HAS-1 genetic polymorphism in sporadic abdominal aortic aneurysm

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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 dilatation 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, 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 capacities.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 history 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 AAs 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.7 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’-GGTTAAGGATCGCAG-3’ (wild type allele), 5’-GGTTAAGGATCGCAGA-3’ (mutant allele) and 5’-CTTTTCTTCAAGCATCAG-3’ (reverse) for each reaction at 0.5 µM. PCR was carried out for 40 cycles.
Table 2. Adjusted odd ratios for the covariates reported in Table 1.

<table>
<thead>
<tr>
<th>HAS-1 genotypes</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds ratio</th>
<th>95% lower limit</th>
<th>95% upper limit</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Common homozygotes</td>
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<td>Rare homozygotes</td>
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<td>0</td>
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<td>0.65</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

50% 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