

GENETIC POLYMORPHISM OF VKORC 1 AND KLOTHO GENES ASSOCIATED WITH ATHEROSCLEROSIS

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Abstract

Atherosclerosis is a complex multifocal arterial disease involving interactions of multiple genetic and environmental factors. Advances in techniques of molecular genetics have revealed that genetic polymorphisms significantly influence susceptibility to atherosclerotic vascular diseases. A large number of candidate genes, genetic polymorphisms and susceptibility loci associated with atherosclerotic diseases have been identified in recent years and their number is rapidly increasing [1].

Genetic studies have suggested that KLOTHO gene polymorphism and vitamin K-epoxide reductase complex subunit 1 gene, VKORC 1 might be associated with vascular atherosclerosis and calcification. In this review we focus on KLOTHO and VKORC 1 gene polymorphisms and their potential role in the physiopathology of atherosclerosis.

Keywords: atherosclerosis, genetic polymorphism, VKORC1, KLOTHO gene.

POLIMORFISMUL GENETIC AL VKORC1 ȘI KLOTHO ÎN ASOCIERE CU ATEROSCLEROZA

Rezumat

Ateroscleroza este o boală arterială complexă ce implică multiple interacțiuni între factorii de mediu și genetici. Tehnicile avansate de genetică moleculară relevă faptul că polimorfismele genetice influențează semnificativ susceptibilitatea de a dezvolta boala vasculară aterosclerotică. În ultimii ani au fost identificate un număr larg de gene și polimorfisme genetice asociate cu ateroscleroza și numărul lor crește rapid.

Studiile genetice sugerează asocierea polimorfismului genei KLOTHO și al genei VKORC 1 cu ateroscleroza și calcificările vasculare.

Acest review se axează pe rolul potențial al polimorfismelor genelor KLOTHO și VKORC 1 în fiziopatologia aterosclerozei.

Cuvinte cheie: ateroscleroză, polimorfism genetic, genele VKORC1, KLOTHO.

BACKGROUND

Atherosclerosis is probably one of the most complex diseases. Although we know only a small fraction of the genes involved in CAD, we can estimate a lower limit of the total number of genes by considering the genetics of the known genetic risk factors for CAD [2]. Genome-wide association studies have identified single nucleotide polymorphisms

associated with coronary artery disease. Rapidly advancing technology has led to expectations that genomics will provide radical insights into cardiovascular disorders, leading to improved diagnostic tools, therapeutics and prognosis. The genetic model underlying this relationship assumes that several variable loci (polymorphisms) on several genes contribute to the variability of known or unknown intermediate phenotypes that affect disease risk. The contributing alleles are supposed to be common and interact with each other and with non-genetic factors on the

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phenotypes [3].

The heritability of atherosclerosis (the fraction of the disease explained by genetics) has been high in most studies, frequently exceeding 50%.

The genetics of atherosclerosis and its complications may be viewed from different perspectives. Classically, epidemiologists are unable to evaluate atherosclerosis directly, as a consequence, they concentrate their efforts on its risk factors, mainly dyslipidemia, hypertension, diabetes, obesity and clinical manifestations, coronary heart disease (CHD), stroke, sudden death, peripheral artery disease. This has led to a considerable number of genetic studies focused on risk factors. The pathophysiological mechanisms that underlie the initiation, evolution and complications of atherosclerosis offer still another perspective for genetic research and in addition reveal the complex architecture of the disease [2].

The most likely candidates for genetic variants predisposing to atherosclerosis are those coding for the lipid transport proteins, but other variants of genetic polymorphisms related to atherosclerosis were studied during the past decade. Among these, we mention VKORC and Klotho alleles.

VKORC AND ATHEROSCLEROSIS

The warfarin-sensitive vitamin K epoxide reductase enzyme complex (VKORC) converts vitamin K epoxide to vitamin K hydroquinone, a required cofactor for the post-translational gamma-carboxylation of several blood coagulation factors and other vitamin K-dependent proteins, such as osteocalcin (bone *Gla* protein, BGP) and matrix *Gla* protein (MGP) [4,5]. MGP has also been found in blood vessel walls and in association with atherosclerotic plaques [6,7].

The vitamin K epoxide reductase complex subunit 1 gene (*Vkorc1*) appears to be a critical component of the VKORC.

Recently, numerous single nucleotide polymorphisms (SNPs) were identified on chromosome 16 in the gene encoding the vitamin K epoxide reductase complex subunit 1 (VKORC1) [4,5] of which several reflect 3 main natural haplotypes of VKORC1. Five SNPs (rs 9934438, rs 9923231, rs 8050894, rs 2359612, and rs 7294) were found to be in strong linkage disequilibrium ($D_{0.9}$ and $r^2_{0.9}$), indicating that any of these could reflect VKORC1 haplotypes. One of these SNPs, rs 993448 or VKORC1 1173C_T, is as informative about coumarin sensitivity as 5 VKORC1 haplotypes which predicted warfarin dose requirement and together accounted for 96% to 99% of the total haplotypes in European-American White populations. The VKORC1 1173C_T SNP is likely to be one of the putative functional SNPs of the VKORC1 gene.

The T-allele of this SNP modifies the effectiveness of coumarins, which reduce the activity of the VKORC1 enzyme. In carriers of the T-allele, additional inhibition

by coumarins had a higher impact on hemostasis than in those with the 1173CC genotype. Beyond hemostatic effects, different studies suggest an influence of vitamin K-dependent proteins on bone mineralization and arterial calcification [8,9]. The key function of MGP is to inhibit calcification in cartilage and arteries. Hereto, MGP has to be activated by carboxylation of its 5 glutamic acid residues, which is mediated by vitamin K hydroquinone. During carboxylation, the hydroquinone becomes oxidized to vitamin K epoxide. Vitamin K hydroquinone is derived from dietary vitamin K intake or by recycling of the epoxide. First, the epoxide is reduced to vitamin K, catalyzed by the Vitamin K epoxide reductase (VKOR). Second, vitamin K is further reduced to the hydroquinone. This second reduction step differs between tissues. VKORC1 seems crucial for reduction of vitamin K in extra hepatic tissues, whereas in the liver also other enzymes such as DT diaphorase mediate further reduction of vitamin K into the hydroquinone. Inhibition of the VKORC1 with coumarins for coagulation factors could be antagonized by dietary vitamin K but not for MGP as extra hepatic protein. Price et al used the implication fundamental difference between tissues on the activation of vitamin K-dependent proteins by giving warfarin in combination with vitamin K to young rats [10]. Thus mineralization of arteries could be promoted without inducing fatal bleeding before measurement of arterial calcification. In human studies the recommended daily allowance for vitamin K was shown to be sufficient for maintaining functional hemostasis, whereas undercarboxylation of at least 1 nonhemostatic protein was observed. More recent studies have shown that, despite their similar in vitro cofactor activity, the 2 forms of vitamin K differ concerning their ability to counteract the effects of warfarin [11]. High doses of vitamin K1 could counteract the effect of coumarins on the coagulation factors in the liver but not in the extrahepatic tissue. In the extrahepatic tissue only vitamin K2 was able to inhibit warfarin-induced arterial calcification. This implicates different effects of VKORC1 activity and of vitamin K1 and K2 intake on coagulation factors as hepatic proteins and on extrahepatic proteins such as MGP.

A diminished functionality of the VKORC1 enzyme is therefore not likely to influence coagulation factors and hemostasis in persons with normal vitamin K1 intake and not using coumarins. A lifelong decreased activity of the VKORC1 enzyme, however, might impair MGP activity and by this increase the risk of vascular calcification. This could be further worsened by reduced intake of vitamin K2. The association between impaired carboxylation of MGP and intimal and medial vascular calcification in humans has been described before [12]. Calcification of the aortic far wall has shown to be a good indicator of vascular calcification [2].

In the past few years many clinical studies were carried out to highlight the role of the VKORC gene in the

atherosclerotic process.

A 2-Center prospective cohort study proved that a single nucleotide polymorphism of vitamin K epoxide reductase complex subunit 1 (VKORC1) was reported to have association with arterial vascular disease and was associated with athero-thrombotic complication after drug-eluting stent implantation [13].

Besides effects on hemostasis, vitamin K-dependent proteins play a role in bone mineralization and arterial calcification. A large Clinical and Population Study (The Rotterdam Study), investigated the association between the VKORC1 1173C>T polymorphism and calcification of the aortic far wall in Whites. The T-allele of this polymorphism was significantly associated with a higher risk of aortic calcification [14].

KLOTHO AND ATHEROSCLEROSIS

Klotho is a transmembrane protein that, in addition to other effects, provides some control over the sensitivity of the organism to insulin and appears to be involved in aging. Its discovery was documented in 1995 by Masuda et al. [15].

The Klotho protein is a novel β -glucuronidase (EC number 3.2.1.31) capable of hydrolyzing steroid β -glucuronides. Genetic variants in *KLOTHO* have been associated with human aging and Klotho protein has been shown to be a circulating factor detectable in serum that declines with age [16].

Klotho-deficient mice manifest a syndrome resembling accelerated human aging and display extensive and accelerated arteriosclerosis. Additionally, they exhibit impaired endothelium dependent vasodilation and impaired angiogenesis, suggesting that Klotho protein may protect the cardiovascular system through endothelium-derived NO production.

Although the vast majority of research has been based on lack of Klotho, it was demonstrated that an over-expression of Klotho in mice might extend their average life span between 19% and 31% compared to normal mice [17].

A functional variant of the *Klotho* gene has been shown to be associated with high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and stroke, suggesting an association of this genetic variation with vascular atherosclerosis [18].

Japanese scientists were the first to learn that variations in klotho, named after the Greek Fate purported to spin the thread of life, made mice age quickly and similarly to humans, developing conditions similar to atherosclerosis and osteoporosis that are practically unheard of in the furry critters.

In a Korean population, Rhee et al. [19] showed that some *Klotho* gene polymorphisms were related to coronary artery disease and hypertension. Kim et al. [20] also reported that *Klotho* gene polymorphisms were risk

factors for ischemic stroke. Notably, the *Klotho* gene G-395A polymorphism was shown to be related to these atherosclerotic diseases in both studies.

Hopkins scientists (Howard Hughes Medical Institute), who first linked Klotho to shorter life expectancy in humans, developed a massive clinical study, their report appeared in the 2003 May issue of the American Journal of Human Genetics [21]. The study includes more than 900 people between the ages of 39 and 59. The scientists determined which klotho variants each participant had, and linked that to the person's clinical diagnosis and risk factors, which had been gathered as part of the older studies.

One of these studies, called SIBS-I, included 520 apparently healthy siblings of hospitalized patients, and 97 of them were discovered to have undetected atherosclerosis. The other, called SIBS-II, included only African Americans and found that 56 of 436 participants had undetected atherosclerosis. For the SIBS-I group, roughly 15 percent of the 373 participants with two "good" copies of klotho had undetected atherosclerosis. Of the 135 people with one copy of the KL-VS version of klotho, about 25 percent had hidden coronary artery disease, as did about 40 percent of the 12 people with two copies of KL-VS. Similar results were seen for SIBS-II.

Overall, those with at least one copy of KL-VS had approximately twice the risk of having atherosclerosis than others. Start adding known risk factors to the presence of KL-VS, and risk really shot up, the researchers discovered.

For example, smokers with at least one KL-VS copy had more than seven times the risk of non-smokers without the gene variant. Smokers who had low (less than 40 mg/dl) "good" cholesterol, or HDL, and at least one copy of KL-VS had about 10 times the risk of comparable individuals without the variant. However, high levels of "good" cholesterol, known as HDL, significantly reduced the risk associated with the KL-VS variant.

How exactly Klotho increases the risk of atherosclerosis, or exacerbates the effects of smoking, is still unknown. The Klotho gene carries the blueprint for a protein that seems related to enzymes known as beta-glycosidases, but no specific target for the klotho protein has been identified. The KL-VS gene results in two changes in the protein's sequence that seem to influence how cells secrete the protein and how well it functions, the researchers say.

CONCLUSIONS

Atherosclerosis, the primary cause of coronary heart disease (CAD) and stroke, is a disorder with multiple genetic and environmental contributions. Genetic and epidemiologic studies have identified a surprisingly long list of genetic and non-genetic risk factors for CAD [2].

Identification of atherogens may allow prediction from an early age of individuals who are predisposed to develop premature atherosclerosis, including premature

coronary artery disease [22].

Important and valid studies evaluated VKORC and the role that this gene plays in atherosclerosis. Besides the well known effects on hemostasis, vitamin K dependant proteins such as vitamin K epoxide reductase complex subunit 1 (VKORC1), play a role in arterial calcification and T allele polymorphism is significantly associated with a higher risk of atherosclerosis.

HDL cholesterol levels are inversely associated with the detrimental effect of a dysfunctional KLOTHO protein in humans [23].

HDL cholesterol levels in the high normal range seem to be completely protective against KLOTHO dysfunction [24,25]. The underlying mechanism of this relationship is still unknown, but is obvious however, that HDL and KLOTHO modulate similar signaling pathways [26].

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