

Genetic Predisposition to Cardiovascular Diseases

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KEY WORDS Cardiovascular risk factors; coronary heart disease; genetic predisposition

ABSTRACT Extensive clinical and statistical studies have identified several factors that increase the risk of heart disease, heart attack and stroke. Major risk factors are those that are associated with a significant increase in the risk of heart and blood vessel disease (CVD). Contributing risk factors are those associated with increased risk of cardiovascular disease, but their significance and prevalence haven't yet been precisely determined. A family history of cardiovascular disease is an important factor in the evaluation of a given individual's cardiovascular risk. Behavioral modes common to members of the same family may increase predisposition to cardiovascular disease at an early age and thereby simulate a genetic risk factor. However, a genetic component of cardiovascular risk has been demonstrated, mainly thanks to twin studies. A new generation of genetic risk factors has become apparent in the past decade. Prominent novel genetic factors for CVD, not related to lipids, include high levels of homocysteine, low activity of paraoxonase, and elevated plasma fibrinogen levels.

INTRODUCTION

Cardiovascular diseases (CVD) occupy the number one position in the morbidity and mortality statistics in most industrialized countries of the world (Tunstall-Pedoe et al. 1995). The underlying cause of most CVD is atherosclerosis, a disease characterized by accumulation of lipids in the intima of large- and medium-sized arteries. The terms "coronary heart disease" (CHD), "ischaemic heart disease" (IHD) and "coronary artery disease" (CAD) are more or less synonymous. The most common form of heart disease is CHD. It can lead to chest pain, called angina, and heart attacks. CHD occurs when the coronary arteries become narrowed or clogged and cannot supply enough oxygen-rich

blood to the heart. Atherosclerosis is characterized by the narrowing of the lumen and hardening of the arterial walls. It is a condition present in many common heart and circulatory diseases, as it effects normal blood pressure, resistance and blood flow. The artery may take decades to develop a localized atheroma, leading to the narrowing and hardening of the artery. A partially blocked vessel however, can become completely occluded within minutes. The lipids that are contained in streaks and plaques are derived from circulating lipoproteins.

The major clinical manifestations of CHD consist of congestive heart failure, conduction defect, arrhythmia, angina pectoris, and myocardial infarction. Laboratory evidence and findings from pathologic studies suggest that chronic inflammation plays an important part in the atherosclerotic process. People are more likely to die from heart disease than cancer, stroke, lung diseases or accidents. The lifetime risk for developing CHD has been estimated to be quite high: one out of every two men and one out of every three women aged 40 and older will develop CHD. At age 70, the risk is still high: one out of every three men and one out of every four women will develop CHD in their remaining years of life. The lifetime risk estimate for CHD is an average value for the general population, but individuals may have higher or lower absolute lifetime risks depending on whether or not they smoke, have high blood pressure, high blood cholesterol, or diabetes, and are sedentary or overweight.

In the first half of this century, cardiovascular diseases were more prevalent in individuals belonging to the richest social class. Later on, their prevalence became higher in individuals belonging to the lowest socio-economic level. In developed countries, this phenomenon is common and independent of traditional risk factors. Eating habits, living

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condition at birth and during childhood, the genetic susceptibility of the population, self-perceived health as a consequence of the social hierarchy are some of the factors which may explain the independent role of socio-economic status on cardiovascular diseases development.

The dramatic developments in identifying genetic risk factors in the last decade have placed genomics at the forefront of life sciences. The discovery of a large number of disease-related genes demonstrates this. The increasing development of molecular genetics, the progress of the Human Genome Project, and the widespread application of its new methods and molecular techniques provide a new perception of coronary heart disease and a better recognition of genetic markers (mutations and polymorphism) related to traditional or new cardiovascular risk factors (Kraus 2000). An example of this has been the major effort made over the last years to evaluate and establish the genetic molecular mechanisms that are the basis of the synthesis of apolipoproteins, lipoprotein processing enzymes and lipoprotein receptors. These are some of the subjects discussed in this review in which the role of polymorphic alleles and isoforms of lipoproteins and other enzyme proteins involved in lipid peroxidation and coagulation in cardiovascular risk and coronary heart disease is stressed.

RISK FACTORS FOR CARDIOVASCULAR DISEASES

Extensive clinical and statistical studies have identified several factors that increase the risk of heart disease, heart attack and stroke (Wood 2001). Major risk factors are those that medical research has shown to be definitely associated with a significant increase in the risk of heart and blood vessel disease (referred here as cardiovascular diseases, CVD). Contributing risk factors are those associated with increased risk of cardiovascular disease, but their significance and prevalence haven't yet been precisely determined. A family history of cardiovascular disease is an important factor in the evaluation of a given individual's cardiovascular risk. The determination of this

factor may be congenital or acquired (Sankaranarayanan et al. 1999; Walter and Zeiher 2000).

Behavioral modes common to members of the same family may increase predisposition to cardiovascular disease at an early age and thereby simulate a genetic risk factor. However, a genetic component of cardiovascular risk has been demonstrated, mainly thanks to twin studies. It is a multigenic factor. The family history of cardiovascular disease covers a large number of common mutations or polymorphisms, the detection of which has been greatly facilitated by the explosion of modern techniques of molecular biology over the last few years. The dissection of these factors of genetic predisposition may help in the understanding of the risk of cardiovascular disease and the interaction between genetic and environmental factors (Hayman 2000; Winkelmann et al. 2000).

Several risk factors for coronary heart disease, which cause heart attack, have been identified (Table 1). Some of them can be changed, treated or modified, and some cannot. But the more risk factors a person has, the greater the chance that he or she will develop heart disease.

High Blood Cholesterol and Tryglyceride Levels

Cholesterol and triglycerides are the major

Table 1: Major risk factors for cardiovascular disorders

Modifiable Risk Factors:

- High plasma total blood cholesterol and triglycerides
- High LDL cholesterol
- Low HDL cholesterol
- Elevated blood pressure
- Physical inactivity
- Overweight/obesity
- Hyperglycemia/diabetes
- Thrombogenic factors (coagulation and fibrinolysis)
- Metabolic factors (homocysteinemia)
- Inflammatory processes
- Stress
- Diet and lifestyle (alcohol and fat intake etc.)

Non-modifiable Risk Factors:

- Gender
 - Age
 - Heredity
1. Family/personal history of CHD
 2. Hyperlipidemia/dyslipidemia
 3. Functional polymorphisms in apolipoproteins/ paraoxonase/MTHFR/angiotensin-converting enzyme
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fats and oils found in the blood. Cholesterol is found in animal foods and produced by the human liver. Triglycerides are dietary and body fats. Our livers produce triglycerides from an excess of calories. These triglycerides are transported in the blood and stored as body fat. Cholesterol and triglycerides are carried through the blood by lipoproteins “LDL” and “HDL”. LDL is the low-density lipoprotein, the major carrier of cholesterol in the blood. High blood cholesterol often means high LDL cholesterol. It is often called “bad cholesterol.” HDL is the high-density lipoprotein that carries cholesterol away from the blood vessels and halts fatty build-up on blood vessels and their walls. HDL is often referred to as “good cholesterol.” Experts agree that high LDL cholesterol and low HDL cholesterol are independent risk factors for CVD (da Silva 1999). High triglycerides are not a proven direct cause of CVD. However, high triglycerides are often associated with obesity, high blood pressure, diabetes and low HDL cholesterol. Some of the causes of high cholesterol and high triglycerides are:

- Genetic, lifestyle, and health factors
- Diets high in total fat, saturated fat and dietary cholesterol
- Medical conditions such as hypertension, obesity, diabetes and kidney disease
- Sedentary lifestyle, alcohol consumption and certain medications

Elevated Blood Pressure

High blood pressure, or hypertension, is defined in an adult as a blood pressure greater than or equal to 140 mm Hg systolic pressure or greater than or equal to 90 mm Hg diastolic pressure. High blood pressure directly increases the risk of coronary heart disease (which leads to heart attack) and stroke (or brain attack), especially along with other risk factors. High blood pressure can occur in children or adults, but is particularly prevalent in middle-aged and elderly people, obese people, heavy drinkers and women who are taking oral contraceptives. Individuals with diabetes mellitus, gout or kidney disease have a higher frequency of hypertension.

Physical Inactivity

Lack of physical activity is a risk factor for coronary heart disease. Regular, moderate-to-vigorous exercise plays a significant role in preventing heart and blood vessel disease. Even moderate-intensity physical activities are beneficial if done regularly and long term. More vigorous activities are associated with more benefits. Exercise can help control blood cholesterol, diabetes and obesity as well as help to lower blood pressure in some people.

Obesity and Overweight

People who have excess body fat are more likely to develop heart disease and stroke even if they have no other risk factors. Obesity is unhealthy because excess weight increases the strain on the heart. It is directly linked with coronary heart disease because it influences blood pressure, blood cholesterol and triglyceride levels, and makes diabetes more likely to develop. Many obese and overweight people are not able to change their condition.

Cigarette and Tobacco Smoking

Smokers’ risk of heart attack is more than twice that of non-smokers. Cigarette smoking is the biggest risk factor for sudden cardiac death: smokers have two to four times the risk of non-smokers. Available evidence also indicates that chronic exposure to environmental tobacco smoke (second-hand smoke, passive smoking) may increase the risk of heart disease.

Hyperglycemia/Diabetes Mellitus

Diabetes seriously increases the risk of developing cardiovascular disease. Even when glucose levels are under control, diabetes seriously increases the risk of heart disease and stroke. More than 80 percent of people with diabetes die of some form of heart or blood vessel disease.

Increasing Age

About four out of five people who die of

coronary heart disease are age 65 or older. At older ages, women who have heart attacks are twice as likely as men are to die from them within a few weeks.

Gender

Men have a greater risk of heart attack than women, and they have attacks earlier in life. Even after menopause, when women's death rate from heart disease increases, it is not as great as that of men's.

Heredity

Familial aggregation of CHD has been well recognized (Higgins 2000; Pereira et al. 2000; Friedlander et al. 2001). Children of parents with heart disease are more likely to develop it themselves. African Americans have more severe hypertension than whites. Consequently, their risk of heart disease is greater. Twin studies have further supported the notion that CHD risk is under genetic influence (Marenberg et al. 1994).

OTHER FACTORS CONTRIBUTING TOWARD INCREASED RISK OF CHD

Individual response to stress may be a contributing factor. Some scientists have noted a relationship between coronary heart disease risk and stress in a person's life, their health behaviors and socio-economic status. These factors may affect established risk factors. For example, people under stress may overeat, start smoking or smoke more than they otherwise would. A family history of high blood pressure, heart disease or stroke also increases the risk of stroke.

TRADITIONAL GENETIC RISK FACTORS

Several investigations have been carried out to identify genetic factors that make a significant contribution to the risk of CVD. The identification of these factors may represent an important step forward a better prediction of individual risk. A tendency toward heart disease or atherosclerosis seems to be hereditary. That means children of parents with heart and blood

vessel diseases are more likely to develop them. A family history of diabetes, gout, high blood pressure or high blood cholesterol also increases the risk of heart disease. Susceptibility to CVD appears to be influenced by "context-dependent effects," which include interactions among genes (genetic epistasis) and among genes and environmental factors (gene-environment interactions).

Lipid and Lipoproteins

Atherosclerosis/coronary artery disease (CAD) is largely a result of genetically linked dyslipidemias that can often be identified in clinical practice. Expression of these genetic traits is highly individual and can be affected by environmental factors such as diet and exercise. Evidence from epidemiological studies, clinical correlations, genetic hyperlipidaemias etc., indicates that lipids and lipoproteins play a key role in the pathogenesis of CVD (da Silva 1999). Dyslipidemia is said to be present when lipid or lipoprotein levels lie within a range which is known from epidemiological studies to be associated with secondary complications, in particular atherosclerosis of the coronary arteries.

In addition, many cases of hyperlipidemia are secondary to other disorders such as hypothyroidism or renal dysfunction. Such disorders may also unmask or exacerbate a genetic lipoprotein disorder. Examples of the latter are the unmasking of type III hyperlipidemia by diabetes mellitus or the exacerbation of familial hypercholesterolemia by hypothyroidism.

The known lipid-related risk factors include:

- High levels of low-density lipoprotein cholesterol (LDL)
- Low levels of high-density lipoprotein cholesterol (HDL)
- High apoB levels (the major protein fraction of the low density lipoprotein particles)
- Elevated levels of Lp(a) lipoprotein
- Apolipoprotein AI-CIII-AIV gene cluster polymorphisms

Lp(a) lipoprotein

High plasma levels of Lp(a) constitute an independent risk factor for coronary artery

disease, acute myocardial infarction, peripheral vascular disease, and stroke as well as aortic aneurysms (Dahlen and Stenlund 1997; Gazzaruso et al. 1999a; Marcovina et al. 1999; Hoogveen et al. 2001). The plasma level of Lp(a) is more than 90% genetically determined and is inversely related to the size of apo(a). In contrast to LDL, Lp(a) contains an additional large specific protein apolipoprotein(a) [apo(a)], disulfide linked to apo B-100, in each Lp(a) particle. Apo(a) is characterized by a genetically determined size and sequence polymorphism with at least 34 isoforms in plasma. Recent studies have shown that in atherothrombosis apo(a) polymorphism could play a role independent of Lp(a) levels. In particular, apo(a) phenotypes seem to have their highest predictive value for coronary heart disease when apo(a) isoforms are detected by high-resolution phenotyping methods and when an adequate operative cut-off of apo(a) polymorphism is used (Gazzaruso et al. 1999a). The analysis of apo(a) polymorphism appears to be particularly useful in healthy subjects with a family history of atherothrombotic diseases, in patients with a high cardiovascular risk (diabetes, hypertension, hypercholesterolemia) and in subjects with conditions modifying the Lp(a) levels (Brazier et al. 1999; Gazzaruso et al. 1999b).

Although lipoprotein(a) [Lp(a)] has been recognized as an atherothrombogenic factor, the underlying mechanisms for this pathogenicity have not been clearly defined. Plasma levels have received most of the attention in this regard; however, discrepancies among population studies have surfaced. Particularly limited is the information on the fate of Lp(a) that enters the arterial wall, in terms of mechanisms of endothelial transport and interactions with cells and macromolecules of the extracellular matrix.

GENETIC POLYMORPHISMS IN LIPOPROTEINS

Many genes regulating lipids and lipoproteins are polymorphic and the variant alleles may have important modulating effects (Cullen et al. 1998; Loktionov et al. 1999; Nassar et al. 1999; Pereira et al. 2000). Although the results have not been uniform across studies, the current research support the concept that

variation at these genes explains a significant, but still rather small, proportion of the variability in responses to dietary interventions.

Apo E Polymorphism

Apolipoprotein E (apo E) plays a central role in lipoprotein metabolism as a ligand for LDL-, VLDL- and remnant receptors (Friedlander and Leitersdorf 1996). It is mainly synthesized by hepatocytes and also by monocytes-macrophages. There are three common codominant isoforms E2, E3 and E4. Apo E2 binds defectively to LDL- and to remnant-receptors. Apo E alleles frequency varies from population to population around the world. In Europe, E4 allele frequency increases from south to north along the cardiovascular disease frequency gradient (Hallman et al. 1991). On the other hand, the association between E2 allele and these diseases remains to be proved except for type III hyperlipoproteinemia. Apo E4 role in atherosclerosis could be explained, at least in part, by its high solubility in apo B lipoproteins (Cubrilo-Turek et al. 1998). The average cholesterolemia of E4/E3 subjects is higher than of E3/E3 subjects and E3/E3 subjects' cholesterolemia is higher than in E3/E2 subjects, probably because of a faster uptake of chylomicrons and VLDL remnants in E4/E3 subjects.

Apolipoprotein AI-CIII-AIV gene cluster

More than 20 different RFLPs have been described in the apo AI-CIII-AVI gene cluster and their association with several dyslipidemias has been claimed in different populations (Ordovas and Schaefer 2000). In particular, six RFLPs in the apolipoprotein AI-CIII-AIV gene cluster, detected with the restriction enzymes XmnI, MspI, PstI, SstI and PvuII, respectively, have been used to study the role of genetic variation at this gene locus in the development of CHD and in the regulation of serum levels of various lipids and lipoproteins.

NEW GENERATION OF RISK FACTORS

Many potential "new" risk factors may predispose to atherosclerotic disease (Folsom 1999). The most promising currently seem to be:

- high levels of homocysteine
- low activity of paraoxonase
- elevated plasma fibrinogen levels
- low vitamin E intake
- insulin resistance
- C-reactive protein

High Levels of Homocysteine

Elevated plasma homocysteine is a known risk factor for atherosclerotic vascular disease, but the strength of the relationship and the interaction of plasma homocysteine with other risk factors are unclear (Refsum and Ueland 1998; Langman and Cole 1999). Homocystinuria is an uncommon genetic disease characterized by a marked increase of serum homocysteine (HCY), an intermediate of methionine metabolism. Homocysteine can damage vascular endothelium, cause proliferation of vascular smooth muscle, activate platelets, promote lipid peroxidation, and activate the coagulation cascade. Recently, several studies have also demonstrated that moderate hyperhomocysteinemia - not necessarily linked to an inborn metabolic defect - may also be considered as an independent risk factor for cardiovascular disease (Folsom 1999). The main mechanisms of HCY atherogenic action are thought to be LDL oxidation, inhibition of vascular endothelium growth combined with stimulation of smooth muscular cells proliferation, and interference with the coagulation and fibrinolytic systems (Folsom et al. 1998; Refsum and Ueland 1998). Cofactors of key enzymes in HCY metabolism, folic acid, vitamin B12 and vitamin B6, may be given, alone or in combination, for the treatment of hyperhomocysteinemia.

A point mutation (cytosine to thymidine substitution, C677T) in the gene encoding methylenetetrahydrofolate reductase (MTHFR) has been associated with elevations in homocysteine levels in homozygous carriers (TT genotype) and is considered as an independent risk factor for vascular diseases (Chambers et al. 2000).

Low Activity of Paraoxonase and CVD

It is known that peroxidation of low-density

lipoprotein (LDL) lipids is a crucial step in favoring lipid deposition in arterial wall and plaque formation. Therefore genes affecting LDL oxidation are excellent candidate genes for CVD. Paraoxonase/arylesterase (PONA) is an enzyme exclusively bound to high-density lipoprotein and has been shown to hydrolyze lipid peroxide, thus protecting LDL from oxidation. PONA is present in two isoforms, A and B. Homozygotes for the A allele have a lower enzymatic activity than homozygotes for the B allele; AB heterozygotes have intermediate values. Given the potential role of PONA in preventing LDL oxidation and the presence of functionally significant genetic polymorphism, PONA Gln-Arg192 polymorphism has been proposed as a useful genetic marker of risk of CVD (Durrington et al. 2001).

Fibrinogen and CVD

There is growing epidemiological, clinical and physiological evidence incriminating fibrinogen in the evolution of cardiovascular disease (Behague et al. 1996; van der Bom et al. 1998). Elevated plasma levels of fibrinogen have been shown to have a major influence on atherosclerotic disease involving the coronary, cerebral and peripheral vasculature. Fibrinogen is a plasma protein that is cleaved to generate fibrin and participates in platelet aggregation. Plasma levels of fibrinogen are strongly influenced by genetic factors. Fibrinogen is also an acute phase reactant that increases after myocardial infarction and in other acute and chronic inflammatory states. Thus there is growing interest in the concept that fibrinogen levels might be used to identify patients at higher risk for cardiovascular events and that interventions to lower fibrinogen levels might reduce the risk of clinical events.

The plasma fibrinogen level of any given individual, and its associated cardiovascular risk, is dependent upon an interaction between environmental and intrinsic (genetic) factors. Most environmental factors associated with elevated fibrinogen levels are also potent cardiovascular risk factors (e.g., cigarette smoking). However, as research into the genotypic influences on both basal and "stimulated" fibrinogen production progresses, high-risk groups may be identified

that may benefit from therapeutic intervention aimed at lowering plasma fibrinogen.

RISK FACTORS FOR CVD IN ASIAN INDIANS

Contrary to popular belief that CHD is uncommon in developing countries, Asian Indians have a relatively high prevalence of CVD. Numerous recent epidemiological studies have revealed that the usual risk factors i.e. hypertension, hypercholesterolemia, obesity, smoking and a family history of CVD, are not common among South Asians. Rather, they possess a different risk factor profile characterized by high triglycerides, low HDL, glucose intolerance, insulin resistance, abdominal obesity and increased lipoprotein(a) levels (Hoogeveen et al. 2001). On account of this difference and the alarming explosion of CVD in India, conventional guidelines for prevention of CVD may not be applicable in Indian population (Singh and Niaz 1999).

MOLECULAR STRATEGIES TO IDENTIFY GENETIC RISK FACTORS FOR CVD

Recent advances in the field of molecular biology have led to a better understanding of the pathological mechanisms of cardiovascular disease (Cambien et al. 1999; Ellsworth et al. 1999). The impact of these findings will shape the future of treatment modalities for cardiovascular disorders. Postulated targets and biological rationale of new techniques are being developed in a race towards molecular therapies for vascular diseases. Whether it is modulation of transmembrane cell receptors or phenotypic changes via vectors that mediate gene transfer, there is no doubt that molecular strategies will be an integral part of the future therapies.

Genetic Testing of CVD Risk Factors

The potential applications for genetic testing are immense, with most diseases having some aspect influenced by, if not directly caused by, changes in the genome of the patient. The translation of genetic information into medical

applications will be influenced by our ever growing understanding of the human genome, the technological advances, and social, ethical, and legal issues surrounding genetic testing of diseases.

Gene Therapy and CVD

Novel therapeutic strategies to treat cardiovascular disease consist of 3 major approaches: (1) changing the biology of vascular disease; (2) intervening in the ischaemic event; or (3) modifying the post-ischaemic course. The development of future therapies depends on continuing advancements in our understanding of the molecular mechanisms of vascular pathobiology. Molecular cardiology aims at applying molecular biological methods for both diagnosis and treatment of cardiovascular disease. Clinical experience has so far only been obtained in patients with familial hypercholesterolaemia and mutations in the LDL receptor. Recent research suggests that somatic cell gene therapy may find application against nearly every form of cardiovascular disease - atherosclerosis, thrombosis, restenosis, myocardial infarction, hypertension, heart failure, and transplant rejection.

PERSPECTIVES

Taken together, CHD is a multifactorial disease that is associated with non-modifiable risk factors, such as age, gender and genetic background, and with modifiable risk factors, including elevated lipids and lipoproteins. Though our improved understanding of the fundamental basis of these important cardiovascular disease processes has established a scientific foundation for diagnostic, prognostic, and therapeutic advances in the mainstream of cardiovascular medicine, our current knowledge of the genetic basis of cardiovascular diseases is still primitive. As we reflect on the enormous progress in the understanding and treatment of cardiovascular diseases in the 20th century, we confront greater opportunities and challenges in the new millennium, in particular the search for candidate genes reflecting genetic susceptibility to CVD.

ACKNOWLEDGEMENTS

Financial support by the Volkswagen-Stiftung, Hanover, and DLR, Bonn is thankfully acknowledged.

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