Genetic susceptibility to myocardial infarction and coronary artery disease

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Atherosclerotic involvement in the coronary arteries, which can result in heart attack and sudden death, is a common disease and prototypic of a complex human trait. To understand its genomic basis, eight linkage studies of sibling pairs have been performed. Although there was limited inter-study concordance of important loci, two gene variants in the leukotriene pathway (ALOX5AP and LTA4) have emerged as susceptibility factors for myocardial infarction (MI). Genome-wide association studies have also been undertaken, and the pro-inflammatory cytokine lymphotoxin-α (LTA), and its key ligand galec-tin-2 (LGALS2) have been identified as genes implicated in predisposition for heart attack. By cueing into the genomic basis for low serum LDL cholesterol levels, much work has been done to advance the importance of the serine protease PCSK9, which modulates LDL receptor function. Lifelong lowered LDL cholesterol associated with PCSK9 point mutations in 2–3% of individuals have been shown to provide marked protection from coronary artery disease (CAD). Most of the success in this field has been with the phenotype of MI, which is considerably more restrictive than CAD. Four principal and interdependent processes—lipoprotein handling, endothelial integrity, arterial inflammation, and thrombosis—have been supported as important via the clustering of genes, thus far implicated in CAD susceptibility. Of note, connecting genes in a single pathway (leukotriene), of a protein and its ligand (LTAα) or from one disease to another [age-related macular degeneration (AMD); complement factor H (CFH)], or even three disease characterized by inflammation (MHC2) have now been reported. Although the population attributable risk for any of the genes identified to date is limited, such discovery is likely to be accelerated in the future.

INTRODUCTION

Although we are still in the early phase of understanding the genomic basis of complex traits, there have been some remarkable recent advances for disease such as AMD (1–3), inflammatory bowel disease (4) and diabetes (5). From the standpoint of public health burden, no complex trait is more important than atherosclerotic coronary artery disease (CAD) and myocardial infarction (MI). Not only is this disease the leading cause of death and disability in Western society, but by 2020 it is expected to be the number one cause of death worldwide (6). The underlying pathologic spectrum is broad, ranging from accumulation of cholesterol deposits in the subendothelial arterial intima, to frank inflammation of the artery wall, and, in some individuals, culminating in a plaque rupture, fissure or erosion of the wall with resultant blood clot formation. Linkage analysis, whole genome association studies and specific genetic epidemiologic studies have, in aggregate, begun to open up this field and provide insights to the genes underlying this common and important condition. The purpose of this article will be to review the recent progress that has been made and to outline the challenges for the future of the field.

Linkage studies: ALOX5AP, LTA4 and MEF2A

Collectively, there have been seven genome wide scans of sibling pairs published for CAD and MI (Table 1) (7–15). Several loci of interest have been identified, but only two...
Table 1. Genome wide scans for CAD and MI

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Families</th>
<th>Age</th>
<th>1st locus</th>
<th>Program</th>
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<td>&lt;55</td>
<td>2q21</td>
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<tr>
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| Found | British Heart Foundation; Euro-Amer, European-American. |

Genes have thus far been directly implicated. Of note, only a single locus has been replicated from one study to the next: 2p11 in the Wang et al. (a secondary locus) and British Heart Foundation studies (11,15). It is unclear why there has not been replication or concordance from one study to another. As possibilities, there are clearcut differences in the ethnicity of the cohorts, the number of families studied, the criteria for enrollment and whether CAD or MI was the main phenotype. The differences in the patients were further affected by the age of the cohorts, ranging from a mean of 44 to 62 years. Even the analysis of the linkage data was processed using a variety of different statistical methods and software programs (Table 1).

Despite general lack of inter-study replication of linkage loci, Helgadottir et al. (13,14) have identified two genes associated with MI—both in the same pathway of leukotriene B4 production. A four-marker SNP haplotype of ALOX5AP—5-lipoxygenase activating protein (FLAP) was identified under the linkage peak at 13q12-13, with an odds ratio (OR) of 1.8 for MI, and 1.9 for stroke. This same haplotype was later independently confirmed for risk of stroke in a Scottish cohort (16), and for MI in a Cleveland population (unpublished). Subsequently, from the same genome-wide scan, Helgadottir et al. (14) used fine mapping to determine that the five to seven SNP marker haplotype leukotriene A4 hydrolase (LTA4H) accounted for the 12q22 linkage peak. Of particular interest with this haplotype was its ancestry-specific incidence and risk for MI. In European-Americans, the relative risk for MI was only 1.2, with population attributable risk (PAR) of 4.6%, whereas among individuals of African ancestry (using genomic control), the relative risk was 3.5 and a PAR of 14% (14). Together, these findings set three important precedents. First, two different genes in the same leukotriene pathway of inflammation were found to be disease-associated via a single genome-wide scan. This pathway had already been invoked by multiple studies in murine experimental atherosclerosis models and human epidemiologic and pathologic studies (17–19). Secondly, a small molecule FLAP-blocker, used in a pilot, placebo-controlled, randomized trial for individuals with the at-risk FLAP or LTA4 haplotype, was shown to reduce both leukotriene production and C-reactive protein (20), an important biomarker for CAD. Thirdly, ancestry-specific risk of MI was demonstrated for a particular gene.

Using a rich pedigree approach in a family with autosomal dominant inheritance pattern of MI or CAD, Wang et al. (21) found a significant locus at 15q26. A candidate gene in this region, MEF2A, had a 21 bp deletion in exon 11 which co-segregated with the trait and we have yet to find a similar deletion in over 500 controls with normal coronary angiograms. Furthermore, transfecting endothelial or smooth muscle cells with the deletion mutant resulted in cytoplasmic trapping, and this failure of nuclear translocation led to markedly diminished transcription with a dominant, negative pattern. Our finding of MEF2A as a gene associated with MI and CAD was further supported by identification of three new point mutations in exons 6 and 7, which were also associated with diminished transcription activity and MI (22). The Pro279Leu exon 7 variant has been independently confirmed by Gonzalez et al. (23) in a Spanish population with an OR of 3.1 in 483 MI cases and 1189 controls.

The MEF2A findings were challenged by Weng et al. (24) with an accompanying commentary by Altshuler and Hirschhorn (25). The finding of the 21 bp deletion in 0.15% of Canadian population, and in individuals without CAD or MI, raised the concern as to whether MEF2A is a CAD disease causing gene. Yet, this was not a definitive assessment because the phenotypic characterization of the individuals presented was incomplete. Furthermore, Weng et al. did identify one point mutation (S360L) only in a case and not controls, and this, like the Pro279Leu, is located in the transcriptional activation domain and may be a functional variant. It remains possible that, like many autosomal dominant traits, there is incomplete penetrance and the potential of modifier gene effects on MEF2A. Further work with a knock-in of the MEF2A 21 bp deletion mutation in mouse models with an atherosclerotic background is in progress as are efforts to further replicate the results in independent populations.

Genome-wide association studies: LTA and LGALS2

There have been two completed ‘genome-wide’ association studies for MI, although neither would fulfill the current state-of-the-art coverage with at least 250 000 informative tag-SNPs. Ozaki et al. (26) assessed 92 788 SNPs in 13 738 genes (only 1491 were non-synonymous SNPs) and identified a locus on chromosome 6p21 which mapped to a 5-SNP haplotype of the lymphotoxin-α gene (LTA). In a very extensive clinical assessment involving 1133 cases and 1878 controls, they showed an OR of 1.8 for MI, $P = 3.3 \times 10^{-6}$. The gain-of-function effect of this LTA variant was shown to enhance vascular adhesion molecule expression and thus considered to be pro-inflammatory and pro-atherogenic. The same group followed on with an important subsequent finding of galectin-2 (LGALS2), a key ligand for LTA (27). The C3279T SNP in exon-1 was associated with a markedly reduced level of the LGALS2 transcript and, in over 4000 MI cases and controls, a significantly reduced risk for MI (relative risk 0.40, $P = 2.6 \times 10^{-6}$). These findings illuminate a potential key inflammation pathway, previously unappreciated inflammatory pathway has been linked to disease, and is strengthened by the identification of genes for both a ligand and its receptor that are both strongly associated with MI.
Shiffman et al. (28) performed a genome-wide association by assessing 11,053 SNPs in 6891 genes, thus providing lesser coverage than the Ozaki study, but using a much higher number of non-synonymous SNPs (7946). The average age of the cases with MI was 62 years. Although there were no functional genomic assessment of these genetic variants, these investigators used three sequential case and control cohorts and identified four gene variants with \( P < 0.05 \) and a false discovery rate of \(< 10\%\); palladin, a cytoskeletal protein (OR = 1.4), ROS1, a tyrosine kinase (OR = 1.75) and two G-protein-coupled receptors—a taste receptor, TAS2R50 (OR 1.6) and OR13G1 (OR 1.40). In a separate study of early-onset MI subjects (average age of 48 years), Shiffman et al. (29) similarly assessed 11,647 SNPs in three sequential case and control cohorts and found two variants with significantly associated MI, with \( P < 0.05 \) and false discovery rate \(< 10\%\). A VAMP8 variant, which is a gene modulating platelet degranulation, had an OR of 1.75 and HNRNPL-1, encoding a ribonuclear protein, had an OR of 1.92. Again, no functional assessment of the gene variants was performed, and independent investigator replication has not yet been reported. There are ongoing state-of-the-art genome-wide association studies for MI using over 250,000 informative SNPs with results expected in the next year.

Genetic studies of special interest: PCSK9, CFH

For decades, the primary determinants of serum LDL cholesterol were thought to be the LDL receptor and apolipoprotein B. However, in the last few years, a cholesterol-regulated gene were thought to be the LDL receptor and apolipoprotein B. For decades, the primary determinants of serum LDL cholesterol drive atherosclerotic disease both in human and animal models. Furthermore, the atherosclerosis predisposition of protective and at-risk alleles of apolipoprotein E (\( \varepsilon2 \) and \( \varepsilon4 \), respectively) correlates well with their effects on LDL cholesterol. We know that PCSK9 loss-of-function mutations yield low levels of LDL and strikingly limit the incidence of MI and CAD. Cohen et al. (39), the same group which advanced our knowledge on PCSK9, examined sequence variations in individuals with low HDL cholesterol and found that rare variants in multiple genes, particularly ABCA1, were associated with low levels of HDL. Low HDL levels are known to be a significant risk factor for MI and CAD. Previously, it had been anticipated that the genetic basis for low HDL, a common endophenotype, would be attributable to several common genetic variants. The findings that multiple rare, non-synonymous variants in three genes—ABCA1, APOA1 and LCAT—were associated with a considerable portion of the population variance of low HDL was indeed unexpected. Only these three genes were sequenced and other genes such as CETP which are known to influence HDL levels were not assessed. Furthermore, only exons and flanking intronic regions were screened. Nevertheless, a major finding from this study is the challenge to the common disease, common variant hypothesis.

Secondly, endothelial integrity appears to be an important pathway that contributes to the biologic basis of CAD or MI susceptibility. In the first high-throughput genotyping study in this field, the thrombospondin family of extracellular matrix proteins was identified to be potentially associated with premature MI (40,41). The A387P thrombospondin-4 variant is common (\( >30\% \) minor allele frequency), and by formation of extracellular deposits of druzen. Linkage studies mapped a gene at 1q32 and through both fine-mapping and a whole genome association study, the Y402H variant of CFH gene was identified as the dominant susceptibility factor for AMD with ORs ranging from 2.5 to 7.4 in five independent studies (1–3,35,36). Interestingly, the environmental risk factors for AMD are cigarette smoking, a high fat diet, obesity, sedentary activity, hypertension and high C-reactive protein (37), which is remarkably similar to the risk factor profile for CAD and MI. With CAD and MI representing an arterial inflammatory process akin to retinal druzen deposits, perhaps it is not surprising to learn of the recent association of CFH genetic variation with MI. Kardys et al. (38), using the Rotterdam study cohort, followed 5530 subjects during a 12-year period and demonstrated a 1.8-fold OR for MI among those individuals harboring the Y402H variant of the CFH gene. Thus, new knowledge of the genetic susceptibility of one complex trait has helped to identify the potential genomic basis of a distinct condition.

Identifying the major pathways

In aggregate, the research to date has identified four distinct and inter-dependent biologic pathways that are implicated in MI and CAD. First, as the classic discovery of familial hypercholesterolemia tagged to LDL receptor deficiency, abnormal lipoprotein handling (production, transport and catabolism) has long been established to promote atherosclerosis and place individuals at risk for developing MI. High levels of LDL cholesterol drive atherosclerotic disease both in human and animal models. Furthermore, the atherosclerosis predisposition of protective and at-risk alleles of apolipoprotein E (\( \varepsilon2 \) and \( \varepsilon4 \), respectively) correlates well with their effects on LDL cholesterol. We know that PCSK9 loss-of-function mutations yield low levels of LDL and strikingly limit the incidence of MI and CAD. Cohen et al. (39), the same group which advanced our knowledge on PCSK9, examined sequence variations in individuals with low HDL cholesterol and found that rare variants in multiple genes, particularly ABCA1, were associated with low levels of HDL. Low HDL levels are known to be a significant risk factor for MI and CAD. Previously, it had been anticipated that the genetic basis for low HDL, a common endophenotype, would be attributable to several common genetic variants. The findings that multiple rare, non-synonymous variants in three genes—ABCA1, APOA1 and LCAT—were associated with a considerable portion of the population variance of low HDL was indeed unexpected. Only these three genes were sequenced and other genes such as CETP which are known to influence HDL levels were not assessed. Furthermore, only exons and flanking intronic regions were screened. Nevertheless, a major finding from this study is the challenge to the common disease, common variant hypothesis.
altering calcium binding has a gain-of-function impact (42). This leads to impaired endothelial cell adhesion and proliferation, along with activation of neutrophils (43). The risk of MI for the A387P SNP has subsequently been confirmed by multiple studies (43,44). Yamada et al. (44) performed a high-throughput screen of genomic DNA from over 4000 cases and controls genotyping 112 SNPs in 71 genes that were previously reported to confer risk of MI. The C1019T connexin 37 gene variant was identified as having significant association with MI in men. Connexin 37 is an endothelial gap-junctional protein which supports the endothelial barrier, preventing untoward diapedesis or penetration of LDL cholesterol. MEF2A is localized to the endothelium and is known to influence vascular permeability (21); and, the autosomal dominant CAD associated with the 21 bp-deletion could possibly exert its effect through disruption of normal endothelial integrity.

Thirdly, the process of arterial inflammation has been implicated through a number of genetic studies. In addition to the findings of LTA and LGALS2 (26,27), and the leukotriene pathway of ALOX5AP and LT4A (13,14), Swanberg et al. (45) found a multihistocompatibility factor (MHC2TA) promoter A168G SNP to be associated with three diseases characterized by inflammation—rheumatoid arthritis, multiple sclerosis and MI. Wang et al. (46) identified Ox40L, from the tumor necrosis family of pro-inflammatory cytokines, as a susceptibility factor for atherosclerosis in mice and confirmed the association in two large epidemiologic cohorts of CAD and control subjects. Another gene SNP of interest from the Yamada study was stromelysin-1 (5A-1171/6A), a key matrix metalloproteinase in the arterial wall, which had an OR of 4.9 for women with MI (44).

Fourthly, thrombosis has been implicated in CAD and MI by multiple studies. In a comprehensive meta-analysis of 66 155 cases and 91 307 controls, the 1691A variant of factor V and the 20210A variant of prothrombin both were associated with an increased CAD risk (47). Beyond these two variants which promote thrombin generation, multiple platelet glycoprotein receptor variants, VAMP-8 and plasminogen activator inhibitor-1 have also been implicated for risk of MI or CAD (29,44,47).

It is clear that these four pathways are indeed interactive (Fig. 2). For example, if endothelial integrity is not maintained or is disrupted, transmigration of macrophages and lipoproteins can facilitate arterial inflammation. Should arterial inflammation proceed, an arterial fissure, erosion or frank plaque rupture could ensue (Fig. 2) which can precipitate thrombosis and an acute MI. While it is possible, and even likely, that other major pathways will be identified, the genomic research to date has been effective in not only implicating specific genes but also honing in on the principal processes.

**Challenges for the future**

To advance the genomics of atherosclerotic coronary disease, there are a number of specific challenges that have become increasingly apparent. The definition of cases and controls is particularly tricky. As a late onset disease, a control may ultimately become a case later in life. The use of invasive techniques to define the disease, such as coronary angiography,
Figure 2. Schematic of the arterial processes in which there has been genetic supportive data for CAD or MI. Top panel, left-plaque accumulation in coronary arterial wall. Top panel, right-plaque rupture, a hallmark of MI. Middle panel, left, endothelial reduced integrity with denudation of cells (genes implicated: MEF2A, THBS4). Middle panel, right-early inflammation with monocyte and oxidized LDL subintimal penetration (genes implicated: PCSK9, apoE4). Bottom panel, left-advanced inflammation with macrophage transformation to foam cells and lymphocyte, neutrophil activation (genes implicated: ALOX5AP, LTA4, LTA, LGALS2, Ox40L, MHC2TA, CFH). Bottom panel, right-prothrombotic with breach of the arterial wall and platelet adhesion and aggregation (genes implicated: VAMP8, Factor V Leiden, Prothrombin).
results in an ascertainment bias, as patients do not undergo such testing if they are free of symptoms. Furthermore, the threshold for categorizing a ‘normal’ coronary angiogram void of significant atherosclerotic burden is difficult, as the angiogram is only characterizing the lumen of the arteries, whereas atherosclerotic plaque is building up in the walls.

The case and control difficulties have made replication challenging, as the definition of cases and controls has varied from one study to the next (21,24). Importantly, although atherosclerosis is generally straightforward to induce in certain mouse strains (48), the experimental model in mice for MI is a very difficult one that involves a double knockout with the scavenger receptor SR-B1 and apoE or SR-B1 −/− and hypomorphic apoER61 (49,50). The breeding of such mice is especially laborious, as they have a high mortality independent of the occurrence of MI. Without access to human tissue, this is especially laborious, as they have a high mortality independent of the occurrence of MI. Without access to human tissue, reliance on murine models makes in vivo functional assessment of genes of interest particularly challenging. Beyond the experimental model hurdle, recent studies with either low LDL or low HDL suggest that many variants in important susceptibility genes might account for a significant proportion of population risk for the common traits of CAD and MI (31–33,39). Altogether, these challenges are formidable and may, in part, explain some of the lag in progress in cardiovascular genetics when compared with cancer genetics.

CONCLUSION

Recent reviews have highlighted in more depth some of the important advances in CAD and MI genomics (51–53). Collectively, some important patterns have emerged. Critical pathways have been identified and specific genes connected with multiple prime examples. Two genes in the leukotriene pathway, first identified as important in susceptibility for murine atherosclerosis (48) have been definitively associated with the risk of MI. The genes encoding LTA receptor and the principal ligand LGALS2 have been demonstrated to confer risk of MI. CFH, the dominant genetic basis for AMD, has now been implicated as having a role in MI, thereby connecting two diseases with known underpinning of inflammation and activation of the complement pathway. Even such diverse conditions as multiple sclerosis, rheumatoid arthritis and MI have converged with the finding of a MHC2 promoter that leads to susceptibility to all three.

Overall, no gene for CAD or MI has been identified with a large population attributable risk akin to the TCF7L2 finding for type 2 diabetes (5). Perhaps, one of the critical insights from the work to date has been the cumulative sense that the phenotype for CAD and MI are quite distinct, and the pathology may also be dissimilar. Nearly all of the progress has been made in MI, with the exceptions of PCSK9 and Ox40L. It is likely that MI represents a more restrictive and possibly ethnicity-specific risk of myocardiial infarction.

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REFERENCES


