A Comparative Histologic Study of the Fibrillin Microfibrillar System in the Lens Capsule of Normal Subjects and Subjects with Marfan Syndrome

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Purpose. To gain a better understanding of the pathogenesis of ectopia lentis and myopia in Marfan syndrome, studies were performed to determine the distribution and structure of fibrillin microfibrils in the lens capsule of normal subjects and of subjects with Marfan syndrome.

Methods. Frozen sections and/or flat mounts of lens capsules were prepared from six autopsy eyes, nine surgical capsulotomy specimens obtained at the time of cataract extraction, and five capsules from patients with Marfan syndrome obtained at intracapsular lens extraction. Avidin-biotin-peroxidase complex (ABC) immunoperoxidase or immunofluorescence staining with monoclonal antifibrillin antibody was used to localize fibrillin in lens capsules. Image analysis was also performed to compare the amount of fibrillin expression in normal and Marfan syndrome capsules.

Results. Based on fibrillin staining patterns, we identified three distinct zones in the equatorial and periequatorial regions of the normal lens capsule. Zone I, a 0.75-mm-wide peripheral ring of the anterior capsule, contained radial bundles of fibrillin fibers. In Zone II, a 1-mm-wide meshwork of fibrillin-rich fibers encircled the equator and served as an insertion platform for zonular fibers. Zone III was composed of radial, 0.1-mm-wide bands arranged in a periodic fashion in the most peripheral part of the posterior capsule. Fibrillin fibers were abnormal and disrupted in all three zones in patients with Marfan syndrome. The amount of fibrillin staining per unit area was significantly reduced in Marfan capsules compared with normal capsules (16-26% versus 49-56% per unit area, respectively; \( P < 0.001 \)).

Conclusions. Fibrillin was a major constituent of the peripheral and equatorial areas of the lens capsule. Zonular fibers, also rich in fibrillin, insert into the equatorial region, primarily in Zone II. Possibly, fibrillin played a role in the ability of the lens to change its configuration during accommodation. The observed qualitative and quantitative abnormalities in fibrillin expression in the lens capsule of patients with Marfan syndrome supported a causal relationship to lens abnormalities in these patients.


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including the normal human lens capsule, are not entirely known. A recent immunohistochemical study in lenses with pseudoexfoliation suggested that abnormal expression or aggregation of fibrillin-containing fibrils contribute to this syndrome. Using antifibrillin antibodies and EM, Streeten and co-workers previously showed that the pseudoexfoliation material was fibrillin positive. 

Using immunohistochemical methods, we determined the localization, distribution, and structure of fibrillin microfibrils in lens capsules from normal subjects and from subjects with Marfan syndrome. The results of these studies allowed us to generate a working hypothesis about the role of fibrillin microfibrillar fibers in the normal functioning of the lens and its interaction with the ciliary zonules. We describe changes in fibrillin that might explain the pathogenesis of ectopia lentis and myopia in Marfan syndrome.

**Materials and Methods**

**Tissue Specimens**

Six normal eyes were supplied by the Medical Eye Bank of Maryland, Baltimore, with a death-to-preservation time of less than 24 hours. Portions of nine anterior capsules were obtained from patients undergoing cataract surgery and known to have no connective tissue disease. Five dislocated lenses from patients with a clinical diagnosis of Marfan syndrome were extracted intracapsularly and made available for immunohistochemical examination (Table 1).

Histologic specimens were prepared using one of the following two methods.

**Conventional Preparation.** Nine lenses were immediately frozen and embedded in compound (OCT; Miles, Elkhart, IN) and stored at −85°C until sectioning. Frozen sections (8 μm) were placed on gelatin-coated slides and fixed for 1 minute in cold (4°C) acetone before storage at −20°C. Three anterior capsulotomy specimens were prepared in the same manner.

**Preparation of Flat Mounts.** Two cadaveric lenses were cut radially into four equal sections, and the capsules were stripped from the bulk of the nucleus and cortex. One lens from a patient with Marfan syndrome was prepared in a similar fashion. The capsules were then flat mounted on gelatin-coated slides and stored at 4°C until staining. Portions of six surgical capsulotomy specimens were also flat mounted.

**Immunostaining**

**Light Microscopy.** Frozen sections and flat mounts were fixed in cold acetone for 5 minutes before rehydration in phosphate-buffered saline (PBS) for 10 minutes at room temperature. All subsequent steps were performed at room temperature as previously described. All antibodies were diluted in PBS. Nonspecific staining was blocked by incubation at room temperature for 15 minutes with normal horse serum (1:50; Vector Laboratories, Burlingame, CA). Tissue sections were incubated for 90 minutes with culture supernatant containing mouse antihuman fibrillin-1 antibody at a 1:50 dilution (a gift from Lynn Sakai, Oregon Health Sciences Center, Portland, OR). For negative control sections, the primary antibody was substituted with normal mouse immunoglobulin G (1:10,000; Vector). Sections of human skin or known fibrillin-positive ocular tissue, such as sclera, were included in each run as positive controls. Following this incubation, the slides were washed twice for 5 minutes in PBS and incubated with biotinylated horse antimouse immunoglobulin G (1:100) for 30 minutes. An avidin-biotin peroxidase reagent (Elite; Vector) was applied for 45 minutes. After PBS washes, the reaction was developed using 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO) as substrate in a solution of dimethyl sulfoxide and 0.01% hydrogen peroxide for approximately 10 to 15 minutes. The tissue sections were counterstained with hematoxylin for 10 seconds and were dipped in tap water substitute (1% magnesium sulfate, 0.2% sodium bicarbonate) to enhance hematoxylin staining. Coverslips were mounted with glycerin-gelatin mounting medium. Slides were examined and photographed using a microscope (Nikon Optiphot; Image Systems, Columbia, MD) with Kodak Ektachrome 100 film.

**Fluorescence Microscopy.** Flat mounts of lens capsules from human cadavers were also stained for fluorescence microscopy. In this procedure, normal goat serum was used at a 1:50 dilution for 15 minutes to block nonspecific staining. The application of the primary antibody was identical with that described for immunoperoxidase staining. After incubation with the primary antibody, the sections were rinsed in PBS and incubated with a rhodamine-conjugated, goat antimouse immunoglobulin G (1:30) for 30 minutes at 37°C. Coverslips were mounted using mounting medium (ProLong; Molecular Probes, Eugene, OR). The sections were photographed using an epifluorescence microscope (Zeiss) with Kodak Ektachrome 400 film.

**Image Analysis**

To more quantitatively describe fibrillin distribution in capsules from normal subjects and patients with Marfan syndrome, cross sections of lens capsules (n = 3 from each group) were digitally analyzed using video analysis software (JAVA; Jandel Scientific Software, San Rafael, CA). A microscope (UXF-II; Nikon) and a color camera (WV-CD 110A; Panasonic) were used with a computer (System 310; Dell) containing a frame grabber board (M8; Targa, Truevision, Santa Clara, CA). Three tissue sections were analyzed for each of the six specimens under identical threshold settings based on the intensity of signal for positive reaction product compared with negatively stained areas at 100× final magnification. For each section, three representative fields of lens capsule were outlined, and the area (μm²) of fibrillin staining and total area were digitally calculated for each of the three fields in each of three sections per specimen. Linear measurements were used to determine the fiber lengths using the same software.

Using SigmaStat (Jandel), the mean percentages of the areas of fibrillin staining per lens capsule were calculated for the normal subjects and patients with Marfan syndrome based on the ratio: Area fibrillin positive / Total outlined area. Student’s t-test was used for all except one comparison, for which the Mann-Whitney rank sum test was used, to determine whether the altered expression of fibrillin in Marfan syndrome lens capsules was significantly different from the expression in normal lens.

**Results**

Three distinct and adjacent zones of the equatorial and periequatorial regions of the lens capsule contained fibers composed of fibrillin. The information leading to the reconstruction of these zones was derived from examination of
immunoperoxidase and immunofluorescence preparations of flat mounts of capsulotomy specimens, cadaveric lens capsules, and cross sections of both. Figure 1 shows the three regions of the lens that contain fibrillin microfibrils. In the following sections we will refer to these as zones I (anterior), II (equatorial), and III (posterior).

**Immunoperoxidase Staining of Flat Mounts of Surgical Capsulotomy Specimens**

Five of the six capsulotomy flat-mounted specimens were devoid of fibrillin staining. To determine whether the absence of staining was caused by the inability of the antibodies to penetrate the entire thickness of the lens capsule, three additional specimens were embedded in OCT compound and cross-sectioned serially in their entirety. None of the sections stained positively for fibrillin.

The sixth and largest capsulotomy specimen contained abundant fibrillin staining in a doughnut-shaped distribution pattern (corresponding to Zone I in Fig. 1). In this specimen, numerous bundles of fibrillin-positive fibers were arranged radially in the peripheral portion of the specimen (Fig. 2). The individual fibers within each bundle were parallel to each other. The lengths and widths of 18 distinct large bundles were measured. The lengths varied from 360 to 970 \( \mu \text{m} \) with a mean of 531 ± 36 \( \mu \text{m} \), and the widths ranged from 60 to 230 \( \mu \text{m} \) with a mean of 124 ± 12 \( \mu \text{m} \). The fibers in each bundle were arranged in parallel and close proximity to each other. Their fine structure was evident at higher magnification (not shown). The thinnest microfibrils, probably corresponding to the reported 10- to 12-nm microfibrils, combined to form larger fibers. Fibers appeared to be anchored in the capsular substance by their individual microfibrillar components, which were evident at each end of the fibers as fine, branching structures (Fig. 3A).

There was another category of smaller and more disordered bundles with lengths of 170 to 390 \( \mu \text{m} \) (mean, 250 ± 70 \( \mu \text{m} \)) and widths averaging 80 ± 6 \( \mu \text{m} \) (Fig. 3B). Some fibers were isolated, not bundled, and were significantly shorter than those in bundles (100 ± 11 \( \mu \text{m} \) compared with 531 ± 30 \( \mu \text{m} \); \( P < 0.001 \)). These microfibrils unraveled at each end and appeared to anchor the fibrils (Figs. 3B, 3C).

There was a wide variation in the thickness of the fibrillin fibers between and within all three arrangements described above. The thickest fibers were present in the large bundles, whereas the thinnest were found in the solitary fibers. Although it was difficult to measure the depth at which these fibers lay within the lens capsule, confocal microscopy demonstrated that the large and small bundles were located in the same plane in the outer lamellae of the capsule (not shown). The fibers did not penetrate the entire thickness of the capsule, and there was a significant fibrillin-negative space between the ends of the fibers and the epithelial cells.

In another limited area of the same capsulotomy specimen, we observed short microfibrillar structures that were distinct from the three groups of fibers described above. Some of these microfibrils emanated from around epithelial cells and appeared to converge or coalesce above each cell (Figs. 4A, 4B). Several epithelial cells in this same area of the capsule and in other areas stained positively for fibrillin; in some specimens, immunoreactivity might additionally have derived from the intercellular matrix. One example of epithelial cell-associated immunoreactivity is shown in Figure 4B.

**Immunoperoxidase and Fluorescence Staining of Flat Mounts of Cadaver Capsules**

The thick bundles of fibrillin fibers described in the capsule segment from the cataract patient were also present in capsules from both cadaver lenses. Although there were fewer fibers in the cadaver capsules than in the capsulotomy specimen, their organization into bundles and their microfibrillar structure were similar to those observed for the capsulotomy specimens. Fibrillin staining of these specimens confirmed that these bundles of fibers in the anterior capsule were located close to the equatorial region.

A second major aggregation of fibrillin fibers was present in the equatorial region of the flat-mounted cadaver lens capsule; P, peripheral anterior capsule. Original magnification, ×10.
Fibrillin in Normal and Marfan Lens Capsule

Capsules (Zone II in Fig. 1). These fibers formed a meshwork, which we believe corresponds, at least in part, to the structure known as the zonular lamella. The meshwork straddles the equator and extends for a short distance anteriorly and posteriorly to the equatorial line. The fibers composing this meshwork were oriented radially and were also organized into bundles. Whereas more central bundles in the anterior capsule were isolated, distinct structures, those in Zone II coalesced to form a meshwork. This meshwork was most evident on fluorescence staining (Fig. 5A). We observed the fibrillin-positive meshwork to extend in a continuous manner over the entire circumference of the lens.

Pre-equatorial zonular fibers, organized into fibrillin-positive bundles of variable lengths and widths, were found attached to the anterior edge of the meshwork. They intersected the meshwork tangentially and became anchored after branching into the capsule. The fibrillin-positive fibers in the central equatorial region appeared to be more integrated into a sheet-like structure. Few intact postequatorial zonules survived processing; the zonules that were present also fused with the fibrillin-positive lamellar meshwork in Zone II. The average width of the lamellar meshwork in one capsule was 531 ± 30 μm (range, 420-680 μm). In the second capsule, the average width of the lamellar meshwork was 925 ± 56 μm (range, 580-1460 μm).

A third array of fibrillin-positive fibers was present in the retroequatorial region of the capsule (Zone III in Fig. 1). This system of fibers consisted of faint, short, and narrow parallel bands aligned with periodicity around the entire retroequatorial region (Fig. 5B). The bands were radial, with one end pointing toward the center of the posterior capsule and the other toward the equator. This group of fibers appeared to lie closer to the epithelium than the fibrils in Zones I and II. The difficulty in achieving a completely flattened orientation of the capsule prevented the measurement of the length of these bands, but their widths were uniform at 10 μm and they were spaced at identical 3-μm intervals (Fig. 5B).

Immunoperoxidase Staining of Flat Mounts of a Marfan Syndrome Capsule

A microscopic examination of a flat-mounted capsule from the lens of a 30-year-old man with Marfan syndrome revealed several distinct differences from non-Marfan capsules: The retroequatorial periodic bands were present in some areas of the posterior capsule, but these bands stained only faintly and irregularly for fibrillin (Fig. 6A). The normal capsule contained larger and more regular segments (Fig. 6B). Thin and scanty zonular fibers of varying sizes were found to extend into the equatorial region. Some of these fibers had irregular, round knob-like terminations, whereas others were hairpin-shaped (Fig. 6C). The radial fiber bundles observed in the normal capsulotomy specimen were totally absent in the anterior capsule from the patient with Marfan syndrome (compare Fig. 3A with Fig. 6C). Instead, a bed of granular fibrillin-positive debris was present in the capsule overlying the lens epithelial cell layer (Fig. 6D). The membranes—cytoplasm of some epithelial cells stained positively for fibrillin (Fig. 6E).

Immunohistochemical Comparison of Cross Sections from Normal and Marfan Syndrome Lens Capsules

Normal Lens Capsules. The pattern of fibrillin localization in serial cross sections of normal lens capsules was consistent with the distribution in flat mounts. In all samples, the central portions of the anterior and posterior capsule were entirely devoid of fibrillin. In the equatorial region, there was abundant fibrillin staining. Irregular short fibrillin-positive segments of varying lengths were present in the outermost capsular lamellae. An intense band of fibrillin staining was present in the superficial lamella. This band overlapped with, and was continuous with, zonular attachments (Fig. 7A). In some sec-
FIGURE 4. Microfibrillar structures associated with the epithelial layer of the lens capsule. (A) Microfibrils adjacent to the lens epithelium had a more disorganized orientation. Several epithelial cells appeared to stain positively for fibrillin (open arrow). Original magnification, ×100. (B) Large radial bundles as in Figures 2 and 3A. B shows the boxed area in A at a higher power of magnification (original magnification, ×250). Some microfibrils appeared to join in a pyramidal fashion after emanating directly from epithelial cells (arrow).

FIGURE 5. Immunofluorescence staining for fibrillin in flat-mounted normal lens capsule. (A) Fluorescence staining of lens capsule with antifibrillin antibody and a tetramethyl rhodamine isothiocyanate-conjugated secondary antibody reveal fibrillin fibers that coalesced into bundles of varying widths forming the meshwork of the zonular lamella (solid arrows). A section of the lamella was bent upward (open arrow). This confirmed its characterization as a true lamella, containing fibrillin bundles parallel to the plane of the lamella. Fibers under the lamella appeared to penetrate the lens capsule and were seen in cross section (asterisk). Original magnification, ×300. (B) In the immediate retroequatorial region of the lens capsule, approximately 10-μm-wide periodic bands of immunoreactive fibrillin are visible. These fibers form Zone III of fibrillin staining shown in Figure 1. No zonular attachments were visualized in Zone III. Sites bound by antifibrillin antibody are gray; negative sites are black. Original magnification, ×480.
FIGURE 6. Fibrillin abnormalities in lens capsules of patients with Marfan syndrome. Flat-mounted capsule specimens were stained by ABC immunoperoxidase for fibrillin. (A) Irregular periodic segments in the retroequatorial (Zone III) region of capsule (arrowhead). Portions of ragged-appearing zonular attachments are seen to the right. Original magnification, ×100. (B) A normal capsule exhibited more intense, regular, and larger periodic segments. These contrasting distributions were supported by the serial-sectioned samples shown in Figure 7. Original magnification, ×100. (C) Zonular fibers were elongated and floppy. Some had a hairpin shape. No staining was observed in the area corresponding to Zone II. Original magnification, ×100. (D) Large bundles normally present in Zone I were absent in the Marfan capsule. Instead, a bed of granular fibrillin-positive material was present over the epithelial cell layer. Original magnification, ×100. (E) Some epithelial cell membranes appeared to be immunoreactive for fibrillin (arrows). Original magnification, ×250.

than in normal capsules. The single band in the superficial lamella, the periodic segments, and the randomly organized punctate segments stained in an overall less intense, consistent, or homogeneous fashion than its counterpart in normal lens capsules (Fig. 7C). The cross-sectional widths of the periodic segments in Marfan capsules were significantly less than those observed in normal capsules, with a mean width of 9 ± 0.6 μm for three Marfan capsules (P < 0.003). Variability in the intensity, homogeneity, and width of the periodic bands was seen among the Marfan specimens.

Image analysis of peroxidase-stained specimens confirmed the reduction in fibrillin expression as follows: The fibrillin
FIGURE 7. Immunoperoxidase staining for fibrillin on a snap-embedded lens capsule. Three distinct patterns of fibrillin staining were observed in the equatorial region of normal lens capsules (A, B). (A) The first pattern consists of a solid band located in the superficial lamella of the capsule (solid arrow). The second consists of random punctate staining, which presumably represents short fibrillin segments (hollow arrow). The epithelial layer is counterstained with hematoxylin. Original magnification, ×250. (B) The third pattern, believed to be present in Zone III only, consists of periodic segments approximately 13 μm in width (solid arrowheads). Zonules inserted superficially into the solid fibrillin band (arrow). Original magnification, ×250. (C) In a Marfan lens capsule, all three patterns of fibrillin staining were less intense, consistent, and organized. The zonular attachments appeared shorter in width (arrow), and the periodicity was more sporadic and lower in expression (solid arrowhead). Original magnification, ×250.

staining per unit area was significantly less in the three Marfan capsules than in the three normal capsules, with 49% to 56% of the unit area positive for normal lens capsules compared with 16% to 26% for Marfan capsules (P < 0.001) (Fig. 8). The results of these analyses were consistent with the linear measurements of individual fiber bundles and insertions described in previous sections.

FIGURE 8. Reduced immunoreactivity for fibrillin in Marfan syndrome lens capsules. Sections of lens capsules from normal subjects and patients with Marfan syndrome were ABC immunoperoxidase-stained for fibrillin, then sections were analyzed for the percentage of multiple randomly-selected areas composed of fibrillin-positive material (n = 3 measurements per section, with three independent sections per patient specimen). Nine fields for each capsule were analyzed as described in Materials and Methods. Data are expressed as the mean percentage of unit area staining positively for fibrillin (± SEM). Values were obtained from microscopic images of histologic sections captured and analyzed with JAVA software. Statistical comparisons were performed with SigmaStat.

Altered Expression of Fibrillin In Normal and Marfan Human Lens Capsule

DISCUSSION

Wheatley et al. confirmed the presence of fibrillin in the normal human lens capsule and showed a periodic staining pattern in the equatorial region of one specimen. The present studies confirm and extend our previous findings and provide the first evidence that abnormal fibrillin expression occurred in the lens capsule of patients with Marfan syndrome. By a combination of studies of conventional serially sectioned lenses and a novel technique of flat-mounted lens capsules, we developed a three-dimensional view of the distribution and structure of fibrillin microfibrils in the normal human lens capsule. These findings were used to define irregularities in fibrillin expression in lens capsules from a few patients with Marfan syndrome.

As a basement membrane, the human lens capsule has a high collagen content (69–8%). Collagen type IV predominates, and its distribution in the lens capsule has been widely studied biochemically and immunohistochemically. Marshall et al. reported an even distribution of collagen types I, III, and IV throughout the thickness of the capsule. The different collagens may have slightly varied roles, but they generally provide strength and structural substance to basement membranes. Collagen interacts with glycoproteins and proteoglycans to form supramolecular assemblies in basement membranes.

We found an elaborate network of fibrillin fibers in three distinct, adjacent zones in the peripheral and equatorial regions of the capsule. The three groups of fibers were oriented in a radial configuration with one end pointed toward the central anteroposterior axis of the lens and the other toward the equatorial line.

In the anteriorly located Zone I, the majority of fibers were organized into bundles of various sizes and thicknesses. This is consistent with previously published electron microscopic observations of microfibrillar fiber organization in connective tissues and with the scanning electron microscopic studies.
of Streeter. This most anterior group of fibers was best observed in a flat mount of one surgical capsulotomy specimen that must have included an unusually large portion of the pre-equatorial region, given the absence of fibrillin staining in the smaller, more central capsulotomy samples. The fiber bundles in this region contained the thickest and bulkiest fibrillin fibers of the entire capsule. No zonular attachments were detected in the region containing these bundles. This does not preclude, however, the possibility that zonules that extend into that region were pulled off as a result of surgical trauma or processing of autopsy specimens. Interestingly, the large bundles observed in the capsulotomy specimen were less prominent in the autopsy specimens, in which mostly single fibers and small bundles were observed. This discrepancy in the number and size of fibers requires further investigation before any generalization of fibrillin fiber distribution and zonular insertion in this most anterior area can be made.

The fibrillin fibers in the equatorial region (Zone II in Fig. 1) appear to contribute to, or form, the meshwork of the zonular lamella. The zonular lamella is a superficial layer of the lens capsule into which the zonules insert. Scanning electron microscopic studies by Streeter identified the zonular lamella as a distinct morphologic structure. Our observations confirm those of Streeter and indeed suggest that the zonular lamella is not only a morphologically, but also a biochemically, distinct structure of the lens capsule. We visualized large fibrillin-positive, pre-equatorial zonular fibers that were morphologically identical with those reported by Streeter and others. Although many of the fibrillin-positive fiber bundles in this region appeared to be zonular attachments, the majority appeared to be an integral part of the lamella.

The third group of fibrillin fibers (Zone III in Fig. 1) was located immediately behind the posterior edge of the meshwork forming Zone II. These fibers were arranged in a distinct pattern of 10-μm-wide periodic segments that we did not find to be associated with zonular insertions. We presume that this is the pattern that Streeter described to be "rippling," which results from the extension of the zonules. Our immunohistochemical results suggest that this pattern of fibrillin staining represents an integral feature of the lens, not folds or ripples. The serial cross sections revealed short periodic segments within the capsule substance whose spacing pattern resembled that observed in the flat mounts. The periodic segments in the frozen sections were clearly not insertions of zonules, but distinct structural components of the lens capsule.

The patterns of fibrillin distribution observed in cross sections of normal human lens capsules were consistent with those in the flat mounts and with the patterns observed by Wheatley et al. The solid band of fibrillin located in the superficial region of the cross-sectioned capsule may correspond to the zonular lamella. Streeter showed that, for the most part, the zonules attached superficially in the lens capsule. We visualized zonules that penetrated both the solid fibrillin band in the cross sections and the meshwork in Zone II of the flat mounts, suggesting that these represent the same structure. The periodic bands of fibrillin seen in the cross sections appear to correspond to the parallel periodic bands located in Zone III. The randomly distributed superficial short segments and excrescences in the cross sections may correspond to the thick, closely aligned bundles of fibrillin fibers of the anterior capsule (Zone I).

Given the wide and varied pattern of distribution of fibrillin microfibrils in the lens capsule and the presence of microfibris in zonular fibers, in lens and ciliary epithelia, and in the capsular substance, it is likely that fibrillin microfibrils have multiple functions in the normal human lens capsule: In the equatorial region, fibrillin microfibrils of the ciliary zonules may function to anchor the zonular fibers to the lens capsule. We suggest that the microfibrils of the equatorial meshwork have a similar and complementary role. Several studies have shown that zonular fibers attach to the zonular lamella. We believe that the fibrillin meshwork in the zonular lamella provides a mechanical and biochemical capsular substrate to which the zonular fibers adhere and integrate. Further ultrastructural and biochemical studies are needed to support this hypothesis. Pre-equatorial zonules branch out as they contact the capsule; this branching may strengthen the attachments by allowing multiple points of root-like contact over a wide area.

The fibrillin meshwork may also have an elastic function. Recent ultrastructural analyses have suggested that normal fibrillin microfibrils possess the capacity to extend and retract. The interlocking structures, of the fibrillin fibers in the equatorial region and the zonular attachments, may contribute to the elasticity required in the dynamic suspension of the lens by the zonules. The circumferential compliance and elasticity of the lens capsule imparted by the fibrillin meshwork may also be key factors in accommodation. In the only specimen in which they were clearly identified, the thick fibrillin fibers composing the most anterior system of bundles (Zone I) had no apparent contact with zonules. The characteristic pattern of distribution and the bulkiness of these fibers suggest an elastic role. Their organization into radial bundles in the peripheral part of the anterior capsule resembles that of the springs of a circular trampoline. These bundles surround, and possibly suspend the central anterior fibrillin-free capsule. It has been suggested that collagen type IV contributes to the flexibility of the lens capsule. Considering the essentially structural and supportive function of collagen and the absence of elastin in the lens capsule, we contend that these anterior fibrillin bundles provide much of the capsular elasticity and may be important in the normal accommodative function of the lens.

The known physiological changes that the lens undergoes during the process of accommodation are consistent with our hypothesis. Most of the changes in lens curvature take place in its anterior surface, with the posterior surface remaining relatively immobile. As the ciliary muscle relaxes, creating tension between the zonules and capsule, the fibrillin bundles would extend and pull on the central disc of fibrillin-free capsule, thereby allowing the lens to flatten, which, in turn, decreases its dioptric power.

It appears that the lens enlarges primarily in its peripheral region with increasing age. This increase in size may also be possible in part because of the elasticity provided by fibrillin in the equatorial region. Defective fibrillin expression in Marfan patients could lead to stunted lens growth and to spherophakia. We have immunohistochemical evidence for abnormal fibrillin expression by lens epithelial cells. Examination of a flat-mount preparation from one patient with Marfan syndrome revealed total absence of the fiber bundles in Zone I in the periphery of the anterior lens capsule. There were, instead, fragments of fibrillin-positive material in the superficial layers of the capsule. The zonular fibers appeared thin,
stretched, and irregular in diameter. They inserted into a faintly stained zonular lamella, which was flanked posteriorly by an irregularly staining row of periodic bands. Comparison of stained cross sections of lens capsule from normal subjects and patients with Marfan syndrome revealed clear qualitative and apparent quantitative differences in the patterns of fibrillin staining. This was most evident in the area of periodic fibrillin-positive segments, which was significantly diminished in length and width compared with normal capsules. Additionally, the total area of fibrillin staining was significantly decreased in Marfan lens capsules when semiquantitative analyses of the amount of immunoreactive material were performed.

The high occurrence of zonular elongation and subsequent lens dislocation in Marfan syndrome has been attributed to anatomic and mechanical aberrations of the globe and ciliary processes in several pathology reports.\(^5\)-\(^7\) Enlargement of the globe was presumed to lead to an increase in the circumferential space, causing the zonules to stretch and the ciliary processes to appear thin and decreased in number.\(^6\) The zonules were described as defective, especially at the point of lenticular attachment, at which there appeared to be a lack of divergence of the zonular fibers into a fan of filaments.\(^9\) In view of the high content of fibrillin in the zonular fibers, they may be less elastic under normal stress in patients with Marfan syndrome and result in elongation over time. Our data suggest that, in Marfan syndrome, lens capsule abnormalities caused by scarce and defective fibrillin deposition into the capsule also contribute to lens dislocation. If the fibrillar fibers that compose the lamellar meshwork are diminished in number, or if they are of reduced structural and biochemical quality, the result may be a weak and defective surface to which the zonular fibers could anchor.

The higher prevalence of spherophakia and increased axial length contribute to the common occurrence of myopia in patients with Marfan syndrome. The reduced number of abnormal zonular fibers with weak lenticular attachments may produce inadequate tension in the zonules and an inability of the capsule to stretch, hence leading to myopia. The absence of myopia in some patients with Marfan syndrome\(^7\) may relate to the differential expression of fibrillin gene mutations.

In summary, we have compared the distribution of fibrillin-containing microfibrils in lens capsules from normal subjects to that in patients with Marfan syndrome. Patients with Marfan syndrome have significantly less fibrillin and aberrant fibrillin-containing fibers in their lens capsules. We propose that fibrillin plays an important role in normal capsular elasticity and, possibly, in the process of accommodation. Aberrant fibrillin protein expression in patients with Marfan syndrome correlates with abnormal capsular and zonular structure and is consistent with the role of mutations in the fibrillin gene in the clinical manifestations of lens subluxation, myopia, and presenile cataract formation.

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References


