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Cardiovascular Genetic Medicine: The Genetics of Coronary Heart Disease

David Seo · Pascal J. Goldschmidt-Clermont

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Abstract Advances in genomic technologies have provided researchers with an unprecedented opportunity to identify genes that may contribute to the development of coronary heart disease. The power of these technologies lies in their ability to survey the entire genome in a nonbiased fashion to find genes and gene variants associated with coronary heart disease. This article reviews different genomic approaches for studying coronary heart disease and the clinical implications of using genetic information.

Keywords Genomics · Cardiovascular Disease · SNPs · Single Nucleotide Polymorphisms · Atherosclerosis · Cardiovascular Genetic Medicine · Linkage Analysis · Coronary Heart Disease · Coronary Artery Disease

Introduction

According to the latest statistics from the American Heart Association, over 650,000 people in the United States died as the result of atherosclerotic coronary heart disease (CHD) events such as myocardial infarctions (MI) [1]. Additionally, for a substantial number of individuals, sudden death or nonfatal MI were their first manifestation of CHD and occurred without warning. Therefore, considerable efforts have gone toward improving diagnostic and prognostic tools and developing more effective treatment strategies.

A new line of research has been to define the genetic component of this disease. Many believe that knowledge of the genes contributing to disease development and progression will provide new opportunities for stratifying people by their individual disease risk, identifying novel therapeutic targets, and tailoring management strategies based on inherent genetic characteristics [2–4]. Thus far, the effort to pinpoint the genetic component of CHD has been difficult owing to the complex nature of the disease, but the application of new genomic methods provides hope of ultimate success. The objective of this article is to provide an overview of the current knowledge regarding the genetics of CHD and the possible clinical implications of the genetic information.

D. Seo (✉) · P. J. Goldschmidt-Clermont
Medicine, University of Miami School of Medicine,
1120 NW 14th Street, Room 1116,
Miami, FL 33136, USA
e-mail: dseo@med.miami.edu

Background

Atherosclerotic CHD results from the interaction between an individual's genetic make up and environmental factors such as smoking and diet [3, 5]. There are rare Mendelian disorders for which single gene changes lead to accelerated atherosclerosis such as familial hypercholesterolemia. In the more common "garden variety" atherosclerosis, however, multiple genes are likely to influence the disease process by enhancing disease susceptibility or by augmenting the impact of environmental risk factors. The genetic component is likely to be multigenic and comprised of a collection of gene variants such as single nucleotide polymorphisms (SNPs). Each individual SNP may have a modest effect on the quantity or function of a translated protein product. However, when individual SNPs are aggregated, the combination of the multiple variants may have a major impact on disease biology. In addition, it is important to consider the interaction between genes and gene variants with environmental risk factors. For instance, a particular combination of SNPs may lead to increased disease

susceptibility in one setting of environmental risk factors but may decrease disease susceptibility in another setting [6]. In such a multigenic disease model, identifying the gene variants associated with atherosclerosis development and progression could be valuable disease biomarkers new diagnostic and prognostic tools. Some of the gene variants may be functional biomarkers that can be used to assess treatment response or serve as targets for novel treatment strategies. The obvious challenge is how one identifies the genes and gene variants that collectively contribute to coronary atherosclerosis. With the advent of broad-based genomic technologies, researchers now have tools for studying the genetic component of multigenic disorders like CHD. What follows is a brief overview of three types of high throughput genomic methods that are well suited for studying CHD and their results compiled to date.

Genomic Studies of Coronary Heart Disease

Linkage Analysis

Linkage analysis is a nonbiased and powerful approach for identifying causative genes for complex diseases [7]. The analysis is conducted without a priori identification of potential candidate genes or their chromosomal locations. Linkage analysis looks for DNA markers that cosegregate with the disease phenotype between affected family members at a rate that is statistically greater than random chance. The chromosomal region containing the DNA markers can, then, be examined in further detail to look for potential candidate genes. This methodology has successfully defined the etiology for many monogenic disorders by studying families with a high prevalence of premature atherosclerotic disease. There have been several successful such studies in CHD. One study by Wang et al. examined a family cohort with a high prevalence of myocardial infarction and found linkage for a region on chromosome 15. [8] The putative candidate gene in this chromosomal region was identified as myocyte enhancer factor 2A (*mef2a*). In another linkage study, Broeckel et al. studied a group of 513 unrelated families in which there was at least one member with a documented myocardial infarction. [9]. In this study, a region on chromosome 14 was linked to the myocardial infarction event; however, the putative causative gene(s) could not be identified further. Another linkage study performed by the Genetics of Early Onset Coronary Artery Diseases (GENECARD) investigators looked at families with early-onset coronary artery disease [10]. In this study, researchers found multiple linked regions on chromosomes 1, 3, 5, 7, and 19, and recently, the GENECARD investigators have identified putative candidate genes within the chromosome 3 regions: the GATA2 transcription factor [11], limbic system-associated membrane protein [12], and Kalirin [13] genes—all of which had never been previously associated with atherosclerosis or CHD.

In considering the three linkage studies discussed, there is a notable lack of agreement in the results. One explanation may be the significant differences in the subject groups and the outcome variable analyzed by each study. Wang et al. performed their analyses for CHD genes in a single family cohort while Broeckel et al. and the GENECARD investigators used hundreds of unrelated families. This may explain why *mef2a* was not found in the other two studies given the focus on a single family, albeit a large cohort. The major findings from Broeckel et al. and the GENECARD investigators were also quite different, possibly due to differences in the populations and phenotypes studied. The probands in the Broeckel study had a confirmed diagnosis of myocardial infarction. Additional affected family members defined as those with a diagnosis of myocardial infarction or history of revascularization by coronary artery bypass surgery or percutaneous intervention. The GENECARD study included a much wider range of study subjects. Affected subjects were defined as those with myocardial infarction, unstable angina, coronary atherosclerosis diagnosed by stress test or cardiac catheterization, and individuals with a documented history of disease or revascularization. So, the population was much broader than the Broeckel study. In addition to differences in the subject groups, the phenotype of interest was different. The investigators in the Broeckel study looked for genes linked to myocardial infarction while the GENECARD investigators identified genes linked to the incidence of early onset coronary artery disease.

Whole Genome Association

In the past, genetic association studies examined a limited, defined number of polymorphisms within a handful of candidate genes for association with a disease phenotype or outcome. While valuable, the need to preselect these polymorphisms was the main limitation of this methodology. However, using the latest genomic technologies, researchers can now assay hundreds of thousands of SNPs simultaneously in a single individual using “SNP chips”. High throughput genotyping using SNP chips with subject cohorts containing thousands of individuals has made it possible to perform genetic association studies in a non-biased fashion [14–16]. There have been two recent studies that performed whole genome association to identify SNPs associated with the development of myocardial infarctions [17, 18]. Both were large case-control studies of thousands of subjects. Using SNP chips, McPherson et al. and Helgadottir et al. found three different SNPs (rs10757278

in Helgadottir and rs10757274 and rs2383206 in McPherson) on chromosome 9p21 adjacent to the tumor suppressor genes, *CDKN2A* and *CDKN2B*, which were highly associated with myocardial infarction. These three SNPs are not located within a known or putative gene, and their functional roles, if any, have not been determined. However, the SNPs may indeed be true biomarkers for myocardial infarction risk as they have been validated by other independent studies [17, 18]. Approximately 20% of Caucasians carry copies of the SNPs, and the lifetime risk of myocardial infarction for homozygous carriers of the SNPs is over 20%. A clinical genetic test evaluating these SNPs is now commercially available.

Other ongoing whole genome association studies for CHD include the Women's Genomic Health Study and the Cardiogenics Project which may confirm and identify new SNPs associated with cardiovascular disease.

Gene Expression Profiling

The use of functional genomics is another approach to identify genes and pathways that contribute to the development and progression of coronary atherosclerosis [2, 19]. This approach analyzes disease in relevant tissues to look for changes in the abundance of transcribed genes or messenger RNAs (mRNA) that correlate with a disease state, clinical outcome, or therapeutic response. Functional genomics quantifies the levels of tens of thousands of mRNAs simultaneously to identify genes with significant differential expression. There have been several large studies of human atherosclerosis to date using gene expression analysis. In one study, Seo et. al. performed gene expression analysis on human aortic tissues collected at the time of organ donation to identify genes associated with the degree of atherosclerotic burden [20]. By comparing aortic tissues with minimal or severe disease, researchers found a number of differentially expressed genes. The candidate genes included many that were previously associated with atherosclerosis as well as a number of novel genes. Another study by Randi et al. performed gene expression analysis of atherosclerotic plaques obtained by atherectomy from patients with stable and unstable coronary artery disease [21]. Analysis revealed differential expression of genes known to be involved in hemostasis and inflammation such as protein S (PROS1), cyclooxygenase 1 (COX1), and interleukin 7 (IL7). Another unique study examined gene expression in the peripheral blood mononuclear cells of patients with and without significant carotid atherosclerosis [22]. They found differential expression in a number of regulatory genes and transcription factors including Finkel–Biskis–Jinkins osteosarcoma protooncogene and dual specificity phosphatase 1. An intriguing implication of this study result is the possibility that blood gene expression could be used as a molecular signature to detect the presence of atherosclerosis.

Clinical Implications of Genetic Information

Clearly, recent research has generated interesting new information regarding genes that are associated with coronary atherosclerosis and the development of CHD events, particularly myocardial infarctions. The key question at this point is how to bring these results to clinical practice.

One way to apply new genetic information is the development of new diagnostic and prognostic tests for CHD. As reviewed elsewhere (Hershberger review in this journal issue), genetic information is already being used clinically for single gene disorders such as channelopathies [23, 24] and Familial Hypercholesterolemia [25, 26]. There is also a growing number of clinical genetic tests for nonMendelian settings such as breast cancer [27, 28] and cardiac transplantation [29, 30]. These clinical genetic tests are being used in two different ways—generating detailed disease characterization or establishing disease susceptibility. Current testing for breast cancer and cardiac transplantation is used for more detailed disease characterization. The tests are performed to generate a highly detailed molecular phenotype of the disease-related tissue to further stratify patients into clinical subgroups. For example, in breast cancer, the traditional approach for categorizing a subject, as either low or high risk for future disease recurrence, is to use clinical factors such as tumor size, lymph node status, and histological grade. The molecular information generated from tumors by genetic testing can be used to identify subgroups within the clinically estimated low risk category who are actually at significantly higher risk for disease recurrence and will have outcomes in line with patients in the high risk category, thus, further refining the diagnostic process. Such knowledge can have a significant impact on clinical decision making with regard to surgery and chemotherapy. In the case of cardiac transplantation, the genetic tests can identify subtle changes in the peripheral blood mononuclear cells that can be used to detect organ rejection much earlier than traditional histopathology. In the examples of breast cancer and cardiac transplantation, the disease diagnosis has already been established, and the genetic testing can be thought of as an extension of currently available biochemical and histopathological testing that aids in clinical decision making or determining prognosis by the virtue of providing more detailed molecular phenotype information. There are no current genetic tests that meet these criteria with respect to CHD.

The second way to apply genetic information is to establish disease susceptibility by defining a person's

individual risk beyond currently available risk assessment tools. There are a number of commercially available tests that fall in this category including tests for susceptibility for diabetes [31, 32], atrial fibrillation [33], and myocardial infarction [17, 18]. The purpose of such genetic testing is to identify individuals at higher risk who would, then, receive more intensive preventive medical treatments in hopes of delaying or preventing disease development. While this is an intriguing opportunity, there are several issues to consider about using this new generation of clinical genetic testing [34, 35]. The first is in the interpretation of the results. Unlike single gene disorders, the results of a positive or negative test provide no concrete diagnostic or prognostic information. A positive test simply means that a patient has an elevated risk for developing the disorder—it is not a near certainty. Similarly, a negative test does not guarantee that the patient will not ultimately develop the disease. The results of the genetic testing are not given in the context of a subject's current traditional risk factor profile. That is to say, clinical and genomic information are not integrated in the diagnostic testing process, and it is not yet clear what additional prognostic information genetic testing provides beyond risk assessments based on standard clinical risk factor models. Another issue about the clinical genetic testing for multigenic complex disorders is what clinical interventions should be administered given the test results. For example, if the genotyping indicates that the individual is at risk for a future myocardial infarction, should intensive medical therapies be prescribed? As indicated above, a positive test only means that there is an increased risk for having a myocardial infarction at some future time. Intensive medical therapies such as blood pressure reduction, augmented antiplatelet therapy, and aggressive cholesterol lowering have all been shown to substantially reduce CHD. While quite effective in reducing risk and being relatively safe, universal administration of these therapies is both financially infeasible and would lead to unnecessary side effects. However, with the use of genomic information, it may be possible to identify disease-susceptible individuals for whom intensive prevention is cost effective. It remains to be seen whether this hypothesis will be borne out. Will the benefits of interventions applied in response to a positive genomic test outweigh the risks of unnecessary treatment and financial costs and result in durable and cost effective improvements in health? On the other hand, given that there is no guarantee of a disease-free future, will a negative test lead to a false sense of security and encourage the maintenance or resumption of prior deleterious behaviors? Therefore, further evaluation of the utility of these tests through carefully designed disease outcome trials need to be performed. Finally, there are potentially problematic legal and ethical issues surrounding clinical genetic testing for complex diseases that go beyond the scope of this review. While the results of the test provide no concrete diagnostic or prognostic information, they may have potentially significant effects on health insurance premiums or employment.

Finally, another way to translate new genetic information into clinical practice is through the identification of targets for drug development or modified uses for currently existing medications. While a straightforward idea, there are still a few hurdles to overcome. The primary one is to provide convincing evidence that a gene contributes to disease development and progression. In the case of linkage studies, sometimes, it is difficult to determine the dominant candidate genes. Often, these studies identify chromosomal regions associated with disease that are 10 cM or roughly ten million nucleotides in length. A region of this size may contain 100–300 potential genes making it challenging to identify the causal gene(s). For whole genome association studies, an associated SNP is not necessarily functionally relevant and may simply be tightly linked with the real unidentified causal gene. In some cases, the SNP may not even lie within a known or putative gene. For candidate genes identified by functional genomics studies, the difficulty is determining whether the gene expression levels are altered because the genes are contributing to disease biology or whether the genes are altered as a consequence of the disease process.

Conclusion

In the recent past, a significant effort was devoted toward developing methods for generating robust genetic information about complex disorders such as CHD. Technical innovations are now enabling researchers to overcome the challenges of dissecting multigenic diseases. The combination of advanced genomic technologies, reduced assay costs, and the availability of large well-characterized study cohorts provide researchers with the unprecedented opportunity to determine the genetic framework for multigenic disorders such as CHD. There are a number of studies in the literature that have used these technologies to generate more detailed information about the genes that contribute to the development and progression of coronary atherosclerosis and its thromboembolic complications such as myocardial infarction. Knowledge of the genes that influence CHD may ultimately lead to the development of novel treatments and more effective diagnostic and prognostic tools. There are commercially available genetic tests for to assess risk for myocardial infarction currently on the market. However, we must work on the next steps to understand how and when to apply genetic information and study whether this new generation of information will lead to true improvements that are cost effective and improve the quality of

health care. But what is certain is that technology is ever improving its speed and accuracy in examining the entire genome, pathways and protein products. Newer analytical methods and tools are also being developed to handle such complicated data and information, allowing for the integration of genetic data into the current knowledge of cardiovascular disease. We have entered a new era of genetic investigation.

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