Isolated Ectopia Lentis: Potential Role of Matrix Metalloproteinases in Fibrillin Degradation

Isolated ectopia lentis is a rare disorder in which no underlying cause for lens subluxation can be found, and it remains primarily a clinical diagnosis. Patients with this disorder may have a variety of ocular complaints, most commonly decreased vision due to lens subluxation.

Matrix metalloproteinases (MMPs) are proteolytic enzymes important in physiologic and pathologic connective tissue remodeling. Proteolytic activity is stringently controlled by a family of natural antagonists, the tissue inhibitors of metalloproteinases (TIMPs). The MMPs and TIMPs are present in the aqueous humor in normal eyes and may interact with the lens zonules.

We describe a patient with lens subluxation associated with positive MMP expression and no demonstrable TIMP immunoreactivity within the lens. To our knowledge, this is the first report of MMP activity, which we postulate leads to isolated ectopia lentis. Understanding the role of these proteases may lead to novel therapies to reduce the progressive nature of lens subluxation affecting the other eye.

Report of a Case. A 60-year-old man was examined across 12 years of follow-up in the Ophthalmology Clinic at Prince of Wales Hospital (Sydney, Australia). The patient initially was seen at our department in June 1990 with a 2-week history of reduced peripheral vision in his right eye because of a rhegmatogenous retinal detachment that was primarily superotemporal. He had sought treatment elsewhere 9 months previously because of sudden reduction in vision; the right crystalline lens was totally dislocated. After the manifestation of right retinal detachment, the best-corrected visual acuity was counting fingers OD and 20/20 OS.

In the left eye, a superonasal lens coloboma was noted in the dilated pupil (Figure, A). The day after he was first seen in our clinic, he underwent retinal detachment repair by means of external drainage of subretinal fluid and a scleral buckling procedure. The dislocated right lens remained in the vitreous cavity.

By September 1998, the dislocated right lens was cataractous, and the patient had abandoned regular use of a contact lens. In anticipation of the possibility of spontaneous total subluxation of the left lens, removal of the right lens before intraocular lens placement was recommended. Right vitreolensectomy and placement of a scleral fixated posterior chamber intraocular 21 OD lens (CZ70BD; Alcon Laboratories Inc, Ft Worth, Tex) were successfully performed. Postoperatively, the best-corrected visual acuity eventually was 20/30 OD.

In May 2001, the patient sought treatment because of a 5-week history of reduced vision in the left eye. There was no history of ocular trauma. Ophthalmological examination results revealed total dislocation of the left crystalline lens. In August 2002, a pars plano vitrectomy was performed, and the dislocated lens was removed from the eye in toto via a superior limbal incision. An intraocular lens (CZ70BD; Alcon Laboratories Inc) was located in the ciliary sulcus and fixed with polypropylene sutures (Alcon Laboratories Inc) to the sclera. The removed lens (Figure, B) was immediately washed twice with phosphate-buffered saline and prepared at room temperature in buffered saline for analysis.

The patient's general medical history included mild aortic regurgitation, gastroesophageal reflux, hypothyroidism after a left thyroid lobectomy, and left-sided deafness that began spontaneously in December 1996. There was no family history of systemic or ocular disease. Findings from a thorough systemic examination and investigation by a cardiologist and general physician and biochemical urinalysis revealed no features of homocystinuria, and the diagnostic criteria for Marfan syndrome were not met; ectopia lentis was the only major criterion present when 2 are needed. The patient's aortic regurgitation was not associated with aortic root dilatation; therefore, it did not fulfill a minor criterion for Marfan syndrome.

In addition to analysis of the extracted left lens, a skin punch biopsy was performed for fibrillin immunoreactivity. The lens was compared with lenses from age-matched and sex-matched normal postmortem eyes (n = 10) obtained from the Lions Eye Bank (Sydney, Australia). Enucleated lenses and the patient's lens were processed as previously described. Eyes with posterior synechiae or posterior subcapsular, nuclear, or other forms of cataract were excluded. In addition, patients with a recent history of trauma, steroid treatment, alcohol abuse, or premature cataract formation were excluded. The 10 subjects from whom the control eyes were obtained had no previous or family history of ophthalmic disease and had not been receiving any medication known to influence cataract formation.

Macroscopically, the lenses (n = 11) were oriented, retroilluminated, and photographed under a microscope. Slitlamp examination (Figure, A) was used to orient the patient's extracted lens (Figure, B). Three experienced and masked observers assessed all photographs for opacification. The photographs were scanned (OpticPro 4830; Plustek, Taipei, Taiwan, Republic of China) and analyzed with a Nikon 2.0 (Nikon Corp, Tokyo, Japan) in Windows 98 (Microsoft Corp, Redmond, Wash).

After orientation, the lenses were weighed and placed on a grid for dissection into 4 quadrants under a microscope. Each quadrant of each lens was weighed individually before and after a small region from each quadrant of the anterior capsule periphery was carefully dis-
A. Clinically observed dislocation of the lens in the left eye viewed with a slitlamp. B. Macroscopic view of extracted lens (original magnification ×5). C. Macroscopic view of a normal age-matched and sex-matched lens (original magnification ×5). Immunolocalization of ectopic lens zonule matrix metalloproteinase (MMP)-1 (D, arrow), MMP-3 (E, arrows), and MMP-9 (F, arrow) (original magnification ×250). Control lens (D, inset, arrow) negative for MMP-1 (original magnification ×125). Lack of ectopic lens zonule staining for tissue inhibitor of metalloproteinase (TIMP)-1 (G, arrow), TIMP-2 (H, arrows), and TIMP-3 (I, arrow) (original magnification ×250). Positive fibrillin immunoreactivity in the patient’s lens zonule (G, inset, arrow) and skin biopsy results (H, inset) (original magnification ×125). A normal age-matched and sex-matched lens revealed no immunoreactivity for MMP-1 (J, arrow), MMP-3 (K, arrow), or MMP-9 (L, arrow) but positive staining for TIMP-1 (M, arrow), TIMP-2 (N, arrow), and TIMP-3 (O, arrow) (original magnification ×250). Control lenses were negative for MMP-1 (J, inset, arrow) and TIMP-1 (M, inset, arrow) (original magnification ×125).
Results. The mean ± SD time from death to fixation of the control lenses was 5.5 ± 1.4 hours (range, 4-9 hours). None of the control lenses was subluxated. There was no significant difference in the weight of each quadrant in all 11 lenses examined. All 10 control lenses were normal macroscopically (Figure, C), and the capsules were normal histologically at hematoxylin-eosin staining. The patient’s left lens macroscopically appeared compacted in the superonasal quadrant and apart from nuclear sclerosis appeared otherwise normal (Figure, B).

None of the normal lens zonules (n=10) had MMP-1 (Figure, J), MMP-3 (Figure, K), or MMP-9 (Figure, L) immunoreactivity, and TIMP-1 (Figure, M), TIMP-2 (Figure, N), and TIMP-3 (Figure, O) staining was observed in all 10 normal lens zonules. Positive staining of the zonules immunolocalized only to the capsular insertion point and lens epithelium in all eyes. Enzyme-linked immunosorbent assay analysis of the normal age-matched and sex-matched lenses revealed low MMP-1, MMP-3, and MMP-9 activity levels (Table), and the results were not different among quadrants. Levels of TIMP-1 and TIMP-2 activity for the whole lenses were more than 11 times higher in normal lenses, as compared with that in the subluxated lens, and were not different among quadrants.

In contrast, MMP-1 (Figure, D), MMP-3 (Figure, E), and MMP-9 (Figure, F) were localized to the degraded lens zonule and lens epithelium in the superonasal area in the subluxated lens. No TIMP-1 (Figure, G), TIMP-2 (Figure, H), or TIMP-3 (Figure, I) staining was found in the dislocated lens. A fibrillin monoclonal antibody was included to identify the lens zonule in the patient’s lens (Figure, G, inset). Sections incubated with isotype control antibodies demonstrated no reactivity in normal lenses or the patient’s lens (Figure, D, inset, J, inset, and M, inset). Enzyme-linked immunosorbent assay analysis (Table) revealed a MMP-1 level 5.6 times higher in the superonasal quadrant of the dislocated lens than in normal lenses and 4.1 times higher than the mean of the other 3 quadrants. In the dislocated lens, there was an unequal distribution among quadrants (P<.001). Similarly, there were 8.0 times greater MMP-3 and 4.2 times greater MMP-9 levels in the superonasal quadrant between the dislocated lens and normal lenses and unequal distribution among quadrants (P<.001) in the dislocated lens. Little TIMP-1 or TIMP-2 was found in the dislocated lens, and levels were not significantly different among quadrants. Skin biopsy results revealed normal fibrillin morphology (Figure, H, inset).

Comment. To our knowledge, this is the first article in which lens-associated immunoreactivity to MMPs and TIMPs in isolated ectopia lentis is quantified. Authors of previous articles have shown that MMPs degrade fibrillin4 and may play a role in lens subluxation in Marfan syndrome.3 The MMPs appear to be stable for as long as 24 hours post mortem in removed ocular tissue.5 To our knowledge, no authors have examined whether MMPs can diffuse into the lens from the aqueous humor or vitreous body or quantified the levels within human lenses. The fibrillin in some cases of ectopia lentis is abnormal.6 We hypothesize that (1) the fibrillin in patients with isolated ectopia lentis is more prone to degradation by MMPs than is normal fibrillin; (2) there is an abnormal zonular protein in ectopia lentis that predisposes fibrillin to MMP degradation, as in Marfan syndrome; or (3) dysregulation of MMPs and TIMPs results in the progressive destruction of lens zonules and subsequent lens subluxation. These data support existing study results that show that excessive proteolysis of the lens zonule results in fibrillin degradation.7 The presence of excessive MMP-1, MMP-3, and MMP-9 and low TIMP-1 and TIMP-2 levels in the superonasal quadrant in this patient correlate with the inferotemporal direction of lens displacement observed clinically before total subluxation.

The lens zonule consists of a series of fibers composed of microfibrils 8 to 12 nm in diameter. The fibrils consist largely of a cysteine-rich microfibrillar component of the elastin system, fibrillin. In other tissues, fibrillin provides a template for elastin deposition.8 Understanding of the functions of the fibrillin-containing microfibrils is still incomplete; correspondingly, no comprehensive theory of the pathogenesis of lens dislocation has emerged.

### Table: Proteolytic Activity in 11 Lenses

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Superonasal</th>
<th>Inferonasal</th>
<th>Inferotemporal</th>
<th>Superotemporal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dislocated Lens (n = 1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-1</td>
<td>56 ± 4.2</td>
<td>20 ± 1.4</td>
<td>15 ± 1.3</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>MMP-3</td>
<td>80 ± 5.3</td>
<td>79 ± 6.4</td>
<td>74 ± 4.4</td>
<td>78 ± 6.4</td>
</tr>
<tr>
<td>MMP-9</td>
<td>63 ± 5.3</td>
<td>40 ± 3.4</td>
<td>32 ± 2.4</td>
<td>20 ± 2.1</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>5 ± 0.4</td>
<td>6 ± 0.3</td>
<td>8 ± 0.4</td>
<td>7 ± 0.2</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>8 ± 0.5</td>
<td>7 ± 0.3</td>
<td>6 ± 0.2</td>
<td>7 ± 0.6</td>
</tr>
<tr>
<td><strong>Normal Lenses (n = 10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-1</td>
<td>10 ± 1.0</td>
<td>10 ± 1.1</td>
<td>9 ± 0.7</td>
<td>8 ± 0.3</td>
</tr>
<tr>
<td>MMP-3</td>
<td>10 ± 0.5</td>
<td>7 ± 0.2</td>
<td>8 ± 0.4</td>
<td>6 ± 0.2</td>
</tr>
<tr>
<td>MMP-9</td>
<td>15 ± 1.2</td>
<td>12 ± 0.2</td>
<td>13 ± 0.2</td>
<td>13 ± 0.2</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>80 ± 6.4</td>
<td>80 ± 8.3</td>
<td>83 ± 6.4</td>
<td>86 ± 7.3</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>86 ± 6.3</td>
<td>81 ± 5.4</td>
<td>83 ± 7.4</td>
<td>84 ± 6.3</td>
</tr>
</tbody>
</table>

Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

*Data are expressed as the mean ± SD nanograms per gram.
Both fibrillin molecules and fibrillin-rich microfibrils are susceptible to degradation by serine proteases, and the amino acid substitutions found in Marfan syndrome change the fragmentation patterns.9 Fibrillin degradation products generated by MMP activity provide conclusive evidence that these enzymes cause specific changes to assembled microfibrils.10 In Marfan syndrome, most of the mutations in fibrillin-1 are found within epidermal growth factor–like motifs and are predicted to disrupt calcium binding. These mutations may render fibrillin-1 more susceptible to proteolytic cleavage.11 Patients with isolated ectopia lentis may also have an increased susceptibility to zonular degradation. Structural modifications in fibrillin-rich microfibrils occur during aging of the human ciliary zonule.10 These age-related changes may account for the increased incidence of ocular disease in older patients with ectopia lentis. The hypothesis regarding the role of MMPs in lens subluxation implies that an imbalance of lens proteases and their antagonists may be involved in the development of ectopia lentis.

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Orbital Metastasis and Intraocular Invasion of Malignant Mixed Tumor (Carcinosarcoma) of the Parotid Gland in a Child

Malignant mixed tumor of the salivary glands is a rare neoplasm, and the majority of these tumors arise from the parotid gland.1 Histopathologically, 3 distinct variants of malignant mixed tumors are recognized, the most common being carcinoma arising from a preexisting pleomorphic adenoma.1,2 The second type is the metastasizing mixed tumor, which has benign-appearing epithelial and stromal components.1,2 The true malignant mixed tumor or carcinosarcoma, an exceptionally rare tumor, is the third subtype and is composed of malignant epithelial and malignant mesenchymal elements.1,2

Less than 5% of all salivary gland neoplasms are seen in patients younger than 16 years and 13% of these tumors are solid, of which only 23% are malignant.1 The most common malignant salivary gland tumor in children is mucoepidermoid carcinoma, followed by rhabdomyosarcoma and acinic cell carcinoma.3 We herein describe a highly unusual patient with a carcinosarcoma of the parotid gland that metastasized to ipsilateral orbit and intraocular structures.

Report of a Case. A 10-year-old boy was seen at another institution because of right orbital and preauricular masses that evolved gradually during a period of 6 months (Figure 1). His medical history included trabeculectomy for right congenital glaucoma at the age of 3 years. His records indicate that there was no evidence of an intraocular mass or iris neovascularization.

Visual acuity in the right eye had remained no light perception since then. Magnetic resonance images showed a right anterior orbital and preseptal mass, as well as a large...

Figure 1. Patient at initial examination, the day after the incisional biopsy was performed.