Mitral Valve Prolapse: A Consistent Manifestation of Type IV Ehlers-Danlos Syndrome

The Pathogenetic Role of the Abnormal Production of Type III Collagen

ALLAN S. JAFFE, M.D., EDWARD M. GELTMAN, M.D., GLEN E. RODEY, AND JOUNI UITTO, M.D., PH.D.

SUMMARY To evaluate whether abnormal production of type III collagen, the characteristic biochemical feature of patients with the type IV Ehlers-Danlos syndrome, consistently predisposes to mitral valve prolapse, we evaluated the family of a proband with classic type IV Ehlers-Danlos syndrome. Production of type III collagen was assessed with the use of cultured skin fibroblasts. Mitral valve prolapse was detected by M-mode and two-dimensional echocardiography. Biochemical abnormalities in the production of type III collagen and echocardiographic findings of mitral valve prolapse were completely concordant. All patients with abnormal production of type III collagen had mitral valve prolapse and all subjects with normal production of type III collagen had entirely normal echocardiograms. Six of the eight patients with abnormal production of type III collagen had subtle cutaneous abnormalities. The consistent association of abnormal production of type III collagen and mitral valve prolapse in this family suggests that this abnormality of collagen may give rise to mitral valve prolapse.

MITRAL VALVE PROLAPSE (MVP) is a heterogeneous clinical syndrome that affects 5% or more of the population. Since the syndrome was described originally, the pathologic anatomy, auscultatory and echocardiographic correlates, symptoms, natural history and several associated abnormalities have been reported. Despite this extensive body of information, the molecular basis and etiologies of mitral valve prolapse are obscure. Speculation about pathogenesis has led to attempts to correlate the etiology of mitral valve prolapse with concomitant cardiac abnormalities or systemic diseases. Several disorders that affect the production or structure of collagen or that involve connective tissue in general, such as the Ehlers-Danlos syndrome, Marfan's syndrome, relapsing polychondritis and lupus erythematosus, have been associated with mitral valve prolapse. These associations and the presence of disordered collagen noted pathologically in the mitral valves of patients with mitral valve prolapse has led to the hypothesis that abnormal connective tissue in the mitral valve apparatus may be of pathogenetic importance in the development of mitral valve prolapse. This view is supported by the absence of type III and A-B collagens in the mitral valve of a patient with mitral valve prolapse who underwent mitral valve replacement.

We recently evaluated 10 members of a family with type IV Ehlers-Danlos syndrome and assessed the relationship between the abnormal production of type III collagen and mitral valve prolapse. Classic type IV Ehlers-Danlos syndrome is characterized by friable tissues, easy bruising, translucent skin, mild hypermobility of the joints and a propensity to aortic and gastrointestinal rupture. The inheritance pattern has been thought to be autosomal recessive, and the characteristic biochemical abnormality, an absence of type III collagen. Recent studies have shown that family members of subjects with this classic presentation may have only a diminution in the production of type III collagen. They may exhibit a continuum of cutaneous manifestations. The pattern of inheritance may be either autosomal dominant or sporadic.

To assess whether abnormalities in type III collagen, a type abundant in mitral valve tissue, would consistently predispose to mitral valve prolapse, we studied the family of a patient with typical type IV Ehlers-Danlos syndrome. We evaluated this kindred for the concordance between abnormal production of type III collagen assessed from cultured skin fibroblasts and mitral valve prolapse diagnosed clinically and by echocardiography.

Methods

Patients

The father, mother, four male siblings and three female siblings of a propositus with clinically diagnosed type IV Ehlers-Danlos syndrome were evaluated. One daughter, age 20 years, was not available for evaluation.
Analysis of Collagen

After the patients gave informed consent, skin biopsies were obtained with the use of a punch biopsy technique and fibroblasts were cultured in confluent monolayers and incubated in Dulbecco's minimum essential medium as reported elsewhere. For analysis of collagen, 10 μCi of tritiated proline were added. Twenty hours later, the incubation medium was removed and rapidly cooled, and protease inhibitors were added. The 3H-procollagen secreted into the medium was then precipitated with 20% ammonium sulfate and the ratio of newly synthesized type I and type III procollagen was estimated by assay of hydroxyproline in the peaks separated by diethylaminoethyl-cellulose chromatography under nondeaturing conditions. We have reported recently the optimal conditions for this technique. Based on assay of (3H) hydroxyproline in the peaks identified to include type I and type III procollagen by cyanogen bromide peptide mapping, it was shown by diethylaminoethyl-cellulose chromatography that type III collagen accounted for 19.6 ± 3.2% (± s.d) of the newly synthesized collagen in 26 normal subjects. Collagen analysis was done and patients were classified as normal or abnormal on the basis of the collagen determinations without knowledge of the results of the clinical or echocardiographic determinations.

Echocardiographic Methods

M-mode echocardiograms were obtained with the subjects supine. The transducer was maintained perpendicular to the plane of the mitral valve and echocardiograms were taken with the left atrium behind the mitral valve leaflets. Mitral valve prolapse was said to be present only when the holosystolic or late systolic buckling pattern was definitely present. Two-dimensional echocardiograms were performed with a Varian 3000 echocardiograph in the long-axis parasternal view, the apical four-chamber view, and the apical short-axis view. Mitral valve prolapse was said to be present if coaptation of the mitral leaflets was displaced posteriorly, if there was movement of the mitral valve leaflet above the mitral ring, or if there was systolic curling of the posterior mitral valve leaflet. All echocardiographic determinations were made by observers without knowledge of the results of analysis of the collagen.

Clinical

A complete cardiovascular physical examination was performed. Rising from a squatting position was included in an attempt to elicit findings suggestive of mitral valve prolapse.

HLA Determinations

HLA-A and B specificities and genotypes were determined by microlymphocytotoxicity testing, with the use of the NIH modifications of the microdroplet procedure of Mitall et al. Seventy antisera were used to detect the following specificities: HLA-A1, A2, A3, A9, A10, A11, A28, A29, Aw30, Aw31, Aw32, Aw33, B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, Bw21, Bw22, B27, Bw35, B37, B40, Bw4 and Bw6. At least two antisera were available for each specificity. HLA-DR typing was performed on T-cell-depleted, B-cell-enriched lymphocytes by extended-incubation microlymphocytotoxicity. Fifty-five antisera were used to detect HLA-DR1, 2, 3, 4, 5, 3 + 6, and 7.

Results

Dermatologic Findings

Two subjects, identical twins who were 16 years old, had marked cutaneous abnormalities, including thin, translucent and hyperextensible skin, easy bruisability, multiple molluscum pseudotumors over the knees, elbows and buttocks and mild hyperextensibility of the digits. Similar findings had been described in the maternal grandfather. One of the twins also had recurrent bilateral pneumothorax and hip dislocation. Six siblings and the mother reported no history of skin abnormalities. In five siblings and the mother no obvious skin abnormalities were detected by several examiners. However, two dermatologists experienced in evaluating patients with Ehlers-Danlos syndrome and unaware of the echocardiographic findings and biochemical analysis evaluated these six subjects independently and found mild hyperextensibility and increased velvetyness of the skin in all six. The results of dermatologic examination in the father and one sibling were considered normal by all examiners. No subject had stigmata of Marfan's syndrome.

Echocardiographic Results

The echocardiographic findings are summarized in table 1. The M-mode echocardiogram and a simultaneous phonocardiogram from the propositus is shown in figure 1A. The two-dimensional echocardiogram of his twin brother is shown in figure 1B. Eight of the 10 members of the family were found to have mitral valve prolapse by two-dimensional echocardiographic examination. In all cases, coapation of one or both mitral valve leaflets was posterior to the mitral valve annulus and two also had systolic curling of the posterior mitral valve leaflet. Of the eight family members with mitral valve prolapse by two-dimensional echocardiography, only the twins and one other sibling had definite mitral valve prolapse detected by M-mode echocardiography. Three of the other subjects had M-mode echocardiograms that were highly suggestive of mitral valve prolapse. In the remaining two family members, only limited views of the mitral valve during systole could be obtained. The twins were the only family members who had additional echocardiographic abnormalities; both had a mildly increased diameter of the proximal aortic root.

Physical Examination

The findings on physical examination are summarized in table 1. The auscultatory findings in the propositus are shown in figure 1A along with a simultaneous M-mode echocardiogram. All subjects with
mitral valve prolapse detected by echocardiography had an early or midsystolic click or a click and a mid-to-late systolic murmur. The father had a normal echocardiographic examination, but a click was elicited by rapid standing from a squat.

Studies of Collagen

Studies evaluating the production of type III collagen are shown in figure 2. Production of type III collagen was 21.53 ± 2.0% (± SD) of total production for eight determinations of control (range 18.8–25.5%). Seven family members had definitely abnormal production of type III collagen (greater than 2 standard deviations below the mean for controls) and two subjects had normal production of type III collagen (less than 2 standard deviations below the mean for controls and higher than production in at least one control subject). One subject had a diminution in the production of type III collagen (17.8% of total production, or 83% of the mean for controls) that was slightly less than 2 standard deviations below the mean for control subjects; however, because this value was less than all of the determinations of control, production of type III collagen was designated as abnormal. The mean production of type III collagen in subjects with abnormal production was 12.08 ± 3.7% (± SD) of total production, or 56.5 ± 18% (± SD) of the mean for controls. Each subject with abnormal production of type III collagen had echocardiographic evidence of mitral valve prolapse and at least subtle cutaneous abnormalities (figure 3). The two subjects with mitral valve prolapse and obvious cutaneous abnormalities produced 11.5% and 7.5% of their total collagen production as type III collagen, or 53% and 35%, respectively, of the mean production for control subjects, whereas patients with mitral valve prolapse and subtle cutaneous changes produced 13.1 ± 4% (± SD) type III collagen, or 61 ± 19% (± SD) of the mean for controls.

Determinations of Haplotype

No consistent pattern of inheritance could be determined by evaluation of A and B antigens. The HLA-A-B13 pattern seen in one other family with type IV Ehlers-Danlos syndrome was absent, as was the A3 antigen, which has been associated with mitral valve prolapse.

---

**TABLE 1. Physical Examination and Echocardiographic Findings**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age (years)</th>
<th>Echocardiography</th>
<th>Physical examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-mode</td>
<td>2-D</td>
<td>Click*</td>
</tr>
<tr>
<td>CB</td>
<td>Normal</td>
<td>Normal</td>
<td>+</td>
</tr>
<tr>
<td>MB</td>
<td>Limited views, nondiagnostic MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>KB</td>
<td>Normal</td>
<td>Normal</td>
<td>O</td>
</tr>
<tr>
<td>GB</td>
<td>Suggestive of MVP MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>DB</td>
<td>Limited views, nondiagnostic MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>JB (propositus)</td>
<td>MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>JB</td>
<td>MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>MB</td>
<td>Suggestive of MVP MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>PB</td>
<td>MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
<tr>
<td>YB</td>
<td>Suggestive of MVP MVP</td>
<td>MVP</td>
<td>+</td>
</tr>
</tbody>
</table>

*Only clicks that moved towards S1 when the patient arose from a squat are included.
† Only murmurs whose duration increased when the patient arose from a squat are included.
Abbreviations: MVP = mitral valve prolapse; + = present; O = absent.

---

**FIGURE 1.** (A) Simultaneous phonocardiogram and echocardiogram from the propositus. The paper speed is 100 mm/sec. An early systolic click (C) follows the first heart sound (S1) by approximately 150 msec. The simultaneous M-mode echocardiogram shows mitral valve prolapse (MVP) during early systole. (B) The two-dimensional echocardiogram from the twin brother of the propositus shows thickening and prolapse of the anterior and posterior mitral valve leaflets (AMVL and PMVL). AO = aorta; LA = left atrium; LV = left ventricle.
prolapse. HLA BW 35, which has also been associated with mitral valve prolapse, was found in three subjects with mitral valve prolapse and in both subjects without evidence of mitral valve prolapse. All siblings inherited the A2 B15 haplotype from the mother, but this inheritance pattern did not discriminate between patients with and without mitral valve prolapse. Typing for DR specificity did not suggest a consistent pattern of inheritance for mitral valve prolapse or the cutaneous abnormalities.

**Discussion**

Ehlers-Danlos syndrome is caused by impaired synthesis of collagen; eight types have been described. Type I and type III, disorders without identified biochemical and ultrastructural abnormalities, have been associated with mitral valve prolapse. Type IV Ehlers-Danlos syndrome is characterized biochemically by an absence or diminution of type III collagen and is thought to be the rarest of the eight subtypes. Recent studies of type IV Ehlers-Danlos syndrome have delineated a continuum of cutaneous abnormalities from obvious to subtle and at least two distinct abnormal ultrastructural patterns of collagen. The extent of the defect of collagen is undefined. To the best of our knowledge, only patients with clinically detectable skin abnormalities have been evaluated biochemically. Although mitral valve prolapse in type IV Ehlers-Danlos syndrome has not been systematically investigated, we are unaware of patients with type IV who do not manifest mitral valve prolapse.

The results of this study show a concordance between mitral valve prolapse and abnormal production of type III collagen in patients with both phenotypically expressed and clinically occult type IV Ehlers-Danlos syndrome, and suggest a pathogenetic link between the characteristic biochemical abnormality of type IV Ehlers-Danlos syndrome and mitral valve prolapse. The probability that two independent autosomal dominant characteristics such as mitral valve prolapse and abnormal production of type III collagen could be inherited without a subject who manifests only one of these characteristics is 1 in 128. Because these and other data clearly show that the expression of the biochemical abnormality may be subtle and because type III collagen is more common in mitral valve tissue than in many other tissues, it is possible that mitral valve prolapse could be the only clinical manifestation of this biochemical abnormality. This expectation is supported in part by the work of Hammer and associates, who evaluated a patient with known mitral valve prolapse who underwent mitral valve replacement. They found an absence...
of type III and A-B collagens in this patient’s mitral valve tissue but did not analyze his skin collagen, although overt cutaneous abnormalities were not present. Hammer et al. mention that the mitral valves from five other patients with mitral valve prolapse had normal levels of type III collagen, but criteria for the diagnosis of mitral valve prolapse were not delineated. In addition, normal values for type III and A-B collagens in mitral valve tissue were not established. This consideration is critical because in that study only 30% of the mitral valve tissue could be solubilized for biochemical examination. Although it is unlikely that limited solubility would account for the apparent total absence of type III and A-B collagens, it is possible that the analysis might not have detected some of our patients, who had a variable (17–71%) diminution in the production of type III collagen. Thus, it may be only in the most severely affected patients, as in the one reported by Hammer et al., that the abnormality of collagen can be recognized by analysis of mitral valve tissue. For this reason, skin analysis may prove to be a practical and more sensitive method to evaluate the importance of the production of type III collagen in the pathogenesis of mitral valve prolapse. Further study of a larger number of patients is necessary to assess this hypothesis and the possibility that mitral valve prolapse may sometimes represent a form fruste of type IV Ehlers-Danlos syndrome. The concordance between mitral valve prolapse and abnormal skin production of type III collagen in the family studied suggests that this collagen abnormality may be important in the pathogenesis of mitral valve prolapse.

Acknowledgment

The authors gratefully acknowledge review of the manuscript by Dr. Burton E. Sobel, assistance from Dr. Michael Cain in studying the patients, and preparation of the manuscript by S. Payne.

References

Mitral valve prolapse: a consistent manifestation of type IV Ehlers-Danlos syndrome. 
The pathogenetic role of the abnormal production of type III collagen. 
A S Jaffe, E M Geltman, G E Rodey and J Uitto

Circulation. 1981;64:121-125
doi: 10.1161/01.CIR.64.1.121

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on 
the World Wide Web at:
http://circ.ahajournals.org/content/64/1/121