

CORONARY ARTERY DISEASE (CAD): INTERPLAY OF NUTRITION, SERUM PARAMETERS AND GENETICS

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Contribution

SUS conceived the idea and designed the review. Data collection and manuscript writing was done by SUS, and S. All the authors contributed equally to the submitted manuscript.

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ABSTRACT

Coronary artery disease (CAD) is a leading cause of death worldwide. The death rate is decreasing in developed countries due to awareness, but the disease burden is increasing in developing countries. Pakistan is a country with high prevalence of CAD. The serum lipids have long been implicated in the development of CAD by the deposition of mainly LDL on endothelial lining resulting in atherosclerosis. Progression to CAD involves environmental as well as genetic factors. The genetic component is due to the contribution from various low to modest effect size variants in many genes. Common variants have been used to construct a genetic risk score (GRS) to calculate the risk of future CAD. In conclusion, lifestyle interventions in concert with the knowledge of genetic predisposition based on family history and use of population data may one day lead to the development of personalized medicine for the treatment and prevention of CAD.

Keywords: Coronary artery disease; genetic risk score, Pakistan

INTRODUCTION

Coronary artery disease (CAD) is a multifactorial chronic disorder of coronary arteries progressing silently and usually has established to an advance stage by the time symptoms start appearing. In CAD, a fatty material known as plaque deposits inside the coronary arteries which narrows their lumen and restricts flow of blood to myocardial muscles, the process is known as atherosclerosis. Atherosclerotic plaques are characterised by the accumulation of necrotised tissue, calcification of dead necrotic material, deposition of oxidized lipids, aggregation of foam cells and other cell types like smooth muscle cells, endothelial cells, dendritic cells, T lymphocytes and a fibrous cap. Blood vessels contain a very thin and smooth lining of cells called endothelium which facilitates an easy flow of blood. Although born with clear coronary arteries having wide lumen, the first streaks of fatty material may appear in the teenagers but the disease is asymptomatic until the plaque has grown enough to compromise the flow of blood. Atherosclerosis is a complex process commonly associated with elevated blood lipid levels especially low-density lipoprotein cholesterol (LDL-C) that affects endothelium by altering its permeability. Atherosclerosis commences with damage to endothelium which can be caused by diverse factors such as bacterial toxins, hyperlipidaemia, hypertension, hyperglycaemia, circulating inflammatory cells and adipokines.¹ Figure 1 depicts stages in the progression of atherosclerosis with age.

PREVALENCE AND EPIDEMIOLOGY OF CAD

During the last century, CAD has become the leading cause of death worldwide. At the beginning of 20th century, it represented 10% of all deaths while by 2020, the mortality rate from CAD is expected to reach to 25 million worldwide which will be greater than the deaths due to infectious diseases.² Despite all measures, CAD remains the single largest killer in the developed countries and is the leading cause of disease burden in developing countries. According to 2006 mortality rate data, approximately 2300 Americans die due to cardiac

diseases every day which corresponds to an average of 1 death per 38 seconds. According to worldwide statistics, CAD caused 247.9 deaths/100,000 individuals and 28.2% of all cause deaths in 2013. In high income countries like North America, Western Europe and Australia, the mortality rate due to CAD declined since 1980 and was shifted to an elder age group whereas, middle- and low-income countries bear three fourth of the global CAD burden.³ In figure 2, average deaths from CAD per year have been highlighted in different regions of the world.

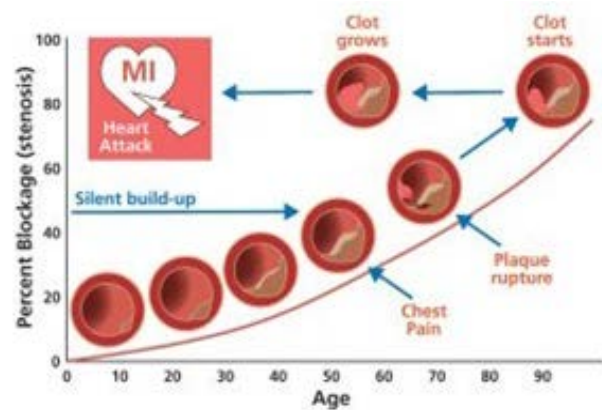


Figure 1. Progression of atherosclerosis and CAD

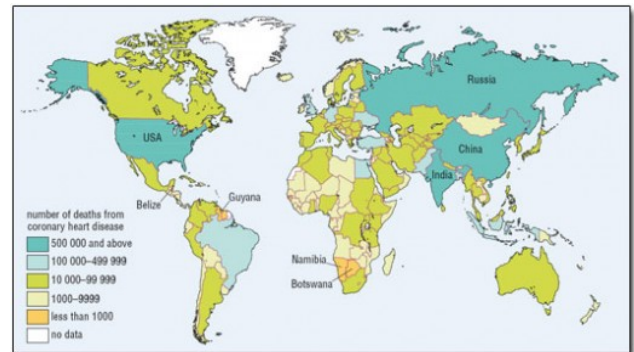


Figure 2. Worldwide death rate due to cardiovascular disease.⁴

The South Asian countries are at a greater risk of developing CAD and the prevalence is 50% to 300% higher than rest of the world. In South Asia, the CAD mortality rate was highest among the world in 2010. Progression of the disease is often severe and

aggressive and it has shifted towards younger age group compared to the rest of the world. South Asians who have migrated to other regions of the world display a CAD prevalence rate which is comparable to their native ethnicity. The diseases pattern of migrants usually blends with the host population after two to three generations but South Asian immigrants to USA, UK, Canada and Africa show a high prevalence of CAD than the host population.⁵

Pakistan which is a country of >180 million people has high burden of CAD like rest of the world. The prevalence of CAD risk factors is high in Pakistani population and more than 30% of the people above 45 years of age are affected by the disease.⁶⁻⁸ A number of studies have indicated that change in the lifestyle and dietary habits has resulted in a continuous increase in the prevalence of CAD in Pakistan.^{9,10} In addition, the cultural and religious norms have resulted in concentration of risk genotypes which have in turned contributed to a further rise in CAD cases.¹¹

ETIOLOGY AND PATHOPHYSIOLOGY OF CAD

Both genetic and environmental factors contribute to the development of disease. The role of hypertension, diabetes, dyslipidemia, obesity, smoking and elder age in the pathogenesis and progression of CAD has been well established. These conventional risk factors (CRF) for the development of CAD can be classified into non-modifiable and modifiable categories. The non-modifiable risk factors are older age, male gender and having a family history of CAD. Modifiable CAD risk factors include hypertension, diabetes, blood lipid levels, obesity, smoking, unhealthy use of alcohol, sedentary life style and psychosocial stress. The non-modifiable factors are innate and cannot be changed whereas; modifiable risk factors can be changed through life style/behavioral changes or drug interventions. Atherogenesis is a complex phenomenon where multiple processes participate. This is an interplay of ROS production, oxidative stress, inflammatory response and inappropriate lipid metabolism. The genes encoding proteins involved in maintaining vascular homeostasis, lipid metabolism and inflammatory responses could be the good candidates for either development or protection from CAD.¹²

LIPID PROFILE AND CAD

The association of lipids with CAD has been explained on the basis of most popular hypothesis that atherosclerosis begins by the oxidation of circulating lipids especially LDL-C. According to this hypothesis, the excess LDL-C first deposits in the sub endothelial space and is modified which induces a chemotactic effect to attract monocytes and macrophages which subsequently cause oxidation of LDL-C (OX LDL-C). The protein part of LDL-C (apo B-100) becomes more negatively charged and is recognized by scavenger receptors on macrophages and is eventually internalized by macrophages to form so called foam cells. In contrast to the native LDL-C, the uptake of this oxidized LDL-C by macrophages is not controlled by feedback mechanism. In addition to enhancing foam cell formation, OX LDL-C is also directly chemotactic for monocytes, stimulating the binding between monocytes and vascular endothelium and prevents the escape of monocytes from sub endothelium. The OXLDL-C is also directly cytotoxic by promoting the release of lysosomal enzymes. Hence, oxidative modification of LDL-C has an important role in atherogenesis and provides a rationale for the role of oxidative stress and antioxidants in the progression of atherosclerosis. The LDL-C oxidation primarily occurs in the vessel wall but circulating LDL-C can also be oxidized. Although the proportion of circulating OX LDL-C is low compared to OX LDL-C present in the vessel endothelium, however an increase in the amount of circulatory OX LDL-C is an indicator of oxidative stress and a CAD risk factor.

The presence of OX LDL-C as a pro-atherogenic marker in healthy individuals also emphasizes its importance as a predictor of CAD in apparently healthy individuals.¹³ A dose dependent reduction in CAD risk has been reported by lowering LDL-C. Each 1mmol/l (38.67mg/dl) reduction in LDL-C corresponds to 20-25% decreased CAD risk. Most recent trials have documented that LDL-C concentration of approximately 70mg/dl has the lowest CAD risk or a 50% LDL-C reduction than the base line value has lowest risk in established CAD patients (31). Low HDL-C is a part of dyslipidemia triad i.e., low HDL-C, moderately elevated TG and high LDL-C/small dense LDL-C particles which is a classical dyslipidemic picture in high CAD risk subjects. Low HDL-C may also lead to hypercholesterolemia due to increased LDL-C. HDL-C <40mg/dl in men and <45mg/dl in women is a CAD risk. Hypertriglyceridemia is an independent CAD risk factor and more risk is associated with a

moderately increased TG levels than very high levels (>900mg/dl) which in turn predispose to other conditions such as pancreatitis. A fasting TG level of >150mg/dl is considered to be a marker for CAD risk.¹⁴ Hypercholesterolemia either genetic or acquired is an independent CAD risk factor. It is estimated that 56% of the total heart diseases may be due to hypercholesterolemia (>200mg/dl) alone.¹⁵

OXIDATIVE STRESS IN CAD

Oxidative stress caused either by the excess production of reactive oxygen species (ROS) or their decreased clearance by antioxidant system is a major contributing factor for the development of CAD. Indeed, other CAD risk factors such as hypercholesterolemia, diabetes mellitus and cigarette smoking by themselves also produce ROS and end up with oxidative stress.¹⁶ Furthermore, the oxidative stress is spurred by the lipid overload and abnormal lipid metabolism. The ROS and oxidative stress are linked to many proatherogenic events like vascular smooth muscle cell migration and proliferation, increased expression of adhesion molecules, lipids peroxidation, irregular vasomotor activity and endothelial cell apoptosis. They also have role in angiogenesis, a final stage of atherogenesis.¹⁷

The systemic inflammation is a pro-atherogenic factor and inflammatory cells like macrophages and T lymphocytes are present in fatty streaks which are the first detectable events in atherogenesis. In particular, inflammation has been suggested to be a mechanistic link between CAD risk factors. Diabetes, dyslipidemia, hypertension and smoking are some of many CAD risk factors. The evidence of molecular and cellular bases of these risk factors to a common outcome (CAD) is limited.¹⁸ It is suggested that oxidative stress signals modulate the expression of genes which regulate vascular inflammatory response. Contrary to this, pro-oxidative or pro-inflammatory stimuli associated with conventional risk factors may directly stimulate vascular cells to produce ROS. The ROS along with OX-LDL transmit these extracellular signals to the genes regulating atherogenic compounds. The induced overexpression of these atherogenic compounds will lead to vascular infiltration by macrophages and T lymphocytes leading to local inflammatory response

and endothelial dysfunction. The interaction of oxidative stress with other factors contributing in the development of CAD can be conceptualized through a flow chart (Figure 3).

An antioxidant system comprising of enzymatic and non-enzymatic molecules is activated in response to inflammation. In the presence of a persistent inflammatory condition, the antioxidant system will get exhausted due to escalation of ROS production. There will be a state of low antioxidants and higher oxidative stress markers in the blood. The endothelial damage is a very first step towards atherogenesis. As a result of this, adhesion molecules are overexpressed in vascular wall which help the inflammatory cells to stick to endothelium.²⁰ Once inside the tunica intima, the macrophages and T lymphocytes influence smooth muscle cells to migrate to tunica intima. Within the intima, these cells proliferate and develop a complex extracellular matrix. As the atheromatous plaque matures, it develops fibrous cap and a core rich in lipid material. It may eventually rupture if an imbalance between plaque forming and plaque stabilizing factors takes place. However, mammalian cells have antioxidant defence mechanism consisting of antioxidant molecules like glutathione (GSH) and antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) to cope the oxidative stress of ROS. The oxidative stress modifies LDL-C to a more atherogenic form. At cellular levels, the main respondent to oxidative stress is endoplasmic reticulum (ER). The ER can work precisely within a narrow lipid range due to low intrinsic cholesterol level and can tolerate only a tight redox control. The ER has role in cell secretions and all secreted proteins have to pass through ER and levels of unfolded proteins correspond to secretory demands. The ROS induced modifications may suppress ER folding ability or may disrupt the protein processing, whereas, the repetitive folding and unfolding of translated proteins to attain their optimum structure is also a source of ROS.²¹ The unfolded protein response is activated through all the phases of atherosclerosis, where it mediates the inflammatory response in endothelial cells and impairs their function by oxidative stress and decreased expression of NOS3 and reduction in contraction and vascular tone.²²

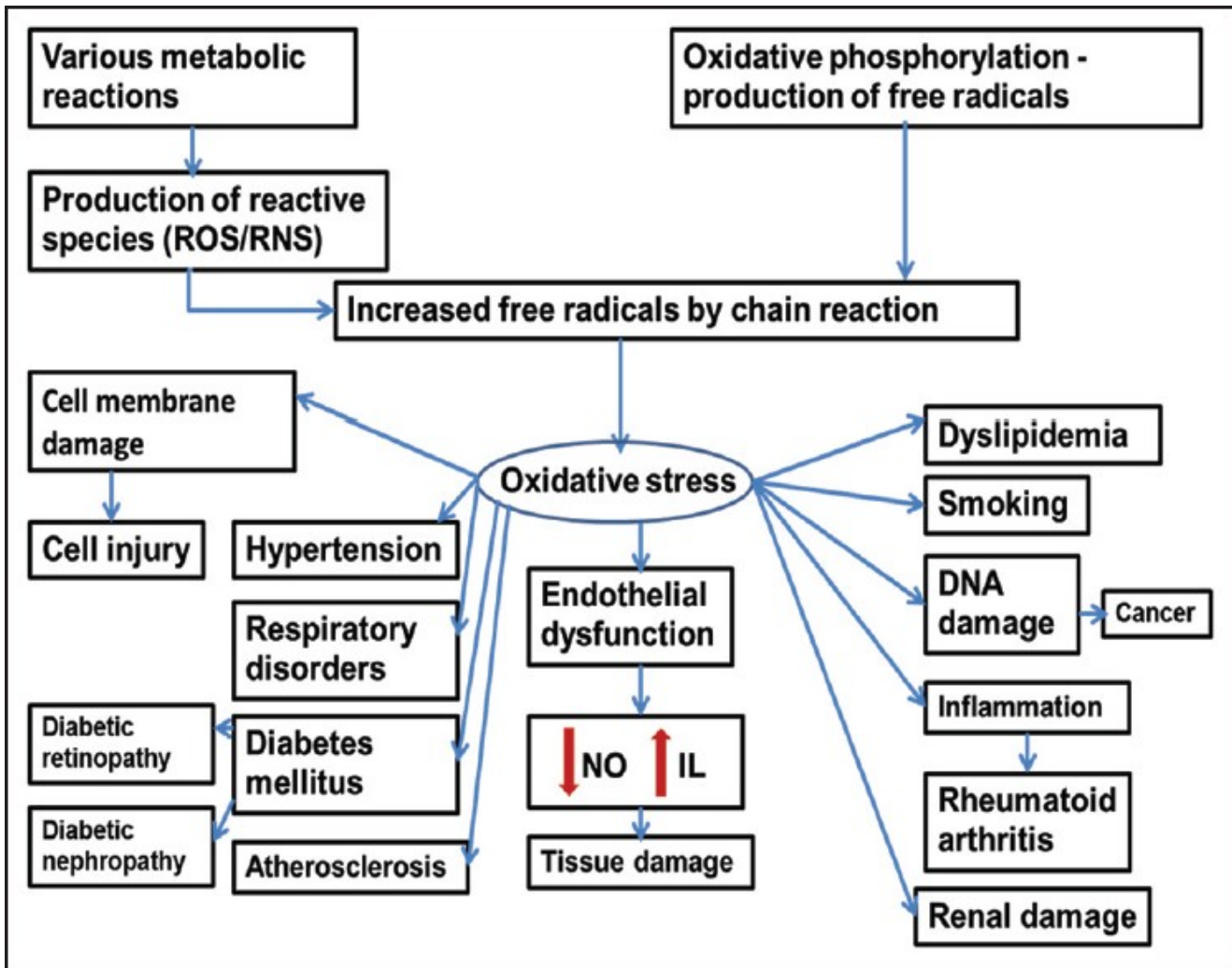


Figure 3. Reactive oxygen species, oxidative stress and other contributing factors of CAD development.¹⁹

ASSOCIATION OF FAMILY HISTORY WITH CAD

A history of having CAD in first degree relatives is a potential risk factor. The subjects exposed to similar environmental conditions exhibit different susceptibility to CAD. This shows that genetics of an individual also strongly contributes in the development of disease and heritability of CAD has been estimated to be 40% to 60%.²³ With the exception of familial hypercholesterolemia which is a monogenic form of CAD, in all other forms multiple genes are involved in the development of CAD and each genetic variant has a mild to moderate

contribution. Being a polygenic disorder, it does not follow a simple Mendelian pattern of inheritance; hence linkage analysis and pedigree analysis are not sufficient to predict the outcome.²⁴ In contrast, multiple loci and the assessment of their contribution to outcome will be required to examine the genetic predisposition of CAD. The heritability of CAD has been known for a long but only a small portion of this heritability could be explained because the genetic variants studied so far have small effect sizes. Except few forms of CAD such as familial hypercholesterolemia and familial defective apoB-100 which are monogenic or Mendelian in nature, majority of CAD are genetically complex and multifactorial.²⁵ In addition, the risk factors for CAD like diabetes, hypertension, obesity are themselves

polygenic in nature. Although heritability of CAD has been proved in twin, family and population studies, unfortunately many of the results have not been reproduced in people from diverse ethnicities making their clinical utility questionable. It is assumed that for multifactorial diseases like CAD, a large number of SNPs play their role.²⁴ The effect of each variant involved is modest and dependent in part on environmental and other genetic components. Neither of these variants is indispensable nor is any one sufficient at its own for the final outcome. The quantitative contribution of each locus in disease outcome is its effect size/magnitude which depends upon the frequency of polymorphism (MAF/RAF) and relative risk (effect size) it contributes to the outcome.²⁶

NUTRITION AND CAD

Nutrition plays a significant role in the development of CAD. There are expanding novel aspects of food on CAD as, fats and lipid content in diet, time of taking meal, influence of diet on gut microbiota, nutritional effect on telomeric length and nutrition-gene interaction. Fruits, vegetable and whole grains slowly releases carbs and fibres²⁷. Use of whole grain reduces the risk of CAD. Legumes and nuts with unsaturated fatty acids, lowering the blood pressure and cholesterol. Consumption of nuts inversely related to the onset of CAD. Fish provide long chain n-3 fatty acids, that are antiarrhythmic in nature. Alcoholic beverages increase the level of HDL-cholesterol. Dairy products contain saturated fatty acids, it is believed that these fatty acids are harmful but studies showed consumption of milk or other dairy products does not increase the risk of CAD rather it has reverse effects.²⁷

GENETIC RISK ANALYSIS OF CAD

The use of genotype information to predict diseases in non-symptomatic carriers, understanding genetic bases of diseases and response to treatment in patients having established disease were the main goals of human genome project. The sequencing of human genome and subsequent mapping in Hap-Map resolved the issue of genetic analysis of CAD like complex diseases.²⁸ It is believed that approximately 3 million single nucleotide polymorphisms (SNPs) are present in human genome and assign a different genetic make up to each individual.²⁹ This genetic variability is responsible for variations in phenotypes as diverse as having a different eye and hair colour to

predisposition to various diseases. The SNPs present in an individual's genome may be neutral, protective or associated with a risk of CAD. Majority of CAD risk SNPs are common in general population with a minimal to moderate relative risk. Most of the SNPs are located in non-coding DNA region implying that they affect by regulating the upstream or downstream genes.³⁰

Another striking feature of CAD risk SNPs is that most of them operate independent of known CAD risk factors. This indicates that many unknown pathways involved in development of CAD still need to be explored. There are potential SNPs in the genes regulating inflammatory response such as *CXCL12* which modulates interleukines. Similarly, *MRAS* which is an oncogene homologue also has many SNPs associated with CAD. To prove that their risk is mediated through inflammation, requires direct functional studies. It is not completely understood that how SNPs modulate the development of disease.³¹ A SNP may be causal or functional such as null alleles caused by premature truncation. Secondly, if itself non-functional, it may be in strong linkage disequilibrium (LD) with a functional SNP or the SNP may be tagging some functional SNP elsewhere in the genome. It is now revealed that majority of the SNPs are in the non-coding or inter-genic regions and act through affecting promoters and enhancers. The multifactorial and polygenic nature of CAD can be conceptualized from Figure 4.

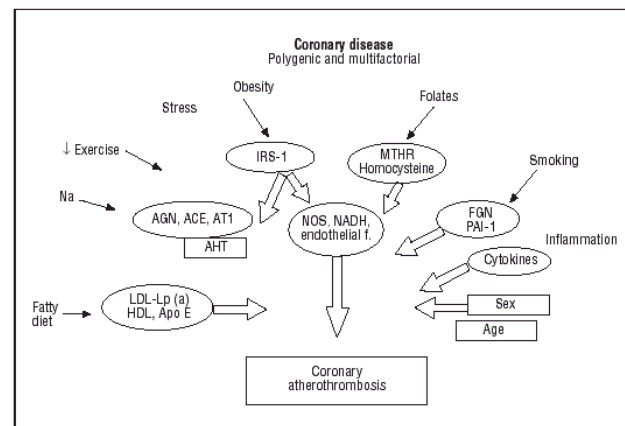


Figure 4. Interaction of genetic and environmental factors in the development of CAD.³²

The benefit of CAD genetic risk analysis of an individual is that the genetic variants need to be genotyped only once because they persist lifelong and do not change with time. The majority of genetic

studies and GWAS have been carried out on European Caucasian population.³³ It has been a routine practice to transpose the results obtained from Caucasians to rest of the ethnicities. There is an immense requirement to extend these studies to other ethnicities also. The LD patterns and allele frequencies may vary widely among different ethnicities e.g., the association of 9p21 region with CAD has not been found in African Americans. Another important aspect to remember is that a genetic biomarker may not be associated with a trait in different ethnicities and in such cases the applicability is limited to only those populations where the genetic defect is clearly seen.³⁴

For the genetic CAD predisposition analysis to be of clinical value, it should provide information over and above the CRFs. The first step of this journey is to identify a set of common variants associated with CAD and traditionally this has been done by the case control studies i.e. to compare the allele frequencies in subjects with disease to that of disease free subjects. The variants associated with CAD in many studies are then confirmed by meta-analysis of large number of studies. Secondly, the effect size of the variants is estimated in prospective studies which are more suitable for this purpose because case controls studies though efficient in gene discovery have the conflict of bias. In addition, the information is pooled on prevalence of RAFs between different countries and by race and ethnicity and whether the effect or allele frequency is affected by environment or any other factor. The SNPs identified may not explain the full genetic spectrum of CAD which may also include rare or private mutations and epigenetic effects due to DNA methylation in a multifactorial disease like CAD, the single genotypes are underpowered and the effect size is modest.³⁵ In case of early CAD there may be the possibility of a monogenic pattern of inheritance such as a mutation in LDLR gene. However, there also remain the chances of coexistence of a large number of risk factors like HTN, DM and high blood lipid levels or the coinheritance of a large number of risk allele with modest effect sizes.

GENETIC RISK SCORE (GRS) AND CAD

CAD is a multifactorial disorder; its onset require interaction between genetic and environmental factors. Conventional CAD risk factors (CRFs) are age, gender, blood pressure, smoking, diabetes. Individuals exposed to same environment or similar CFRs but show variability in onset of disease, is due to variation in their genetic makeup. Many scientists discriminate genetic testing over CRFs. Family

history of CAD is a well-known risk factor due to heritability of genes. Scientist calculated GRS by adding risk allele present on loci including in a specific study. In a previous study risk score of 21 variants was calculated by using genetic risk analysis in Pakistani people. GRS of those 21 variants suggested strong association of CAD with lipid profile of individual.³⁶

PERSONALIZED MEDICINE FOR CAD

In the modern era, especially in developed countries, there happened two major advancements in the field of genetics of complex diseases like CAD. Firstly, modern array technology and high throughput genotyping techniques have made it possible to genotype hundreds of SNPs at the same time. Secondly, collaborations among research groups and large consortia have brought together the groups of phenotypically well characterized individuals together. With respect to CAD, these efforts have identified a number of common variants in individual studies and large genome wide studies many of them reaching the genome wide significance. Unluckily, this approach is still lacking in developing countries due to the lack of awareness and technically and academically sound man power. This may also be secondary to their low GDP and less financing in the field of research and development.³⁷

The genetic analysis does not only apply to the incident diseases, rather can also be used to find the prognosis, severity and response to treatment of disease. Although still very remote, but the dream of personalized medicine is coming to its reality in the field of human medicine. A full biochemical and genetic profile of CAD patients may help the implementation of personalized medicine in this field also. The concept of more specific individualized medications targeted towards subpopulations of patients is gaining more popularity and acceptance from researchers, drug developers, and regulatory agencies rather than developing some 'block buster drugs' for use in the entire population.³⁸ It takes into account the understanding of many interrelated genomic, epigenomic, environmental factors as well as the drug interactions (Figure 5).

This changing perspective has led to the development of a new field in pharmaceutical industry, i.e., Pharmacogenomics, which is the study of the variation in the human genome that can affect the response to drug therapy. Identification of the genomic factors causing variability in drug response relies on identifying variations in a number of genes

encoding either drug-metabolizing enzymes, drug transporters, drug targets, drug receptors or signal transduction molecules. Some of such hallmarks have been identified in cardiovascular diseases also, however, they have not been widely used for treatment. The efficiency of antidiabetic, antihypertensive and cardioprotective drugs is affected by polymorphisms in genes encoding drug-metabolizing enzymes. These findings are, however, based on retrospective studies, and in future larger prospective studies are needed with a sufficient power to determine the precise influence of such polymorphisms on treatment response.³⁹

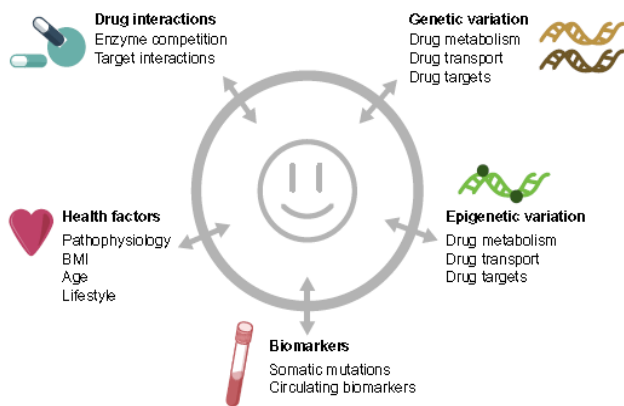


Figure 5. Interacting factors in Personalized Medicine.³⁷

One such example is use of lipid lowering drug statin in subjects with high LDL-C. There is also growing interest in dealing with the adjustment of concentration-response relationship of oral anticoagulants, a classic example of this being the use of warfarin. Although the predictive markers are scarce, cardiovascular medicine has many prognostic markers which are known to increase risk, including previous cardiovascular events, presence of diabetes/obesity, family history, levels of HDL/LDL cholesterol, and C-reactive protein. Thus, the current state of personalized medicine in cardiovascular disease is largely limited to classical approaches such as pharmacokinetic adjustments and dose titration to a pharmacodynamics marker. Among the causes of high prevalence of CAD worldwide are, a tremendous increase in human population since the last century and reduction in mortality due to death rates caused by infectious diseases and other communicable diseases. Another reason is increase in average life expectancy due to low death rates and as a result of improvement in public health policies and medical care. Another reason may be decrease in death rates due to malnutrition, maternal and infant death rates. Since elder age is also a CAD risk factor, in a

population of elderly people more people will feel the victim of the disease.⁴⁰

CONCLUSION

In conclusion, the coronary artery disease, as the leading cause of mortality worldwide, has to be explored in detail. The situation is further complicated due to the fact that it is a multifactorial disorder that has an environmental component in addition to the genetic contribution. The use of high cholesterol diet in Asian countries as a convention contributes greatly to heart diseases. The recent change to a more sedentary lifestyle, lack of physical activity, resistance to exercise, use of vehicles has further resulted in the aggravation of the mild disease to a clinical form. The genetic factors are important at the same time because of the socio-cultural norms that result in the concentration of risk genotypes. It is therefore very difficult to dissect exactly the real cause, but the use of various algorithms has made it possible to identify the high-risk individuals in order to treat them. Recently, the genetic risk scores have been added to the conventional risk factors based on diet and lifestyle) to improve risk prediction. However, the ultimate achievement would be to develop personalized medicine based on environmental, genetic and population data.

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