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RESEARCH ARTICLE

Genetic Risk Stratification and the Primary Prevention of Coronary Artery Disease

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ABSTRACT

Coronary artery disease (CAD) is the number cause of death in the world. It is estimated that 50% of Americans will experience a cardiac event in their lifetime. The underlying pathology leading to coronary artery disease and its clinical manifestations, such as angina, myocardial infarction, and sudden death is coronary atherosclerosis. While the disease is not usually manifested clinically until the sixth or seventh decade, the underlying pathology is initiated as early as the second or third decade. Numerous randomized clinical trials have shown cardiac morbidity and mortality can be prevented by lowering the risk of known conventional risk factors for CAD such as decreasing plasma cholesterol or controlling hypertension. Secondary prevention of these conventional risk factors has been very effective; however, primary prevention has been shown to be even more effective. A major barrier to primary prevention is the lack of markers to detect among young asymptomatic individuals those at risk for CAD. The conventional risk factors are often not present until the sixth or seventh decade which could be late for primary prevention. Genetic predisposition accounts for 50% of the risk for CAD. Recently over 200 genetic risk variants predisposing to CAD have been discovered. Based on these variants, one can express the genetic risk for CAD in a single number referred to as the Polygenic Risk Score (PRS). The PRS has been evaluated in over one million individuals and shown that those with high genetic risk have the highest incidence of heart disease and can be reduced by 40-50%, utilizing drugs (statins and PCSK9 inhibitors) or lifestyle changes (favorable diet and increased exercise). The genetic risk for CAD is determined at conception and thus can be predicted anytime from birth onward. The PRS detection of young asymptomatic individuals based on the PRS enables one to implement early primary prevention. Adoption of the PRS to risk stratify for CAD could represent a paradigm shift in the prevention of this pandemic disease.

Introduction

The 1980s ushered in the Golden Era for pursuit of genes responsible for single gene disorders such as hypercholesterolemia and familial hypertrophic cardiomyopathy[1]. These are rare disorders inherited according to Mendelian patterns which are amenable to genetic linkage analysis.[1] The analysis is based on detecting DNA markers that cosegregate with affected individuals more commonly than by chance. It requires pedigrees of two or three generations and requires only one hundred to two hundred DNA markers. This is in mark contrast to common disorders such as coronary artery disease (CAD) which are known to be polygenic in origin and also associated with environmental and acquired factors. The technology to pursue polygenic disorders did not become available until 2005[2] and would require a very different design, mainly that of the Case-Control Association Study(CCAS)[3,4]. The Case-Control Association Study would require thousands of DNA markers spanning the human genome and a large sample size[3–5]. This review will briefly discuss the genetics of CAD and the application of genetic risk variants as a genetic risk score to identify individuals at high risk for CAD who would benefit most from primary prevention.

The Human Genome

The human genome continues to evolve as part of our genetic ability to adapt to new environments. Each individual inherits approximately 60 novel mutations[6,7]. Over 90% of these mutations are in the form of single nucleotide polymorphisms (SNPs). The source of these mutations is copying errors resulting from replication of DNA by the germinal cells with most of the errors coming from the paternal source[8,9]. The DNA is replicated one base at a time and thus, the most likely error is a single nucleotide. It is well recognized that the sequence of the human genome is 99% identical across all humans.[10] The remaining 1% is responsible for the unique attributes distinguishing each individual. It is not surprising that 80-90% of these attributes whether it be the color of your eyes or predisposition to disease are due to single nucleotide polymorphisms (SNPs)[6,11,12]. These SNPs are distributed throughout the human genome averaging one SNP per 300 base pairs.[13] The number of SNPs per individual is relatively constant at 5 million per genome.[10]

Genome-Wide Association Study and the Development of a Genetic Risk Score

In the 80s and 90's, scientific advancements enabled the discovery of the genes responsible for single-gene disorders[1]. Utilizing genetic linkage analysis, the genes and the precise mutations responsible for rare genetic disorders such as familial cardiomyopathies and familial atrial fibrillation were discovered[14]. Polygenic disorders such as CAD posed a greater challenge due to being caused by numerous genes, each contributing only minimally to the phenotypic expression of the diseases[3,4,13]. The phenotype of polygenic disorders does not follow a Mendelian pattern and is not amenable to linkage analysis utilized to pursue single gene disorder[3,13]. Furthermore, these diseases are also impacted by the environment and lifestyle[3]. As a result, it became apparent that a more appropriate study for polygenic disorders would be a Case Controlled Association Study[3,4,13]. The CCAS is based on the following principle of comparing the frequency of a DNA marker in cases to that of controls[13]. A DNA marker that occurs statistically more frequently in cases than controls would indicate the marker is a risk mutation or is in close physical proximity to that of the causative mutation. It was also postulated by Kruglyak that for the CCAS to be most sensitive it would require a DNA marker every 3,000 base pairs[15]. The DNA markers that came close to the ideal were SNPs since they are distributed throughout the genome. Furthermore, HapMap[13,16,17] was making available the precise chromosomal location of millions of SNPs distributed throughout the human genome. Utilizing the CCAS to analyze SNPs distributed throughout the genome lead to the term Genome-Wide Association Study (GWAS) which was first utilized to discover the gene responsible for macular degeneration[18]. The unbiased approach utilizing millions of markers required a statistical correction. If one used a P-value of 0.05 to determine the difference in frequency between cases and controls, it would lead to 50,000 false positives. The consensus[19] was made to utilize a Bonferroni p-value of 10^{-8} which would subsequently be referred to as genome-wide significant. In addition, it was recommended that all SNPs found to be genome-wide significant had to be replicated in an independent population[20]. Two independent groups later discovered the first genetic risk variant, 9p21[21,22]. This variant was confirmed in populations of different cultural ethnicities and races[23]. Furthermore, the increase in risk for CAD was approximately only 25% per copy. 9p21 has a high prevalence being present in approximately 75% of the population, providing support to the hypothesis that polygenic disorders, such as CAD, are the result of variants that occur frequently in human populations and are associated with only a minimal increase in the predisposition to the disease[21,22]. The common frequency and associated

minimal risk for CAD further confirmed the necessity of even larger sample sizes to discover genetic variants that predispose to CAD. Hence, the international consortium -CARDIoGRAM - was founded[24] and later joined by the Coronary Artery Disease consortium (C4D), to become CARDIoGRAMplusC4D[25]. This collaboration along with many other investigators have played a major role in discovering genetic variants that predispose to CAD. These efforts which have been comprehensively reviewed recently has led to the discovery of over 200 genetic risk variants all of which are genome-wide significant and have been replicated in independent populations[23,26,27].

Calculation of the Genetic Risk Score

A major impetus for pursuing the discovery of genetic risk variants for CAD was its potential to risk stratify asymptomatic individuals for early primary prevention of CAD. It has been longed recognized that about 50% of the risk for CAD is genetic[28]. The genetic risk for CAD can be expressed as a single number and may be referred to as the Genetic Risk Score (GRS) or the Polygenic Risk Score (PRS). The PRS has become the more commonly used term since CAD is a polygenic disease. The genetic risk is determined by the number of genetic risk variants predisposing to CAD inherited by that individual. The PRS is the sum of the product of the number of copies of each risk variant times the odds ratio[26,29].

Primary Prevention of Coronary Artery Disease and the Role of a Genetic Risk Score

Coronary artery disease is a preventable disease. Multiple clinical trials designed to lower the risk associated with conventional risk factors such as plasma Low Density Lipoprotein- Cholesterol (LDL-C) have been shown to significantly reduce cardiac events[30]. A meta-analysis of multiple trials designed to decrease the plasma LDL-C resulted in a decreased incidence of cardiac events by 30-40%[31]. A similar decreased incidence of cardiac events was observed with primary prevention. There are barriers with using conventional risk factors (hypertension, HLD) for primary prevention since often they do not reach a clinical threshold until the fifth or sixth decade of life, which is often too late for primary prevention[32]. Primary prevention is more effective when initiated in the early asymptomatic phase. Navar et al. demonstrated that LDL-C increases early in one's lifetime and the risk of CAD doubles with each decade of exposure[33,34]. The risk for Coronary Artery disease based on genetic risk variants can be determined at birth or anytime thereafter. The genetic risk is determined at conception and does not change throughout one's lifetime, thus is not age dependent. The PRS provides the opportunity to risk stratify for CAD in young asymptomatic individuals. This enables those individuals at high genetic risk for CAD to receive early primary prevention. An alternative approach would be to treat everyone with increased plasma LDL-C. Based on current data most individuals in the Western World, male or female, have markedly increased plasma LDL-C by the age of 40[35,36]. Epidemiological observations have noted that approximately 50% of the population will experience a cardiac event in their lifetime[37]. As a result, treating everyone while effective would be unnecessary in approximately 50%. It would be more desirable to treat only the 50% at risk.

Evaluation of the Polygenic Risk Score as a Means to Stratify for Coronary Artery Disease Risk

Initial studies evaluating the PRS as a means to risk stratify for CAD were retrospective. These studies utilized blood samples collected during clinical trials designed to assess the effect of cholesterol lowering agents on cardiac events. One of the first studies by Mega et al[38] involved a sample size of 48,421 individuals representing four clinical studies assessing statin therapy as primary or secondary prevention. These trials were: Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER), Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), Cholesterol and Recurrent Events (CARE), and Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction (PROVE-IT-TIMI). Individuals with the highest PRS had the highest risk for CAD and received the most benefit from statin therapy. This observation was true for both primary and secondary prevention. A second large clinical trial, assessing the effect of statin therapy on cardiac events, the West of Scotland Coronary Prevention Study (WOSCOPS), was also genotyped with 57 genetic risk variants for CAD[39]. Risk stratification based on the PRS showed individuals with the highest PRS had 44% reduction of cardiac events versus only 24% reduction in the intermediate and low risk groups. Utilizing the PRS as a means to risk stratify was more potent than conventional risk factors as evidenced by the observation; the number of individuals required to be treated in the high genetic risk group to prevent one cardiac event was 13 versus 38 in the low PRS group. This is in contrast to over one hundred individuals being the number required to be treated to prevent one cardiac event

based on CAD risk stratification utilizing conventional risk factors.

The PRS was used to risk stratify for CAD in two recent randomized clinical trials assessing the effect of lowering plasma LDL-C with PCSK-9 inhibitors on cardiac events. The FOURIER[40] (Further Cardiovascular Outcomes Research with PCSK9 inhibition in subjects with elevated risk) and The ODYSSEY[41] (Evaluation of Cardiovascular outcomes after an acute coronary syndrome during treatment with Alirocumab). Evolocumab, a PCSK-9 inhibitor, was evaluated in the FOURIER trial with a sample size of 27,564 patients. A strong correlation between individuals with intermediate and high PRS and cardiac events was observed with odds ratio of 1.23 and 1.65 respectively. Individuals receiving Evolocumab therapy had a 13% reduction in risk in the group stratified by conventional risk factors without high PRS and a 31% reduction in the high PRS groups with or without conventional risk factors. Individuals with the highest PRS had the most benefit from Evolocumab and were independent of conventional risk factors. A total of 11,953 individuals were enrolled in the ODYSSEY trial. Individuals with the highest PRS had the highest risk for CAD. Alirocumab treatment in the group with the highest PRS was associated with a 37% reduction in cardiac events vs. a 13% reduction in the group with the lowest PRS. These results confirm that the PRS is effective in identifying those at greatest risk who will benefit most from lowering plasma cholesterol whether it is achieved by statins or PCSK9 inhibitors.

Another approach to evaluate the PRS was to genotype individuals already collected and phenotyped in various biobanks. Abraham et al[42] evaluated five prospective cohorts in a total population of 16,082 subjects. In this study, genotyping was performed with a microarray containing 49,310 variants. It was noted that those with the highest PRS were strongly associated with coronary artery disease and other cardiac events, independent of conventional risk factors. In a study by Khera et al[43] a microarray was utilized that contained over 6.6 million SNPs. In this population of 288,978 individuals, 8% had a 3-fold increased risk and 0.5% had a 5-fold increased risk for CAD. It was also observed that most individuals would not have been identified as high risk had they been stratified by traditional risk factors since only 20% had hypercholesterolemia, 28% hypertension, and 35% a family history. A trial by Inouye et al[44] used 1.7 million variants on a microarray to genotype just over 500,000 individuals within the UK biobank. Individuals in the top 20% of the polygenic risk score had a 4-fold increased risk for CAD.

Genetic Risk for Coronary Artery Disease is Reduced by Favorable Lifestyles

In an analysis of 55,685 individuals, Khera et al[45] calculated the genetic risk for CAD with the use of 50 genetic risk variants. Upon stratifying for genetic risk, it was shown that those with the highest PRS, the top 20%, had more than a 90% increase in the probability of a cardiac event compared to individuals with a low PRS. In the group with the highest genetic risk score, a favorable lifestyle was associated with a 40% lower risk for cardiac events compared to those with an unfavorable lifestyle. Utilizing a population from the UK biobank, Tikkanen et al.[46] genotyped 468,096 individuals and risk stratified for CAD based on the PRS. The endpoint of the study was to determine how exercise (hand grip for 3-seconds and oxygen consumption test on a stationary bike) influences one's genetic risk for CAD. Individuals with the high genetic risk score who exercised had a 49% reduction in risk for CAD compared to those who were at high genetic risk and did not exercise.

Limitations of Polygenic Risk Score

Most studies evaluating the PRS as a means to risk stratify for CAD have shown the PRS to have an advantage over conventional risk factors. However, two studies[47,48] noted only a minor advantage of the PRS over conventional risk factors. One study[47] utilizing the UK biobank genotyped 352,660 individuals while the other study[48] genotyped a population of 7,237 individuals in the United States. Both of these studies utilized a microarray of 6 million genetic risk variants and found the PRS had only minimal statistical improvement compared to traditional risk factors. The investigators also noted that while the statistical difference was minimal in a population with a mean age in their 50s it would be advantageous in a younger population. The population appeared to be similar to that of previous investigators, however, some differences may have resulted from the pretest sample being differently characterized than in previous studies.

The PRS is based on genetic risk variants discovered in a population that is 77% of European descent[49]. This may be a limitation since the evolutionary pressure for improved survival may induce genetic adaptations associated with SNPs unique to populations in Africa or Asia as indicated in a recent review[50]. In a study examining the genetic risk scores for CAD in different ethnic populations, Iribarren et al[51] genotyped 11,242 individuals consisting of 4804 East Asian, 4349 Latino, and 2089 African. Using 51 genetic risk variants that were derived from individuals of European descent[49] a genetic risk score was calculated for

each individual. The genetic risk scores for these populations were compared to the 10-year risk for CAD determined from the Framingham risk score. This resulted in a 10% reclassification of individuals within the intermediate-risk group. While the PRS showed an improvement over conventional risk factors, the investigators did not recommend utilizing the PRS in clinical practice. A study by Dikilitas et al[52] genotyped a population consisting of 45,645 participants of European ancestry, 7597 of African ancestry, and 2493 with a Hispanic heritage. The hazard ratio standard deviation was 1.53 in Europeans, 1.53 in Hispanics, and 1.27 in African Americans. Results showed a strong association between the PRS and CAD in all three populations, however, this association was less strong in African Americans.

Future Application of Polygenic Risk Score

The use of the PRS to risk stratify CAD is in keeping with the desire to implement precision medicine. It is estimated that about 50% of the population will experience a cardiac event in their lifetime[37]. Utilizing the PRS would enable one to treat those at highest risk and more likely to benefit from therapy as opposed to treating everyone knowing only half of them will not benefit from therapy. The PRS can be determined from blood, saliva, or tissue and is currently costing between two to three hundred dollars per test. This sample can be collected anywhere in the world and transported over great distances since the DNA is stable for months at room temperature. The sample for DNA does not require fasting, is not affected by drugs, and does not change with time. More wide spread use of PRS in clinical practice would be expected to decrease the cost significantly. The test for PRS only needs to be assessed once in a lifetime since one's genetic risk for CAD remains the same. The discovery of genetic risk variants for CAD and their incorporation into a genetic risk score could provide a paradigm shift in the primary prevention of CAD.

Conclusion

Coronary artery disease is the most common cause of death in the world[37]. Secondary prevention has had a major effect on both cardiac morbidity and mortality and is in large part responsible for the 50% reduction in cardiac mortality observed in the U.S.[53,54]. Primary prevention is more effective and probably necessary to significantly decrease the pandemic prevalence of this disease. Currently there are no biomarkers to detect risk for CAD in young asymptomatic individuals. The current conventional risk factors used to identify individuals at risk for CAD, with the exception of plasma cholesterol LDL-C, are often not present until the sixth or seventh decade of life[32]. This is late for primary prevention since coronary atherosclerosis is initiated early in life even in the second and third decade of life[55,56]. Genetic risk accounts for about 50% of the predisposition for CAD. The genetic risk for CAD summarized in the PRS has been evaluated in over one million individuals. Those individuals with a high genetic risk score are associated with increased risk of cardiac events. Furthermore, the genetic risk was shown to be significantly decreased with the use of drugs (statins and PCSK9 inhibitors) and changes in lifestyle (favorable diet and increased physical activity). The genetic risk for CAD is determined at conception and thus, enables one to identify individuals who are young, asymptomatic, and at risk for CAD. The genetic risk can be determined at birth or anytime thereafter since it does not change throughout one's lifetime. It is hoped that as further data and experience utilizing the PRS is acquired it will be considered for incorporation into the Clinical Cardiology Guidelines.

Declarations

Author's contributions

Dr. Robert Roberts contributed to the conception and design of the study, and Esperanza Acuna and Jacques Fair contributed to literature search and writing.

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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