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*Review*

# GENETIC POLYMORPHISMS, PLASMA LEVELS OF LIPOPROTEIN (A) AND ITS POSSIBLE LINKS WITH DEGENERATIVE AORTIC STENOSIS

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## Summary

Degenerative aortic stenosis is the second most common acquired valvular heart disease in adults (after mitral insufficiency) and the second most common cause for cardiac surgery (after coronary heart disease). The reasons for the occurrence of these diseases (congenital abnormality of the valve: bicuspid aortic valve disease, advanced renal failure, impaired calcium-phosphorus metabolism) have been established only in a small portion of these patients. The absence of a specific reason, causing calcification and narrowing of the aortic valve in recent years has challenged researchers to start investigating genetic factors that may correlate with the development of degenerative aortic stenosis. Regardless of the conducted studies, knowledge and identification of predictive genetic factors in the occurrence and progression of aortic stenosis are still insufficient. It is assumed that a specific genetic variant in the Lipoprotein (a) locus (LPA locus), reflected by the Lipoprotein (a) [Lp(a)] plasma levels, is connected to the pathology of aortic stenosis in multiethnic groups. The study of the genetic nature of aortic stenosis and significance of Lp(a) plasma levels and genetically determined variations of its structure associated with the manifestation and progression of valvular calcification in the future might provide predictive intervention. Similar studies relating to genetic polymorphisms in LPA locus, plasma concentrations of Lp(a) and their correlation with aortic stenosis have not been conducted in Bulgaria so far.

**Key words**: aortic stenosis, genetic polymorphisms, rs1544410, LPA locus, valvular calcification, "kringle" IV repeat.

## Introduction

Degenerative aortic stenosis is the second most common acquired valvular heart disease in adults (after mitral insufficiency) and the second most common cause of cardiac surgery (after coronary heart disease). However, only in an insufficient number of patients with congenital abnormality of the valve (bicuspid aortic valve disease), advanced renal failure, impaired calcium-phosphorus metabolism the occurrence can be attributed to a particular reason. The absence of a specific reason that leads to calcification and narrowing of the aortic

valve has, in the last few years, made researchers investigate genetic factors that may correlate with the development of degenerative aortic stenosis. Regardless of the studies conducted, the knowledge and identification of predictive genetic factors in the occurrence and progression of aortic stenosis are still insufficient. It is assumed that a specific genetic variant in the Lipoprotein (a) locus (LPA locus), reflective of Lipoprotein (a) [Lp(a)] plasma levels is connected with the pathology of aortic stenosis in multiethnic groups. According to Vahanian et al., aortic stenosis occurs in 2% to 7% of the population over 65 [1], while in other studies the frequency reported varies in a wider range – 2.6% to 21-29 % [2]. These differences are most likely due to the use of different evaluation criteria applied by research teams, i.e. whether they included only clinical manifestations of degenerative aortic stenosis (severe or moderate), or whether the sub-clinically ongoing degenerative valvular lesions were taken into account, including those with high calcium levels without any formation of hemodynamically significant obstruction of the left ventricular ejection, as assessed by CT or MRI.

Aortic stenosis has a high social significance not only because of its prevalence but also because it may progress for a long period without any clinical manifestation (7-8 years on the average). With the onset of severe aortic stenosis and the accompanying clinical symptoms, the probability of surviving for two years without surgical intervention is 50% [3]. Even in the case of mild symptoms, patients with aortic stenosis are at increased risk of cardiovascular complications, such as coronary artery disease and myocardial infarction, whether or not directly correlated with the disease. All of the above contribute to increase the risk of cardiovascular mortality in people with degenerative aortic valve disease without hemodynamically significant stenosis by 50% [4-7].

The most common morphological form of aortic stenosis in patients over the age of 50 is the calcification of the aortic valve, which occurs in 80% of cases. For a long time it was thought that this medical condition was due to the gradual wear of the aortic valve associated with age. Recently, however, it has been suggested that this is an active process of chronic inflammation involving biochemical, genetic and humoral factors [8, 9]. In many cases, it resembles atherosclerosis but there are some significant differences. For example, in valvular calcification, an increased level of specific inflammatory markers has been observed, such as cytokines and leukocytes in the area of the lesion [antibodies against oxidized low density lipoprotein (LDL), cytokines - transforming growth factor-β1, interleukin-1β, monocytes, macrophages, T-lymphocytes, foam cells, etc.]. Factors that are associated with the development of this pathological process are not entirely clear, but it is assumed that the starting point is mechanical damage of the aortic valve endothelium, with subsequent transition of lipids in the interstitium of the valve and initiation of the process of inflammation. A group of lipoproteins playing an important role in the process of atherogenesis – LDL and Lp(a) are found in the initial lesions, which subsequently undergo oxidative modification. Oxidized lipoproteins are highly cytotoxic and stimulate inflammatory response and the mineralization that follows [10-12]. The increased expression of endothelial cell adhesion molecules such as E-selectin leads to migration of inflammatory cells (macrophages and activated T-lymphocytes) to the subendothelial area, where they release enzymes such as matrix metalloproteinases that break down collagen, elastin and proteoglycans in the aortic valve leaflet.

The process of mineralization is typical for both inflammation and calcified valvular lesions. It is usually localized close to the area of inflammation and it is observed in all stages of the disease. Some of the histopathological signs of surgically excised samples resemble the processes of osteogenesis.

The processes of calcification and atherosclerosis appear to be similar, but they differ in their cellular and mineral composition of the lesions. Lipid-enriched smooth muscle cells and macrophages are the leading features of vascular atherosclerotic plaques, but they are not common in the valvular lesions seen in aortic stenosis. Mineralization (calcification) occurs earlier and is more apparent in degenerative aortic stenosis, as compared to vascular atheromas. These differences confirm the hypothesis that although the two processes bear a strong resemblance, they are not identical. Moreover, this partly explains why only 40% of the patients with severe degenerative aortic stenosis have significant coronary artery disease, and why the majority of patients with significant coronary stenosis do not show signs of aortic valvular calcification [2, 4]. Another important

clinical evidence for the differences between these two processes is the effect of lipid-lowering therapy with statins, which influences the course of the atherosclerotic process in the vessels, but does not affect the progression of degenerative aortic stenosis [13]. This fact was confirmed by a meta-analysis including nine clinical studies that demonstrated the effectiveness of statin therapy in non-rheumatic aortic stenosis. The results obtained showed no significant improvement in the average annual change of transvalvular aortic gradient, as regards the rate of lumen narrowing between the patients receiving statins and the one receiving placebo [14]. According to recent research, the studied polymorphisms can be divided into groups depending on the level of evidence for their relationship with degenerative valvular stenosis.

## Discussion

The genetic polymorphisms associated with apolipoprotein B (ApoB), angiotensin-converting enzyme (ACE), interleukin-10 (IL-10), and Lp(a) correlate with the development of calcific valvular stenosis with a relatively high level of evidence provided. Another group of polymorphisms within the oestrogen receptor α, calcium sensing receptor, IL-10, paraoxonase/arylesterase 1, myosin VIIA, angiotensin II receptor type 1, elastin, interleukin 36 gamma, genes have moderate correlation with the risk of developing valvular calcification. The third group of polymorphisms with a low level of correlation with development of degenerative valvular calcification includes rs1544410 polymorphism within the vitamin D (1, 25-dihydroxyvitamin D3) receptor gene, E2 and E4 alleles within apolipoprotein E gene, rs6254 polymorphisms within the parathyroid hormone gene and rs1800871 polymorphisms within the IL-10 gene [15].

A comprehensive 2013 study, initiated by the "Heart and Aging Research in Genomic Epidemiology" (CHARGE) showed correlation between numerous genetic factors and the development of mitral annular calcification and aortic stenosis [15]. This meta-analysis included three cohorts of patients enrolled in the Framingham Heart Study (FHS), the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS) and white European participants in the Multi-Ethnic Study of Atherosclerosis (MESA) – a total of 6942 individuals with CT-confirmed aortic valvular calcification and 3795 individuals with calcification of the mitral ring. Those findings were then tested in additional European and multiethnic cohorts, including white European participants in the Heinz Nixdorf Recall Study (HNR), African-Americans, Chinese-Americans and Spanish-Americans participants in MESA study, and Swedish and Danish participants in the aortic stenosis studies Malmö Diet and Cancer Study (MDCS) and the Copenhagen City Heart Study (CCHS) [15, 16].

A single nucleotide polymorphism (SNP) in LPA locus (rs10455872) appears to be significantly associated with the presence of aortic valve calcification (odds ratio allele 2.05; $p=9.0x10^{-10}$), these findings being confirmed by additional cohorts of white Europeans, African-Americans and Spanish-Americans ($p<0.05$ for all comparisons). Genetically determined plasma levels of Lp(a) are also associated with aortic valvular calcification, which suggests a causal role of Lp(a) in this process [15, 17-20]. In multiple prospective analyses, the LPA genotype is associated with cases of aortic stenosis [hazard ratio allele 1.68; 95% confidence interval (CI) 1.32-2.15] and aortic valve replacement (hazard ratio 1.54; 95% CI 1.05-2.27) in a large Swedish cohort; an independent Danish cohort study also confirmed this association. The relationship between rs10455872 polymorphism in the LPA locus and clinical manifestations of aortic stenosis remains probable after adjustment for age, smoking, body-mass index (BMI) – a positive predictive value of 90% [21, 22]. Researchers believe that this genetic variation in the LPA locus – reflected by plasma levels of Lp(a) is associated with clinical aortic valve calcification in multiethnic groups [16-25].

Lipoprotein (a) belongs to a subclass of plasma lipoproteins. It is synthesized by the liver and is composed of a LDL-like particle and a specific Lp(a), a glycoprotein with high molecular weight, which is covalently attached via a disulfide bond to one molecule of apoB. The physiological and pathological role of Lp(a) remains largely unclear. Research has suggested that Lp(a) is the link between cholesterol transport and modulatory function of fibrinolytic system in the process of coagulation and fibrinolysis [26, 27]. Genetic and numerous epidemiological studies suggest that Lp(a) is a risk factor for numerous developing cardiovascular and coronary diseases [28, 29]. Plasma concentrations of Lp(a) are hereditary and controlled by the gene encoding Lp(a) on

chromosome 6q26-27. Apolipoprotein (a) protein has various dimensions due to the polymorphism Kringle IV-2 variable number tandem repeats (VNTR) repeated in the LPA gene. Variations in the size of the gene are manifested at protein level, leading to the presence of apolipoproteins (a) with a variable number of "kringle" IV repeats, between 10 to 50 [30-31]. Apolipoprotein (a) proteins with varying sizes are called apo (a) isoforms. Kringle domains are autonomous protein domains which, upon formation of the tertiary structure, bend and form large loops stabilized by three disulfide bridges. These are key domains, involved in protein-protein interactions with coagulation factors. Such domains are also found in plasminogen, hepatocyte growth factor, prothrombin and Lp(a) [32]. It is assumed that they act as mediators in various interactions between membranes, proteins, phospholipids, and also as regulators of the proteolytic activity of different enzymes [33]. The locations and mechanism of the Lp(a) catabolism is unknown. Uptake by LDL receptor is not a major mechanism of Lp(a) metabolism [34]. It has been established that the kidneys are responsible for the plasma clearance [35]. Plasma concentrations of Lp(a) vary from <0.2 to >200 mg/dl. Similar variability was observed in all tested populations. However, individuals with none or very low levels of Lp(a) were healthy, indicating that plasma Lp(a) is not essential, at least under normal conditions [36]. The structure of Lp(a) is similar to those of plasminogen and tissue plasminogen activator (tPA), and therefore Lp(a) competes with plasminogen for its receptor, leading to reduced fibrinolysis. Furthermore, Lp(a) stimulates the secretion of plasminogen activator inhibitor-1 (PAI-1), which results in increased thrombogenesis. Lipoprotein (a) also carries cholesterol, thus contributing to the development of atherosclerosis [37].

## Conclusions

Since the biological significance of this lipoprotein is still unclear and controversial, we can only assume that genetically determined modifications in the structure of Lp(a) are relevant to the development of degenerative aortic stenosis and, generally, of valvular calcification (including calcification of the mitral valve). One possibility is that its pathophysiological effects are due to its specific structural variation rather than its plasma levels.

Studying the genetic nature of degenerative aortic stenosis and the significance of Lp(a) plasma levels, and genetically determined variations of its structure resulting in the manifestation and progression of valvular calcification, could open up new horizons for research. The outcomes could eventually lead to the development of an effective treatment and preventive measures of this common and socially significant disease, whose treatment is limited at this point to the relief of symptoms and valve replacement.

Studies on genetic polymorphisms in LPA locus, plasma concentrations of Lp(a) and their correlation with calcified aortic stenosis in Bulgaria are yet to be conducted.

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## References

1. Joint Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology (ESC), European Association for Cardio-Thoracic Surgery (EACTS), Vahanian A, Alfieri O, Andreotti F, Antunes MJ, et al. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J. 2012;33(19):2451-96.
2. Kamenova Z, Grigorov M, Ingeliev M, Petrova G, Pesheva B. [Correlation between degenerative aortic stenosis and coronary disease]. MedInfo. 2012;4(9):22-6. Bulgarian.
3. Rosenhek R, Binder T, Porenta G, Lang I, Christ G, Schemper M, et al. Predictors of outcome in severe, asymptomatic aortic stenosis. N Engl J Med. 2000;343(9):611-7.
4. Trayanov I. [Aortic stenosis and aortic regurgitation]. Cardio D. 2007;4(2):34-41. Bulgarian.
5. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. N Engl J Med. 1999;341(3):142-7.
6. Cosmi JE, Kort S, Tunick PA, Rosenzweig BP, Freedberg RS, Katz ES, et al. The risk of the development of aortic stenosis in patients with "benign" aortic valve thickening. Arch Intern Med. 2002;162(20):2345-7.

7. Carabello B, Paulus W. Aortic stenosis. Lancet. 2009;373(9667):956-66.
8. Koleva K, Zlatareva N, Gudev A. [A new look at degenerative aortic stenosis]. MP – SSZ. 2007;38(4):14-9. Bulgarian.
9. Hughes B, Chhoud G, Mehta JL. Aortic stenosis: is it simply a degenerative process or an active atherosclerotic process? Clin Cardiol. 2005;28(3):111-4.
10. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, et al. Lipoprotein (a) as a cardiovascular risk factor: current status. Eur Heart J. 2010;31(23):2844-53.
11. Clarke R, Peden F, Hopewell C, Kyriakou T, Goel A, Heath SC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med. 2009;361(26):2518-28.
12. Bozbas H, Yildirir A, Atar I, Pirat B, Eroglu S, Aydinalp A, et al. Effects of serum levels of novel atherosclerotic risk factors on aortic valve calcification. J Heart Valve Dis. 2007;16(4):387-93.
13. Parolari A, Loardi C, Mussoni L, Cavallotti L, Camera M, Biglioli P, et al. Nonrheumatic calcific aortic stenosis: an overview from basic science to pharmacological prevention. Eur J Cardiothorac Surg. 2009;35(3):493-504.
14. De Vecchis R, Di Biase G, Esposito C, Ciccarelli A, Cioppa C, Giasi A, et al. Statin use for nonrheumatic calcific aortic valve stenosis: a review with meta-analysis. J Cardiovasc Med. 2013;14(8):559-67.
15. Thanassoulis G, Campbell CY, Owens DS, Smith JG, Smith AV, Peloso GM, et al. Genetic associations with valvular calcification and aortic stenosis. N Engl J Med. 2013;368:503-12.
16. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. Circ Cardiovasc Genet. 2009;2(1):73-80.
17. Gotoh T, Kuroda T, Yamasawa M, Nishinaga M, Mitsuhashi T, Seino Y, et al. Correlation between lipoprotein(a) and aortic valve sclerosis assessed by echocardiography (the JMS Cardiac Echo and Cohort Study). Am J Cardiol. 1995;76(12):928-32.
18. Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. J Am Coll Cardiol. 1997;29(3):630-4.
19. O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. Arterioscler Thromb Vasc Biol. 1996;16(4):523-32.
20. Gladera CA, Birgander LS, Söderberg S , Ildgruben HP, Saikku P, Waldenström A, et al. Lipoprotein(a), Chlamydia pneumoniae, leptin and tissue plasminogen activator as risk markers for valvular aortic stenosis. Eur Heart J. 2003;24:198-08.
21. Thanassoulis G, Massaro JM, Cury R, Manders E, Benjamin EJ, Vasan RS, et al. Associations of long-term and early adult atherosclerosis risk factors with aortic and mitral valve calcium. J Am Coll Cardiol. 2010;55(22):2491-8.
22. Kutikhin AG, Yuzhalin AE, Brusina EB, Ponasenko AV, Golovkin AS, Barbarash OL. Genetic predisposition to calcific aortic stenosis and mitral annular calcification. Mol Biol Rep. 2014;41(9):5645-63.
23. Ortlepp JR, Schmitz F, Mevissen V, Weiss S, Huster J, Dronskowski R, et al. The amount of calcium-deficient hexagonal hydroxyapatite in aortic valves is influenced by gender and associated with genetic polymorphisms in patients with severe calcific aortic stenosis. Eur Heart J. 2004;25(6):514-22.
24. Gaudreault N, Ducharme V, Lamontagne M, Guauque-Olarte S, Mathieu P, Pibarot P, et al. Replication of genetic association studies in aortic stenosis in adults. Am J Cardiol. 2011;108(9):1305-10.
25. Owens DS, Katz R, Johnson E, Shavelle DM, Probstfield JL, Takasu J, et al. Interaction of age with lipoproteins as predictors of aortic valve calcification in the multi-ethnic study of atherosclerosis. Arch Intern Med. 2008;168(11):1200-7.
26. Kronenberg F, Utermann G. Lipoprotein(a): Resurrected by genetics. J Intern Med. 2013;273(1):6-30.
27. Koschinsky L. Novel insights into Lp(a) physiology and pathogenicity: more questions than answers? Cardiovasc Hematol Disord Drug Targets. 2006;6(4):267-78.
28. Kamstrup R, Tybjærg-Hansen A, Nordestgaard G. Lipoprotein(a) and risk of myocardial infarction-genetic epidemiologic evidence of causality. Scand J Clin Lab Invest. 2011;71(2):87-93.
29. Berg K. A new serum type system in man-the Lp system. Acta Pathol Microbiol Scand. 1963;59:369-82.
30. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature. 1987;330(6144):132-7.
31. Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes. Inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. J Clin Invest. 1987;80(2):458-65.
32. Patthy L, Trexler M, Banyai L, Varadi A, Vali Z. Kringles: modules specialized for protein binding. Homology of the gelatin-binding region of

fibronectin with the kringle structures of proteases. FEBS Lett. 1984;171(1):131-6.
33. Atkinson RA, Williams RJ. Solution structure of the kringle 4 domain from human plasminogen by 1H nuclear magnetic resonance spectroscopy and distance geometry. J Mol Biol. 1990;212(3):541-52.
34. Knight BL, Perombelon YF, Soutar AK, Wade DP, Seed M. Catabolism of lipoprotein(a) in familial hypercholesterolaemic subjects. Atherosclerosis. 1991;87(2-3):227-37.
35. Albers JJ, Koschinsky ML, Marcovina SM. Evidence mounts for a role of the kidney in lipoprotein(a) catabolism. Kidney Int. 2007;71(10):961-2.
36. Rath M, Pauling L. A unified theory of human cardiovascular disease leading the way to the abolition of this disease as a cause for human mortality. J Orthomol Med. 1992;7(1):5-15.
37. Sotiriou SN, Orlova VV, Al-Fakhri N, Ihanus E, Economopoulou M, Isermann B, et al. Lipoprotein(a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin. FASEB J. 2006;20(3):559-61.