)	6.2×10^{-11}
12	*SH2B3*	rs3184504	T/C	0.42	1.07 (1.04 – 1.09)	1.0×10^{-9}
12	*KSR2*	rs11830157	G/T	0.36	1.12 (1.08 – 1.16) **	2.1×10^{-9}
13	*COL4A1/A2*	rs4773144	A/G	0.26	1.07 (1.05 – 1.09)	1.8×10^{-10}
14	*HHIPL1*	rs10139550	G/C	0.42	1.06 (1.04 – 1.08)	1.4×10^{-8}
15	*ADAMTS7*	rs4468572	C/T	0.59	1.08 (1.06 – 1.10)	4.4×10^{-16}
15	*SMAD3*	rs56062135	C/T	0.79	1.07 (1.05 – 1.10)	4.5×10^{-9}
15	*MFGE8-ABHD2*	rs8042271	G/A	0.90	1.10 (1.06 –1.14)	3.7×10^{-8}
17	*BCAS3*	rs7212798	C/T	0.15	1.08 (1.05 –1.11)	1.9×10^{-8}
18	*PMAIP1-MC4R*	rs663129	A/G	0.26	1.06 (1.04–1.08)	3.20×10^{-8}
19	*LDLR*	rs56289821	G/A	0.90	1.14 (1.11 – 1.18)	4.4×10^{-15}
19	*APOE*	rs4420638	G/A	0.17	1.10 (1.07 – 1.13)	7.1×10^{-11}
19	*ZNF507-LOC400684*	rs12976411	T/A	0.09	0.67 (0.60 – 0.74) **	3.2×10^{-8}
21	*KCNE2*	rs28451064	A/G	0.12	1.14 (1.10 – 1.17)	1.3×10^{-15}
22	*POM121L9P-ADORA2A*	rs180803	G/T	0.97	1.20 (1.13 – 1.27)	1.2×10^{-10}

Only loci exceeding the genome-wide threshold for significance ($p=5.0\times10^{-8}$) from the meta-analysis by CARDIoGRAMplusC4D Consortium [7••] are shown.

* All ORs refer to allele that increases risk of CAD except for the *ZNF507-LOC400684* locus on chromosome 19.

** Derived from an analysis assuming recessive inheritance.

Table 2

Genes in Which Rare Variants Are Associated with Risk of CAD.

Discovery Approach	Gene	Type of Mutation	Effect on CAD Risk	Biological Mechanism(s)	Ref.
Family-based	*GUCY1A3*	Loss-of-function	Increase	Decreased nitric oxide signaling, increased blood pressure and thrombus formation	[39]
	CCT7	Loss-of-function	Increase	Decreased nitric oxide signaling and increased thrombus formation	[39]
Targeted sequencing	*PCSK9*	Loss-of-function	Decrease	Lower LDL levels	[40]
	APOC3	Loss-of-function	Decrease	Lower triglyceride levels	[42]
	ANGPTL4	Loss-of-function	Decrease	Lower triglyceride levels	[43]
	SCARB1	Loss-of-function	Increase	Higher HDL levels	[44•]
Exome sequencing	*APOC3*	Loss-of-function	Decrease	Lower triglyceride levels	[45]
	NPC1L1	Loss-of-function	Decrease	Decreased intestinal cholesterol absorption and lower LDL levels	[46]
	APOA5	Loss-of-function	Increase	Higher triglyceride levels	[47]
	LDLR	Loss-of-function	Increase	Higher LDL levels	[47]
Exome chip	*LPA*	Gain-of-function	Increase	Higher Lp(a) levels	[50]
	PCSK9	Loss-of-function	Decrease	Lower LDL levels	[50]
	ANGPTL4	Loss-of-function	Decrease	Lower triglyceride levels	[50]
	LPL	Gain-of-function	Decrease	Lower triglyceride levels	[50]
	LPL	Loss-of-function	Increase	Higher triglyceride levels	[50]
	SVEP1	Unknown	Increase	Increased blood pressure and possibly other unknown pathways	[50]
Whole-genome sequencing	*ASGR1*	Loss-of-function	Decrease	Glycoprotein homeostasis and lower non-HDL levels	[48]