Intracranial aneurysms and connective tissue disorders
van den Berg, J.S.P.

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Type III collagen deficiency in a family with intracranial aneurysms

JSP van den Berg, M Limburg, G Pals, F Arwert, and A Westerveld.

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Introduction

Rupture of an intracranial aneurysm may result in a subarachnoid haemorrhage (SAH) with a high chance of death, or serious sequelae in surviving patients.¹ Formation of intracranial aneurysms appears to be a multifactorial process with intrinsic (e.g. vessel wall weakness) and extrinsic factors (e.g. arterial hypertension). In some patients the intrinsic factor may be a type III collagen deficiency.² Patients with Ehlers Danlos Syndrome (EDS) vascular type, a genetically determined type III collagen deficiency, have an increased likelihood of harboring intracranial aneurysms.³ Type III collagen protein is abundant in the vessel wall, and responsible for the tensile strength of the arteries.⁴ About 10% of patients have a positive family history for intracranial aneurysms.⁵ Some of the familial aneurysms may be due to a type III collagen deficiency. However, in 5 patients with familial intracranial aneurysms no deficiency of type III collagen was observed suggesting that other factors play a role.⁶,⁷

We describe a family with aneurysmal subarachnoid haemorrhages, and a type III collagen deficiency in some members. DNA analysis was performed to investigate the underlying cause of this deficiency.

Patients and methods

After informed consent blood samples were taken from the mother of patient III-24 (II-9), and skin biopsies were taken for fibroblast culture from the three patients (III-9, III-12, III-24) with a ruptured intracranial aneurysm, and the healthy twin sister of patient III-24 (III-25). Type III collagen production (type III / type I collagen ratio) was determined, as described previously.²

DNA analysis. DNA extracted from white blood cells or fibroblasts, SSCP/heteroduplex analysis of PCR-amplified fragments from the α-helix of type III collagen cDNA, and DNA sequence analysis of the complete N-propeptide and C-propeptide of type III collagen were performed as published previously.⁸ A DNA tandem repeat polymorphism in the COL3A1 gene was typed to determine the segregation of the COL3A1 alleles in the family.⁹
Results

Three patients had a ruptured intracranial aneurysm (see figure 1). None smoked cigarettes or had polycystic kidney disease. Patient II-9 died of carcinoma of the pancreas one year after blood samples were taken for our study.

One (patient III-9) experienced sudden headache, and drowsiness at the age of 21 years. She was admitted, and underwent surgery. The physicians told her that she had a ruptured intracranial aneurysm. This happened approximately 41 years ago, and unfortunately all medical records have been destroyed.

Patient III-24. A 31 year old woman, without relevant medical history, was admitted with serious headache and loss of consciousness. At examination she was drowsy, without focal neurological deficit. Computed tomography of the brain demonstrated blood in the subarachnoidal spaces, fourth ventricle, and basal cisterns. Angiography revealed an aneurysm of the right carotid artery at the junction of the middle and anterior cerebral arteries. The aneurysm was successfully clipped in an operative procedure. She was discharged in good health.

Patient III-12. At the age of 44 years she was admitted for subarachnoid haemorrhage. She had hypertension for 4 years for which she took medication. Angiography revealed an aneurysm of the right internal carotid artery. The aneurysm was clipped, and she was discharged in good health. Three years later she was readmitted for another subarachnoid haemorrhage. There was an aneurysm of the left internal carotid artery at the junction of the left posterior communicating artery. The aneurysm was clipped, and she was discharged in good health. After review of the first angiography the second aneurysm could not be detected.

Protein and DNA analysis. The collagen type III / type I ratio was below 0.06 in patients III-12 and III-24 indicating a reduced protein production of type III collagen in these patients (see table 1 and figure 2). In normal controls this ratio ranges from 0.06 to 0.2.² SSCP/heteroduplex analysis of PCR-amplified cDNA fragments encoding type III collagen α-helix revealed no fragments with an altered electrophoretic mobility in any patient. Sequence analysis of the
N-propeptide and C-propeptide of type III collagen demonstrated no DNA sequence variations in any of the three patients.

To investigate whether the patients shared one or eventually two type III collagen genes we studied a highly variable 16 base pairs tandem repeat marker in intron 25 of the COL3A1 gene. Typing revealed two similar alleles (242/242) in patient III-24 and her mother (II-9) while the patients III-9 and III-12 had different alleles (256/272).

Figure 1. Pedigree of the family.

Ο, female; □, male; ●, had SAH; ☋, ☋ deceased.
**Table 1. Protein and DNA analysis results**

<table>
<thead>
<tr>
<th>Proband</th>
<th>Type III / Type I collagen ratio</th>
<th>Marker intron 25 of COL3A1</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-9</td>
<td>0.139</td>
<td>256/272</td>
</tr>
<tr>
<td>III-12</td>
<td>0.045</td>
<td>256/272</td>
</tr>
<tr>
<td>II-9</td>
<td>not determined</td>
<td>242/242</td>
</tr>
<tr>
<td>III-24</td>
<td>0.053</td>
<td>242/242</td>
</tr>
<tr>
<td>III-25</td>
<td>0.113</td>
<td>242/242</td>
</tr>
</tbody>
</table>

**Figure 2.** Polyacrylamide gels electrophoresis of patient III-24 (A) with a collagen type III / type I ratio of 0.053, healthy twin sister of patient III-24 (B) with a collagen type III / type I ratio of 0.113, patient III-12 (C) with a collagen type III / type I ratio of 0.045, patient III-9 (D) with a collagen type III / type I ratio of 0.139, and a normal control (E) with a collagen type III / type I ratio of 0.10. Arrow 1 = type III collagen α1 (α1(III)); arrow 2 = type I collagen α1 (α1(I)); arrow 3 = type I collagen α2.
Type III collagen deficiency has been demonstrated in some patients with sporadic intracranial aneurysms. However, the underlying mechanism has not been elucidated. Leblanc et al did not observe a type III collagen deficiency in 5 patients with familial intracranial aneurysms. Now we present two patients with a ruptured intracranial aneurysm from the same family with a type III collagen deficiency.

We screened the complete type III collagen coding sequence for mutations using SSCP/heteroduplex analysis, but found no pathogenetic mutations to explain the type III collagen deficiency. It is noted that SSCP-heteroduplex analysis will detect most but not all sequence variations, missing some mutations.

For the formation of the triple helix in fibrillar collagens the globular part of the C-propeptide is essential. If formation fails it may lead to intracellular breakdown of mutated and normal type III procollagen through a dominant negative effect. However, the DNA sequence of the C-propeptide of the type III collagen gene did not reveal any sequence variation (data not shown).

Using a highly variable 16 base tandem repeat marker in intron 25 of the COL3A1 gene we found that the two family members with a type III collagen deficiency have different alleles encoding type III collagen, indicating that even if mutations were present these would have to be different mutations. This indicates that type III collagen deficiency is not likely the result of a common mutation in the type III collagen gene.

One patient had multiple intracranial aneurysms, occurring with a time interval of about three years. De novo symptomatic aneurysms occur in approximately 1% of patients with subarachnoid hemorrhages, with an interval between first and second bleed ranging from 4 to 34 years. In one study all patients with a recurrent de novo aneurysm had hypertension, as did our patient. The patient with a recurrent de novo aneurysm had a type III collagen deficiency. A type III collagen deficiency is possibly associated with recurrent intracranial aneurysms, as has been suggested in the case of recurrent carotid dissections. A study of 105 patients with cervical arterial dissections, demonstrated that 2 of 3 patients with recurrent cervical arterial dissections of the same vessel had EDS vascular type. However, in a previous study we
found no association between type III collagen deficiency and multiple intracranial aneurysms.²

In some patients with familial intracranial aneurysms a type III collagen deficiency may be observed. Our data indicate that in the family described here this is probably not due to a mutation in the type III collagen gene, but that it may be related to defects during post-translational modification or an altered collagen metabolism.

References

Type III collagen deficiency has been demonstrated in a number of conditions, including pulmonary hypertension and congenital heart disease. However, the specific cause of such cases is often unclear. The role of collagen type III deficiency remains to be elucidated.

We analyzed the complete type III collagen coding sequence for mutations using SSCP/heteroduplex analysis, but found no pathogenetic mutations to explain the type III collagen deficiency. It is noted that SSCP -heteroduplex analysis detected a novel intron 1 duplication in an infant with severe hypotrichosis. For the detected mutations, we performed fibroblast protocols and ultrastructural analysis, but no significant differences were observed.

A dominant negative effect has been observed in the keratinocyte of the type III collagen gene. This might have resulted from an extracellular mutation that could affect keratinocyte function. However, specific studies are required to confirm this hypothesis.

One patient had multiple intracranial aneurysms. De novo symptomatic aneurysms occur in approximately 15% of patients with unruptured intracranial aneurysms, with an interval between first and second bleed ranging from 4 to 34 years. In one study, all patients with a recurrent de novo aneurysm had hypertension, as did our patient. The patient with a recurrent de novo aneurysm had a type III collagen deficiency. In type III collagen deficiency, type III collagen deficiency is usually associated with recurrent intracranial aneurysms. In the case of recurrent intracranial aneurysms, a study of 105 patients with cervical arterial dissections demonstrated that 3 of 3 patients with symptomatic cervical arterial dissections had type III collagen deficiency.