

HAS-1 genetic polymorphism in sporadic abdominal aortic aneurysm

Chiara Armani,¹ Stefano Landi,² Marco Nuti,¹ Rossella Di Stefano,¹ Alberto Balbarini¹

¹Cardiac Thoracic and Vascular Department, University of Pisa, Pisa; ²Genetics, Department of Biology, University of Pisa, Pisa, Italy

Abstract

The hyaluronan synthase 1 (*HAS-1*) gene encodes a plasma membrane protein that synthesizes hyaluronan (HA), an extracellular matrix molecule. Accumulating evidence emphasizes the relevance of HA metabolism in an increasing number of processes of clinical interest, including abdominal aortic aneurysm (AAA). The existence of aberrant splicing variants of the *HAS-1* gene could partly explain the altered extracellular matrix architecture and influence various biological functions, resulting in progressive arterial wall failure in the development of AAA. In the present study, we assessed the hypothesis that *HAS-1* genetic 833A/G polymorphism could be associated with the risk of AAA by performing a case-control association study, involving AAA patients and healthy matched donors.

Introduction

Human abdominal aortic aneurysm (AAA) is the most common type of aneurysm in humans. It is characterized by abnormal dilation of the aorta and is associated with

histopathological changes of the arterial wall. Although the presence of atherosclerotic lesions is a common feature in AAA, the destruction of the arterial wall is a crucial point in the development of aneurysm.¹

Effective matrix macromolecules, such as elastin, collagen and glycosaminoglycans/proteoglycans, are responsible for the integrity of the arterial wall. Hyaluronan (HA) is a large, nonsulfated glycosaminoglycan (GAG) produced in the vasculature by smooth muscle cells and endothelial cells. It is synthesized at the inner face of the cell membrane by hyaluronan synthase (HAS) encoded by three genes to different chromosome localizations,² followed by translocation to the outer surface and the intercellular space. Each isoenzyme synthesizes different sizes of HA molecules that exhibit not completely overlapping functions.³

Recent *in vitro* investigations have shown that HA influences important cellular functions such as proliferation, migration and secretory capabilities.⁴ Alterations in hyaluronan metabolism, distribution and function have been documented in many diseases e.g. arthritis, immune and inflammatory disorders, pulmonary and vascular diseases, as well as cancer.⁵

There seems to exist a delicately regulated balance between production and removal of HA that is central to its biological functions under normal conditions. If this balance is disturbed, it has been hypothesized that disease may develop.⁶ We hypothesize that changes in HA content or aberrant variants of HA make the wall more susceptible to atherosclerotic stimuli, thus contributing to the AAA development. We therefore, carried out a case-control association study in order to verify the association between the 833A/G polymorphism, located on an exonic splicing enhancer motif of the *HAS-1* gene, and risk of AAA.

Materials and Methods

Sample collection

Blood was collected from 146 AAA patients and 156 control subjects with a negative his-

tory of vascular diseases. Patients with connective tissue disorders or known inflammatory or malignant disease were excluded. Controls were collected among volunteer healthy blood donors and among people hospitalized for traumatic accidents to avoid the use of hospitalized controls. All samples were collected in the Cisanello Hospital of Pisa (Tuscany, Italy). The Ethics Committee of the institution approved the study. Participants gave their informed consent for the study, according to the Helsinki declaration.

Clinical diagnosis of AAA was confirmed by echographic and spiral computed tomographic (CT) scan evaluations. AAA was defined as a focal dilation of the abdominal aorta at least 50% larger than expected normal diameter (antero-posterior and lateral aortic diameter values were increased to 50.78±11.66 mm and 50.54±13.35 mm, respectively). All AAAs underwent surgical repair.

We recorded demographic and clinical data including age, gender, BMI (Body Mass Index), smoking habits (S=smoker, EX=ex-smoker, NS=no smoker; patients were considered ex-smokers if they had stopped smoking at least five years before inclusion in the study), the presence or absence of hypercholesterolemia (defined as total plasma cholesterol level ≥220 mg/dL or cholesterol-lowering drug therapy), the presence or absence of hypertension (defined as diastolic blood pressure >95 mmHg and/or systolic blood pressure >160 mmHg), the presence or absence of diabetes. Moreover, a detailed history of cardiovascular risk factors was obtained from each patient.

DNA extraction and genotyping

Genomic DNA was isolated from EDTA blood samples by a modified salting-out procedure.⁸ Allele-specific oligonucleotide PCR (ASO-PCR) method was carried out for *HAS-1* single nucleotide polymorphism (SNP) 833 A/G genotyping in a 20 µL reaction mixture and 1 U of Taq polymerase (Eurobio-Labtek, FR). Primers of our own design were used: 5'-GGGTAAAGGATCCGCACG-3' (wild type allele), 5'-GGGTAAAGGATCCGCACA-3' (mutant allele) and 5'-CCTTCTCTCAAGCATCAGC-3' (reverse) for each reaction at 0.5 µM. PCR was carried out for 40 cycles

Correspondence: Chiara Armani,
Cardiac Thoracic and Vascular Department,
University of Pisa, via Paradisa 2,
56124 Pisa, Italy
E-mail: armani.chiara@yahoo.it

Key words: aneurysm, genetics, hyaluronan synthase, SNP, susceptibility.

Received for publication: 17 July 2008

Accepted for publication: 18 July 2008.

This work is licensed under a Creative Commons Attribution 3.0 License (by-nc 3.0).

©Copyright Chiara Armani et al., 2009
Heart International 2009; 4:e1
doi:10.4081/hi.2009.e1

consisting of an initial denaturation step at 95°C for 15' followed by 20 cycles at 95°C for 1', 68°C for 30" (-1°C/cycle), 72°C for 1'5" and to 20 cycles at 95°C for 1', 51°C for 30", 72°C for 1'5", with a final extension at 72°C for 10'. The 178 base-pair (bp) PCR product was visualized on a 2% agarose gel (Eurobio-Labtek, FR) stained with ethidium bromide.

Statistical analysis

We tested the Hardy-Weinberg equilibrium of genotype distributions separately among case patients and control subjects by χ^2 test. All the covariates were analyzed by χ^2 or Fisher's exact test. We used unconditional multivariate logistic regression analysis to examine the association between the genetic polymorphism (dominant model: heterozygotes+rare homozygotes *vs.* common homozygotes), and the risk of AAA. The calculation of the Odds Ratio and its 95% confidence interval was performed adjusting the analysis for the other covariates. A type-I error of 0.05 was used and all the tests were two-tailed.

Results

Significant differences were observed in demographic factors between groups of AAA patients and control subjects, including age, gender, diabetes, hypertension, and smoking status (Table 1). Patients showed an elevated percentage of males, higher prevalence of diabetes and hypertension compared to controls. As expected, the group of AAA cases was older than controls. The genotype distributions for the HAS-1 polymorphism were consistent with the Hardy-Weinberg equilibrium ($p>0.05$). The simple analysis with a χ^2 test between the status (case/control) and HAS-1 genotypes revealed a trend in the co-dominant model (three genotypes separated, $p=0.077$) and a statistically significant association in the dominant model ($p=0.042$, with Yates' correction). However, the multivariate logistic analysis, after the adjustment for the above mentioned covariates, showed that the association between the risk of AAA and HAS-1 genotype was not statistically significant, as reported in Table 2.

Table 1. Demographic data.

	Control (N=156)	AAA (N=146)	<i>p</i>
Age (years)	55 (50-69)	74 (30-94)	<0.0001 ^a
BMI (kg/m ²)	26.4±2.8	27.1±3.5	0.074 ^a
Male sex	107	139	<0.0001 ^b
Hypertension	16	56	<0.0001 ^b
Hypercholesterolemia	22	25	0.94 ^b
Diabetes	2	10	0.04 ^c
	S 18	S 28	
Smoking status	EX 30	EX 60	<0.0001 ^b
	NS 49	NS 25	

AAA: abdominal aortic aneurysm; BMI, body mass index; S: smokers; EX: ex-smokers; NS: no smokers. Age data represent median and range. BMI data represent mean ± SD. ^aStudent's *t* test; ^b χ^2 test; ^cFisher's exact test.

Discussion and Conclusions

Hyaluronan (HA) is an acidic glycosaminoglycan made entirely of a repeating disaccharide. Its molecular weight is often very high mass, ranging from about 10⁵-10⁷ Da, depending upon the tissue, but it can also exist as smaller fragments and oligosaccharides under certain physiological or pathological conditions. Originally thought to be an inert molecular "stuffing" in connective tissues, HA mediates, by specific receptors, many important functional activities e.g. cell motility, invasion and proliferation. These receptors can also interact with smaller forms of HA. Indeed, several studies suggest that fragmentation of HA enhances its ability to activate cell-signaling pathways.^{9,11}

HA is present in the extracellular matrix, on the cell surface and inside the cell. HA functions depend not only on its size but also upon its cellular distribution. The notion that HA is likely to take part in the development of vascular pathologies has been promoted by a number of observations, which show marked changes in the content and distribution of HA in several arterial disease entities such as diabetic angiopathy, atherosclerosis and restenosis. Early biochemical studies showed that HA concentration of human atherosclerotic plaques generally decreases with increasing severity of atherosclerosis.¹²

Human AAA is always accompanied by

advanced atherosclerotic lesions. In AAA, matrix molecules, such as elastin, collagen and glycosaminoglycans/proteoglycans (GAG/PG), are all responsible for the integrity of the arterial wall. One of the most critical events implicated in the development of AAA, is the mechanical failure of collagen and elastin¹³⁻¹⁵ and although many investigations have focused on these components, our understanding of the metabolism of GAG/PG in AAA is extremely limited.^{16,17}

Some studies on AAA report a decrease in the amount of HA that may be attributed either to HA-degrading enzymes released by inflammatory cells or to an altered synthesis of HA related to polymorphisms in the promoter gene. This latter hypothesis could be explained by the existence of splicing variants of *HAS-1* that could activate specific inflammatory cell-signaling pathways and increase the protease production.

In our study, a significant difference in the genotype distribution of HAS-1 polymorphism in AAA patients, compared to controls was not seen.

We are aware that the present study is underpowered, however, our results show implicitly that the effect of the polymorphism is too weak to confer a decisive risk in the etiology of AAA. Probably, other polymorphisms within HAS-1 could have a stronger impact on the risk of the disease.

Table 2. Adjusted odd ratios for the covariates reported in Table 1.

HAS1 genotypes	Cases	Controls	Odds ratio	95% lower limit	95% upper limit	<i>p</i>
Common homozygotes	117	107	1			
Heterozygotes	21	41				
Rare homozygotes	3	0	0.65	0.13	3.15	0.58

95% Odds ratio (OR) and 95% confidence intervals for the risk of AAA. OR is adjusted for the covariates reported in Table 1. Dominant model: the rare homozygotes are pooled with the heterozygotes. Reference category: common homozygotes.

References

1. Reilly JM, Tilson MD. Incidence and etiology of abdominal aortic aneurysms. *Surg Clin North Am.* 1989; 69:705-11.
2. Itano N, Sawai T, Yoshida M, et al. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. *J Biol Chem* 1999; 274:25085-92.
3. Spicer AP, McDonald JA. Characterization and molecular evolution of a vertebrate hyaluronan synthase gene family. *J Biol Chem* 1998; 273:1923-32.
4. Wight TN, Kinsella MG, Qwarnstrom EE. The role of proteoglycans in cell adhesion, migration and proliferation. *Curr Opin Cell Biol* 1992; 4:793-801.
5. Turley EA. Extracellular matrix remodeling: multiple paradigms in vascular disease. *Circ Res* 2001;88:2-4
6. Toole BP, Wight TN, Tammi MI. Hyaluronan-cell interactions in cancer and vascular disease. *J Biol Chem* 2002; 277:4593-6. Epub 2001 Nov 20.
7. Tammi MI, Day AJ, Turley EA. Hyaluronan and homeostasis: a balancing act. *J Biol Chem* 2002; 277:4581-4. Epub 2001 Nov 20.
8. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
9. Camenisch TD, McDonald JA. Hyaluronan: is bigger better? *Am J Respir Cell Mol Biol* 2000; 23:431-3.
10. Horton MR, McKee CM, Bao C, et al. Hyaluronan fragments synergize with interferon-gamma to induce the C-X-C chemokines mig and interferon-inducible protein-10 in mouse macrophages. *J Biol Chem* 1998; 273:35088-94.
11. Lokeshwar VB, Selzer MG. Differences in hyaluronic acid-mediated functions and signaling in arterial, microvessel, and vein-derived human endothelial cells. *J Biol Chem* 2000; 275:27641-9.
12. Kolodgie FD, Burke AP, Farb A, et al. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: insights into plaque erosion. *Arterioscler Thromb Vasc Biol* 2002; 22:1642-8.
13. Dobrin PB, Mrkvicka R. Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation. *Cardiovasc Surg* 1994; 2:484-8.
14. Menashi S, Campa JS, Greenhalgh RM, Powell JT. Collagen in abdominal aortic aneurysm: typing, content, and degradation. *J Vasc Surg* 1987;6:578-82.
15. Campa JS, Greenhalgh RM, Powell JT. Elastin degradation in abdominal aortic aneurysms. *Atherosclerosis* 1987;65:13-21.
16. Tamarina NA, Grassi MA, Johnson DA, Pearce WH. Proteoglycan gene expression is decreased in abdominal aortic aneurysms. *J Surg Res*1998; 74:76-80.
17. Theocharis AD, Tsolakis I, Tsegenidis T, Karamanos NK. Human abdominal aortic aneurysm is closely associated with compositional and specific structural modifications at the glycosaminoglycan level. *Atherosclerosis* 1999;145:359-68.