Review Article

Genetics and Coronary Artery Disease: Present and Future

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During the last decade we have witnessed remarkable development in the field of genetics. This was due to great technological progress in the laboratory, through mapping and analysing the human genetic substrate and its association with diseases, and a better understanding of molecular and genetic mechanisms, allowing a more detailed comprehension of the role of genetics and environment in determining the risk of disease. It was inevitable that geneticists would focus their attention on ischemic cardiovascular disease, since this and its underlying pathological processes (atherosclerosis and thrombosis) are the leading cause of morbidity and mortality in the world. Coronary heart disease (CHD) is the leading cause of mortality worldwide, in both high- and low-income countries, according to the latest data of the World Health Organization. In a report updated in 2011, the death rate from ischemic heart disease worldwide amounted to 12.8%.[1] In the USA, according to a recent report of the American Heart Association, there was a reduction of cardiovascular mortality by 32.7% in the decade 1999-2009. But despite this decrease, deaths from cardiovascular disease in 2009 amounted to 32.3% of all deaths. For coronary disease *per se*, a coronary event occurs every 34 seconds and a death every minute, while one out of every six deaths is attributed to CHD.[2]

Evidence of genetic predisposition to CHD

A genetic predisposition to CHD has been documented for many years through numerous studies, since the CHD phenotype often exhibits a remarkably inheritable pattern. Family history is the most direct evidence for the estimation of genetic susceptibility to CHD. The Framingham study reported that a family history of CHD or stroke is associated with CHD independently of other cardiovascular risk factors, showing a 2.4-fold increase in risk for men and a 2.2-fold increase in risk for women.[3] Other studies reported that CHD in first-degree relatives raised the risk by 2-3 times more.[4,5] A study in Danish twins revealed a higher incidence of CHD and deaths among monozygotic (44%) rather than dizygotic (14%) twins.[6] Another study in twins highlighted the strong genetic basis of CHD and its main complication, myocardial infarction, in addition to other modifiable risk factors.[7] Also, in a study of 20,966 Swedish twins over 36 years, the heritability of fatal incidents was estimated; the risk amounted to 0.57 for men and 0.38 for women.[8] In the INTERHEART study, a family history of CHD raised the risk to 1.55 after adjustment for age, sex, smoking, and geographic region, while after correction for nine known factors it was slightly reduced to 1.45.[9] Finally, the PROCAM study al-

so found family history of myocardial infarction to be an independent risk factor. After adjustment for age, systolic blood pressure, cholesterol, smoking, triglycerides, and lipoprotein(a), the risk was 1.67.[10]

Brief history of genetic engineering

Genetic association studies with cardiovascular disease using DNA markers have been carried out over the last 30 years. The evolution of technology led to a burst of studies, especially during the last five years. In 1970, it was impossible to study human DNA directly, but only via major cytogenetic abnormalities visible microscopically. Nevertheless, many serological markers that were often studied, such as ABO blood groups and types of leukocyte antigens checked for their connection to disease, in fact reflected the level of genetic alterations of DNA and represented it in a way.[11]

The transition from protein representatives to DNA polymorphisms marked the beginning of the genome study. In 1980, the construction of a genetic linkage map (Southern blot analysis) was attempted using restriction fragment length polymorphisms of DNA (RFLPs). This was printed on radiology film and later converted into gene scores to check for connections with disease.[12] This method assisted in the mapping of rare diseases on a genetic basis. During the 1980s, Southern blot analysis was succeeded by the revolutionary technique of DNA polymerase chain reaction (PCR), which allowed the replication of small targeted DNA sequences billions of times in a test tube. The arrival of PCR facilitated the analysis of polymorphisms and mutations, reduced the time and cost of genetic studies, and still remains a fundamental laboratory technique.[13]

In 2000, technological progress provided scientists with the method of the DNA microarray, also called the "single nucleotide polymorphism (SNP) chip", which allowed the simultaneous examination of millions of polymorphisms throughout the genome. Multinational studies, such as the International Hap Map Project, recorded over two million polymorphisms in multinational populations.[14] In 2005, the first computerized DNA microarray appeared, and the technology has been constantly developing ever since. This, combined with high penetration platforms, reached the goal of analyzing the DNA samples of hundreds of thousands of patients, as well as healthy individuals.[15]

CHD genetics

In the last 20 years, with developing improvements in genotyping, several methods have been used to link the influence of a genetic polymorphism with a phenotypic effect. Considering the genetic basis of CHD, it is necessary to distinguish between the rare single-gene causes and the common type, also called multigenic, whose pathogenesis involves various factors. Multigenic CHD is of significant interest, given the important role it plays in public health. Nevertheless, several elements in our understanding of the common types of genetic variation were derived from research into single-gene diseases characterized by a low incidence of genetic changes and considerable phenotypic effect. This last category includes examples such as hypertrophic or dilated cardiomyopathy, channelopathies (Brugada syndrome and long-QT syndrome), as well as familial dyslipidemias.[16] Indeed, research into single-gene CHD preceded interest in the multigene type by some decades. With the passage of time, two main methods developed and are listed below.

Candidate gene method

The candidate gene method was the first and simplest used. It was based on the *a priori* assumption that a gene is involved in the mechanisms and plays a role in determining an intermediate phenotype, such as in molecular and cellular function through proteins, or a clinically evident result. In this gene, genetic changes occur that affect the abovementioned operations and eventually result in a high risk of CHD. Early studies focused on single polymorphisms and single genes for research and identification of functional changes in the associated proteins. Frequencies of a small number of selected genetic variants were compared between groups of patients (cases) and healthy subjects (controls) (Figure 1).[17]

The first candidate gene study in cardiovascular disease was published in 1992. It made clear that homozygotes who carry a deletion in the gene region for angiotensin-converting enzyme, which had already been linked to higher levels of circulating enzyme, are at higher risk of myocardial infarction. This association was much more evident in people who had no other documented (non-genetic) risk factors and supported the significance of studying genetic factors.[18] Subsequently, studies were conducted that included genotyping research on more than a single polymorphism in the same gene. The first such study

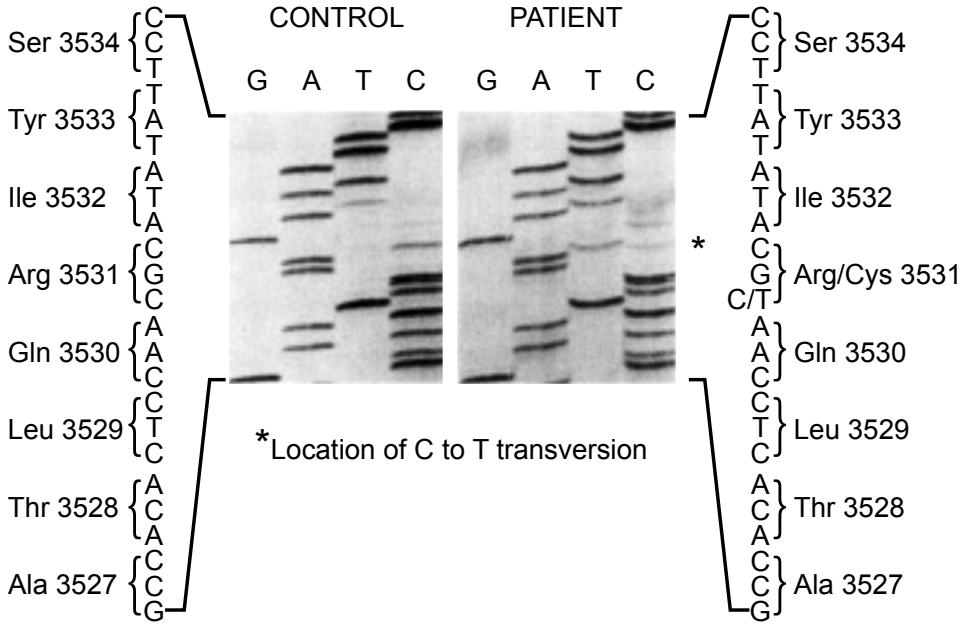

Figure 1. DNA sequencing gel showing the single nucleotide change leading to the Arg3531`Cys substitution. An asterisk indicates the T for C substitution in codon 3531. Single-stranded DNA prepared by asymmetric PCR (United States Biochemical Corp., Cleveland, OH).

published addressed two polymorphisms in distant regions of the gene encoding the coagulation factor VII. The results demonstrated that individuals carrying these polymorphisms are at higher risk of myocardial infarction, perhaps through thrombotic activity of the elevated levels of factor VII and its antigens that are present.[19] In this case, as previously, there are some intermediate endpoints that act as a link between the genotype and the disease. A brilliant example of a single gene disease predisposing to early coronary disease is familial hypercholesterolemia. It is characterized by an excessive plasma concentration of low-density lipoprotein (LDL) cholesterol and early-onset myocardial infarction. It is caused by mutations in genes encoding proteins that regulate LDL plasma levels.[20] People suffering from familial hypercholesterolemia exhibit eightfold higher mortality compared to the general population between the ages of 20-59 years.[21] This is mainly due to CHD, and the risk can be reduced to the general population levels by early intensive statin therapy.[22] Not only patients, but also their relatives should be monitored. If they also carry relevant mutations, various recommendations should be made, including lifestyle changes and an early initiation of statin treatment.

The candidate gene method has several limitations. One of them was monitoring one or a few polymorphisms in a given gene, while many others, seated even thousands of base pairs away from the gene, could also potentially affect the observed effect. Therefore, given the enormous variability of the human genome, genetic variables acting strongly in the determination of the structure and function of proteins could escape linkage to the disease. Moreover, the limited numbers of people monitored in these studies, as well as the heterogeneity of the samples, exhibited a number of false positives, leading to reduced statistical power and questionable publications. Since 1980, thousands of studies using the candidate gene method have been published without consistent findings.[23] Many of the early correlations were neither repeated nor confirmed by other researchers. However, genetic variations of a limited number of genes, acting mainly on LDL cholesterol, were repeatedly and reliably associated with the risk of CHD.[24] In any case, considering an overall review of the results, the limited success of the candidate gene method was not surprising, though it triggered further research on DNA.

Genome-wide association study

A genome-wide association study (GWAS) detects any genetic variants throughout the whole human ge-

nome, trying to identify genetic correlations with observed traits or diseases. The method does not require a preselected gene, but investigates a huge number of polymorphisms for any possible association between some of them and various phenotypes. A genotyping record is achieved for hundreds of thousands of people in a fast and efficient process. Lately, it is widely used in studies on variations or genes of many complex diseases, including CHD.

GWAS uses methods of standard design. People who carry the disease are compared to non-carriers (case-control studies). The frequency or the intensity of appearance of a genetic polymorphism (biallelic gene) is calculated for both carriers and controls. A statistically higher incidence among people who carry the disease marks it as a risk allele. The estimation of this risk is based on calculation of the odds ratio (Figure 2).[25] The second type of GWAS design includes a quantitative correlation of a trait, such as cholesterol or blood pressure, which is constantly monitored in a sample of the general population. All individuals are divided into genetic categories according to their allele (homozygotes or heterozygotes). The mean value of the quantitive characteristic is statistically compared for each and every genetically defined group (linearly or with correlation coefficient b). The specific polymorphism is the independent variable, while the dependent variable is the trait. The correlation between a genotypic polymorphism and the measurement of the quantitative trait provides an assessment of the effect magnitude.[11] When specifying the association of a polymorphism with a disease or a disease trait, the position of this variable within the genome is studied as well as the proximal genomic environment, which is described as the place (locus).

The first GWAS studies in CHD were independently and simultaneously published in 2007. All

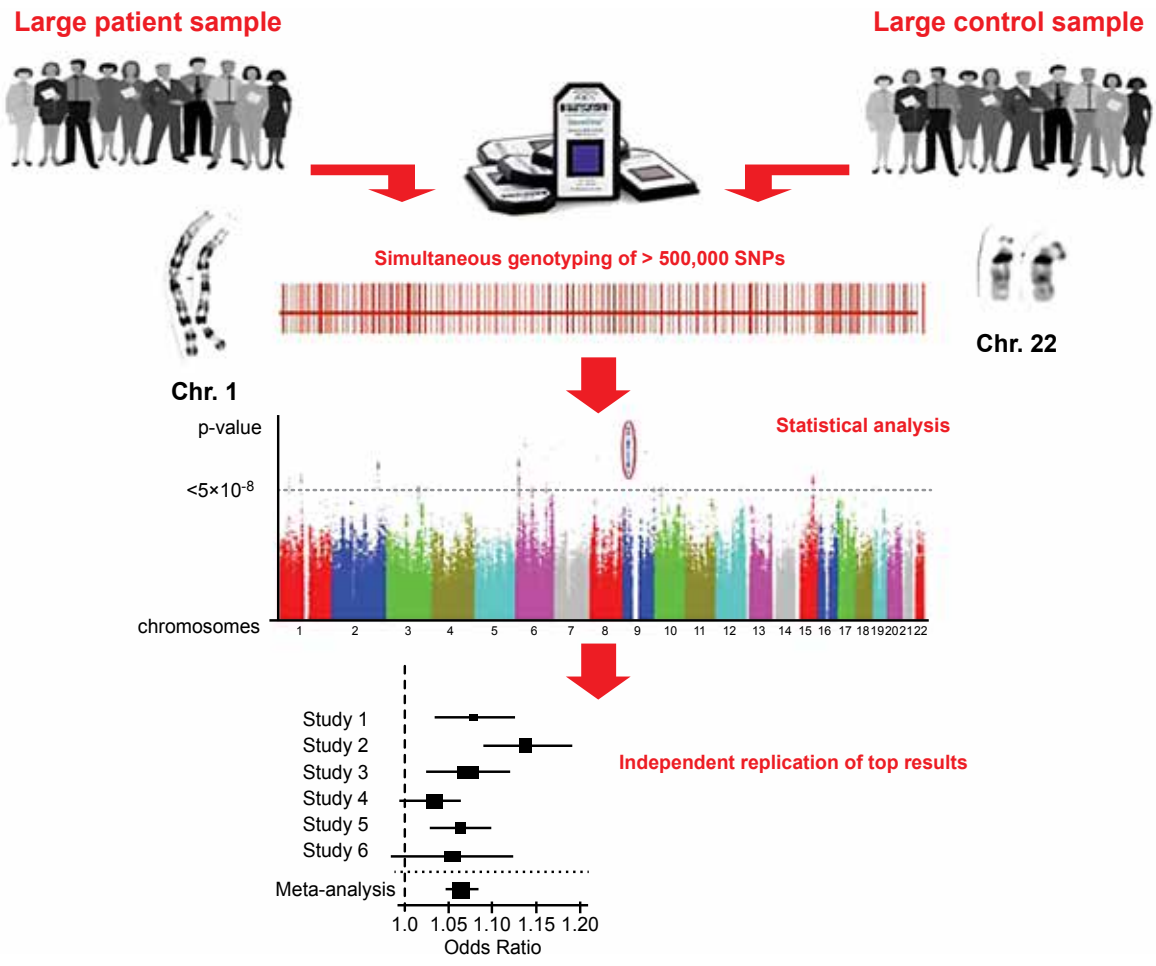

Figure 2. Principle of genome-wide association studies. SNP – single nucleotide polymorphism.

Glossary

Gene: A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism.
Single nucleotide polymorphism: A genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered. SNPs are usually considered to be point mutations that have been evolutionarily successful enough to recur in a significant proportion of the population of a species.
Genetic Variants: Variants of genomes between members of species, or between groups of species.
Allele: One member of a pair or series of genes that occupy a specific position on a specific chromosome.
Locus: The position of a gene on a chromosome.
Mutation: A change in the nucleotide sequence of the genome.

these studies determined only a few polymorphisms linked to CHD, all located in the 9p21.3 genome region.[26-28] In a short time several groups worldwide confirmed the results regarding the 9p21 region, while 11 new high-risk genetic variants for CHD were also mapped. These studies made clear that each of these genetic variables could lead to a moderate or minimal risk effect. Besides, the sample size to reliably detect most of these variables should have exceeded that initially planned, according to statisticians.[29] Responding to this observation, researchers with previously published successful results combined their sources and data into international formations. This partnership was the greatest collaboration in cardiology, since CARDIoGRAM[30] focused on a sample of 86,995 people (22,233 cases and 64,762 controls) and a validation sample of 56,682 people, while the IBC50K CAD Consortium[31] included 57,594 subjects (15,596 cases and 34,992 controls) and a validation sample of 57,594 people. Thereby, new genetic risk variables were detected and others were confirmed, while in a short period of 5 years a total of 36 genetic risk variables associated with a high risk for CHD were identified. Seven of them act through lipoproteins, two through blood pressure, and one on myocardial infarction, while 23 genetic loci act independently of all known risk factors.[32] These variables are more common than expected, since more than half appear in >50% of the population and ten of them appear in >75% of the population. The majority of genetic variables exhibit minimal risk, ranging from 6% to 17%, whereas >66% of them express their risk independently of other known risk factors, such as cholesterol. The fact that 23 of the 36 risk variables for CHD do not act through any of the known risk factors indicates unknown mechanisms that need to be elucidated.[33] In another review of 35 common variables that affect CHD, the risk for disease did not exceed 13% of the total heritability of CHD.[34] All these findings suggest that there are still many things to be discovered. However, preventive treatment of CHD is unlikely to be achieved without complete clarification, consideration and prevention of the genetic predisposing factors. The advantage of DNA risk variables versus blood biomarkers is that they are not altered during an individual's lifetime; they are not affected by age, nor by meals, medications or environmental stimuli. Therefore, the DNA genotype at birth is stable and can be used for the entire lifetime.

All GWAS studies for CHD used the phenotype of either ≥50% damage to one or more coronary vessels, identified by angiography, or proven myocardial infarction. However, some genes predispose people particularly to the process of atherosclerosis and others to myocardial infarction. Regarding the genetic variables, the extensively studied 9p21 acts on the vascular wall and contributes to the pathogenesis of atherosclerosis, without being involved in the acceleration and occurrence of myocardial infarction.[34-36] Only 2 of 11 polymorphisms associated with myocardial infarction affect well-known traditional risk factors and involve LDL cholesterol and lipoprotein(a).[37] A recent study proved that a risk variable for myocardial infarction is associated with the DNA position responsible for ABO blood group. GWAS studies consistently confirm that genes defining blood groups A and B are associated with myocardial infarction, having no connection with coronary atherosclerosis.[38] This raises a concern as to whether, in the future, individuals with blood groups A, B, or AB should receive antiplatelet therapy preventively, especially those who suffer from CHD or have undergone cardiac surgery. It is clear that clinical studies are required to assess the scale of the problem and the possibility of reversing the effects through the application of this particular treatment.

Clinical evaluation and utility

The clinical evaluation and utility of the outcomes resulting from genetic studies in CHD are still difficult and fall far from the desired goal. The main reason is the contradiction between the high significance and correlation of genetic variables with CHD in meta-analyses of large samples of individuals and

the quite low actual risk effect of each variable, as measured using the odds ratio. A typical example is the most extensively studied locus, 9p21.3, which is statistically important for large populations but displays an almost negligible risk effect on the individual patient (homozygote allele for 9p21.3 exhibits about 1.5-fold risk for CHD). Furthermore, calculations including the 9p21.3 genotype do not alter the risk degree of common algorithms.[39] However, using the 9p21.3 risk locus significantly improves the prediction of mortality for patients after coronary artery bypass surgery.[40] In contrast, other studies have shown that a combination of multiple genotypes in a single sum of genetic risk scores failed to enhance the traditionally known risk factors.[41-43,46] Additionally, meta-analyses of the effectiveness of the genetic risk score associated with cardiovascular disease gave controversial findings.[44,45]

The concept of genetic risk score aims to identify the additional risk for individuals that has not been revealed by the algorithms of traditional risk factors. At present, all researchers take for granted that any heredity calculation involves multiple factors not yet discovered, while even the way of action of those already known remains obscure. The answer to this issue requires further implementation of large-scale genomic research and meta-analyses, as well as targeted polymorphisms with high-fidelity mapping to specific genetic loci, and is still a challenge for all researchers-geneticists.

Future perspectives

GWAS studies had considerable interest in identifying new genetic loci for CHD and myocardial infarction, whereas the responsible genes provided new insights into the genetic architecture of these diseases. Although these studies highlighted powerful markers and offered immediate improvement in the determination of risk for patients with CHD, the original expectations have now come to a halt. Regarding the findings so far, there are limitations based on the small percentage of disease risk, the unknown mechanisms of most variables, the possible interactions among them, and the discovery of new ones. These clearly give the impression that there are many missing parts to complete the puzzle of heredity. After more than 5 years of high-profile cellular and molecular experiments, the mechanism through which the 9p21.3 locus increases the risk of CHD remains undetermined.[46,47] Further clarification and crucial information is expected as a result of next-generation DNA sequencing techniques.[48,49] In this kind of genome sequencing, platforms from either the whole genome or selected regions of it are cut into small fragments, thus providing the potential for several different readings. This new strategy calls for the development of new bioinformatics tools to enable analyses of the large amount of resulting data. Nowadays various bioinformatics programs for different sequencing platforms are available.

Generally the assessment of genetics in CHD is still a tough, yet highly promising conundrum. Constantly increasing value through research and development will ultimately allow routine genetic screening, aiming at an attempt to reduce CHD risk using pharmaceutical or other approaches.

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