Spontaneous spinal cerebrospinal fluid leaks and minor skeletal features of Marfan syndrome: a microfibrilopathy

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Object. Spontaneous spinal cerebrospinal fluid (CSF) leaks are increasingly recognized as a cause of postural headaches. The authors examined a group of patients suffering from spontaneous spinal CSF leaks who also had minor skeletal features of Marfan syndrome for abnormalities of fibrillin-containing microfibrils.

Methods. Patients with spontaneous CSF leaks were evaluated for the clinical characteristics of connective tissue disorders. Skin biopsies were obtained in three patients with skeletal manifestations that constitute part of the Marfan syndrome phenotype. Cultured fibroblasts were studied for fibrillin-1 synthesis and incorporation into the extracellular matrix (ECM) by performing quantitative metabolic labeling and immunohistochemical analysis. Among 20 consecutive patients found to have spinal CSF leaks, four (20%) exhibited minor skeletal features of Marfan syndrome, but lacked any ocular or cardiovascular abnormalities. The mean age of these patients (30 years) was lower than that of the 16 patients without skeletal abnormalities (44 years; p = 0.01). Abnormalities in fibrillin-1 metabolism and immunostaining were detected in all three patients with the skeletal abnormalities who underwent examination, but not in a control patient without these skeletal manifestations.

Conclusions. Twenty percent of patients who experience spontaneous spinal CSF leaks have minor skeletal features of Marfan syndrome. The authors demonstrated abnormalities in fibrillin-1 protein deposition in all patients examined, but only one person was found to have a fibrillin-1 abnormality typically found in classic Marfan syndrome. The results indicate that there is a heterogeneous involvement of other components of ECM microfibrils at the basis of this cerebrospinal manifestation. In addition, the authors identified a connective-tissue etiological factor in a group of disorders not previously classified as such.

KEY WORDS • fibrillin • headache • intracranial hypotension • cerebrospinal fluid leak • Marfan syndrome • microfibrilopathy • pulse-chase analysis • fibrillin immunofluorescence

S PONTANEOUS spinal CSF leaks resulting in intracranial hypotension are increasingly recognized as a cause of postural headaches. The clinical features of spontaneous spinal CSF leaks are identical to those occasionally seen following a lumbar puncture and consist of headaches exacerbated by the upright position, which may be associated with nausea, vomiting, cranial nerve palsies, photophobia, visual blurring, tinnitus, and neck stiffness. Characteristic findings on cranial MR imaging include pachymeningeal enhancement and downward displacement or “sagging” of the brain.

Using widely available technologies such as CT myelography, MR imaging, or radionuclide cisternography, one can identify spinal CSF leaks in the majority of patients with spontaneous intracranial hypotension. The exact cause of a spontaneous spinal CSF leak often remains unknown, but a combination of a trivial precipitating event and an underlying weakness of the spinal meninges is generally suspected. In patients with Marfan syndrome, an autosomal-dominant disorder that is defined by pleiotropic manifestations in the cardiovascular, ocular, musculoskeletal, dermal, and integumental systems, weakness of the dura mater may lead to dural ectasia, an abnormality that is probably underrecognized in asymptomatic patients due to its mode of diagnosis. In symptomatic patients who undergo MR or CT imaging studies, a localized or general widening of the dural sac is frequently identified.

Marfan syndrome is caused by mutations in the FBN1 gene, which is located on chromosome 15 and expressed throughout the human body. Fibrillin-1 is the main element of microfibrils and, together with collagen, elastin, and numerous other proteins, forms the extracellular connective tissue matrix. The microfibrils are associated with elastin in

Abbreviations used in this paper: CSF = cerebrospinal fluid; CT = computerized tomography; DHPLC = denaturing high-performance liquid chromatography; ECM = extracellular matrix; MR = magnetic resonance.
tissues such as skin and blood vessels, but can also be found in an environment devoid of elastin, such as the ocular ciliary zonules that suspend the lens. Microfibrils have been ascribed functions including maintenance of tissue elasticity, providing a stable network for the accumulation of tropoelastin during developmental stages, and of tissue elasticity, providing a stable network for the accumulation of tropoelastin during developmental stages, and the complex organization of the connective tissue matrix.

**Clinical Material and Methods**

**Patient Population and Specimen Gathering**

The study population consisted of a consecutive cohort of 20 adult patients with intracranial hypotension in whom a spontaneous spinal CSF leak was confirmed by CT myelography or radionucleide cisternography. All patients were clinically examined for features of connective tissue abnormalities by one of the authors (W.I.S.). Skin samples were obtained from the edge of the incision at the time of surgery, two leaking meningeal diverticula. Dermal fibroblast cultures were established using standard procedures.

**Pulse-Chase Analysis of Fibrillin**

The pulse-chase assay was performed in the exact manner that has previously been described. Briefly, fibroblasts from affected and control patients were maintained in a hypoperconfluent state for several days and then were metabolically labeled by incorporation of 35S-cysteine for 30 minutes, after which they were chased with unlabeled cysteine for up to 20 hours. Proteins from the soluble cell lysis and the insoluble ECM fractions were separated by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Labeled fibrillin bands were quantified using phosphorimaging analysis. The results were interpreted for assignment to defined phenotypic groups as previously described.

**Fibrillin Immunofluorescence**

Samples selected for immunofluorescence studies were analyzed in a blinded fashion. Immunostaining of hyperconfluent fibroblast cultures was performed in a manner previously described. Briefly, chamber slides (Nalge Nunc International, Rochester, NY) containing 2.5 x 10^6 cells were incubated for 2 days and then fixed in −20°C acetone and stained with an fibrillin-1-specific monoclonal antibody. Secondary antimouse antibody conjugated to phycoerythrin was used for indirect immunofluorescence. The slides were examined and photographed with the aid of an Olympus BH-2 fluorescence microscope (model BH-2; Olympus, Melville, NY) and film (Ektachrome 400; Eastman-Kodak, Rochester, NY). Exposures were timed to 16 seconds, at standard magnification and field of view, for comparison of fluorescence intensity and fibril structure.

**Screening for FBN1 Mutation**

Genomic DNA was amplified using primers for each of the 65 FBN1 exons. We searched for heteroduplexes, indicating the heterozygous presence of sequence variants, by using DHPLC, as described previously. Amplicons with DHPLC peak profiles distinct from the homoduplex peaks of control samples were sequenced (ABI Prism 377 DNA sequencer; Applied Biosystems, Foster City, CA).

**Statistical Analysis**

For statistical analysis, the chi-square and Wilcoxon rank-sum tests were used to compare clinical features of patients with and without the skeletal manifestations of Marfan syndrome.

**Results**

**Clinical Studies**

During examination, four (20%) of the 20 patients with spontaneous spinal CSF leaks were found to have minor skeletal features of Marfan syndrome. Clinical manifestations of three of these four patients (Cases 1–3) have previously been published. Clinical radiographic details in these patients are summarized in Table 1 and demonstrated in Figs. 1 and 2. The skeletal abnormalities were subtle and mainly consisted of tall stature, a high and narrow palate, marked hypermobility of the finger joints, and long, slender fingers. Other skeletal features commonly seen in patients with Marfan syndrome, such as dolichoostenomelia (arm span greater than height) and scoliosis, were not present in these patients.

At the time of surgery, two leaking meningeal diverticula were discovered in one patient (Case 1) and a single leak-

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**TABLE 1**

*Characteristics of patients with spontaneous spinal CSF leaks and minor skeletal features of Marfan syndrome*

<table>
<thead>
<tr>
<th>No.</th>
<th>Location of CSF Leak</th>
<th>Clinical Manifestations</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>T-11 &amp; T-12 meningeal diverticula</td>
<td>tall stature (height 206 cm [&gt;95th percentile], weight 90 kg), joint hypermobility</td>
</tr>
<tr>
<td>2</td>
<td>T-11 meningeal diverticulum</td>
<td>tall stature (height 176 cm [&gt;95th percentile], weight 50 kg), high arched palate, mild joint hypermobility</td>
</tr>
<tr>
<td>3</td>
<td>C-7*</td>
<td>tall stature (height 172 cm [90th percentile], weight 65 kg), long &amp; slender fingers</td>
</tr>
<tr>
<td>4</td>
<td>Upper lumbar spine</td>
<td>tall stature (height 172 cm [90th percentile], weight 51 kg), mild pectus excavatum, long &amp; slender fingers</td>
</tr>
</tbody>
</table>

*This patient harbored several small thoracic and lumbosacral meningeal diverticula.*
ing diverticulum in another (Case 2). In the third patient (Case 3), the exact cause of the leak could not be determined. Although this patient displayed a focal CSF leak at C-7, a meningeal diverticulum was not detected at that site during surgery. Nevertheless, myelography revealed several small meningeal diverticula at other sites. The fourth patient (Case 4) was treated nonsurgically with multiple spinal epidural blood patches. In none of the patients was the characteristic, generalized dural ectasia of Marfan syndrome exhibited on CT myelography studies.

The results of ophthalmological and echocardiographic examinations performed to screen for additional features of Marfan syndrome in Cases 1, 2, and 3 were normal. The fourth patient (Case 4) was treated in an out-patient setting and was unavailable for further studies. All four patients had one or more first-degree relatives with tall stature, but none had a family history of Marfan syndrome, ocular lens dislocations, cardiac valvular disease, aortic aneurysms or dissections, spinal CSF leaks, or other connective tissue abnormalities.

A comparison of clinical and imaging characteristics of patients with and without the minor skeletal features of Marfan syndrome is presented in Table 2. The only significant difference between the two groups was an earlier age at onset of symptoms in those patients who exhibited minor skeletal abnormalities in addition to their CSF leaks.

Evaluation of fibrillin synthesis and deposition into the ECM as well as fibrillin immunofluorescence assays was performed on fibroblast samples obtained in three patients (Cases 1, 2, and 3; Table 3), and in one control patient who experienced a spontaneous spinal CSF leak but did not exhibit any features of Marfan syndrome. A search for the FBN1 mutation was conducted in two patients (Cases 1 and 2).

**Pulse-Chase Analysis of Fibrillin-1**

The quantitative pulse-chase assay follows the process of fibrillin molecule formation in cultured fibroblasts and the increase in deposition of this protein into the preexisting ECM over time. According to a previously established classification,29 patients with a fibrillinoethy can be subdivided into four groups. Individuals in Groups I and II both exhibit reduced synthesis (<70% of control), but the fibrillin deposition in Group II is lower (levels <35% of control) than that found in Group I (levels >35%). In Groups III and IV there is normal synthesis, but reduced deposition: 35 to 70% in Group III and less than 35% of control in Group IV. A fifth group, in which both synthesis and deposition are maintained at a level higher than 70%, compared with normal controls, is considered to be within the normal range (100% ± two standard deviations).

In our biochemical evaluation of three patients with spontaneous spinal CSF leaks and minor features of Marfan syndrome, one control patient who experienced a spontaneous spinal CSF leak but did not have skeletal anom-
alies, and one unaffected control volunteer, fibrillin synthesis was determined after 30 minutes of incubation with \( ^{35} \)S-labeled cysteine and expressed as a percentage of synthesis in the control fibroblast culture that was included in the same experiment. Matrix deposition was determined after 8 hours and again after 20 hours, when the maximum accumulation of fibrillin in the ECM is achieved (Fig. 3).\(^{22}\) Fibrillin synthesis in the patient in Case 2 was reduced to 53% of normal, and extracellular fibrillin incorporation was severely reduced, by 3% after an 8-hour chase period and by 12% after a 20-hour period. These levels are compatible with a biosynthetic Group II designation. In contrast, the other two patients (Cases 1 and 3) exhibited a Group III protein phenotype. Fibrillin synthesis was 103% and 89%, and deposition was 48% and 43% at 8 hours and 46% and 52% at 20 hours, respectively, when compared with values measured in control fibroblasts. The pulse-chase assay of fibroblasts obtained from the control patient who experienced a spontaneous spinal CSF leak but lacked features that are commonly associated with Marfan syndrome revealed normal fibrillin metabolism, resulting in a Group V classification of protein phenotype (Table 3).

### Fibrillin Immunofluorescence

Results of fibrillin immunofluorescence are shown in Fig. 4. The specimen from the control patient (Fig. 4A) displayed a normal pattern of fibrillin immunostaining. The abnormal immunostaining pattern of the specimen from the patient in Case 2 (Fig. 4B) was similar to that seen in individuals with classic Marfan syndrome.\(^{14,16}\) The specimens from the patients in Cases 1 and 3 (Figs. 4C and D, respectively) displayed a somewhat reduced, that is, intermediate, pattern of fibrillin immunofluorescence. The pattern was not the typical abnormal pattern observed in more than 95% of individuals with Marfan syndrome, which is similar to the pattern shown in Fig. 4B and did not resemble the expected pattern of a healthy control individual. In all cases, however, the morphological characteristics of the fibrils appeared normal.

### Screening for the FBN1 Mutation

All 65 exons of the \( FBN1 \) gene found in two patients (Cases 1 and 2) were amplified and analyzed by means of DHPLC. Amplicons with abnormal peak profiles were sequenced, but no mutation was detected. In the patient in Case 2, in whom reduced fibrillin synthesis had been revealed by pulse-chase studies, the genetic structure was found to be heterozygous for a single nucleotide polymorphism in exon 13, suggesting that this individual does not have a genomic deletion of the entire \( FBN1 \) gene.

### Discussion

In this series of 20 consecutive patients with a neuroimaging-confirmed diagnosis of spontaneous spinal CSF leakage, all patients with the clinical features of Marfan syndrome had abnormal fibrillin metabolism. The patient in Case 2, with reduced fibrillin synthesis and severe reduction in its deposition, was classified as a Group II. The other patients (Cases 1 and 3) exhibited a Group III protein phenotype. Fibrillin synthesis was 103% and 89%, and deposition was 48% and 43% at 8 hours and 46% and 52% at 20 hours, respectively, when compared with values measured in control fibroblasts. The pulse-chase assay of fibroblasts obtained from the control patient who experienced a spontaneous spinal CSF leak but lacked features that are commonly associated with Marfan syndrome revealed normal fibrillin metabolism, resulting in a Group V classification of protein phenotype (Table 3).
leaks and intracranial hypotension, one fifth displayed minor skeletal features of Marfan syndrome, including joint hypermobility, arachnodactyly, and tall stature. In our experience, approximately one third of patients with spontaneous spinal CSF leaks are found to have a leaking meningeal diverticulum at the time of neurosurgical repair. In the remainder, it is postulated that the CSF leak results from a simple dural tear or from a small meningeal diverticulum below the level of resolution of the imaging study. The exact cause of the meningeal defect generally remains unknown, although in addition to the Marfan syndrome, both spontaneous spinal CSF leaks and meningeal diverticula have been described in other disorders that involve connective tissue components, such as autosomal-dominant polycystic kidney disease, neurofibromatosis Type 1, and Lehman syndrome. Our patients did not display the characteristic stigmata of these or other connective tissue syndromes.

Spontaneous spinal CSF leaks have been reported in two patients who suffered spontaneous retinal detachments at an early age. Although not as characteristic as ectopia lentis, spontaneous retinal detachment is a well-recognized complication of Marfan syndrome and has been ascribed to both a high level of myopia and microfibril fragility. These two patients did not have the typical features of Marfan syndrome or those of any other syndrome that has been associated with spontaneous retinal detachment, such as Stickler syndrome, Ehlers–Danlos syndrome, osteogenesis imperfecta, or Knobloch syndrome. The presence of skeletal features associated with Marfan syndrome or of spontaneous retinal detachments in patients with spontaneous spinal CSF leaks indicates that generalized connective tissue disorders are important contributors to disease states in this patient population, whereas the nature of the connective tissue defect is likely to be heterogeneous.

Because these patients displayed phenotypic manifestations, although minor, in two of the five organ systems typically affected in Marfan syndrome (that is, the skeletal system and spinal meninges), we conducted a search for the fibrillin protein by applying a quantitative pulse-chase method and a qualitative immunofluorescence assay. These independent assays were performed at separate laboratories and their results were consistent. Pulse-chase analysis demonstrated that the most severe

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**Fig. 4.** Photomicrographs demonstrating fibrillin immunostaining of fibroblast cultures in patients whose clinical data are detailed in Table 1. A: Staining of fibroblast cultures from a control patient with a spontaneous spinal CSF leak but no other clinical manifestations of connective tissue abnormalities showing a prominent meshwork of fibrillin-immuno-stainable fibrils. This pattern is also seen in normal control fibroblasts. B: Case 2. A significant reduction in accumulation of fibrillin-stainable fibrils in cultured cells. This pattern is similar to that observed in more than 95% of people with the Marfan syndrome. C and D: Cases 1 (C) and 3 (D). Fibrillin immunostaining revealing moderate reductions in fibrillin-stainable fibrils. In neither case was the decrease reminiscent of the abnormal pattern seen in Marfan syndrome fibroblast cultures. Original magnification × 50.
fibrillin phenotype was harbored by the patient in Case 2 (Fig. 3), in whom cultured fibroblasts exhibited reduced synthesis of normal-size fibrillin molecules (53% of control) and severely reduced fibrillin deposition (only 12% of control after 20 hours), resulting in a Group II classification. Assuming that only one fibrillin allele is affected, which is consistent with both the autosomal-dominant pattern of inheritance in patients with Marfan syndrome and related disorders and with the level of fibrillin synthesis in this patient, the amount of fibrillin accumulation in the ECM is disproportionately low. This severe protein phenotype is commonly associated with classic Marfan syndrome. Considering the mild disease course in the patient in Case 2, the symptoms of which consisted of a meningeal diverticulum, tall stature, mild joint hypermobility, and a high arched palate (Table 1), this biochemical outcome is surprising. This patient is only 22 years old, however, and enlargement of the aortic root may still ensue.

Pulse-chase analysis of specimens from Cases 1 and 3 revealed normal intracellular fibrillin synthesis and moderately decreased fibrillin accumulation in the ECM, which is consistent with a Group III classification. This is the only one of the four abnormal biochemical groups that could possibly result from a genetic defect in a connective tissue component other than fibrillin. Interaction of normal fibrillin molecules with other defective connective tissue proteins could lead to reduced microfibril assembly. Such a disorder would be classified as a microfibrillopathy, in contrast with a fibrillinopathy, in which the fibrillins are defective.

In agreement with the results of the pulse-chase studies, fibrillin immunostaining was most severely reduced in only one patient (Case 2). In the other two patients (Cases 1 and 3) there was a moderate reduction in apparent fibrillin accumulation. Whereas the findings in Case 2 resemble those in classic Marfan syndrome, the patterns in Cases 1 and 3, while reproducible, are less suggestive of a primary fibrillin abnormality. Such intermediate levels of fibrillin staining have been observed in pseudoxanthoma elasticum, running the gamut from normal to moderate accumulation, to a significant reduction in fibrillin-stainable fibril. In addition, although fibrillin-2 is not expressed in dermal fibroblasts, abnormal fibrillin-1 immunostaining has also been observed in individuals with mutations in FBN2, suggesting that coordinated interaction between multiple connective tissue constituents is central to assembly of microfibrils.

The severe protein phenotype demonstrated in Case 2, which was observed by using both pulse-chase and immunofluorescence studies, is indicative of a primary fibrillin abnormality; however, an FBN1 mutation was not detected by DHPLC, the most sensitive method available to date. The mutation detection rate for classic Marfan syndrome is only approximately 70%, however, because some mutations are not detected by this screening method. On the other hand, fibrillin protein abnormalities are identified in almost all patients with classic Marfan syndrome and in some individuals with isolated connective tissue features, including abnormalities in the musculoskeletal, ocular, and cardiovascular systems. Because researchers in quantitative fibrillin protein studies have concentrated on individuals with defined connective tissue syndromes, correlations between other connective tissue phenotypes and protein abnormalities are only now beginning to emerge. Specimens from individuals with isolated connective tissue abnormalities may more frequently exhibit moderately reduced fibrillin staining detected by immunofluorescence analysis, causing these cases to be assigned to protein phenotype Group III when studied by means of metabolic labeling. In contrast, specimens from individuals who meet the criteria of Marfan syndrome commonly fall into protein phenotype Groups II or IV, display severely reduced fluorescent signals, and are associated with higher risk of cardiovascular involvement at an early age. Although the spontaneous spinal CSF leaks described in two patients (Cases 1 and 3) in this report may not be due to specific mutations in FBN1, the moderately reduced accumulation of fibrillin-stainable fibrils and subclassification into protein phenotype Group III suggest an etiological role of ECM microfibrils in the development of this neuropathological manifestation. A modifying effect on phenotypic expression could be caused by genes that directly interact with fibrillin in the ECM, which provides the matrix scaffold during early development, microfibril-associated protein, one or more of the latent transforming growth factor binding proteins, and enzymes that process profibrillin into the mature fibrillin protein. The eventual characterization of the full complement of these matrix molecules and their interactions will enable a more specific answer.

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References

Microfibrillopathy in spinal cerebrospinal fluid leaks


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