Heart and Cardiovascular Disorders

Review Article

ISSN: 2631-9969

The multifaceted role of genetic polymorphisms in atherosclerosis

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Abstract

Atherosclerosis is one of the most investigated disorders among cardiovascular anomalies and its clinical manifestations are responsible for the deaths of over 17 million patients worldwide. Atheromatous plaques block the blood flux and bring about long-term serious consequences. Although the precise cause is still unknown, atherosclerosis onset is related to risk factors such as high cholesterol levels, high blood pressure, obesity, diabetes, smoking, imbalanced diet, family history and genetic predisposition. The inherited risk of atherosclerosis is increased in families with multiple affected members. Therefore, family history of atherosclerosis account for genetic counseling and genetic testing. In the present review, we explore the influence of genetic polymorphism of target genes (*eNOS*, *GSTM1*, *GSTT1* and *TP53*) and on atherosclerosis. Here we show the possible influence of genetic polymorphisms and atherosclerosis. Knowledge of genetic variants that increase susceptibility of atherosclerosis may launch more efficient methods of prevention, diagnostic, treatment, and genetic counseling in the important field of cardiology. Although discrepancies are seen, each target gene show promisor activity as atherosclerosis markers that could be used in diagnostic and treatment.

Introduction

Atherosclerosis is the most common disorder among cardiovascular anomalies and its clinical manifestations are responsible for the high rate of mortality (17 million deaths) observed worldwide [1-3]. The main characteristic of the disease is the progressive narrowing of artery lumen due to the development of atheromatous plaques, which limits the flow of oxygen-rich blood to organs and other parts of the body. The blood flux blockage performed by atheromatous plaques can affect large and medium-size arteries leading to severe consequences. Although the precise cause is still unknown, atherosclerosis onset is related to risk factors such as high cholesterol levels [4], high blood pressure [5], obesity [6,7], diabetes [8,9], smoking [10,11], imbalanced diet [7], family history and genetic predisposition [12,13].

Risk factors act synergistically and stimulate the synthesis and release of proinflammatory cytokines, oxidative degradation of lipids and oxidative stress. The outcome is increased cell adhesion [14] and endothelium-derived relaxing factors and anticoagulants a reduction [15,16]. Atherosclerosis is asymptomatic at first [17], but it might evolve to peripheral [18] or coronary [19] artery disease, ischemic stroke [20], myocardial infarction [21], severe thrombosis [22], artery stenosis [23], kidney [24,25], liver [26,27], lung [28] and intestines [29] injuries. Atherosclerosis is a complex condition, usually starts early in life and progresses as people age [26]. In addition, several works have shown the influence of DNA damage to atherosclerosis [30-32].

Inflammation is intimately linked to atherosclerosis lesion formation and progression [33]. The endothelial dysfunction observed in atherosclerosis is responsible for the proinflammatory and prothrombotic environment generated by atheromatous plaques, lesions and inflammation, which might lead to severe clinical manifestations of the disease [16]. Atheromatous plaques comprises macrophages, monocytes, low-density lipoproteins (LDL), cholesterol crystals, smooth muscle cells, cellular debris and extracellular matrix. Atherosclerosis initiates as monocytes from blood adhere to the endothelium and then diapedeses between endothelial cells to enter the sub-endothelial region, differentiate into macrophage and accumulate lipoprotein [34]. LDL is then oxidized leading to oxygen reactive species formation (ROS), lesion and consequently inflammation response driven by cytokine production. Next, macrophages are recruited, they phagocyte oxidized LDL turning into foam cells. The inflammatory estate is aggravated by death of foam, a process which will recruit more macrophages and lead to the production of more foam cells [35,36].

Atherosclerosis has a complex genetic trait. Hundreds of genes have been reported to be related to atherosclerosis and they regulated a myriad of physiological processes, including macromolecules metabolism, coagulation, detoxification, endothelial function and coagulation. Several of those genes are polymorphic and variations in their sequence contribute to the molecular pathogenesis of the disease [37-41]. The inherited risk of atherosclerosis is increased in families with multiple affected members [42]. Therefore, family history of atherosclerosis account for genetic counseling and genetic testing. In the present review, we explore the influence of genetic polymorphism of target genes (eNOS, GSTM1, GSTT1 and TP53) and on atherosclerosis.

Endothelial nitric oxide synthase

Endothelial dysfunction is characterized by apoptosis, down regulation of the endothelial nitric oxide synthase (eNOS), a reduction of nitric oxide levels and an increase in ROS formation. These features lead to inflammation and are highly related to atherosclerosis onset

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Key words: *atherosclerosis, eNOS, GSTM1, GSTT1, TP53*

Received: August 01, 2018; **Accepted:** August 28, 2018; **Published:** August 31, 2018

and progression. The most important eNOS function is the regulation of vascular tonus and integrity [43], producing nitric oxide (NO), an important relaxing factor that acts on vasodilation [44,45], leukocyte adhesion [46], antithrombotic [47] and antioxidant processes [48], and platelet aggregation [49]. NO diffuses freely across vascular smooth muscle cell membranes and activates a cascade pathway resulting in vasodilation [50]. eNOS is highly polymorphic and studies have showed that variations in eNOS gene might increase the susceptibility to vascular [37,38] and other diseases such as diabetes [51], retinopathies [52] and erectile dysfunction [51,53].

Among the most frequent polymorphisms of the eNOS gene are on exon 7 (894 G/T), intron 11(-30 A/G), intron 18(27A/C), intron 23(10G/T) within the coding region and T786C, A922G and T1468A in the flanking region of the gene [54]. G894T and T786C are the most investigated eNOS polymorphisms.

The relation between the T786C eNOS polymorphism and risk of atherosclerosis has been contradictory. Ethnic groups may respond differently regarding susceptibility to disease [55]. T786C polymorphism features the substitution of thymine (TT genotype) to cytosine (CC or TC genotypes) at the 786 locus of the gene. Carriers of the mutant allele (homozygotes or heterozygotes) might have increased susceptibility to vascular diseases. Higher frequencies of the wild type has been identified in the Brazilian [37,56], Greek [57], Chilean [58] and Italian [59] population with no statistically significant result for the influence of the polymorphism on cardiovascular diseases. Conversely, the mutant allele was more frequent in the Turkish [60] and Ukrainian [61] populations, for which the polymorphism may be an increased risk factor for atherosclerosis.

The replacement of guanine for a thymine at position 894 of the eNOS gene characterizes the G894T polymorphism. G894T carriers show less NO production and it may be a factor that increases patients' susceptibility to atherosclerotic disease. The frequency of the mutant allele was more frequent in the Brazilian [62], Mexican [63] and Italian [64] populations. The wild type allele is more frequent in England [54], Germany [65], Turkey [66], South Africa [67] and in the African-American population [68]. The wild type is also predominant in the Asia population such as Korea [69], Japan [70] and India [71]; but interestingly, TT the genotype is practically absent. Although, the distribution of these polymorphisms vary among populations, eNOS gene seems to be related to the pathogeny of artherosclerosis. Future studies are required to shed some light on observed discrepancies and bring a better understanding of the relation between polymorphisms and disease.

Glutathione S-transferase

Glutathione S-transferase (GST) belong to a family of genes and proteins from phase II metabolic isozymes. They catalyze the reduced form of glutathione into xenobiotic substrates, turning such compounds more water-soluble in order to fulfill their function of detoxification [72]. GSTs polymorphisms are linked to innumerous diseases and anomalies such cancer [73], drug resistance [74], diabetes [75], atherosclerosis [39,40], and other inflammatory [76] and autoimmune diseases [77,78].

Glutathione S-transferase Mu 1 (GSTM1) and glutathione S-transferase theta-1 (GSTT1) take part in the detoxification of carcinogens, drugs, xenobiotics and products generated by oxidative stress. GSTs from the mu and theta classes are particularly polymorphic. Null mutations in GSTM1 have been associated with a large variety of diseases and an increased susceptibility to toxins [39,73]. Null mutations in GSTM1 increases the susceptibility diseases as well [40,75]. The GSTM1 present genotype was identified in a higher frequency in the Brazilian [39], Turkish [79] and British [80] population. They found statistically significant difference comparing the null and present genotype in healthy and atherosclerotic patients, suggesting that the null genotype is a risk factor for the disease.

GSTT1 present genotype has been found to be more frequent in certain populations from Brazil [40,81], India [82] and Serbia [83]. Interistingly, a study conducted in a Turkish population has shown that GSTT1 null genotype was nine-fold more frequent in atherosclerotic patients compared to healthy ones [79]. Another interesting fact is the difference between frequencies of null and present GSTT1 genotypes in males and females, it has been found that the present genotype is more frequent in males [40,84-86].

Understanding the polymorphisms that affect the GST family, especially GSTM1 and GSTT1, is important in order to elucidate their connections with onset and progression of atherosclerosis. Moreover, the results from worldwide indicates that having GSTM1 and GSTT1 as molecular markers of atherosclerosis in order to establish better prevention, diagnostic, treatment, and genetic counseling in cardiovascular diseases.

Tumor protein 53

Tumor protein 53 (TP53) gene is classified as an oncogene (tumor suppressor gene). The protein coded by TP53 (p53) exerts a function of protector of the genome, in a way to maintain the genetic material stability [87]. The protein p53 regulates cell cycle and cellular growth [88], apoptosis [87], transcription [89] and angiogenesis [90]. The TP53 location within the human genome is 17p13.1. The gene is proximately 20 kb and comprises 11 exons. The nucleotide sequence is highly conserved and structurally homologous across species from different kingdoms [91]. DNA damage induces TP53 expression which consists of cell cycle arrest, repair or apoptosis [87].

Mutations on p53 alters the capacity of the cell to control mitosis [92]. This single nucleotide polymorphism changes de structure of the protein, losing its capacity to bind to DNA [93] and consequently increasing the risk of disease. The most frequent p53 polymorphism is characterized by a substitution of the base guanine by a cytosine at codon 72 and consequently within the protein the amino acid arginine is substituted for proline. There are three possible genotypes for the TP53 (arg/arg, arg/prol, prol/prol).

Polymorphic studies about p53 polymorphisms and atherosclerosis show some discrepancies. Studies performed with Italian [94,95], Kwait [96], Brazilian [41,97] did not find any relation between the polymorphic p53 and occurrence of atherosclerosis. Conversely, studies performed in different population from Italy [98,99], Chile [100] and Japan [101] have shown an intrinsic influence of p53 codon 72 polymorphism on atherosclerosis. Clearly, the large number of function exerted by TP53 and its protein is somehow related to a series of inflammation disorders such as atherosclerosis, however, more studies should be performed in order to identify what causes lie underneath this influence. Large samples studies and wide genome association studies may shed some light on answered questions.

Concluding remarks

Knowledge of genetic variants that increase susceptibility of atherosclerosis may launch more efficient methods of prevention, diagnostic, treatment, and genetic counseling in the important field of cardiology. Molecular biology and molecular genetics have contributed

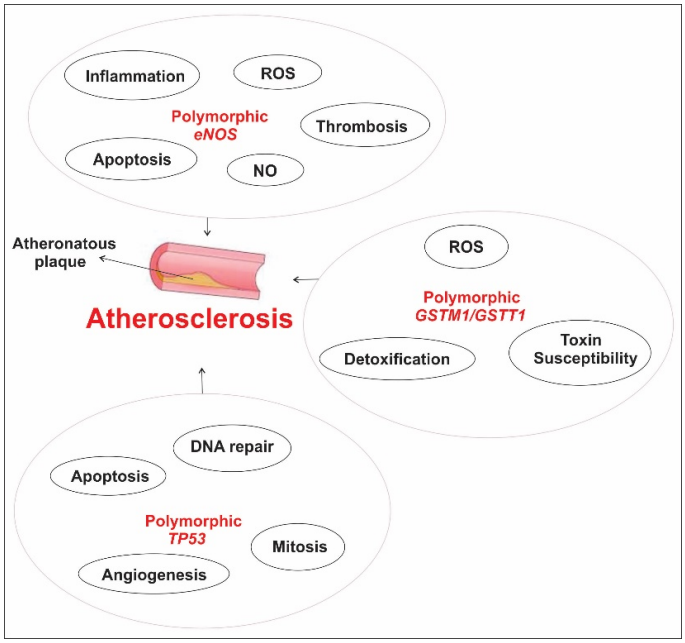

Figure 1. A schematic view of important polymorphic genes related to atherosclerosis. The *eNOS* gene produces nitric oxide (NO), important to prevent reactive oxygen species formation. Polymorphic *eNOS* induces inflammation, ROS and thrombosis and reduces apoptosis rate and NO production. *GSTM1* and *GSTT1* are related to detoxification processes and mutations in their sequence lead to increased susceptibility to toxins and carcinogens. Polymorphic *TP53* promotes cell division control loss, reduced apoptosis and angiogenesis rate and affects DNA repair

to our understanding of the complex and multifactorial diseases such as cardiovascular disorders. The most important polymorphic genes related to atherosclerosis, eNOS, GSTM1, GSTT1 and TP53 (Figure 1) may show some conflicting results but they have definitely shown a relationship with onset and progression of atherosclerosis.

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