

Role of Genetic Changes in the Progression of Cardiovascular Diseases

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ABSTRACT

This review aims to investigate the role of genetic changes in the development of cardiovascular diseases [CVD]. Oxidation of Low density Lipoprotein (LDL) and mutations in LDL receptors gene are a trigger for numerous of atherogenic events. Also, endothelial nitric oxide synthase (*eNOS*) plays an important role in vasodilatation of blood vessels through synthesis of nitric oxide. Three single base pair changes, 786T/C, 922A/G, and 1468T/A, have been identified in the promoter region of the *eNOS* gene and are associated with coronary spasm. Moreover, two distinct variable nucleotide tandem repeats (VNTRs) in introns 4 and 13 have been detected. The presence of a minimum of 38 CA repeats in intron 13 has been associated with an independent 2.2-fold increase in the risk of coronary artery disease [CAD]. Plasma glutathione peroxidase (*GPx-3*) maintains the vascular bioavailability of nitric oxide (NO), through depletion of reactive oxygen species. Mutation(s) or polymorphism(s) in the plasma *GPx-3* gene promoter may predispose to a thrombotic disorder, and constitute a genetic risk factor for thrombotic cerebrovascular disease. Hyperhomocysteinemia is another independent risk factor for atherosclerosis and arterial thrombosis. Severe hyperhomocysteinemia could be caused by cystathionine- β -synthase enzyme deficiency but it could be due to homozygosity of a common 677C/T point mutation in the coding region of the methylenetetrahydrofolate reductase (MTHFR) gene as a 3-fold increase in risk of CAD is associated with the MTHFR 677TT genotype. A second common variant in MTHFR 1298A/C is associated with decreased enzyme activity *in vitro* and *in vivo*, especially when occurring simultaneously with the 677 C/T polymorphism. Elevated fibrinogen, an essential component of the coagulation system, has been most consistently associated with arterial thrombotic disorders. Several polymorphisms (148C/T, 455G/A, and -854G/A) have been identified in the genes encoding the 3 pairs of fibrinogen polypeptide chains. The -455G/A, and -854G/A substitutions are the most physiologically relevant mutations. In addition the -455A allele has been associated with the progression of atheroma, and also with a 2.5-fold increase in risk of multiple lacunar infarcts in a cohort of elderly patients with stroke. It is concluded that genetic changes in the previously mentioned genes could play a significant role in the initiation and progression of CVD. This review provides useful information for both physicians and medical students whom are interested in human genetics which is related to cardiovascular diseases. (*Int J Biomed Sci* 2011; 7 (4): 238-248)

Keywords: cardiovascular diseases; genetic polymorphisms of nitric oxidesynthase; hyperlipidemia; hyperhomocysteinemia polymorphisms; paraoxonase polymorphisms

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INTRODUCTION

More than 1.5 million individuals have a myocardial infarction each year and coronary artery disease (CAD) is responsible for about 500,000 deaths with coronary atherosclerosis being the major cause of CAD (1, 2). The atherosclerotic process is an organized, active, lifelong process involving elements of chronic inflammation followed by repair in the artery wall (3, 4). The endothelium and many growth factors, cytokines, and vasoregulatory molecules are involved in the atherosclerotic process (3). Atherosclerosis begins with the appearance of fatty streaks in the intima (5). The fatty streaks can then be transformed into fibrous plaques by accumulation of lipid in smooth muscle and connective tissue. These plaques undergo vascularization, intraplaque hemorrhage, rupture, ulceration, and calcification thereby impeding arterial blood flow. Superimposition of thrombosis on a lesion can lead to myocardial infarction or sudden death (5, 6). Comprehensive studies to date have identified risk factors for cardiovascular diseases (CVD) which include older age, male sex, elevated total cholesterol levels, elevated low-density lipoprotein cholesterol levels, reduced high-density lipoprotein cholesterol levels, hypertension, diabetes mellitus, smoking, obesity, physical inactivity, and genetic changes in some enzymes (7-9). In this review, we are focusing on the genetic changes of genes which contribute to cardiovascular diseases susceptibility.

Genetics changes as risk factors affecting cardiovascular diseases

Genetics of Dyslipidemia. Serum cholesterol concentrations are profoundly influenced by the composition of dietary fat, with saturated fatty acids (SFAs) being the major determinant of serum cholesterol (10, 11). There are wide interindividual variations in total, LDL, and HDL cholesterol and lipoprotein responses (12-14). The existence of consistent hypo- and hyperresponders supports the hypothesis that the degree of responsiveness is related to genetic variation (15-17). Single-gene disorders are the most dramatic effects of the genetic contributions to atherosclerosis (9, 18, 19). However, most forms of the CVD are the product of many gene changes with small environmental effects. Cardiovascular disease has a multifactorial etiology with many established risk factors (20). Many studies have investigated this possibility and have largely focused on genes whose products affect lipoprotein metabolism, e.g. apolipoproteins, enzymes, and receptors (20).

In the last few years, much has been added to our understanding of the genetic basis of dyslipidemias and the role of different molecular players in the pathogenesis of these disorders. New information has also emerged regarding the efficacy and safety of intensive lipid-lowering therapies from many large-scale randomized clinical trials (21). Hypercholesterolemia as well as hypertriglyceridemia in the vast majority of patients are either secondary to diet, obesity, medications, or other disorders affecting lipoprotein metabolism, or are polygenic in origin. All the known genes defective in patients with monogenic hypercholesterolemias are involved in the receptor-mediated uptake of low-density lipoproteins (LDL) by the LDL receptor (LDLR) in hepatocytes (22, 23). Autosomal dominant familial hypercholesterolemia (FH) has been well recognized to be due to mutations in *LDLR* gene and is one of the most common inherited metabolic diseases (22). Previous studies revealed that *LDLR* mutations have been reported in 52-76% of patients meeting the clinical criteria for monogenic hypercholesterolemia (24). Loss of function or reduced LDLR number in the hepatocytes results in reduced clearance of plasma LDL and a 2- to 3-fold elevation in LDL cholesterol levels in heterozygous FH patients. Approximately half of these patients develop tendon xanthomas, xanthelasmas, premature corneal arcus, and coronary heart disease (CHD) which occurs in the fourth or fifth decades of life. Homozygous or heterozygous patients have a greater than 5-fold increase in plasma LDL cholesterol levels and often develop severe atherosclerosis before the age of 20 years (25).

Familial defective apolipoprotein B, an autosomal co-dominant disorder due to mutations in apolipoprotein B (*APOB*) gene, is indistinguishable from FH due to *LDLR* mutations. Certainly, the frequency of *APOB* mutations in patients with hypercholesterolemia phenotype is reported to be much less than *LDLR* mutations and varies from 0-11% in 40-year-old subjects in Slovakia (26). Currently, eight more missense mutations in the LDLR binding domain of the APOB have been identified (27-29). Previous studies revealed that familial defective apolipoprotein B-100 [FDB] patients have 20-25% lower LDL cholesterol levels compared with FH patients with *LDLR* mutations (30), and have lower risk of CHD (31). Recently, variants in *LDLR* gene have been classified as pathogenic and non-pathogenic in familial hypercholesterolemia [FH] (32). They have found that 862G>A, and 895G>A variants are causative of FH and pathogenic in the Spanish population (Etxebarria *et al.*, 2011). In another study, *LDLR* gene mutations: 81T>G, 1775G>A, 517T>C, 858C>A, 1352T>C,

1285G>A, 761A>C, 1195G>A, 1646G>A, and a deletion mutation in exon 4 have been detected in 164/254 Greek patients (33). In addition, no functional mutations in ARH, Apo B and PCSK9 genes have been detected in 40 patients with no LDLR gene defect (33). Moreover, Twenty-five mutations (24 LDLR, 1 APOB) were identified in 38/62 Mexico patients (34) and also a novel PCSK9 mutation, c.1850 C>A (p.Ala617Asp) was detected (34). In France, mutations in the LDLR gene were identified in 1003 ADH subjects representing 391 unique events with 46.0% missense, 14.6% frameshift, 13.6% splice, and 11.3% nonsense mutations, 9.7% major rearrangements, and 3.8% small in frame deletions/insertions mutations (35). Furthermore, mutations in the APOB gene were identified in 89 probands and in the PCSK9 gene in 10 probands. The respective contribution of each known gene to ADH in French cohort was: LDLR 73.9%, APOB 6.6%, PCSK9 0.7% (35).

Proprotein convertase subtilisin-like kexin type 9 (*PCSK9*) has been involved in autosomal dominant hypercholesterolemia since PCSK9 binds and favors degradation of the low-density lipoprotein receptor (LDLR) and thereby modulates the plasma levels of LDL-cholesterol. Interestingly, some loss-of-function in *PCSK9* variants has been reported to be associated with low levels of plasma LDL cholesterol and reduced risk of CHD (36, 37, 38). The clinical features of PCSK9 mutations in these patients are also similar to FH patients with *LDLR* mutations (39). LDLR adaptor protein (LDLRAP1 or ARH) promotes the clustering of LDLRs into the clathrin-coated pits on the basolateral surface of hepatocytes by coupling the cytoplasmic tail of LDLR to structural components of the clathrin coated pit (40) and thus is essential for LDLR-mediated endocytosis. Inactivating mutations in LDLRAP1 lead to retention of LDLRs on the apical surface, thereby severely reducing LDL uptake. Serum LDL cholesterol levels in ARH patients have been reported to be lower or similar to those seen in patients with homozygous *LDLR* mutations (41), but no differences were seen in the prevalence of planar, tuberous, or tendon xanthomas. Interestingly, ARH patients had markedly reduced prevalence of CHD compared with those with homozygous *LDLR* mutations (41). Another rare autosomal recessive disorder in which elevations of serum cholesterol might be observed along with markedly elevated plasma levels of plant sterols is sitosterolemia or alternatively called phytosterolemia. Mutations in two adjacent ATP-binding cassette transporters, ABCG8 (sterolin-2) and ABCG5 (sterolin-1) that regulate sterol transport at the apical surface of hepatocytes and enterocytes have been identified in patients with sitosterol-

emia (42, 43). ABCG5 and ABCG8 dimerize to form a functional complex necessary for efflux of dietary cholesterol and noncholesterol sterols from the intestine and liver (44). Thus, mutations in either of these two genes lead to increased absorption and accumulation of cholesterol and plant sterols.

Genetic polymorphisms of paraoxonase. Human paraoxonase/arylesterase (PON-1) (EC3.1.1.2) is a calcium-dependent glycoprotein that is present bound to HDL particles (45). Paraoxonase-1 (PON1) is an HDL-associated protein of 354 amino acids with a molecular mass of 43 000 Da. It is synthesized in the liver, and in serum is almost exclusively associated with HDL (46). The paraoxonase (PON) gene family is composed of three members (PON1, PON2, PON3) that share considerable structural homology and are located adjacently on chromosome 7 in humans (47). By far the most-studied member is PON1, a high-density lipoprotein-associated esterase/lactonase, also endowed with the capacity to hydrolyze organophosphorus insecticides, but all the three proteins prevent oxidative stress and fight inflammation (48). High-density lipoprotein (HDL) is an independent protective factor against cardiovascular disease (45). The enzyme paraoxonase-1 (PON-1) contributes to the antiatherogenic effects of HDL. The antiatherogenic potential of paraoxonase is derived from its capacity to hydrolyze oxidized lipids, phospholipids, and cholesterol ester hydroperoxides, thus preventing them from accumulating in LDL particles (45).

Oxidized LDL has been reported to trigger a number of putatively atherogenic events including the induction of proinflammatory molecules that lead to an increase in recruitment of inflammatory cells to the artery wall (46). HDL, on the other hand, is antiatherogenic, and its protective effects have been ascribed primarily to its ability to shuttle excess cholesterol from peripheral tissues to the liver in the reverse cholesterol transport pathway and also to its capacity to protect against LDL oxidation (47, 48). These protective effects of HDL have been attributed to the presence of various proteins associated with HDL in the circulation including apolipoprotein AI, lecithin cholesterol-acyl transferase (LCAT), and serum paraoxonases (PONs). The activity of plasma PON was lower in individuals with PON1 55 LM genotype than those with LL genotype (49). Gene polymorphisms of PON1 55 Met/Leu, PON2 148 Ala/Gly are involved in the morbidity of CHD by influencing the plasma activities of PON (49, 50). As a result, research in these fields has blended, with the goal of further elucidating the role of this polymorphic enzyme in disease and in an individual's response to exogenous

agents. Four additional polymorphisms have been found in the 5'-regulatory region of PON1 (51), but they have a lesser effect on PON1 protein levels (52). Complete re-sequencing of PON1 from several individuals has led to the identification of nearly 200 new single nucleotide polymorphisms, some in the coding regions and others in introns and regulatory regions of the gene (51). Though most of the new polymorphisms have not yet been characterized, a few have already provided explanations for discrepancies found when comparing PON1 status and PCR analysis of codon 192 (51). Variation in serum PON1 activity by its polymorphisms in both the coding and the 5'-regulatory regions of PON1 gene should also be considered (53, 54). A functional genomic analysis, however, provides a much more informative approach, as direct measurement of an individual's PON1 function (plasma activity) takes into account all polymorphisms that might affect activity.

Genetic polymorphism of endothelial nitric oxide (NO) synthase. Nitric oxide (NO), a product of the normal endothelium, has a variety of physiologic effects that converge to maintain normal endothelial function and an antithrombotic intravascular milieu. NO is a smooth muscle relaxant and inhibits the adhesion, activation, and aggregation of platelets (55). NO-dependent endothelial dysfunction is now accepted as a key initial step in atherothrombogenesis. NO is produced by the constitutive endothelial isoform of the nitric oxide synthases (NOSs) as a by-product of the conversion of L-arginine to L-citrulline (56). The endothelial NOS gene (*eNOS*) is located on chromosome 7q35-36 and comprises 26 exons (57). Numerous polymorphic sites have been identified in the *eNOS* gene, most of which are intronic. Two distinct variable nucleotide tandem repeats (VNTRs) in introns 4 and 13 have been examined in association with vascular disease. The intron 4 VNTR is characterized by the presence of either 4 (minor allele) or 5 (major allele) copies of a 27-bp repeat. A mild but significant reduction in plasma levels of nitrogen oxides had been observed in homozygotes of the minor allele (58). In intron 13, between 17 to 44 copies of a CA repeat have been described, and the presence of a minimum of 38 repeats has been associated with an independent 2.2-fold increase in the risk of CAD (59). Three strongly linked, single base pair changes, 786T/C, 922A/G, and 1468T/A, have been identified in the promoter region of the *eNOS* gene and associated with coronary spasm in the Japanese population (60). The authors found that the substitution at position 786T/C resulted in a significant reduction in *eNOS* gene promoter activity, whereas the other 2 polymorphisms had no effect. Japanese carriers of the 786CC genotype

have also been shown to have reduced cerebral blood flow, yet this effect was only observed among smokers (61). Two Italian studies found an association of the 786C allele with angiographically defined CAD, as well as with endothelial dysfunction among hypertensive individuals (62). However, numerous other studies have failed to confirm these observations. A G/T base-pair change at position 894 in exon 7 predicts a Glu298Asp substitution, which is the only polymorphism that alters the primary structure of the protein (63). This amino acid change influences enzyme stability, the 298Asp isoform being degraded more rapidly than its 298Glu counterpart. Numerous studies have identified the role for this polymorphism in atherothrombotic disease and risk of myocardial infarction (64-66).

Polymorphisms of Glutathione Peroxidase (GPx-3) Gene. There are 5 known isoforms of glutathione peroxidase [GPx]. Glutathione peroxidase (GPx-3) is a major antioxidant enzyme in plasma and scavenges reactive oxygen species (ROS) such as hydrogen peroxide and organic (lipid) hydroperoxides produced during normal metabolism or after oxidative insult (67, 68). Nitric oxide (NO), a major vasorelaxant and inhibitor of platelet function, can be inactivated rapidly by ROS. Therefore, GPx-3 contributes significantly to maintain the vascular bioavailability of nitric oxide level. In addition, the reduction of oxidant stress by GPx-3 activity also protects against post-translational modifications of fibrinogen by ROS and NO-derived oxidants that increase its thrombogenicity (69, 70). Two studies of families with idiopathic childhood stroke have provided clinical evidence for the importance of GPx-3 in the modulation of NO bioavailability and thrombosis (71, 72). The patients in these studies had hyperreactive platelets, and their plasma impaired the normal inhibition of platelet activation by NO. These findings were determined to be a consequence of a familial reduction in GPx-3 activity that correlated with decreased protein expression of this enzyme (73). Consistent with this role, functional transcription site of the GPx-3 gene showed that it is regulated by oxygen tension and redox state (73). Therefore, it is hypothesized that mutation(s) or polymorphism(s) in the plasma GPx-3 gene promoter may be responsible for the reduction in enzyme activity and predispose to a thrombotic disorder, thus constituting a genetic risk factor for thrombotic cerebrovascular disease. Supporting this hypothesis, it has been found that direct sequencing of GPx-3 promoter fragments obtained from CVT patients and controls revealed the presence of 8 promoter polymorphisms (-942 A3C, -927 T3C, -861A3T, -302 A3T, -284 T3A, -568 T3C, -518 T3C, and -65 T3C (74-77). A small series of

reports has demonstrated decreased *GPx-3* activity among patients with CAD (78, 79). Taken together, this evidence points to the *GPx-3* gene as a candidate gene for cardiovascular disease risk.

Another isoform of glutathione peroxidases is called GPx1, the key antioxidant enzyme in vascular endothelial cells and plays a protective effect against the presence of coronary artery disease [CAD] (80). It has been found that subjects with 198Pro/leu and 198Leu/leu variants genotype had a significantly higher risk of CAD compared to the 198Pro/Pro carriers in Chinese population (80). In addition, it has been found that the presence of Pro197Leu substitution of the GPx-1 gene may play a crucial role in determining genetic susceptibility to coronary-arteriosclerosis in type II diabetic patients (81).

DNA alterations in glutathione S-transferase (GSTM1 genotype). The glutathione (GSH) system is a major player in protection against a broad variety of toxic agents involved in the pathogenesis of several chronic degenerative diseases (82, 83). Most of the biological functions of this tripeptide (γ -glutamyl-L-cysteinyl glycine) depend on the reactivity of the thiol group of its cysteinyl residue (84). The reaction rate of GSH with electrophiles is mainly dependent on GSH-S-transferases (GST), which catalyze conjugation processes resulting in detoxification and excretion of water-soluble conjugates (85, 86). Genetic polymorphisms have been detected within this broad family of phase II drug metabolism isoenzymes. In particular, two of these encoding genes, identified as *GSTM1* (m) and *GSTT1* (u), have a null genotype in humans due to the deletions of both paternal and maternal alleles, resulting in lack of active proteins (87). Polymorphisms within *GSTT1* and especially *GSTM1* have been associated with cancer in various organs (88, 89). In addition, the null *GSTM1* genotype has been associated with elevated levels of DNA adducts, which are promutagenic and procarcinogenic lesions, in leukocytes, bronchi, and lung tissue (90, 91). DNA adducts have consistently been demonstrated in smooth muscle cells (SMC) of human abdominal aorta affected by atherosclerotic lesions (92). All of the surgical samples analyzed contained DNA adducts as measured by ^{32}P post labeling; and these adduct levels were correlated significantly with known atherogenic risk factors including age, number of currently smoked cigarettes, arterial pressure, blood cholesterol (total/high density lipoproteins), triglycerides, and oxidative DNA damage in the same cells (92). These findings were further supported by a study evaluating 30 thoracic aortic samples taken at autopsy; DNA adduct levels were

significantly correlated with total and low density lipoproteins and cholesterol and were higher in subjects with frequent atherosclerotic changes as compared to subjects with rare lesions (93).

Glucose-6-phosphate Dehydrogenase. Nitric oxide (NO) has been shown to modulate angiogenesis by mediating growth factor-stimulated endothelial cell migration and proliferation (94). NO is permissive for endothelial cell migration and enhances directional migration by inducing a switch from a stationary to a mobile phenotype (95, 96). In addition, NO promotes endothelial cell proliferation, and further, proliferating endothelial cells demonstrate increased expression of the endothelial isoform of nitric-oxide synthase (eNOS) compared with quiescent cells (97). Glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme in the pentose phosphate pathway, is the principal intracellular source of NADPH which in turn, is utilized directly as a cofactor for eNOS and, indirectly serves to maintain levels of another important cofactor, tetrahydrobiopterin, via de novo synthesis and the dihydrofolate reductase salvage pathway. In this manner, G6PD regulates both eNOS activity and NO levels (98). The association between G6PD activity and cell proliferation has been well described in several non-vascular cell types (99, 100). Growth factors, such as platelet-derived growth factor and epidermal growth factor, have been shown to increase cell proliferation by increasing both basal G6PD activity and G6PD expression (99). Adenoviral gene transfer of G6PD increases G6PD expression 2-3-fold in COS-7 cells and results in a marked increase in (^3H)thymidine incorporation (99). Similarly, NIH 3T3 cells transfected with human G6PD cDNA exhibited contact- and anchorage-independent growth (101). These findings demonstrated that G6PD activity was associated with cell proliferation in non-vascular cell lines. New evidence indicates that G6PD activity is associated with vascular endothelial cell proliferation. The mechanism by which G6PD enhances acquisition of an angiogenic phenotype has been characterized (102). G6PD activity modulates endothelial cell migration, proliferation, and tube formation by mediating NO levels. Therefore, G6PD serves as a novel regulatory determinant of the angiogenic phenotype (102). It has been demonstrated that intracellular NADPH levels, and not the production of ribose moieties by G6PD, are the critical determinant of cell growth (99). This suggests that one mechanism by which G6PD regulates endothelial cell migration, proliferation and tube formation is by synthesizing NO via eNOS, which has an absolute requirement for NADPH as a cofactor. The relationship between G6PD

and NOS activity has been demonstrated in several cell lines. For example, NO production is impaired in G6PD-deficient granulocytes stimulated with lipopolysaccharide or phorbol 12-myristate 13-acetate. Interestingly, this response resulted from a decrease in activity, not expression, of the inducible form of NOS (103). In vascular endothelial cells, it has been shown previously that G6PD influences eNOS activity by regulating substrate availability. Furthermore, gene transfer of G6PD to increase G6PD expression and activity in aortic endothelial cells resulted in enhanced NADPH levels, which in turn increased eNOS activity and NO generation measured as cGMP, nitrate, and nitrite levels (104).

Hyperhomocysteinemia Polymorphisms. Extensive epidemiological studies have indicated that elevated levels of homocysteine, a sulfur containing amino acid formed as an intermediary compound during methionine metabolism (8), are an independent risk factor for atherosclerosis and arterial thrombosis (8). The adverse effects of homocysteine are manifold but ultimately lead to endothelial dysfunction with associated platelet activation and thrombus formation (105). Although severe hyperhomocysteinemia can be caused by inborn errors of metabolism resulting - from cystathionine β -synthase deficiency. Elevation of homocysteine levels can be also due to homozygosity of a common 677C/T point mutation in the coding region of the methylenetetrahydrofolate reductase (*MTHFR*) gene. It may also be caused by nutritional deficiencies in the vitamin cofactors required for homocysteine metabolism (e.g. folate, vitamin B12, and vitamin B6). An inverse correlation between serum levels of these cofactors and homocysteine has been clearly documented (8, 106). Despite constituting the most common genetic cause of mild to moderate hyperhomocysteinemia, homozygosity for the *MTHFR* 677T allele, which leads to the substitution of valine by alanine in a potential folate-binding site and thermolability of the enzyme, has not been clearly associated with atherothrombotic disease (107). Other studies have shown a 3-fold increase in risk of CAD is associated with the *MTHFR* 677TT genotype; yet others found no association (108, 109). Brattstrom and colleagues pooled the results of 12 case-control studies and determined that individuals homozygous for the *MTHFR* 677T allele generally had fasting homocysteine levels higher than in heterozygous or normal individuals. Nevertheless, 8 of these studies did not report an increased risk of cardiovascular disease associated with the homozygous *MTHFR* 677TT genotype, and it was concluded that this polymorphism is a modest risk factor for arterial thrombosis. Similar results

were obtained in a meta-analysis of 40 observational studies involving 11,000 CAD patients and 12,000 controls: the overall carriers were 677TT genotype. The heterogeneity of findings was interpreted as being secondary to the interaction of the *MTHFR* 677C/T polymorphism and folate status. Evidence from previous reports supports this hypothesis: Jacques and colleagues determined that among individuals homozygous for the *MTHFR* 677T allele, homocysteine levels were only elevated when plasma folate concentrations were low (110). There was no significant difference in plasma homocysteine levels between 677TT homozygotes who had normal folate concentrations and individuals with the 677CC genotype (8, 110). Similarly, homozygotes with the lowest plasma folate concentrations had the highest homocysteine levels (111). Thus, folate levels can confound the interpretation of genotype association studies. Therefore folate supplementation might be a means of preventing atherothrombotic events in these patients. A second common variant in *MTHFR* 1298A/C is associated with decreased enzyme activity *in vitro* and *in vivo*, especially when occurring simultaneously with the 677 C/T polymorphism (112). Fasting homocysteine levels are significantly higher in individuals heterozygous for both substitutions compared with individuals who carry only the 677C/T variant (113). These polymorphisms are in linkage disequilibrium, rarely forming the *MTHFR* 677T/1298C haplotype, and their role in arterial thrombosis has not been studied (114).

Polymorphisms in Fibrinogen. Among the components of the coagulation system, elevated fibrinogen has been most consistently associated with arterial thrombotic disorders. Several mechanisms explain the association of increased fibrinogen with arterial thrombotic disease (115). In addition, high fibrinogen concentrations lead to the formation of a fibrin clot with thin and tightly packed fibers that has high thrombogenicity, possibly because the small pore size restricts access of fibrinolytic enzymes (116). Fibrinogen levels are strongly correlated with traditional vascular risk factors, including age, physical inactivity, hypertension, smoking, and features of the insulin resistance syndrome. Furthermore, fibrinogen is an acute-phase reactant, in part owing to its upregulation via activation of interleukin-6-responsive elements in the promoter of all 3 fibrinogen chains; the acute-phase response is strongly implicated in the development of arterial disease and may arise from e.g. viral infection, inflammatory stimuli, and smoking. Alternatively, elevated fibrinogen might reflect the inflammation associated with atherosclerosis rather than being a causal risk factor (117). Genetic factors are

estimated to contribute to 50% of the total variability in fibrinogen levels. Several polymorphisms have been identified in the genes encoding the 3 pairs of fibrinogen polypeptide chains (α , β , and γ); however, because the synthesis of the β -chain is rate-limiting *in vitro*, most studies have focused on this gene. The main β -chain variants include the Arg448Lys, BclI, -148C/T, -455G/A (*Hae*III), and -854G/A polymorphisms. The promoter polymorphisms are in strong linkage disequilibrium with each other. The -455G/A and -854G/A substitutions are the most physiologically relevant, because the respective alleles have distinct nuclear protein-binding properties and, reporter gene studies in HepG2 cells showed an increased rate of basal transcription in the less common -455A and -854A alleles (118, 119). Of the β -chain polymorphisms, the -455G/A has been the most extensively studied clinically. The -455AA genotype is present in 10% to 20% of the population and is correlated with fibrinogen levels that are 10% higher than in individuals with the GG genotype. Nevertheless, the relation between the -455G/A variant and the risk of arterial thrombotic disease is controversial; only limited case-control studies have been performed (120, 121). In a pooled analysis of inherited hemostatic risk factors and the risk of acute MI, homozygosity for the fibrinogen -455A allele was significantly though only marginally associated with a decreased risk of MI (122). In addition, the -455A allele has been associated with the progression of atheroma (123). In a recent cohort of elderly stroke patients, the presence of the -455A allele was associated with a 2.5-fold increase in risk of multiple lacunar infarcts but not with large-artery strokes. It has been suggested that elevated fibrinogen levels might predispose to the development of thrombosis primarily in small arteries (124). The association of other β -chain polymorphisms and arterial thrombosis remains unclear. In addition to the β -chain polymorphisms, a variant in the α -chain codes for a Thr312Ala substitution within its carboxy-terminal end (125), a region important for factor XIII-dependent processes, including α/α -chain cross-linking. Clots generated *in vitro* in the presence of the Ala312 fibrinogen isoform have more extensive α -chain crosslinking and in consequence, thicker fibers (126). Although in 1 study the Ala312 variant had a gene dose-related influence on poststroke mortality rates in subjects with arterial fibrillation (127, 128). More recent studies indicate that this α -chain polymorphism has a more relevant role in the pathogenesis of venous thrombo embolism (129). Taken together, these results suggest that Ala312-induced changes in clot structure predispose to embolization in both the arterial and venous vascular systems.

It is concluded that genetic changes in dyslipidemia, paroxonase, glutathione peroxidase, nitric oxide synthase, tetrahydrofolate reductase, glucose-6-phosphate dehydrogenase, and glutathione S-transferase could play a significant role in the initiation and progression of CVD.

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REFERENCES

1. Karasek J, Widimsky P, Ostadal P, Hrabakova H, *et al.* Acute heart failure registry from high-volume university hospital ED: comparing European and US data. *Am. J. Emerg. Med.* 2011 Jun 2. PMID: 21641150 (Epub ahead of print).
2. Orlova IA, Nuraliev EY, Yarovaya EB, Ageev FT. Prognostic value of changes in arterial stiffness in men with coronary artery disease. *Vasc. Health Risk. Manag.* 2010; 4 (6): 1015-1021.
3. Ross R, Fuster V. The pathogenesis of atherosclerosis. In: Fuster V, Ross R, Topol EJ, eds. *Atherosclerosis and coronary artery disease.* Philadelphia, PA: Lippincott-Raven. 1996; p441-601.
4. Okazaki S, Furukado S, Abe Y, Tanaka M, *et al.* Association of inflammatory markers and carotid intima-media thickness with the risk of cardiovascular events in high-risk patients. *Cerebrovasc. Dis.* 2010; 30 (2): 180-187.
5. Smet BJ, Zijlstra F, Rihal CS, Holmes DR, Mahmoud KD Sudden cardiac death: epidemiology, circadian variation, and triggers. *Curr. Probl. Cardiol.* 2011; 36 (2): 56-80.
6. Kyrle PA, Rosendaal FR, Eichinger S. Risk assessment for recurrent venous thrombosis. *Lancet.* 2010; 376 (9757): 2032-2039.
7. Falcão-Pires I, &Leite-Moreira AF. Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment. *Heart Fail Rev.* 2011. PMID: 21626163 (Epub ahead of print).
8. Ajabnoor MA, AL-Ama MN, Banjar Z, Rafee AA, *et al.* Homocysteine level and other biochemical parameters in cardiovascular disease patients with diabetes mellitus. *Med. Sci. Monit.* 2003; 9 (12): CR523-527.
9. Chen Q, Reis SE, Kammerer C, Craig W, *et al.* Association of anti-oxidized LDL and candidate genes with severity of coronary stenosis in the Women's Ischemia Syndrome Evaluation study. *J. Lipid. Res.* 2011; 52 (4): 801-807.
10. Sheweita SA. Risk factors of cardiovascular diseases: Hyperlipidemia, smoking, and diabetes mellitus. *J. Egypt. Societ. Cardiovasc. Dis.* 2010; 2: 23-35.
11. Czernichow S, Thomas D, Bruckert E. n-6 Fatty acids and cardiovascular health: a review of the evidence for dietary intake recommendations. *Br. J. Nutr.* 2010; 104 (6): 788-796.
12. McNamara DJ, Kolb R, Parker TS, *et al.* Heterogeneity of cholesterol homeostasis in man: Response to changes in dietary fat quality and cholesterol quantity. *J. Clin. Invest.* 1987; 79: 1729-1739.
13. Schaefer EJ, Lamon-Fava S, Ausman LM, *et al.* Individual variability in lipoprotein cholesterol response to National Cholesterol Education Program Step 2 diets. *Am. J. Clin. Nutr.* 1997; 65: 823-830.
14. Reiner Z, Sonicki Z, Tedeschi-Reiner E. Public perceptions of car-

- diovascular risk factors in Croatia: The PERCRO survey. *Prev. Med.* 2010; 51 (6): 494-496.
15. Katan MB, Beynen AC, de Vries JH, Nobels A. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. *Am. J. Epidemiol.* 1986; 123: 221-234.
 16. Beynen AC, Katan MB, van Zutphen LFM. Hypo- and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. *Adv. Lipid Res.* 1987; 22: 115-171.
 17. Talmud PJ, Drenos F, Shah S, Shah T, et al. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. *Am. J. Hum. Genet.* 2009; 85 (5): 628-642.
 18. Nabel, E.G. Cardiovascular disease. *N. Engl. J. Med.* 2003; 349: 60-72.
 19. Yong EL, Li J, Liu MH. Single gene contributions: genetic variants of peroxisome proliferator-activated receptor (isoforms alpha, beta/delta and gamma) and mechanisms of dyslipidemias. *Curr. Opin. Lipidol.* 2008; 19 (2): 106-112.
 20. Masson LF, McNeill G, Avenell A. Genetic variation and the lipid response to dietary intervention: asystematic review. *Am. J. Clin. Nutr.* 2003; 77: 1098-1011.
 21. Garg A, Simha V. Update on Dyslipidemia. *J. Clin. Endocrinol. Metab.* 2007; 92 (5): 1581-1589.
 22. Goldstein JL, Brown MS. The LDL receptor locus and the genetics of familial hypercholesterolemia. *Ann. Rev. Genet.* 1979; 13: 259-289.
 23. Eden ER, Naumova RP, Burden JJ, McCarthy MI, et al. Use of homozygosity mapping to identify a region on chromosome 1 bearing a defective gene that causes autosomal recessive homozygous hypercholesterolemia in two unrelated families. *Am. J. Hum. Genet.* 2001; 68 (3): 653-660.
 24. Van Aalst-Cohen ES, Jansen AC, Tanck MW, Defesche JC, et al. Diagnosing familial hypercholesterolaemia: the relevance of genetic testing. *Eur. Heart J.* 2006; 27: 2240-2246.
 25. Marais AD, Firth JC, Blom DJ. Homozygous familial hypercholesterolemia and its management. *Semin. Vasc. Med.* 2004; 4 (1): 43-50.
 26. Gasparovic J, Basistova Z, Fabryova L, Wsolova L, et al. Familial defective apolipoprotein B-100 in Slovakia: Are differences in prevalence of familial defective apolipoprotein B-100 explained by ethnicity?. *Atheroscl.* 2007; 194 (2): e95-107.
 27. Millan E, Ascaso JF, Carmena R. Evaluation of clinical diagnosis criteria of familial ligand defective apoB 100 and lipoprotein phenotype comparison between LDL receptor gene mutations affecting ligand-binding domain and the R3500Q mutation of the apoB gene in patients from a South European population. *Transl. Res.* 2008; 151 (3): 162-167.
 28. Kotzka J, Knebel B, Janssen OE, Schaefer JR, et al. Identification of a gene variant in the master regulator of lipid metabolism SREBP-1 in a family with a novel form of severe combined hypolipidemia. *Atheroscl.* 2011. PMID: 21645898 (Epub ahead of print).
 29. Soufi M, Sattler AM, Maerz W, Starke A, et al. A new but frequent mutation of apoB-100-apoB His3543Tyr. *Atheroscl.* 2004; 174: 11-16.
 30. Fouchier SW, Kastelein JJ, Defesche JC. Update of the molecular basis of familial hypercholesterolemia in The Netherlands. *Hum. Mutat.* 2005; 26: 550-556.
 31. Fouchier SW, Defesche JC, Kastelein JJ, Sijbrands EJ. Familial defective apolipoprotein B versus familial hypercholesterolemia: an assessment of risk. *Semin. Vasc. Med.* 2004; 4: 259-264.
 32. a, Schaefer JR, Scharnagl H, Baumstark MW, Schweer H, et al. Homozygous familial defective apolipoprotein B-100. Enhanced removal of apolipoprotein E-containing VLDLs and decreased production of LDLs. *Arterio. Thromb. Vasc. Biol.* 1997; 17: 348-353. b, Etxebarria A, Palacios L, Stef M, Tejedor D, et al. Functional characterization of splicing and ligand-binding domain variants in the LDL receptor. *Hum. Mutat.* 2011. doi: 10.1002 [Epub ahead of print].
 33. a, Maxwell KN, Fisher EA, Breslow JL. Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. *Proc. Natl. Acad. Sci. USA.* 2005; 102: 2069-2074. b, Diakou M, Miltiadous G, Xenophontos SL, Manoli P, et al. Spectrum of LDLR gene mutations, including a novel mutation causing familial hypercholesterolaemia, in North-western Greece. *Eur. J. Intern. Med.* 2011; 22 (5): e55-59.
 34. a, Timms KM, Wagner S, Samuels ME, Forbey K, et al. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. *Hum. Genet.* 2004; 114: 349-353. b, Vaca G, Vázquez A, Magaña MT, Ramirez ML, et al. Mutational analysis of the LDL receptor and APOB genes in Mexican individuals with autosomal dominant hypercholesterolemia. *Atheroscl.* 2011; 218 (2): 391-396.
 35. Marduel M, Carrié A, Sassolas A, et al., Molecular spectrum of autosomal dominant hypercholesterolemia in France. *Hum. Mutat.* 2010; 31 (11): E1811-1824.
 36. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, et al. Low, LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat. Genet.* 2005; 37: 161-165.
 37. Cohen JC, Boerwinkle E, Mosley Jr. TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N. Engl. J. Med.* 2006; 354: 1264-1272.
 38. Tibolla G, Norata GD, Artali R, Meneghetti F, et al. Proprotein convertase subtilisin/kexin type 9 (PCSK9): From structure-function relation to therapeutic inhibition. *Nutr. Metab. Cardiovasc. Dis.* 2011; 21 (11): 835-843.
 39. Haddad L, Day IN, Hunt S, Williams RR, et al. Evidence for a third genetic locus causing familial hypercholesterolemia. A non-LDLR, non-APOB kindred. *J. Lipid Res.* 1999; 40: 1113-1122.
 40. Garuti R, Jones C, Li WP, Michaely P, et al. The modular adaptor protein autosomal recessive hypercholesterolemia (ARH) promotes low density lipoprotein receptor clustering into clathrin-coated pits. *J. Biol. Chem.* 2005; 280: 40996-41004.
 41. Pisciotto L, Oliva CP, Pes GM, Di Scala L, et al. Autosomal recessive hypercholesterolemia (ARH) and homozygous familial hypercholesterolemia (FH): a phenotypic comparison. *Atheroscl.* 2006; 188: 398-405.
 42. Berge KE, Tian H, Graf GA, Yu L, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Scien.* 2000; 290: 1771-1775.
 43. Lee MH, Lu K, Hazard S, Yu H, et al. Identification of a gene, ABCG5 important in the regulation of dietary cholesterol absorption. *Nat. Genet.* 2001; 27: 79-83.
 44. Hazard SE, Patel SB. Sterolins ABCG5 and ABCG8 regulators of whole body dietary sterols. *Pflugers Arch.* 2007; 453: 745-752.
 45. Rodríguez F, Yarié E, Trujillo H, Macías A, et al. Concerning the Significance of Paraoxonase-1 and SR-BI Genes in Atherosclerosis. *Rev. Esp. Cardiol.* 2006; 59 (2): 154-164.
 46. Soran H, Younis NN, Charlton-Menys V, Durrington P. Variation in paraoxonase-1 activity and atherosclerosis. *Curr Opin Lipidol.* 2009; 20 (4): 265-274.
 47. Deakin S, Leviev I, Gomaschi M, Calabresi L, et al. Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high-affinity, saturable, desorption mechanism. *J. Biol. Chem.* 2002; 277: 4301-4318.
 48. a, Précourt LP, Amre D, Denis MC, Lavoie JC, et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. *Atheroscl.* 2011; 214 (1): 20-36. b, Costa LG, Cole TB, Jarvik GP, Furlong CE. Functional genomics of the paraoxonase (PON1) polymorphisms: effect on pesticide sensitivity, cardiovascular disease and drug metabolism. *Ann. Rev. Med.* 2003; 54: 371-392.
 49. Chi DS, Ling WH, Ma J, Xia M, et al. Study of the association between paraoxonase1 55 Met/Leu, paraoxonase2 148 Ala/Gly and manganese

- superoxide dismutase (MnSOD) 9 Ala/Val genetic polymorphisms and coronary heart disease. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2006; 27 (9): 808-813.
50. Tomás M, Latorre G, Sentí M, Marrugat J. The antioxidant function of high density lipoproteins: a new paradigm in atherosclerosis. 2004; 57 (6): 557-569.
 51. Jarvik GP, Jampsa R, Richter RJ, Carlson CS, *et al*. Novel paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status. *Pharmacogen*. 2003; 13: 291-295.
 52. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, *et al*. Effects of 5' regulatory - region polymorphisms on paraoxonase gene (PON1) expression. *Am. J. Hum. Genet*. 2001; 68: 1428-1436.
 53. Dahabreh IJ, Kitsios GD, Kent DM, Trikalinos TA. Paraoxonase 1 polymorphisms and ischemic stroke risk: A systematic review and meta-analysis. *Genet Med*. 2010; 12 (10): 606-615.
 54. Sanghera DK, Manzi S, Minster RL, Shaw P, *et al*. Genetic variation in the paraoxonase-3 (PON3) gene is associated with serum PON1 activity. *Ann. Hum. Genet*. 2008; 72 (Pt 1): 72-81.
 55. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. *Prog. Cardiovasc. Dis*. 1995; 38: 87-104.
 56. Rekká EA, Chrysselis MC. Nitric oxide in atherosclerosis. *Mini Rev Med Chem*. 2002; 2 (6): 585-593.
 57. Marsden PA, Heng HH, Scherer SW, Stewart RJ, *et al*. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J. Biol. Chem*. 1993; 268: 17478-17488.
 58. Tsukada T, Yokoyama K, Arai T, Takemoto F, *et al*. Evidence of association of the eNOS gene polymorphism with plasma NO metabolite levels in humans. *Biochem. Biophys. Res. Commun*. 1998; 245: 190-193
 59. Cheng J, Liu J, Li X, Yu L, *et al*. Effect of polymorphisms of endothelial nitric oxide synthase on ischemic stroke: a case-control study in a Chinese population. *Clin. Chim. Acta*. 2008; 392 (1-2): 46-51.
 60. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, *et al*. T(-7863C) mutation in the 5-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulat*. 1999; 99: 2864-2870.
 61. Yamada M, Huang Z, Dalkara T, Endres M, *et al*. Endothelial nitric oxide synthase dependent cerebral blood flow augmentation by L-arginine after chronic statin treatment. *J. Cereb. Blood Flow. Metab*. 2000; 20: 709-717.
 62. Rossi GP, Taddei S, Virdis A, Cavallin M, *et al*. The T-786C and Glu298Asp polymorphisms of the endothelial nitric oxide gene affect the forearm blood flow responses of Caucasian hypertensive patients. *J. Am. Coll. Cardiol*. 2003; 41: 938-945.
 63. Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, *et al*. A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. *Hum. Genet*. 1998; 103: 65-69.
 64. Ellul J, Markoula S, Marousi S, Galidi A, *et al*. Association of endothelial nitric oxide synthase polymorphism G894T with functional outcome in acute stroke patients. *Neurol. Res*. 2011; 33 (8): 835-840.
 65. Shankarishan P, Borah PK, Ahmed G, Mahanta J. Prevalence of endothelial nitric oxide synthase (eNOS) gene exon 7 Glu298Asp variant in North Eastern India. *Indian J. Med. Res*. 2011; 133 (5): 487-491.
 66. Bhanoori M. Endothelial nitric oxide synthase (eNOS) variants in cardiovascular disease: pharmacogenomic implications. *Indian J. Med. Res*. 2011; 133 (5): 464-466.
 67. Maddipati KR, Marnett LJ. Characterization of the major hydroperoxidoreducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase. *J. Biol. Chem*. 1987; 262: 17398-17403.
 68. Takahashi K, Avissarm N, Whitinm J, Cohenm H. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. *Arch Biochem Biophys*. 1987; 256: 677- 686.
 69. Upchurch GR Jr, Ramdev N, Walsh MT, Loscalzo J. Prothrombotic consequences of the oxidation of fibrinogen and their inhibition by aspirin. *J. Thromb. Thrombolysis*. 2004; 5: 9-14 .
 70. Vadseth C, Souza JM, Thomson L, Seagraves A, *et al*. Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species. *J Biol Chem*. 2004; 279: 8820-8826.
 71. Freedman JE, Loscalzo J, Benoit SE, Valeri CR, *et al*. Decreased platelet inhibition by nitric oxide in two brothers with a history of arterial thrombosis. *J. Clin. Invest*. 1996; 97: 979-987.
 72. Kenet G, Freedman J, Shenkman B, Regina E, *et al*. Plasma glutathione peroxidase deficiency and platelet insensitivity to nitric oxide in children with familial stroke. *Arterioscler. Thromb. Vasc. Biol*. 1999; 19: 2017-2023.
 73. Bierl C, Voetsch B, Jin RC, Handy DE, *et al*. Determinants of human plasma glutathione peroxidase (GPx-3) expression. *J. Biol. Chem*. 2004; 279: 26839-26845.
 74. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. *Prog. Cardiovasc. Dis*. 1995; 38: 87-104.
 75. Dogru-Abbasoglu S, Kanbagli O, Bulur H, *et al*. Lipid peroxides and antioxidant status in serum of patients with angiographically defined coronary atherosclerosis. *Clin. Biochem*. 1999; 32: 671-672.
 76. Voetsch B, Jin RC, Bierl C, *et al*. Promoter Polymorphisms in the Plasma Glutathione Peroxidase (GPx-3) Gene : A Novel Risk Factor for Arterial Ischemic Stroke Among Young Adults and Children. *Stroke*. 2007; 38: 41-49.
 77. Voetsch B, Jin RC, Bierl C, Deus-Silva L, *et al*. Role of Promoter Polymorphisms in the Plasma Glutathione Peroxidase (GPx-3) Gene as a Risk Factor for Cerebral Venous Thrombosis. *Stroke*. 2008; 39: 303-307.
 78. Aykac-Toker G, Uysal M. Lipid peroxides and antioxidant status in serum of patients with angiographically defined coronary atherosclerosis. *Clin. Biochem*. 1999; 32: 671-672.
 79. Muzakova V, Kandar R, Vojtisek P, Skalicky J, *et al*. Selective antioxidant enzymes during ischemia/reperfusion in myocardial infarction. *Physiol. Res*. 2000; 49: 315-322.
 80. Tang NP, Wang LS, Yang L, Gu HJ, *et al*. Genetic variant in glutathione peroxidase 1 gene is associated with an increased risk of coronary artery disease in a Chinese population. *Clin. Chim. Acta*. 2008; 395 (1-2): 89-93.
 81. Nemoto M, Nishimura R, Sasaki T, Hiki Y, *et al*. Genetic association of glutathione peroxidase-1 with coronary artery calcification in type 2 diabetes: a case control study with multi-slice computed tomography. *Cardiovasc. Diabetol*. 2007; 6: 23-30.
 82. Meister A. Metabolism and function of glutathione. In *Glutathione: Chemical, Biochemical and Medical Aspects* (Dolphin D, Poulson R, Avramovic O, eds). *New York: John Wiley*. 1989; p361-374.
 83. Sheweita SA, Tilmisany AK. Cancer and phase II drug-metabolizing enzymes. *Curr. Drug Metab*. 2003; 4 (1): 45-58.
 84. Chasseaud LF. The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. *Adv. Cancer Res*. 1979; 29: 175-274.
 85. Ketterer B, Harris JM, Talaska G, Meyer DJ, p361-374. The human glutathione S-transferase supergene family, its polymorphism, and its effect on susceptibility to lung cancer. *Environ. Hlth. Perspect*. 1992; 98: 87-94.
 86. Hayes JD, Pulford DJ. The glutathione-S-transferase supergene family: regulation of GST and the contribution of isoenzyme to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol*. 1995; 30: 445-600.

87. Seidegård J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. *Proc. Natl. Acad. Sci. USA.* 1988; 85: 7293-7297.
88. Hou SM, Ryberg D, Falt S, Deverill A, et al. GSTM1 and NAT2 polymorphisms in operable and non-operable lung cancer patients. *Carcinog.* 2000; 21: 49-54.
89. D'Errico A, Taioli E, Chen X, Vineis P. Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomark.* 1996; 1: 149-173.
90. Perera FP. Environment and cancer: who are susceptible? *Scie.* 1997; 278: 1068-1073.
91. Rojas M, Cascorbi I, Alexandrov K, Kriek E, et al. Modulation of benzo(a)pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogen.* 2000; 21: 35-41.
92. De Flora S, Izzotti A, Walsh D, Degan P, et al. Molecular epidemiology of atherosclerosis. *FASEB J.* 1997; 11: 1021-1031.
93. Binkova' B, Strejc P, Boulbeli' k O, Sta'vkova' Z, Chva'talova' I, et al. DNA adducts and human atherosclerotic lesions. *Homeostas.* 1999; 39: 168-173.
94. Noiri E, Peresleni T, Srivastava N, Weber P, et al. Nitric oxide is necessary for a switch from stationary to locomoting phenotype in epithelial cells. *Am. J. Physiol.* 1996; 270: C794-C802.
95. Noiri E, Lee E, Testa J, Quigley J, et al. Podokinesis in endothelial cell migration: role of nitric oxide. *Am. J. Physiol.* 1998; 274 (1 Pt 1): C236-244.
96. Noiri E, Hu Y, Bahou WF, Keese CR, et al. Permissive role of nitric oxide in endothelin-induced migration of endothelial cells. *J. Biol. Chem.* 1997; 272 (3): 1747-1752.
97. Zollner S, Aberle S, Harvey SE, Polokoff MA, et al. Changes of endothelial nitric oxide synthase level and activity during endothelial cell proliferation. *Endothelium.* 2000; 7: 169-184.
98. Presley T, Vedam K, Druhan LJ, Ilangovan G. Hyperthermia-induced Hsp90{middle dot}eNOS Preserves Mitochondrial Respiration in Hyperglycemic Endothelial Cells by Down-regulating Glut-1 and Up-regulating G6PD Activity. *J. Biol. Chem.* 2010; 285 (49): 38194-38203.
99. Tian WN, Braunstein LD, Pang J, Stuhlmeier KM, et al. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. *J. Biol. Chem.* 1998; 273: 10609-10617.
100. Di Monaco M, Pizzini A, Gatto V, Leonardi L, et al. Role of glucose-6-phosphate dehydrogenase inhibition in the antiproliferative effects of dehydroepiandrosterone on human breast cancer cells. *Br. J. Cancer.* 1997; 75: 589-592.
101. Kuo WY, Tang TK. Effects of G6PD overexpression in NIH3T3 cells treated with tert-butyl hydroperoxide or paraquat. *Free Radic. Biol. Med.* 1997; 24: 1130-1138.
102. Leopold JA, Walker J, Scribner AW, Voetsch B, et al. Glucose-6-phosphate Dehydrogenase Modulates Vascular Endothelial Growth Factor-mediated Angiogenesis. *J. Biol. Chem.* 2003; 278 (34): 32100-32106.
103. Tsai KJ, Hung IJ, Chow CK, Stern A, et al. Impaired production of nitric oxide, superoxide, and hydrogen peroxide in glucose 6-phosphate-dehydrogenase-deficient granulocytes. *FEBS Lett.* 1998; 436: 411-414.
104. Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. *Free Radic Biol. Med.* 2009; 47 (12): 1673-1706.
105. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N. Engl. J. Med.* 1998; 338: 1042-1050.
106. Selhub J, Jacques PF, Wilson PW, Rush D, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA.* 1993; 270: 2693-2698.
107. Kang SS, Zhou J, Wong PW, Kowalisy J, et al. Intermediate homocysteinemia, a thermolabile variant of methylenetetrahydrofolate reductase. *Am. J. Hum. Genet.* 1988; 43: 414-421.
108. Fletcher O, Kessling AM. MTHFR association with arteriosclerotic vascular disease? *Hum. Genet.* 1998; 103: 11-21.
109. Kelly PJ, Rosand J, Kistler JP, Shih VE, et al. Homocysteine, MTHFR 677C3T polymorphism, and risk of ischemic stroke: results of a meta-analysis. *Neurol.* 2002; 59: 529-536.
110. Jacques PF, Bostom AG, Williams RR, Ellison RC, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulat.* 1996; 93: 7-9.
111. Ma J, Stampfer MJ, Hennekens CH, Frosst P, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulat.* 1996; 94: 2410-2416.
112. Weisberg I, Tran P, Christensen B, Sibani S, et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol. Genet. Metab.* 1998; 64: 169-172.
113. Weisberg IS, Jacques PF, Selhub J, Bostom AG, et al. The 1298A3C polymorphism in methylenetetrahydrofolate reductase (MTHFR): *in vitro* expression and association with homocysteine. *Atheroscler.* 2001; 156: 409-415.
114. Ogino S, Wilson RB. Genotype and haplotype distributions of MTHFR677C3T and 1298A3C single nucleotide polymorphisms: a meta-analysis. *J. Hum. Genet.* 2003; 48: 1-7.
115. Folsom AR. Hemostatic risk factors for atherothrombotic disease: an epidemiologic view. *Thromb. Haemost.* 2001; 86: 366-373.
116. Collet JP, Soria J, Mirshahi M, Hirsch M, et al. Dusart syndrome: a new concept of the relationship between fibrin clot architecture and fibrin clot degradability: hypofibrinolysis related to an abnormal clot structure. *Blood.* 1993; 82: 2462-2469.
117. Sakkinen PA, Wahl P, Cushman M, Lewis MR, et al. Clustering of pro-coagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. *Am. J. Epidemiol.* 2000; 152: 897-907.
118. Brown ET, Fuller GM. Detection of a complex that associates with the B₂ fibrinogen G-455-A polymorphism. *Blood.* 1998; 92: 3286-3293.
119. Hamsten A. Two common, functional polymorphisms in the promoter region of the fibrinogen gene contribute to regulation of plasma fibrinogen concentration. *Arterioscler. Thromb. Vasc. Biol.* 1999; 19: 3063-3070.
120. Bhague I, Poirier O, Nicaud V, Evans A, et al. Fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction: The ECTIM Study. Etude Cas-Temoins sur l'Infarctus du Myocarde. *Circulat.* 1996 ; 93 : 440-449.
121. Endler G, Mannhalter C. Polymorphisms in coagulation factor genes and their impact on arterial and venous thrombosis. *Clin. Chim. Acta.* 2003; 330: 31-55.
122. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, et al. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. *Circulation.* 2001; 104: 3063-3068.
123. de Maat MP, Kastelein JJ, Jukema JW, Zwinderman AH, et al. 455G/A polymorphism of the -fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men: proposed role for an acute-phase reaction pattern of fibrinogen. REGRESS group. *Arterioscler Thromb. Vasc. Biol.* 1998; 18: 265-271.
124. Martiskainen M, Pohjasvaara T, Mikkelsen J, Mantyla R, et al. Fibrinogen gene promoter 455 A allele as a risk factor for lacunar stroke. *Stroke.* 2003; 34: 886-891.

125. Baumann RE, Henschen AH. Human fibrinogen polymorphic site analysis by restriction endonuclease digestion and allele-specific polymerase chain reaction amplification, identification of polymorphisms at positions A-312 and B-448. *Blood*. 1993; 82: 2117-2124.
126. Standeven KF, Grant PJ, Carter AM, Scheiner T, *et al.* Functional analysis of the fibrinogen Thr312Ala polymorphism: effects on fibrin structure and function. *Circul*. 2003; 107: 2326-2330.
127. Carter AM, Catto AJ, Grant PJ. Association of the -fibrinogen Thr312Ala polymorphism with poststroke mortality in subjects with atrial fibrillation. *Circul*. 1999; 99: 2423-2426.
128. Curran JM, Evans A, Arveiler D, Luc G, *et al.* The fibrinogen T/A312 polymorphism in the ECTIM study. *Thromb. Haemost.* 1998; 79: 1057-1058.
129. Carter AM, Catto AJ, Kohler HP, Ariens RA, *et al.* -Fibrinogen Thr312Ala polymorphism and venous thromboembolism. *Blood*. 2000; 96: 1177-1179.