Role of genetics in prediction of coronary artery disease.

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Introduction:

      Coronary arteries disease (CAD) is a leading cause of death in United States and rest of the world. It mostly involves atherogenic formation within the walls of the coronary arteries, which in turn restricts the adequate perfusion to the heart muscle. This leads to myocardial infarction and sudden death. In the past few decades the theories of coronary arteries disease pathogenesis have changed. The facts reveal that the onset of the disease can develop as early as childhood. The degree of the disease gradually progresses in stages and it is regarded as a complex, ongoing inflammatory process that begins with initial endothelial dysfunction. There are multiple risk factors that directly and indirectly impacts the development and progression of CAD such as hypercholesterolemia, smoking, hypertension, diabetes, stress , high fat meals, lack of exercise, alcohol abuse, obesity and recently chronic infections were added to the list as well. Moreover, we realize now that coronary artery disease runs in the family and there are some risk factors that can not be altered or controlled such as age, sex and heredity. We know that the first-degree relatives of people who suffers from coronary artery disease at the early age are at much greater risk that others. Coronary artery disease is considered to be highly complex heterogeneous disease that results from blended effect of multiple genes. In recent years, researchers have identified the involvement of about 400 genes that might contribute to or to protect against development of disease.(Dzau and Liew 2007). Although, the definite conformation that even half of those is actually involved is still a long way from determination. The bigger question arises from all this information. Can we take an individual genomic profile and translate it into something that's clinically useful? Rather, than discuss all 400 genes that have been implicated, this paper will focus on several of the best-understood genes in order to find their practical application. Among them are disease-causing genes, susceptibility genes and disease-linked genes. It is important to keep in mind that this situation is rapidly changing and hopefully in future genetic approaches will shape the practice of medicine in fundamental ways.

Normal structure and physiology

The heart functions as a pump that delivers blood to the rest of the body. It consists of the three main layers: endocardium, myocardium and epicardium which surrounds by pericardium. Just like all other organs it has its own blood supply which delivered via two coronary arteries that branches of the aorta. The right coronary artery (RCA) lies in the coronary sulcus and supplies blood to the right ventricle, right atrium, and some portion of posterior wall of left ventricle as well as sinoatrial (SA) and atrioventricular (AV) node. The left coronary (LCA) immediately branches into the left anterior descending artery (LAD) and the circumflex artery (Cx). The LAD primarily supplies blood to the wall of the left ventricle ventricular septum as well as to the anterior of the right ventricle. The Cx encircles the heart lies in the anterior interventricular sulcus and supplies blood to left atrium, lateral wall of the ventricle and partially supplies SA node and posterior wall of the left ventricle **(Fig 1)**. Delivery of blood to myocardium is complicated by compression of intramyocardial vessels during systole, which results in the retrograde blood flow in coronary arteries during diastole. Therefore, subendocardial layer of the myocardium is more susceptible to hypoperfusion because ventricular diastolic pressure opposes the driving pressure for flow. Development of CAD will further complicate myocardial perfusion.
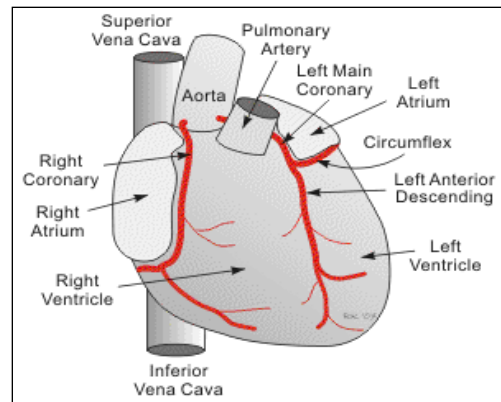
Coronary arteries consist of three layers: the innermost tunica intima, tunica media, and the outermost tunica adventitia. The tunica intima forms an inner lining of a artery and is in direct contact with blood. It is comprised of single layer of squamous epithelial cells, called endothelium that is embedded in an extracellular matrix. The internal elastic lamina separates the tunica intima from the tunica media (Fig 2).
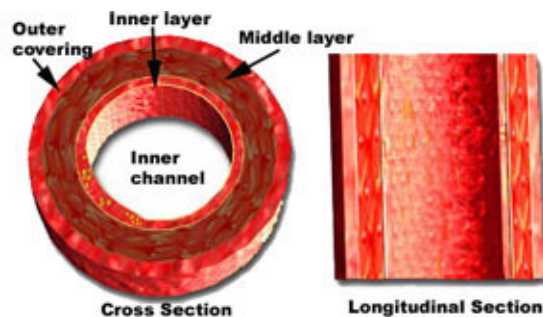
Figure 1. coronary circularion

www.cvphysiology.com/Blood%20Flow/BF001.htm

Figure 1. cross-sectional view of coronary artery
www.chelationtherapyonline.com/articles/p169.htm

Endothelial cells secret locally acting chemical mediators that influence the contractile state of the overlying smooth muscle that play a major role in the control of the vascular tone. These molecules include vasodilators such as prostacyclin, endothelial-derived relaxing and hyperpolarizing factors (EDRF), (EDHF). It also produces vasoconstrictor substances, such as endothelin and prostanoids. In addition, endothelial cells synthesize and secrete heparans and growth factor that regulates smooth muscle cell proliferation. Endothelium remains healthy by secreting different substances that prevent it from platelets aggregation or adhesion of other molecules. The media is relatively thick layer comprised mainly of smooth muscle cells and

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elastic fibers. It's primary role to control the diameter of the lumen by smooth muscle cell contraction or relaxation. Tunica externa is the most outer layer and consists of elastic and collagen fibers. It contains numerous nerve ending and tiny vessels that supply tissue of the cell wall itself as well as connects the artery to the surrounding tissue. This layer separates from media by network of elastic fibers, called external elastic lamina.

Pathophysiology of CAD   Atherosclerosis is the major cause of CAD. Theories of the pathophysiology of atherosclerosis underwent big changes over time. Few decades ago it was looked as mostly disorder of lipid storage, and later it was considered to be disorder of lipid metabolism. Further, CAD was looked as disease of the smooth muscle proliferation. With the discovery of inflammatory cells and molecule in plaques led to conclusion that disease might be inflammatory in nature. Current views on pathogenesis of atherosclerosis are as follows. The development of an atherosclerotic plaque is a multi step process. The presence of risk factors for CAD, such as hypercholesterolemia, cigarette smoking, hypertention, diabetes mellitus and shear stress are associated with endothelial dysfunction. These conditions gradually damage healthy and smooth lining of endothelium. This is the first step in development of atherosclerosis and it results in increased permeability of endothelium **(fig 3)**.

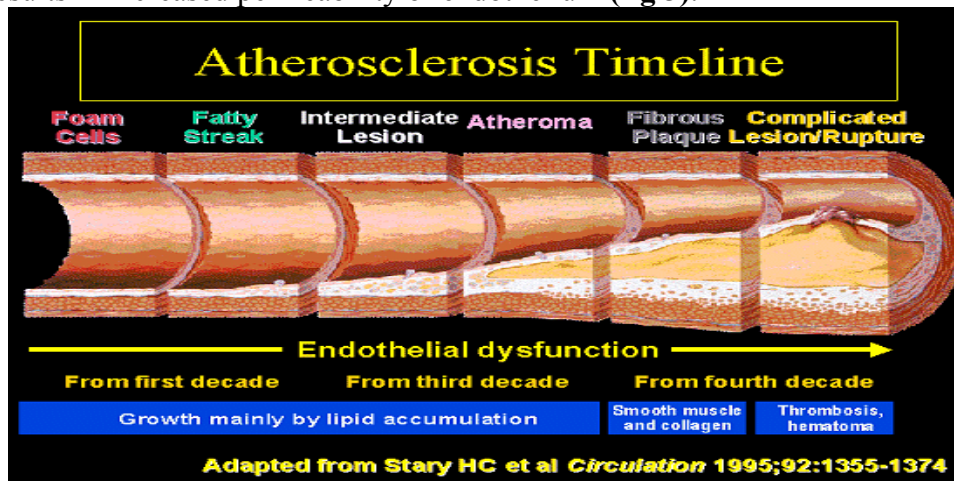
Figure (3) Atherogenesis

www.vhn.ca/images/atherosclerosistimeframe.gif

 Once the layer is damaged, the excess low density lipoproteins and other cellular waste product can easily get into the inner layer of an artery wall. While entrapped low density lipoprotein is progressively accumulates and undergo oxidation. This initiates inflammatory response and stimulates smooth muscle cells proliferation. In response to invasion, endothelium and smooth muscle cells release mediators to initiate recruitment of leukocytes, T-cells and monocytes into the developing lesion. Furthermore, intralesional monocytes become macrophages. They eat and digest large amounts of surrounding oxidized low density lipoprotein. As of the result of this process, these lipid filled macrophages are becoming foam cells, due to their foamy appearance. Together with T cells and monocytes, they form the bulk of "fatty streaks". ". The next stage in development of atherosclerosis is a formation of "intermediate or fibrofatty lesion" which is more complex in composition. The combination of fatty streaks growth and ongoing inflammation, stimulate smooth muscle cells to multiply and migrate to an injury site. As these cells become noncontractile and fibrous they migrate to the top of the atherosclerotic plaque, forming a cap over it. These lesions might not be immediately harmful and may be viewed as protective response to injury. As this plaque continues to grow, the coronary arteries gets thicker,

harder and looses its elasticity. Calcium ions binding to the site of the plaque formation might contribute to lack of elasticity as well. As plaque expands into the lumen of a coronary artery, blood flow is greatly reduced through that artery. At this stage physical symptoms such as chest pains and shortness of breath may occur which associates with angina pectoris. However, it is the physical disruption of the plaque exhibits an acute coronary event such as myocardial infarction. The rupture of the plaque usually occurs due to increased pressure in a narrow artery. It might cause the complete occlusion due to blood clot formation cascade and manifest MI or sudden death.

Genomic approach to CAD

The biggest challenge for scientists of a new era of genetics is to identify the genes and gene expression changes that are responsible for pathological process of CAD. Two main projects: human genome project and international hapmap project provided a crucial data for genetic analysis in a complex heterogenic disease such as atherosclerosis and coronary artery disease. To study such as complex diseases, scientists recently have been using two general categories of study designs such as association studies and linkage studies. In Linkage studies, the goal is to identify the inheritance of the disease in large families throughout few generations. It is possible if we are able to identify single nucleotide polymorphism (SNP) on the fragment of the chromosome that is inherited by family who is affected by a disease but not those who is disease free. Furthermore in this design, we concentrate on identifying the gene variation of our interest after narrowing down the section of a chromosome. This methodology is found to be helpful especially in identifying diseased genes that inherited in a Mendelian fashion. Gene disease associated study was introduced in 1996. It is designed to identify genetic variation of available genome in associations with specific trait or disease of interest. Two independent groups are usually participate in these studies. Those groups are, "cases" which are those individuals who has been diagnosed with a disease and "control" are those who are similar people within same parameters that are disease free. After carefully calculated results, if frequencies of genetic variation are greater in individuals who are affected by a disease then it said to be associated with a disease. Positive association cannot be interpreted as causation, but only statistical association for the most part. Both of these methods have their advantages and disadvantages just like any other studies.

Throughout years of genetic study, molecular biologists have identified several disease-causing genes for disorders in lipid metabolism. They are directly related to development of coronary artery disease. In order to understand dyslipidemia disorders, it's important to understand the physiology of lipid metabolism. Lipids are hydrophobic and therefore have to be transported by lipoproteins in blood. Different lipoproteins have variety of integrated and peripheral apoproteins that perform a different functions such as structural integrity, receptor binding, and enzyme activation. Lipoproteins are categorized by their density of protein content. They divide into two major groups high density lipoproteins (HDL) and non-high density lipoproteins. Non-high density lipoproteins are further subdivided into chylomicrons, very-low density lipoprotein, intermediate density lipoproteins and low-density lipoproteins. They transport lipids from sites of absorption or synthesis to sites of their utilization. High-density lipoproteins are considered as "good cholesterol". They are responsible for transport of excess lipids in circulation back to the liver.

For example Apoprotein B mostly found on non-high density lipoprotein and involves in assembly and packaging of ingested triglycerides and cholesterol into non-high density lipoproteins. Apoprotein E involves in receptor binding to hepatocytes via (LDL receptor).

Through this mechanism, liver uptakes the necessary lipid contents from non-high density lipoprotein. Apoprotein C binds to adipocytes and skeletal muscle cell via lipoprotein lipase, which acts as the receptor to facilitate lipid absorption.

Disease-causing genes have been identified for disorders in lipid metabolism such as familial hypercholesterolemia, familial defective Apolipoprotein B, Apolipoprotein A deficiency, Tangier disease, Autosomal recessive hypercholesterolemia, Sitosterolemia ect.

Familial hypercholesterolemia (LDL)receptor

Familial hypercholesterolemia is an autosomal dominant disease defined by the presence of mutation in the low-density lipoprotein receptor gene. It characterized by severely elevated plasma level of low density lipoprotein (Saint-Jore B et al., 2000) Heterozygouse familial hypercholesterolemia is the most prevalent and affect about 1 in 500 people. Affected heterozygous individual exhibit plasma cholesterol levels in the range of 300-400 mg/dl. Clinically, patients develop severe atherosclerosis involving multiple vascular territories but especially in coronary arteries. This leads to premature coronary atherosclerosis in fourth decade of life and often requiring bypass surgery early in life. Familial hypercholesterolemia patients were found to have one copy of mutated low density lipoprotein gene, which normally is located on chromosome 19p13. A variety of point, deletion and splice mutation have been described. Effect of this mutation leads to loss of its function. The mutant low density lipoprotein receptor has reduced affinity for removal of apolipoprotein B and E from the circulation. (Marian Ali,2000) Because Familial hypercholesterolemia is not only relatively common and associated with a high risk of early coronary artery disease but is treatable with LDL-C-lowering strategies, this genetic disorder meets the World Health Organization criteria for systematic screening. It has been estimated that familial hypercholesterolemia is properly diagnosed in only 15% of affected Canadians and as many as 30% of the patients do not survive their first myocardial infarction. Therefore, early detection of the disease has the potential to save life and prevent morbidities (Yuan et al.,2006)

Apolipoproteins are important components of lipoprotein particles. Current data shows that measurements of various apoliportroteins may help to predict the risks of coronary artery disease (Walldius & Jungner, 2004).Their involvement in synthesis and metabolism of lipoproteins are gradually being defined. In addition to stabilizing lipoprotein structure, some of lipoproteins act as ligands to tissue receptors, while others activate or inhibit enzymes involved in metabolic steps in circulation or tissues.

Apolipoprotein A deficiency.

Apolipoprotein A has two major forms A1 and A2. Apolipoprotein A1 is associated with high density lipoprotein cholesterol, and may be largely responsible for determining the plasma level of it. In addition apolipoprotein A1is the ligand for ATP-binding cassette protein and hence is involved in the docking procedure by which excess cholesterol in peripheral cells is externalized to high density liporprotein (Walldus & Jungner,2004). Its been reported that deficiency of apolipoprotein A1and low levels of high density liporprotein cholesterol were significantly related to an increased risk of cardiac mortality in patients with coronary artery disease(Walldus & Jungner,2004). Gene coding for apolipoprotein A1is located on the long arm of chromosome 11. Two polymorphism of apolipoprotein A1gene namely G to A substitution at -75bp of transcription start site and C to T substitution at +83 bp have been implicated in susceptibility to coronary artery disease and shown to influence plasma lipid levels (Taranjit et al.,2008) Many studies are controversial about the effect of particular gene polymorphism on pathogenesis of

atherosclerosis and coronary artery disease, but in combination with other polymorphic variants may be better predictor of disease risk in future.

Familial Defective Apolipoprotein B

Plasma lipoproteins are important determinants of atherosclerosis. Apolipoprotein B is a large amphipathic glycoprotein that exists in two forms and plays an important role in lipid metabolism. Both forms are produced by the same apolipoprotein gene: apolipoprotein b-48, which is required for chylomicron production in the small intestine and apolipoprotein; b-100, which is required for very low density lipoprotein production in the liver. In addition, apolipoprotein b-100 is the ligand for low density lipoprotein-receptor-mediated endocytosis of low density lipoprotein particles.(Whilfield et al.,2004) Mutation in apolipoprotein b-100 is characterized by increased plasma low density lipoproteins and very low density lipoprotein levels and has clinical features of hypercholesterolemia and premature coronary artery disease(Marian.Ali.2000). Familial defective apolipoprotein b-100 is a second relatively common cause of severe autosomal dominant hypercholesterolemia (Dzau. 2007). It is relatively common, with estimated frequency of 1 out of 500 in normal population and clinically not possible to distinguished from heterozygous familial hypercholesterolemia (Whilfield et al.,2004). The most common mutation is R3500Q which involves in substitution of a glutamine for arginine at the position 3500 (Whilfield et al.,2004). Other point mutation, R3500W and R3500C are rare cause of familial defective apolipoprotein b-100 (Real et al.,2003). Low density lipoproteins of affected individual will have a 90% decrease in affinity for low density lipoprotein receptor and low density lipoproteins clearance will be markedly impaired. Therefore routine screening is feasible and should be performed not only in subjects with clinical phenotypes but also in asymptomatic subjects with elevated low density lipoprotein levels.[Ali.2000] Treatment is similar to that of familial hypercholesterolemia with reliance on statin therapy, which both decrease very low density lipoprotein production and enhances clearance of very low density lipoprotein remnants[Dzau.2007]

Other mutations of Apolipoprotein b-100 may cause familial hypobetalipoprotenimia, which is characterized by hypocholesterolemia and resistance to atherosclerosis and coronary artery disease (Whitfield et al 2004).

Autosomal recessive hypercholesterolemia is caused by mutation in a putative adaptor receptor protein called ARH, that function in the internalization of low density lipoprotein receptor and cargo. This recessive disorder is characterized by severe hypercholesterolemia, xanthomatosis and premature coronary artery disease (Arca et al., 2002). Autosomal recessive hypercholesterolemia did not associated with the low density lipoprotein receptor or apoliporpotein b genes and exhibited an autosomal recessive inheritance, with the parents of the affected subjects having normal low density lipoprotein cholesterol values [Dzau.2007]. Interestingly, the function of low density lipoprotein receptor is near to normal in their cultured fibroblast but is impaired in lymphocytes, macrophages and hepatocytes. Low density lipoprotein receptor protein is present in these cells within normal limits but dispersed unevenly with most of the receptor residing on the plasma membrane, where it binds to low density lipoprotein but fails to internalize and degrade its cargo [Dzau.2007]. This specific disorder is rare and prevalent in Sardinia, Italy. Two ARH mutation were present in all 17 unrelated Sardinians families with ARH: a frameshift mutation (C432insA) in exon 4(ARH1) and nonsense mutation (C65G->A) in exon 1(ARH2) (Arca et al., 2002) . Screening of the coding

regions of ARH with similar phenotype in other random parts of the world revealed that only four out of 40 of them were positive for ARH1 mutation and all happened to be Italian.

Low circulating serum levels of high density lipoprotein cholesterol seem to be a frequent lipoprotein disorder in coronary artery disease and can be caused by either genetic or lifestyle factors. Many gene abnormalities can cause this lipid disorder.

Tangier disease is a rare disorder of lipid metabolism, characterized by extremely low levels of high density lipoprotein cholesterol. It is caused by mutation in the adenosine triphosphate-binding cassette transporter A1 (ABCA 1), also known as the cholesterol efflux regulatory protein (Kolovou et al.,2006). ABCA1 is a member of ATP-binding cassette transporter family. These integral proteins use ATP as a source of energy to transport variety of molecules including ions, vitamins, lipids, peptides proteins and number of hydrophobic compounds across intracellular and plasma membranes (Kolovou et al.,2006). Complete loss of ABCA1 function leads to severely decreased cellular cholesterol efflux and cholesterol ester accumulation in macrophages and other cells of the reticuloendothelial systems (Rust et al.,1999). Due to impaired ability to transport cholesterol out of the peripheral cells, it accumulates in body tissues. Therefore clinically, Tangier disease patients present with hepatosplenomegaly, enlarged tonsils, neurapathy and most important in our case atherosclerotic lesions. Studies suggested that carriers of defective adenosine triphosphate–binding cassette transporter A1 bear a moderately increased risk for coronary artery disease.

Sitosterolemia is a rare autosomal recessive disorder. It characterizes by markedly increased intestinal absorption of all sterols including cholesterol, shellfish and plant sterols and impaired resecretion of those sterols into bile. Affected individual have expanded body pools of cholesterol and very elevated plant-sterol levels. Affected individual will frequently develop accelerated atherosclerosis and premature coronary artery disease (Lu et al.,2001) In the studies, it has been mapped that this condition results from over 25 mutation in either ABCG5 or ABCG8 genes which located on chromosome 2 in band 2p21. These genes encode for adenosine triphosphate-binding cassette transporter protein (ABC) transporters, steroline-1 and steroline -2. Their functions are to limit intestinal absorption and stimulate excretion of noncholesterol sterols by liver into bile. Mutation of these transporters predispose to sterol accumulation and early artheroscrosis. Sitosterolemia can clinically present similar to homozygous familiar hypercholesterolemia in terms of elevated LDL-cholesterol especially in childhood around 300 mg/dl or 8 mmol/l. Therefore, it a possibility that sitosterolemia is frequently misdiagnosed with hyperlipidemia.

Although, all mentioned above lipid metabolism disorders are rare, they are high risk factors and directly related to incidence of coronary artery disease. Studies of these disorders gave researches a better understanding of lipoprotein and cholesterol metabolism. Whether hypercholesterolemia is caused by mutation in one gene or cumulative effect of several genes defects, early diagnosis is essential to start appropriate therapy. Most notable was development of Statins drugs which is a class of medications that was developed to lower plasma low density lipoprotein cholesterol levels.

MEF2A

Recently, the first non-lipid related disease causing gene was identified and proposed to be responsible for an autosomal dominant form of coronary artery disease (adCAD). However, when association is detected, it is still difficult to prove direct causality. False positive association may arise due to differences in ethnicity, age or sex [Dzau, 2007]. Several studies were performed to identify the role of human myocyte enhancer factor-2A (MEF2A) and its mutation in early coronary artery disease development .MEF2A is a protein that is coded by MEF2A gene which is located on chromosome 15q26. The seven-amino acid deletion of the human transcriptional myocyte enhancer factor-2A was reported to be a functional mutation. It disrupted nuclear localization of MEF2A, reduced MEF2A-medieted transcription activation. In addition it abolished synergistic activation by MEF2A and by transcriptional factor GATA-binding protein which involved in cell growth, with which MEF2A interact to regulate the expression of the downstream target genes, by dominant –negative mechanism (Wang et al.,2003). One study was dealing with large family with 13 patients who had an autosomal dominant patterns of CAD. Nine of 13 patients later developed acute myocardial infarction. Further genome wide association study linked data to chromosome 15q26. MEF2A also demonstrate its relevance to expression of coronary arteries endothelium (Wang et al.,2003). Another independent case-control studies was performed from 2003 to 2007 involving 726 individuals in China (Han et al.,2007). The results of that study suggested the disease-causing relationship between MEF2A and coronary artery disease. In subsequent study, Weng et al who report sequencing MEF2A in 300 patients with premature coronary artery disease and control, only 1 CAD patient was reported to have the mutation that wasn't found in the control group (Altshuler & Hirschhorn, 2005). Also another study in large Irish population showed no association between variation in the MEF2A gene and coronary artery disease (Horan et al.,2006).However, the absence of CAD in a control group wasn't confirmed by angiography. As science progresses it is becoming much easier to collect data but it also causes many controversy in its analysis. MEF2A plays an important role in cardiovascular biology, but its genetic variation contribution to pathophysiology in CAD should be further validated in order to make it the criteria for genetic testing for CAD.

Susceptability genes are those that increase or decrease the risk of developing disease and in combination with environmental factors and other genetic factors may contribute to pathogenesis of a disease. These genes might have less predictive values for developing a disease, but contribute a lot to understanding of pathophysiology process.

eNOS gene.

Nitric oxide (NO) is gas and is identified as endothelium derived relaxant factor. NO is synthesized from substrate L-arginine by endothelial cell nitric oxide synthase (eNOS) which encoded by nitric oxide synthase 3 gene (NOS3) that is located on 26-exon on chromosome 7. NO plays a few important roles in healthy endothelium such as regulation of vascular tone in coronary arteries, prevents leukocytes adhesion and platelets aggregation and inhibits vascular smooth muscle migration and proliferation. Biochemical and/or physical shear stress damages endothelium and impairs production of mediator such as nitric oxide. This might lead to vasoconstriction, loss of elasticity and promote platelets activation. Indirectly decrease in nitric oxide activity affect conditions such as hypertension and hypercholesterolemia which predispose to atherosclerosis [Dzau.2007]. Few clinical studies indicated relationship between different

eNOS gene polymorphism and atherosclerosis development. The most commonly studied variant is Glu298Asp which is conversion of glutamate to aspartate at the position 298 that results from G894T substitution within exon 7. In English study sample Glu298T variance has been reported to be associated with atherosclerotic coronary arteries. The mechanism is unknown but by altering mature protein activity can affect enzyme activity and decreased local nitric oxide synthesis. As a result of case-control study involve Iranian population (Salimi et al., 2006), we observed that the genotype frequencies differ from allele frequencies significantly between control and CAD patients. However, controversy brought up by other small studies that do not support these findings. Therefore the relevance of this data remains unclear. It might be because of subjects were picked from different ethnicity and genetic background. Further larger studies are needed in order to prove major effect of Glu298Asp polymorphism on disease development but for now it can be considered as indirect genetic marker associated with the disease.

TNF-alpha

Tumor necrosis factor alpha (TNF-alpha) is a pro-inflammatory cytokine that plays a role in pathogenesis of CAD. It was localized in atherosclerotic plaque in pathological studies. Macrophage derived TNF-alpha has important pro-inflamatory autocrine and paracrine effect. Stimulation of the cells by TNF-alpha, activate the cascade of events that lead to upregulation of various adhetion molecules. Overall contribution of TNF-alpha believed to be activation of influx of additional inflammatory cells and involvement in endothelial dysfunction (Dzau 2007). TNF-alpha has two receptors through which it exerts its effect TNFR1 and TNFR2. Polymorphisms in TNF-alpha and TNF receptor genes are hypothesized to affect secretion of this cytokine and relate to presence or severity of CAD. To investigate this matter scientist conducted a study 259 patients with angiographiclly confirmed CAD and control group (Allen et al., 2001). As a result of the statistical analysis no significant differences were found between polymorphism frequencies. Another European study was performed to investigate association of TNF-alpha gene polymorphism and its relationship with CAD via polymerase chain reaction-single-strand confirmation polymorphism and sequencing (Herrman et al.,1998). Although, TNF-alpha gene polymorphism have been associated with number of inflammatory diseases it is unlikely to contribute to development of CAD

Diseased-linked genes are those genes whose expression is linked or connected to the disease but the cause and effect relationship is not established. They might serve as biomarkers for the disease. A big chunk of genes were newly identified as disease-linked genes for CAD. The most studied include intracellular adhesion molecules-2, E-selectine gene, ect. The reason for interest in studying disease-linked genes is the fact that about half of the patients who are diagnosed with CAD do not have traditionally established risk factors. Therefore, the search for other biomarkers was proposed in order to help identify individuals who might be at risk.

Cellular adhesion molecules.

 Cell adhesion molecules (CAMs) are transmembrane proteins that involve in binding of cells with other cells or extracellular matrix. In healthy endothelium, expression of adhesion molecules is very limited. In diseased coronary artery, transendothelial T-cells and monocytes migration are mediated by these molecules **(figure4) Transendothelial migration**.
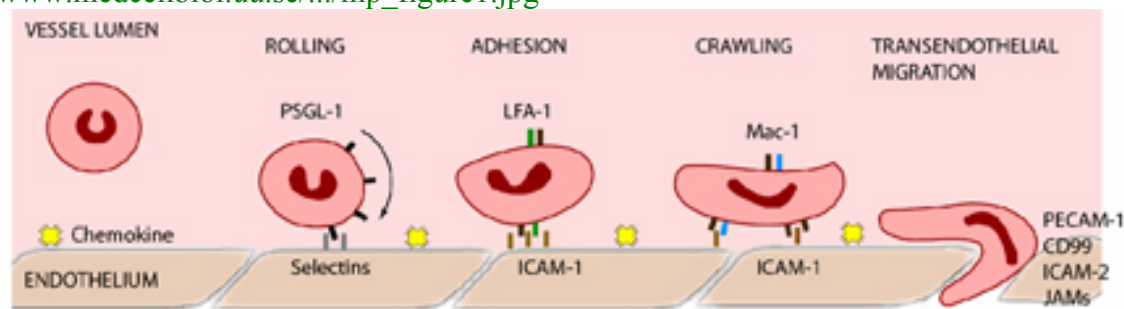
One of the CAMs category belong to Immunoglobulin superfamily. They are intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Animal and human studies earlier show that overexpression of adhesion molecules and endothelial cell activation causes accumulation of leukocytes in coronary tunica intima. According to latest reports, expressions of cellular adhesion molecules is stimulated by cytokins such as interleukin-1, tumour necrosis factor (TNF) and interferons-gamma (INF) (Ohta et al., 1999) Furthermore, the increased expression of cellular adhesion molecules in atherosclerosis shows in early stages of atherosclerotic development which presented by fatty streaks in inner layer of an artery as well as the latest fibro-fatty lesions. Linkage of levels of ICAM-1 and VCAM-1 to coronary artery disease that was established by study and can be considered as an independent risk predictor (Hajilooi et al.,2003).Although, genetic aspect of these molecules remain poorly known, recent multi-ethnic study was conducted to investigate relationship of 12/ICAM-1 and 7/VCAM-1 single nucleotide polymorphism (SNP) and coronary artery calcium and ICAM-1 SNP and circulating levels of soluble ICAM-1. It showed no significant association of ICAM-1 and VCAM-1 and coronary artery calcium in any ethnic groups, but polymorphism in ICAM-1 showed significant association with soluble ICAM-1 levels and was identified as a marker for atherosclerosis (Bielinski et al ., 2008).

Other family of cell adhesion molecules consists of E-selectin and P-selectin. Both of them suggested to play an important role in early and advance stages of coronary arthrosclerosis from earlier double-knockout mouse experiment. P-selectine is important in the transient attachment of leucocytes to endothelial cells and platelets (Carter et al., 2003). When endothelial cells get activated via mediators such as histamine and thrombin in inflammatory process, P-selectine translocates from internal to endothelial cell surface. Just like prior mentioned cell adhesion molecules, P-selectine also contributes to development of atherosclerosis by facilitating adhesion of leukocytes to injured endothelium and platelets. Soluble P-selectins were found in plasma in multiple conditions including heart disease.   Number of polymorphism has been identified in gene encoding P-selectin. THR715Pro polymorphism for example was reported to be associated with myocardial infarction in previous studies. In 2003 the study was performed that compared levels of soluble P-selectin in 249 individuals with CAD and 252 healthy control group and investigated the relationship between polymorphism of P-selectin and circulated soluble P-selectin levels and cardiovascular risk (Carter et al., 2003). It showed that levels of soluble P-selectin were higher in affected individuals than control group after adjustment for age sex and smoking status. Moreover, no significant association was found between P-selectin polymorphism in CAD affected patients and healthy control group. This study suggested that the relationship between Thr715Pro polymorphism and levels of soluble P-selectins exist but its mechanism is still unclear.

E-selectin also involved in adhesion of leukocytes to activated endothelium. Mutation of E-selectin gene, due to G to T mutation (G98T) in untranslated region of exon 2, was found to be significantly higher in frequency in patients with CAD than control group and thus suggests association with premature coronary artery disease (Zheng et al.,2001).Even after adjustment for other multifactor, mutation of G98T may be an independent risk factor for premature CAD. Another study was performed in 2006 within Saudi Arab population. It attempted to investigate amino acid change from serine to arginine at codon 128 (S128R), which corresponds toA>C nucleotide change at the position 561 (A561C), in the epidermal growth factor-like domain of the E-selectin gene that was previously implicated in pathogenesis of CAD in few ethnic groups (Abu-Amero et al., 2006). The frequency of mutation in 128r allele was found to be much higher in patients with CAD than control group, but lost association with adjustment for other CAD risk factors. The most recent study that investigated Association of E-selectin gene (S128R) polymorphism in patients with CAD within Indian population was consisted with previous findings. It revealed the increased frequency of E-selectin polymorphism in CAD patients but its interaction with other risk factors showed that in fact the significant determinant of coronary artery disease were presents of diabetes, hypertension, smoking habit, elevated serum triglycerides and low HDL level.

The involvement of cell adhesion molecules was established to play an important role in development of atherosclerosis. Overall studies suggest that neither the measurements of soluble adhesion molecules, nor screening for their gene polymorphisms cannot be an independent predictor of CAD. It may only offer some additional marker of atherosclerosis but cannot go beyond the assessment of conventional coronary artery disease risk factors.

Conclusion:
Throughout last decade significant advances in genetic studies were made which gave us a fair insight on pathogenesis of coronary artery disease. It no longer viewed as a simply degenerative disease or gradually accumulating lipid disease. Current views regard CAD as complex disease that involved in active process of alteration in cell intercommunication and cell signaling. At this point in time we can only speculate on assumed biological role of genetic polymorphisms that has been identified in relationship with CAD. Field in genetic studies is promising and expects to accelerate as future studies will focus on identifying new genetic polymorphism in connection to CAD. However, at this time coronary artery disease can't be simply diagnosed through genetic testing, but can only look for variants that increase the risk of developing it. Prediction of CAD via genetic polymorphism may eventually lead to clinical utilization, but now only physician can diagnose the disease based on a medical history, family history, risk factors and the results of medical tests. Genetic testing can not clearly tell if disease is going to develop or not, but if certain genetic markers are present, it may help us to modify our estimated risk of developing it. When considered genetics along with other individual's information including other risk factors may help individual to reduce the risks that we can control such as lifestyle, diet and exercise. Moreover, if clinician knows about increased genetic predispositions, they may stronger recommend lifestyle changes. In addition, high index of suspicion might result in early diagnostic application and close monitoring of patient with marked genetic polymorphism by their doctors. Currently a few of screening tests are available for asymptomatic individuals who are at risk for premature atherosclerosis and CAD such as computed tomography, ultrasound ect.

Hopefully in near future genetic studies will lead us to revolutionary changes in further understanding, ability to diagnose, prevent and treat the wold's biggest killer.

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A1 gene polymorphisms in the risk for coronary artery disease

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