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Review article

Clinical Application of Genetic Prediction in the Management of CAD

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Abstract

Sequencing of the human genome followed by the HapMap project made possible the unbiased genome-wide association studies that led to the discovery of hundreds of genetic risk variants predisposing to CAD. The total genetic risk for CAD can be expressed in a single number based on the number of variants inherited. A GRS derived from genotyping with microarrays containing these risk variants has been evaluated in over 1 million individuals. Risk stratification for CAD based on the GRS was shown to be superior to conventional risk factors. Placebo-controlled clinical trials showed individuals with high genetic risk had a 40-50% reduction in cardiac events with a favorable lifestyle, and cholesterol-lowering drugs. The risk of CAD based on conventional risk factors such as hypertension are age-dependent, occurring primarily in the sixth or seventh decade which is too late for primary prevention. The GRS is independent of age and can be determined at birth if needed. Incorporation of the GRS into clinical practice would transform the primary prevention of CAD, the number one killer.

Keywords: ***Genetics, Coronary Artery Disease, Genetic Prediction***

Introduction

The terms personalized medicine, and more recently, precision medicine have become household names although their impact in clinical management is meager. The ultimate form of precision medicine is arguably therapy based on one's genome. The technology facilitating linkage analysis [1] of pedigrees to map single gene disorders revolutionized the discovery of genes responsible for rare cardiac disorders such as familial hypercholesterolemia and familial hypertrophic cardiomyopathy [2]. However, common diseases such as coronary artery disease (CAD) and hypertension, postulated to be due to multiple common genetic variants, required a different analysis of a large population of unrelated individuals by a technique referred to as a Case-Control Association Study [3,4]. To pursue an unbiased case-control association study (CCAS) requires hundreds of thousands of DNA markers that span the human genome. Such well-defined DNA markers and the computerized platforms to rapidly genotype and analyze the results did not become available until 2005 [5,6]. In this review, we will briefly summarize the discovery of genetic risk variants predisposing to CAD, their subsequent utilization to develop a genetic risk score (GRS) and the results of the application of GRS to risk stratify for primary prevention of the number one killer, CAD.

Genetic predisposition to CAD

It has been claimed for several decades that 50 to 60% of susceptibility to common chronic polygenic disorders such as CAD are due to genetic predisposition [7]. The evidence for genetic predisposition was derived in large part from identical and fraternal twin studies [8], together with family clusters. The hereditary component can be very powerful as shown in a Utah study in which 14% of the patients have a family history of heart disease and it is in this cohort that 72% of all premature myocardial infarctions and 48% of all coronary events occur [9]. It is claimed that first degree relatives of individuals with CAD have a 2-3 fold increase in risk for CAD [10-12].

The Human Genome and the Origin of mutations

The Human Genome is a double helix, with each strand containing 3.2 billion bases. The DNA molecule consists of a repeat of 4 bases (Thymine, Adenine, Guanine, and Cytosine). Attached to each base are sugar and phosphorous molecules with the combination being referred to as a nucleotide. The DNA sequence of each human genome is 99% identical [13]. The 1% difference in sequences is due to polymorphic sequences which consist of large structural variants and single nucleotide polymorphisms (SNPs) [14-16]. The number of single nucleotide polymorphisms per genome is fairly constant, at about 5 million. These SNPs are somewhat evenly distributed throughout the genome averaging about one SNP per 3000 bases [17]. Each individual inherits 40-60 unique mutations [18] of which

90% of them are SNPs. These mutations originate from DNA copying errors [19,20] and are in large part responsible for environmental adaptations and evolution. It is not surprising that over 80% of the unique features of each individual such as the color of one's eyes, or hair, including predisposition to disease, are claimed to be due to these SNPs [21]. The SNPs as DNA markers are close to ideal for spanning the genome in search of DNA regions predisposing to CAD.

Genome-Wide Association Studies

The '80s and the '90s are often referred to as the golden era for rare single-gene disorders. The application of genetic linkage analysis to pedigrees with affected individuals led to the discovery of genes responsible for several rare disorders, including familial cardiomyopathies, familial hypercholesterolemia, familial atrial fibrillation, and Wolf-Parkinson white syndrome [2]. It was apparent that this approach would not be appropriate for the discovery of genes responsible for polygenic disorders such as CAD. Common polygenic disorders such as CAD were postulated to be due to multiple genes scattered throughout the genome with each gene associated with only minimal risk [3]. A more appropriate approach would be that of the Case-Control Association Study [22] which would require a large sample size of unrelated individuals with (cases) or without (controls) the disease of interest. The technology and markers to perform a genome-wide search were not available in the '90s. Investigators adopted a direct approach in which candidate genes based on their function and relationship to atherosclerosis were selected and evaluated in cases and controls. A candidate gene occurring more frequently in cases than controls would be considered to have a predisposition to CAD. This was a biased approach since one was a priori selecting the candidate gene. We now know that over 100 such candidates were claimed for CAD and prospectively, none were confirmed [23,24].

The unbiased approach was made possible by the development of several new technologies which led to the first workshop on Genome-Wide Association Study (GWAS) in 2005 [25]. The Human Genome Project sequenced the first human genome which was published in 2000 [26]. This was followed by the HapMap which identified over 1,000,000 single nucleotide polymorphisms (SNPs) [27]. This enabled one to saturate the human genome with SNPs as markers every 3000-6000 base pairs. The subsequent development of microarrays and computerized platforms enabled rapid genotyping and analysis using hundreds of thousands of DNA markers [28]. A marker occurring more frequently in cases than in controls would signify a region that predisposes to the disease. It is important to recognize that the particular SNP used as a marker is not necessarily causative of the disease but rather is in close proximity to SNPs that do predispose to the disease. This eliminated the bias associated with selecting candidate genes simply on the basis of their function. There was, however, the issue of what constituted statistical significance if one utilized 1,000,000 markers. If one accepted a p value of 0.05, there would be 50,000 false positives. A statistical correction was necessary and investigators commonly agreed to use the Bonferroni correction, which meant 0.05/1,000,000 to give a p value of 10^{-8} [29]. This would require much larger sample sizes than expected. It would require thousands of cases and controls. Furthermore, it was also recommended that those SNPs with a p-value of 10^{-8} would be further confirmed by replication in an independent population [30,31].

The first genetic risk variant now referred to as 9p21 was discovered simultaneously by two independent groups [32,33], this variant predisposing to CAD, as indicated, was located on the small arm of chromosome 9 at bands 2-1. The 9p21 risk variant for CAD was confirmed by several groups throughout the world involving multiple ethnic and racial groups [34]. It was also shown by all of the groups that the 9p21 risk variant was associated with only about 25% increased risk per copy and was present in 75% of the world's population. This confirmed our initial hypothesis that a polygenic disease such as CAD would be due to commonly occurring variants in the genome and each would be associated with only minimal risk for the disease. The 9p21 risk variant was shown to mediate its risk for CAD independent of all known conventional risk factors. This implies the 9p21 genetic risk variant mediates its risk for CAD through some unknown molecular pathway that contributes to the pathogenesis of Coronary Artery Disease.

Encouraged by the new findings of the 9p21 risk variant, along with rapidly developing and improved technologies, there was a major movement to perform GWAS, not just for CAD, but for many chronic polygenic disorders. Recognizing the need for a large sample size stimulated the formation of international consortiums. For CAD, this was led by CARDIoGRAM [35] which had available over 80,000 cases and controls. The coronary artery disease (C4D) [36] genetics consortium joined CARDIoGRAM to become CARDIoGRAMPlusC4D, which became the leading force for the discovery of genetic risk variants for CAD. The results of these international consortiums and other individual investigators led to the discovery of 173 genetic risk variants predisposing to CAD which are comprehensively reviewed in several recent reviews [37-39]. All of these genetic risk variants satisfied the p-value of 10^{-8} and were replicated in an independent population.

Features of Genetic Risk Variants Predisposing to CAD

Over 80% of the genetic risk variants predisposing to CAD occur in regions of the genome that do not code for protein [37]. Thus, these genetic risk variants mediate their risk through a regulatory role on upstream or downstream protein-coding regions either in close proximity (cis-acting) or through interactions on other chromosomes (Trans interacting). Secondly, over half of the genetic risk variants mediate their risk independent of known conventional risk factors for CAD. Thirdly, the variants as predicted occur throughout the genome and each is associated with only minimal risk. Fourthly, the genetic risk variants occur commonly with over 50% of them occurring in over 50% of the population. Despite the common occurrence of these genetic risk variants, the mechanisms whereby most of these variants mediate their risk for CAD remain unknown. Elucidation of the pathways mediating their risk will be rich fodder for investigators searching for new targets for the development of novel drugs. Furthermore, elucidation of these pathways will significantly increase our insight into the pathogenesis of coronary atherosclerosis.

The advantage of a Genetic Risk Score for CAD

It became evident as more and more genetic risk variants for CAD were discovered, that one potential immediate application would be risk stratification for the prevention of CAD. Coronary atherosclerosis, the underlying cause of CAD is very common and starts early in the teenage years and gradually increases, particularly in the proximal coronary vessels. The peak time for clinical manifestations such as myocardial infarction or angina in

males is in their late 50's and in females about a decade later [25]. It is claimed that in the US, of those living a normal lifespan about 50% will experience a cardiac event [25]. Secondary prevention, reducing conventional risk factors in individuals who have symptoms, have been shown to be very effective [40-42]. Primary prevention has also been shown to be effective but selecting those who would benefit from primary prevention is more difficult. Plasma cholesterol, the main culprit causing coronary atherosclerosis, increases log linearly, and is significantly increased above recommended levels by the third or fourth decades [43-45]. Lifestyle changes and cholesterol-lowering agents are very effective in decreasing plasma cholesterol [42]. However, other conventional risk factors such as diabetes, and hypertension are age-dependent and often are not present until the sixth or seventh decade, which is too late for primary prevention. The presence of increased plasma cholesterol without other risk factors for CAD creates a major public health conundrum. If one treats increased plasma cholesterol as a sole risk factor, a significant percentage of individuals treated would be without need or benefit, perhaps up to 50% [25]. Detecting those at increased risk remains a problem. The inadequacy of utilizing the Current Cardiology Guidelines based on conventional risk factors to screen for those at risk for primary prevention has been the subject of several recent reviews [39,46-48]. Assessing risk for CAD based on genetic predisposition would have the advantage of not being age-dependent and could be determined as early as birth. Genetic risk is transmitted by one's DNA at conception and is not expected to change throughout one's lifetime.

Development of a Genetic Risk Score for CAD

The total genetic risk burden for CAD is proportional to the total number of genetic risk variants inherited by that individual. Since each variant contributes only minimal risk to CAD it is important to genotype for all known genetic risk variants. The overall genetic risk for CAD can be expressed in a single number. The number of copies of a genetic risk variant for CAD inherited by an individual will vary from none (no copy in either parent) to one copy (Parent Heterozygous) to two copies (Parents Homozygous). The risk associated with each variant inherited can be determined by the product of the number of copies and the derived odds ratio, the summation of which provides for the total burden of risk for CAD [38,49].

Evaluation of the genetic risk score in Clinical Trials

Initial risk assessment using only 12 genetic risk variants [50] for CAD showed statistical benefit over conventional risk factors, but the clinical benefit was minimal [51]. The use of 27 genetic risk variants by Mega et al. in 2015 [52] showed significant improvement over conventional risk factors. All of these genetic risk variants were genome-wide significant and had been replicated in an independent population. Mega et al. retrospectively genotyped 4 large clinical trials performed previously to evaluate the effect of statin therapy on cardiac events. Two of these studies were primary prevention and the other two secondary. The total sample size was 48,421 individuals. The genetic risk score categorized the individuals into low, intermediate, and high risk. The group with the highest genetic risk score coincided with the group that received the most benefit from statin therapy. This confirmed the individuals with the highest GRS were associated with the highest risk for CAD. Furthermore, to prevent one cardiac event required only 25 individuals to be treated which is several-fold more potent than using conventional risk factors. Similar results were observed on genotyping 10,456 individuals recruited into the West of Scotland Coronary Prevention Study (WOSCOP) [53]. In the WOSCOP study, the high GRS score had a risk reduction of 44% compared to a risk reduction of 24% in others. One needed to treat only 13 individuals with a statin versus 38 individuals in the low-risk group to prevent one cardiac event. Risk stratification for CAD utilizing traditional risk factors requires the statin treatment of 100 individuals to prevent two cardiac events [54].

Prospective analysis of the Genetic Risk Score Utilizing millions of Genetic Risk Variants

Utilizing the GWAS studies, 173 genetic risk variants for CAD were discovered and confirmed in an independent population [38]. However, this group of genetic risk variants for CAD accounted for only about 38% of expected inheritability [38]. Several approaches were taken to increase the number of genetic risk variants. Abraham et al. [55] utilized a microarray of 49,310 SNPs based on the CARDIOGRAMPLUSC4D consortium. To obtain these variants, statistical requirements were lowered to include SNPs that were less than genome-wide significant but less than a 5% false discovery rate. It was recognized for some time that using the Bonferroni correction might be too stringent. The other approach taken by Khera et al [56] utilized a computerized algorithm LDpred [57] to predict genetic variants that associate with a predisposition to CAD. Analysis was performed to confirm that all SNPs were in linkage equilibrium to avoid redundancy of markers [56]. Statisticians and investigators all recognized that many of these risk variants predicted from such programs would overestimate the actual number that would predispose to CAD. Nevertheless, statisticians and mathematicians confirm that including variants without effect would not dilute the power to predict risk [58]. Secondly, any variant with even very minimal association with risk for CAD would improve the predictive power.

Abraham et al. [55] using the microarray of 49,310 variants genotyped 5 prospective population cohorts. In this study, individuals with the higher GRS were at higher risk for CAD compared to those with low GRS scores. The observation was similar to the results of trials evaluating statin therapy [52,53]. Inouye et al. [59] utilized a microarray containing 1.7 million risk variants for CAD to genotype nearly 500,000 individuals selected from the UK biobank. The top 20% risk group of the GRS had a four-fold increased risk for CAD. Khera et al. [56] genotyped 288,978 individuals utilizing a microarray with 6.6 million genetic risk variants for CAD. Their analysis showed that 8% of the population inherited a 3 fold increased risk for CAD, and 0.5% inherited a five-fold increased risk for CAD. Individuals with the highest genetic risk score also had the highest risk for CAD. They concluded that most of these individuals at high risk would not have been identified using traditional risk factors for CAD. Those with increased risk for hypercholesterolemia accounted for only 20% of the high genetic risk group and only 28% had hypertension. It is of note that a family history of CAD in the high-risk group was observed in only 35%. Four observations were gleamed to be in common with all of the studies. One, the genetic risk score consistently identified individuals at high risk who would benefit most from statin therapy. Secondly, the genetic risk score was relatively independent of conventional risk factors. Thirdly, the larger the number of genetic risk variants, the greater the power of prediction for CAD risk. Lastly, the genetic risk score was

consistently observed to be more discriminatory in predicting risk for CAD than that observed based on conventional risk factors.

Lifestyle Changes and Cholesterol-Lowering Drugs Reduce Cardiac Events in Those at High Genetic Risk

It has been a common myth that if the problem is in your genes, there is nothing one can do about it. This, of course, is incorrect and for some time we have successfully treated genetic predisposition the same as we treat acquired predisposition. Statin therapy, which inhibits the activity of the rate-limiting enzyme 3-hydroxy-3-methylglutarylcoenzymeA inhibits the synthesis of cholesterol and indirectly blocks the function of the gene encoding for this enzyme. The development of a sensitive program to predict the genetic predisposition of CAD has major implications for the prevention and management of this disease. Previously we indicated that the genetic risk score in clinical trials assessing statin therapy indicated the GRS detected those at the highest risk and would benefit most from statin therapy. Its discriminatory power to detect those who benefit most from statin therapy was greater than those of conventional risk factors [52,53]. Recently, additional studies have been performed assessing the discriminatory power of GRS to stratify the risk of CAD. The FOURRIER trial [60] enrolled 14,298 patients and genotyped with either a microarray having 27 risk variants or 6 million variants predisposing to CAD. Patients with intermediate and high genetic risk for CAD had 1.23 and 1.65 fold increased risk for coronary events respectively. The group receiving Evolucumab had a 13% relative risk reduction in the group with traditional risk factors but without high genetic risk and 31% relative risk reduction in patients with high genetic risk regardless of clinical risk factors. Individuals with the highest genetic risk score also had the greatest risk and the greater benefit from lowering of plasma cholesterol with Evolocumab. A similar study was performed in the ODYSSEY Trial [61] using a microarray with 6 million genetic variants and a sample size of 11,953. The highest risk group for CAD also had the highest GRS. The relative reduction of cardiac events by Alirocumab was 37% in the high GRS versus 13% reduction in the low GRS group. These studies consistently show that lowering of plasma LDL cholesterol, whether it be with statin therapy or PCSK9 inhibitors, the genetic risk score is superior to that of conventional risk factors in identifying those who will benefit most from therapy.

A major interventional preventative therapy for CAD has long been that of changes in lifestyle such as smoking and intake of red meat. In a randomized clinical trial by Khera et al. [62] 55,685 individuals were enrolled and a genetic risk score was derived from genotyping 50 genetic risk variants for CAD. Roughly one-half of the individuals had a favorable lifestyle versus the other half, an unfavorable lifestyle. A favorable lifestyle consists of no obesity, a healthy diet, frequent exercise, and no current smoking. An unfavorable lifestyle had at least two of these unfavorable components. Analysis showed that those with a high GRS in the top 20% had a 91% higher risk of cardiac events than those with a low GRS. A favorable lifestyle and a high GRS was associated with a 40% lower risk for cardiac events than an unfavorable lifestyle. This is a significant clinical trial since it has always been difficult to maintain lifestyle changes long enough to have statistical significance.

Assessing the effect of physical activity on cardiac events has always been difficult in part because it is difficult to quantify physical activity. Tikkanen et al. [63] performed genetic risk stratification for CAD as the basis to assess the effect of physical activity on genetic risk for CAD. They genotyped a population from the UK biobank of 468,095 individuals. Exercise consisted of handgrip for 3 seconds and a cardiorespiratory test of exercise on a stationary bicycle during which oxygen was monitored. The genetic risk for CAD was categorized into low, intermediate, and high. Individuals with the highest GRS also had the most benefit from exercise with a 49% lower risk for CAD.

Limitations of the current genetic risk score

While most of the studies indicate a genetic risk score has an advantage over conventional risk scores for CAD risk stratification, all of the previous studies indicated that GRS is relatively independent of conventional risk factors for CAD. Our current risk scores including the Framingham risk score, the Pooled Cohort Equation, and the Reynolds equation, are all based on traditional risk factors. The GRS appears to be primarily influenced by factors other than these conventional risk factors. Nevertheless, two recent studies evaluating the genetic risk score have concluded the advantage of the GRS over conventional risk factors is minor. Both studies genotyped with a microarray having 6 million genetic risk variants. One study [64] genotyped a UK Biobank population of 352,660 and the other, a US population [65] of 7,237. These studies concluded risk stratifying for CAD by the GRS was statistically better than traditional risk factors, but the difference was so small it may not be of clinical relevance. The investigators recognize that even though the GRS is equal to conventional risk factors in a population with a mean age in the 50's it would have an advantage in the younger population over that of conventional risk factors. An accompanying editorial [66] was in agreement with these studies and recommended the GRS not be used currently for clinical management.

It is difficult to reconcile these results with the results from previous studies. The investigators themselves could only suggest that some of the differences might be due to the pretest sample being more appropriately characterized than in previous studies. The population utilized, in one of the studies the UK biobank population which was similar to that used by Inouye et al. and Khera et al. Nevertheless, it is important to recognize that the results of these two less favorable studies still recognize the advantage of the GRS over conventional risk factors to risk stratify young asymptomatic individuals for CAD risk who could benefit from primary prevention.

The GRS is still evolving and has other limitations. It is evident there are many more genetic risk variants predisposing to CAD to be discovered. The data clearly indicate the more genetic variants one has to evaluate, the more accurate the test will be in identifying those at higher risk. Ongoing studies will clearly discover more risk variants for CAD. A more immediate concern at this time is the realization that almost all of these genetic risk variants have been discovered by GWAS performed in individuals primarily of European descent [37] it is important that GWAS be performed to identify risk variants unique to different ethnic groups.

The importance of genetic variants predisposing to CAD is exemplified in the recent study by Wang et al. [67] In this study, the investigators genotyped a sample size of 11,220 South Asians of which originated three different countries. 7,244 were of South Asian origin obtained from the UK Biobank, 491 from Bangladesh, and the remaining 3,485 individuals from India. The populations were genotyped and a GRS was determined using 6.6 million derived by the LDpred computational algorithm, primarily derived from individuals of European descent. The GRS was determined by

genotyping the same 6.6 million microarrays plus an enrichment of 575,778 genetic variants derived from a South Asian population. The results show as, in previous studies of European descent, the risk of CAD in all three South Asian populations is driven by the GRS. Optimizing the GRS for South Asians by imputing risk variants obtained from a South Asian population, there was a 3.22 to 3.91 fold increase in risk for CAD comparing the highest to the lowest quintiles across three independent populations. The microarray containing risk variants from primarily a European population genotyped in the South Asian population showed odd's ratios per standard deviation of 1.58 to 1.66 which is only slightly attenuated from the odd's ratio of 1.72 obtained in the European population [56]. The investigators found the GRS to be relatively independent of traditional risk factors. Several previous studies have made a similar observation, namely, the GRS for CAD is relatively independent of traditional risk factors [52–61].

Future Implications

The GRS has been evaluated in over 1 million individuals and shown to be superior to risk scores based on conventional risk factors predisposing to CAD. In placebo clinical trials, cardiac events in controlled individuals with high genetic risk for CAD were markedly reduced by favorable lifestyle changes and cholesterol-lowering agents. A risk score for CAD based on genetic risk variants has the advantage of being independent of age since these risk variants are randomly assigned at conception and do not change in one's lifetime. One can determine genetic risk for CAD at birth if needed which would transform primary prevention. The GRS will continue to expand to include more variants particularly those specific to ethnic or racial groups. It is our belief the GRS should be incorporated into the CCCPG for further assessment. Some may think it is premature for clinical application, which is understandable. If one employed the GRS into clinical practice and simply provided preventative therapy to the top 10-20% at risk, it would be a paradigm shift in the primary prevention of CAD. We have data from Mendelian Randomization [68,69] studies that lowering plasma cholesterol earlier in life is associated with nearly 3-fold greater benefit from cardiac events than from doing so later. The GRS is inexpensive as is the proven preventative therapy for CAD. Risk stratification of CAD for primary prevention utilizing the GRS in its present form could do much to halt the pandemic of CAD, the number one cause of death in the world.

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