Increased Extracellular Deposition of Fibrillin-Containing Fibrils in Pseudoexfoliation Syndrome

Ursula Schlötz—Schrehardt,* Klaus von der Mark,† Lynn Y. Sakai,‡ and Gottfried O. H. Naumann*

Purpose. To localize the distribution of fibrillin-containing microfibrils in normal human anterior segment tissues and to characterize the role of fibrillin in the pathogenesis of pseudoexfoliation syndrome.

Methods. Anterior segment tissues were obtained from 10 eyes with pseudoexfoliation syndrome and 10 normal eyes and investigated by indirect immunofluorescence and electron microscopic immunogold labeling using a monoclonal antibody to fibrillin-1.

Results. In addition to labeling of zonular fibers, fibrillin-immunoreactive microfibrillar bundles generally were found in the corneal stroma; the stromal connective tissues of conjunctiva, ciliary body, and iris, especially in the iris root area; the periphery of Schlemm's canal, the scleral spur, and the most anterior portion of the trabecular meshwork; the ciliary muscle, and the dilator and sphincter muscles of the iris; the basement membranes of peripheral corneal epithelium, conjunctival epithelium, ciliary pigmented epithelium, and the lens capsule. The microfibrillar bundles were found to be isolated or in association with elastic fibers and cellular basement membranes. In pseudoexfoliation eyes, an additional strong immunoreaction was localized to pseudoexfoliation fibers and their microfibrillar subunits in close proximity to surfaces of cells involved in pseudoexfoliation fiber production.

Conclusions. The fibrillin-containing microfibrillar system in normal ocular tissues is suggested to have a substantial role in the maintenance of tissue integrity by providing tensile strength and flexibility to mechanically strained tissues. The findings further provide evidence for fibrillin as an intrinsic component of pseudoexfoliation fibers, suggesting the possibility that enhanced expression of fibrillin or abnormal aggregation of fibrillin-containing microfibrils may be involved in the pathogenesis of pseudoexfoliation syndrome.

The pseudoexfoliation (PEX) syndrome is characterized by the widespread production and progressive accumulation of an abnormal extracellular fibrillar material in many ocular and extraocular tissues, including skin and connective tissue portions of various visceral organs.1,2 Despite its wide prevalence among the older population3 and its clinical importance, the pathogenetic mechanism of PEX syndrome and the exact composition of the abnormally produced material remain unknown. The available histochemical and immunohistochemical data suggest a complex glycoprotein—proteoglycan structure containing both non-collagenous basement membrane components4 and epitopes of the elastic fiber system.5-7 This abnormal systemic process of the extracellular matrix has been, therefore, suggested a disorder of disturbed basement membrane metabolism8 or a type of elastosis especially affecting the elastic microfibrillar component.9,10 Recent developments have advanced our knowledge of elastin-associated microfibrils, which are distributed widely in elastic and nonelastic tissues throughout the body.11-13 The glycoprotein fibrillin, the main and most well-characterized component of elastic microfibrils,14,15 has a widespread distribution in many connective tissues and has been shown previously by light microscopic immunohistochemistry in most tissues and the zonular fibers of the normal hu-
man eye. Fibrillin further was shown to be involved in certain connective tissue diseases with ocular manifestations like that of Marfan's syndrome. The ocular problems in Marfan's syndrome largely arise from compromise in the anchoring function of the defective fibrillin-containing microfibrils of the zonular apparatus that lead to dislocation of the lens in approximately 60% of the patients. A high incidence of phakodonesis, spontaneous lens dislocation, and vitreous complications during extracapsular cataract extraction from five patients (77 ± 7.3 years) with PEX syndrome were obtained during blepharochalasis operations from five patients with PEX syndrome (mean age, 74 ± 3.6 years) with clinically diagnosed PEX syndrome were enucleated because of painful absolute glaucoma with open angles and were fixed immediately after removal. Additionally, 10 normal-appearing, age-matched control eyes without any known ocular disease were obtained at autopsy (mean age, 77 ± 8.3 years) and fixed between 5 and 12 hours of death; the eyes had no history or morphologic evidence of glaucoma. Two eyes of patients (mean age, 74 ± 3.6 years) with clinically diagnosed PEX syndrome were enucleated because of painful absolute glaucoma with open angles and were fixed immediately after removal. Additionally, 10 normal-appearing, age-matched control eyes without any known ocular disease were obtained at autopsy (mean age, 77 ± 8.3 years) and fixed between 4 and 7 hours after death. Upper eyelid biopsy specimens were obtained surgically during blepharochalasis operations from five patients with PEX syndrome (mean age, 79 ± 1.4 years) and five patients without PEX syndrome (mean age, 74 ± 6.2 years). Central anterior lens capsules were obtained during extracapsular cataract extraction from five patients (77 ± 7.3 years) with PEX syndrome. Informed consent to eye and tissue donation was obtained and the study followed the tenets of the Declaration of Helsinki.

METHODS

Anterior segment tissues were obtained from 10 eyes with PEX syndrome. Seven eyes with PEX were obtained at autopsy (mean age, 79 ± 1.4 years) and fixed between 5 and 12 hours of death; the eyes had no history or morphologic evidence of glaucoma. Three eyes of patients (mean age, 74 ± 3.6 years) with clinically diagnosed PEX syndrome were enucleated because of painful absolute glaucoma with open angles and were fixed immediately after removal. Additionally, 10 normal-appearing, age-matched control eyes without any known ocular disease were obtained at autopsy (mean age, 77 ± 8.3 years) and fixed between 4 and 7 hours after death. Upper eyelid biopsy specimens were obtained surgically during blepharochalasis operations from five patients with PEX syndrome (mean age, 79 ± 2.6 years) and five patients without PEX syndrome (mean age, 74 ± 6.2 years). Central anterior lens capsules were obtained during extracapsular cataract extraction from five patients (77 ± 7.3 years) with PEX syndrome. Informed consent to eye and tissue donation was obtained and the study followed the tenets of the Declaration of Helsinki.

Indirect Immunofluorescence

Tissue specimens were embedded in OCT compound and snap frozen in isopentan–liquid nitrogen. Six micrometer cryostat sections were fixed in cold acetone, incubated in a 10% solution of normal rabbit serum in phosphate-buffered saline (PBS), and incubated in primary antibody diluted 1:300 in PBS overnight at 4°C. The monoclonal antifibrillin antibody (MAB 201) was produced by Sakai et al.; it recognizes fibrillin-1 and does not cross-react with fibrillin-2 (Sakai L, personal communication, 1996). Antibody binding was detected by incubating the sections with fluorescein isothiocyanate-conjugated secondary antibody (Dako, Hamburg, Germany) 1:50 in PBS for 30 minutes at room temperature.

Immunogold Labeling

Postembedding Labeling. Tissue specimens were fixed in a freshly made solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 to 2 hours at 4°C. After being rinsed, the specimens were dehydrated serially to 70% ethanol at —20°C, embedded in LR White resin, and polymerized for 24 hours at 45°C. Ultrathin sections were incubated successively in drops of Tris-buffered saline (TBS), 0.05 M glycine in TBS, 0.5% ovalbumin, and 0.5% fish gelatin in TBS, antifibrillin antibody diluted 1:200 in TBS–ovalbumin overnight at 4°C, and 10 nm gold-conjugated secondary antibody (BioCell, Cardiff, United Kingdom) diluted 1:30 in TBS–ovalbumin for 1 hour at room temperature. After being rinsed, the sections were stained with uranyl acetate and examined with an EM 9A electron microscope (Zeiss, Oberkochen, Germany).

Preembedding Labeling. Freshly obtained anterior lens capsule specimens were incubated in primary antibody diluted 1:20 in PBS containing 1% bovine serum albumin overnight at 4°C, followed by the 10-nm gold-conjugated secondary antibody diluted 1:30 in PBS–bovine serum albumin for 1 hour at room temperature. After being washed, the specimens were fixed in 2.5% glutaraldehyde in PBS for 30 minutes and then in 1% osmium tetroxide in PBS for 1 hour at 4°C. The specimens were dehydrated in an ethanol series and embedded in Epon according to standard procedures. In negative control specimens, the primary antibody was replaced by PBS–TBS or nonimmune mouse serum.

RESULTS

Despite considerable interindividual differences in labeling intensity, the general pattern of immunoreactivity was similar in normal eyes and in eyes with PEX syndrome, which disclosed an additional strong immunoreaction to fibrillin at all sites of PEX material accu-
Immunofluorescence for fibrillin is detected in the basement membrane (arrows) of the peripheral epithelium (Ep) and in the corneal stroma (St). Bl = Bowman’s layer. Magnification, X440. (b) A laminar pattern of fluorescence is evident on the posterior surface of Descemet’s Membrane (Dm) in some PEX specimens; the endothelium is artificially detached. St stroma. Magnification, X460. (c,d) The immunogold reaction for fibrillin is localized to microfibrillar bundles between Descemet’s membrane (Dm) and corneal endothelium (En) as well as within the fissures of Hassall–Henle warts (H). Magnification bars = 0.3 μm.

At the electron microscopic level, the gold marker for fibrillin generally was localized to bundles of microfibrils, approximately 10 nm in diameter, in ocular tissues and skin specimens of normal eyes. In tissues of eyes with PEX, the gold marker also was associated clearly with typical PEX fibers, 20 to 45 nm in diameter and a main banding periodicity of approximately 50 nm, and their intermingled microfibrillar subunits, approximately 10 nm in diameter. The abnormal fibrillar PEX material accumulated adjacent to various cell types and close to elastic fibers and collagen fibers within connective tissues.

We found ultrastructural evidence of local production of PEX fibers by most cell types of the iris, ciliary nonpigmented epithelial cells, preequatorial lens epithelial cells, and trabecular meshwork cells. Indications for a focal synthesis included abnormal fibers arising from membrane invaginations of the cell surfaces, interrupting the continuity of the basement membrane; clusters of cytoplasmic coated vesicles fusing with the cell membrane in the region of the invagination and opening toward the extracellular matrix; and an extracellular maturation process from microfibrils to typical thick, fuzzy PEX fibers.

Cornea

In the normal corneal stroma, a weak immunoreactivity for fibrillin could be localized to patchy isolated bundles between the collagen lamellae (Fig. 1a).
FIGURE 2. Trabecular meshwork of normal (a,c to e) and pseudoexfoliation (PEX) specimens (b,f). (a) Immunofluorescence microscopy shows fibrillin staining in the walls of Schlemm’s canal (Sc) and in the scleral spur (Ss); the trabecular meshwork (Tm) shows no staining. Ac = anterior chamber. Magnification, ×230. (b) Additional fibrillin immunoreactivity is present in PEX deposits in the trabecular meshwork and in the uveal meshwork (arrows) of a glaucomatous PEX specimen. Ac = anterior chamber. Magnification, ×230. (c,d) The gold label for fibrillin is located along microfibrillar bundles connecting Schlemm’s canal (Sc) endothelium (En) and extracellular plaque material (Pl). Bar = 0.3 μm. (e) Fibrillin-reactive microfibrillar bundles are present in the extracellular matrix in the transitional zone to the cornea. Ac = anterior chamber; En = trabecular endothelium. Bar = 0.5 μm. (f) Dense gold labeling of PEX fibers in the juxtacanalicular tissue underneath Schlemm’s canal (Sc) endothelium (En). Bar = 0.5 μm.
Whereas corneal epithelium, endothelium, and Descemet's membrane showed a negative immunoreaction, the epithelial basement membrane showed a positive immunoreaction in its peripheral portions only (Fig. 1a). This reactivity corresponded at the ultrastructural level to diffusely distributed gold particles, marking single microfibrils or delicate bundles in the periphery of keratocytes, between collagen fibers, and in the region of the epithelial basement membrane.

In some corneal specimens of one normal and four PEX eyes, an additional laminar staining pattern was observed occasionally on the posterior surface of peripheral Descemet's membrane (Fig. 1b). This could be electron microscopically related to thick immunoreactive microfibrillar bundles, localized between endothelial cells and Descemet's membrane (Fig. 1c) and within fissures and channels of Hassall–Henle warts of the peripheral cornea (Fig. 1d).

**Trabecular Meshwork**

In frozen sections of normal trabecular meshwork specimens, positive immunostaining for fibrillin was confined to the entire periphery of Schlemm's canal with an increase in staining density toward the posterior part of the
meshwork, to the scleral spur, and to the most anterior portion of the meshwork adjacent to Schwalbe’s line (Fig. 2a). The corneoscleral and uveal meshwork tissue proper essentially was negative. Trabecular meshwork specimens of PEX eyes contained, in addition, heavily stained extracellular plaques of irregular size, which predominantly were located in the periphery of Schlemm’s canal and in the uveal portion of the meshwork and which were presumed to represent PEX material accumulations (Fig. 2b). These fibrillin-positive plaques particularly were prominent in the trabecular meshwork of glaucomatous PEX eyes.

At the ultrastructural level, the gold marker clearly was associated with isolated microfibrillar bundles localized directly underneath the endothelium of Schlemm’s canal along its inner and outer walls (Fig. 2c). The microfibrils appeared to be attached to the endothelial cells on one side and to extracellular plaque material (sheath-derived plaque material) or cells of the juxtacanalicular connective tissue on the other side (Fig. 2d). In the most posterior part of the meshwork, these microfibrillar bundles were not limited to the subendothelial space but were dispersed in the juxtacanalicular tissue. At the corneolimbal attachment of the trabecular meshwork, fibrillin-containing microfibrillar bundles were found scattered within the extracellular matrix of the most anterior trabecular beams (Fig. 2e). In the scleral spur, similar fibrillin-positive microfibrillar bundles were present between the collagen fibers, in association with fibrocytes and with the periphery of elastic fibers.

In the trabecular meshwork specimens of PEX eyes, most PEX material deposits were located in the juxtacanalicular tissue adjacent to the inner and outer walls of Schlemm’s canal, where they appeared to arise from local production by the endothelial cells and to replace the normal subendothelial microfibrillar bundles. Although showing regional differences in staining intensities, the PEX fibers generally were labeled strongly for fibrillin, particularly in close proximity to the endothelial cell surface (Fig. 2f). Similarly, PEX material accumulations in the uveal meshwork and adhering to its inner surface showed strong, but variable, staining patterns.

Iris

Immunofluorescent labeling with anti-fibrillin antibody showed a diffuse, faintly labeled fiber network in the iris stroma of normal eyes. This network was particularly concentrated in the regions of the dilator and sphincter muscles, in blood vessel walls, and, to a minor extent, in the anterior border layer. Especially thick and prominent fibrillin-positive bundles could be observed consistently in the iris root area (Fig. 3a). In PEX specimens, extracellular plaques supposed to represent PEX deposits exhibited an additional strong immunoreaction both on the surface of the posterior pigment epithelium and within the stromal connective tissue, predominantly in the periphery of blood vessels and in the anterior border layer (Figs. 3b, 3c).

The immunogold label for fibrillin could be localized to small sporadic microfibrillar bundles within the stroma that was arranged loosely, which occurred either isolated or in close association with fibrocytes. Within blood vessel walls, these bundles often could be observed in association with the basement membranes of endothelial cells, pericytes, and smooth muscle cells as well as with elastic fibers (Fig. 3d). In the dilator and sphincter muscle tissues, the microfibrillar bundles were present mostly at the interface between muscle and surrounding connective tissue, but also interspersed between the smooth muscle cells (Fig. 3e). The microfibrils frequently were found to be attached to the basement membranes of the muscle cells. The PEX specimens exhibited a marked, yet variable, labeling of PEX fibers both on the pigment epithelial surface and in all intrastromal locations (Figs. 3f, 3g). The gold marker was evident predominantly on PEX fibers adjacent to cell membranes and arising from cellular surface invaginations.
Ciliary Body

In normal specimens, marked fluorescence was detected in the zonular fibers inserting into the basement membrane of the nonpigmented ciliary epithelium and, to a minor extent, in the basement membrane of the pigmented epithelium, whereas the basement membrane of the nonpigmented epithelial layer and the epithelial cells were negative (Fig. 4a). Correspondingly, electron microscopy showed the presence of fibrillin along the zonular fibers in a dense and homogenous distribution (Fig. 4c) and in a rather patchy distribution pattern within the basement membrane of the pigmented epithelium. In the latter, the gold marker could be localized to areas of dispersed fibrogranular material within the amorphous basement membrane of the pigmented layer.

Strongly labeled cords were found in the ciliary body stroma of the pars plicata and pars plana regions, particularly prominent in the inner connective tissue layer between ciliary epithelium and ciliary muscle (Fig. 4a). These fibrillin-immunoreactive bundles were observed to run either parallel to the longitudinal axis or in cross-connection between the basement membrane of the pigment epithelium and the outer ciliary muscle fibers. Similar cords were seen traversing and coursing within the connective tissue separating the muscle bundles. The walls of stromal and muscular blood vessels contained a condensed, radially arranged fiber network (Fig. 4a). At the ultrastructural level, the presence of fibrillin in the ciliary stroma and muscle tissues could be immunolocalized to numerous large microfibrillar bundles occurring either separately (Fig. 4c) or in association with the basement membranes of connective tissue and muscle cells (Fig. 4f), with the periphery of elastic fibers (Fig. 4g), and with blood vessel walls. The bundles sometimes could be found running in parallel to cell surfaces, in close apposition to the basement membrane or the cell membrane (Fig. 4f).

Ciliary body specimens of PEX eyes additionally disclosed an extensive labeling of PEX deposits on the epithelial surface, occasionally appearing as a bright band of fluorescence along the basal aspect of the nonpigmented epithelial layer in the anterior portion of the pars plicata (Fig. 4b). Electron microscopically, the gold label decorated the PEX fibers on the epithelial surface, with a particularly high affinity for PEX fibers within surface indentations of the nonpigmented epithelial cells (Fig. 4d).

Lens

The findings of normal lenses stained with antifibrillin antibody were negative, except for the heavily labeled zonular lamella on the surface of the preequatorial and postequatorial lens capsule and a delicate lamellar pattern of staining in the prequatorial capsular regions. The immunogold label was localized correspondingly to the zonular fibers on the capsular surface (Fig. 5a) and to horizontal fibrogranular inclusions within the prequatorial lens capsule (Fig. 5b). Almost no reaction product was noticed in the lens epithelial cells and lens fibers.

Lenses with PEX disclosed an additional positive immunofluorescence of PEX material accumulations on the lenticular surface and on zonular fibers as well as a narrow band of fibrillin positivity along the inner aspect of the prequatorial lens capsule corresponding to the deep fibrogranular zone of intracapsular PEX material (Figs. 5c, 5d). By electron microscopy, an intense labeling of PEX fibers was apparent on the capsular surface in the peripheral granular zone, in the prequatorial region of zonular attachment, and in the region of the central disk. Whereas the postembedding technique yielded a rather homogenous staining of PEX fibers throughout the capsular deposit, a denser but mostly superficial staining of the PEX clumps could be obtained with the preembedding technique (Figs. 5e, 5f). However, by both labeling techniques, the gold marker was associated predominantly with the periphery of mature PEX fibers, frequently in a linear pattern of arrangement, and with the microfibrillar components (Figs. 5e, 5f). A period-
Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrillin in Pseudoexfoliation Syndrome

Fibrill.
connective tissue. In eyelid skin specimens of patients with PEX, PEX aggregates in the dermal connective tissue exhibited a moderate staining with focal variations in labeling density (Fig. 6c).

**Conjunctiva**

The connective tissue stroma of the normal limbal conjunctiva stained intensely for fibrillin, forming a fiber network that extended perpendicularly toward the epithelial-stromal junction. The basement membrane of the epithelium was found to be positive. Additional irregular plaques of strong immunofluorescence were found in the conjunctival stroma of most PEX specimens, probably representing PEX material accumulations. The immunogold label was present on normal microfibrillar bundles and on PEX fibers and their microfibrillar subunits in close apposition to fibroblast-like cells (Fig. 6d) and to elastic and collagen fibers.

**DISCUSSION**

**Fibrillin-1-Containing Microfibrillar System in Normal Human Ocular Tissues**

The findings of the current study are consistent with previous studies in showing an extensive network
FIGURE 5. Lens capsule of normal (a,b) and pseudoexfoliation (PEX) specimens (c to h). (a) The gold label for fibrillin is present along the zonular lamella (Zo) on the surface of the lens capsule (Ca). Bar = 0.3 μm. (b) Gold particles are restricted to horizontal fibrogranular inclusions within the capsule (Ca). Le = lens epithelium. Bar = 0.3 μm. (c) Strong fluorescence is shown on PEX material accumulations on the capsular surface, on the zonular lamella (Zo), and along the inner aspect (arrows) of the preequatorial lens capsule (Ca). Magnification, ×440. (d) Prominent fluorescence on PEX accumulations covering the equally positive zonular fibers (Zo). Magnification, ×260. (e) Gold particles are associated with PEX fibers on the surface of the capsule (postembedding technique; bar = 0.5 μm). (f) Association of the gold marker with the periphery of PEX fibers and microfibrils on the capsular surface (preembedding technique; bar = 0.3 μm). Periodic array of the gold label along a PEX fiber at 50 to 55 nm (arrows). Bar = 0.1 μm. (g) Association of gold particles with intracapsular (Ca) PEX fibers emerging from a pit of a lens epithelial cell (Le). Bar = 0.3 μm. (h) Extension of cytoplasmic processes of preequatorial lens epithelial cells (Le) into the capsule (Ca) in a PEX specimen; the lumen of the channels above the processes is filled with fibrillin-positive material. Bar = 1 μm.

FIGURE 6. Conjunctiva and eyelid skin. (a) Extensive fibrillin-positive network extending perpendicular toward the epidermal-stromal junction in a normal skin specimen (Ep epithelium; magnification, ×440). (b) Immunoreactive microfibrils (arrow) are attached to the basement membrane (Bm) of the epithelial cells (Ep) in a normal skin specimen. (c) Pseudoexfoliation (PEX) aggregates in the connective tissue of eyelid skin specimens exhibit a moderate labeling density (Ef elastic fiber). (d) Dense gold labeling of PEX fibers and microfibrils in the conjunctival stroma (Fi fibroblast). (Magnification bars in all electron micrographs = 0.3 μm.)
Apart from their implication in elastogenesis, microfibrillar insertion of elastic microfibrils into the respective basement membranes of various cell types and to the periphery of elastic fibers were found in proximity to basement membranes. In the trabecular meshwork, the fibrillin-positive microfibrillar network, located immediately underneath the endothelial lining of Schlemm's canal, may be of particular functional importance, because the juxtanaculcular tissue generally is regarded as the most important site for outflow resistance and for the genesis of primary open-angle glaucoma. The fibrillin-containing microfibrils may be important for the maintenance of a proper cell-matrix interaction and for the organization of a normal tissue architecture in the juxtanaculcular region, acting as a flexible anchorage for the endothelial cells. They might correspond to the “connecting fibrils” described by Rohen et al. that link the elastic-like fiber plexus in the juxtanaculcular tissue to the inner wall of Schlemm’s canal.

Possible Role of Fibrillin-1 in the Pathogenesis of Pseudoexfoliation Syndrome

Because of their central structural role and their wide distribution throughout the body, fibrillin-containing microfibrils are supposed to be involved in certain diseases of the connective tissue. An increase in microfibrils was shown in skin specimens from patients with localized and systemic scleroderma and in photodamaged skin. A significant reduction of microfibrils has been reported in skin specimens and dermal fibroblast cultures from patients with Marfan’s syndrome. The Marfan’s syndrome is caused by mutations in the fibrillin-1 gene on chromosome 15 that lead to decreased and disordered incorporation of fibrillin into the connective tissue matrix. The hallmark of ocular involvement in Marfan’s syndrome is the dislocation of the lens in approximately 60% of the patients. Apart from other histopathologic abnormalities of ocular tissues in these patients, the zonular fibers are attenuated and broken. A high incidence of phakodonesis, spontaneous dislocation of the lens, and an increased risk of intraoperative complications due to a weak zonular apparatus also are well known in eyes with PEX syndrome. Pathologic alterations of the zonular fibers and their attachment sites into ciliary body and lens were shown and proposed to account for these complications, indicating that lens-dislocating disorders, such as PEX syndrome, may be attributed to defects in the zonular-elastic microfibrillar system.

Abnormal aggregation of glycoproteins associated with the zonular-elastic microfibrillar system in a type of elastosis has been suggested in the pathogenesis of PEX syndrome. This well-founded theory was based on the premise that abnormal fibrillin-containing microfibrillar inclusion is a critical component in the pathogenesis of Pseudoexfoliation Syndrome.
on the frequent structural association of PEX fibers with elastic components in connective tissues and with zonular fibers within the eye, on ultrastructural indications for the development of degenerating elastic microfibrils into PEX material, on similar histochemical staining properties of PEX fibers and the zonular-elastic microfibrillar system, and on a similarity of amino acid composition between PEX material and elastic microfibrils. This view was supported further by a marked, localized elastosis of the elastic fibers in the lamina cribrosa of PEX eyes and the immunohistochemical demonstration of various epitopes for elastic components in PEX material. The epitopes shown to be present include fibrillin, the microfibril-associated glycoprotein MAGP, tropoelastin and α-elastin, as well as amyloid P component and vitronectin, proteins that become bound noncovalently to the periphery of elastic fibers, and possibly the elastin-associated glycoprotein gp 115 corresponding to emilin. Finally, the causes of death of the two patients, in whom the systemic occurrence of PEX material has been established, were dissecting aneurysm of the abdominal aorta and rupture of the esophagus, possibly indicating an underlying generalized defect in the elastic fiber system. This faintly may resemble the association of aortic aneurysm in patients with Marfan’s syndrome.

Together with previous studies on lenticular PEX aggregates, the immunohistochemical findings of the current study provide evidence for fibrillin-1 as an intrinsic constituent of PEX material and, therefore, further evidence for an elastic tissue relation. A strong immunoreaction for fibrillin was localized to PEX material accumulations in all intraocular sites as well as in extraocular conjunctival and skin specimens in addition to the normal fibrillin-positive microfibrillar network, indicating a generalized, increased production and deposition of abnormal fibrillin-containing extracellular material. At the electron microscopic level, the gold marker was associated clearly with the PEX fibers and their microfibrillar subunits, sometimes in a linear periodic array, and often in immediate proximity to the surfaces of cells obviously involved in PEX production. The structure of the microfibrillar subunits of PEX material resembled the structure of connective tissue microfibrils, which have a diameter of 10 to 12 nm, a tubular cross-section, and an average but variable periodicity of 50 to 55 nm. A clear periodicity of 50 to 55 nm of the gold marker along the PEX fibers could be observed only in our specimens by application of the preembedding labeling technique by incubating unfixed tissue specimens with the fibrillin antibody. Nevertheless, the monoclonal anti-fibrillin antibody had a marked affinity for PEX fibrils in the extracellular matrix, irrespective of the labeling technique applied, whereas intracellular structures or cytoplasmic vesicles fusing with the cell membrane in areas of PEX fiber formation remained unstained, suggesting that fibril assembly essentially is an extracellular event or that some processing of the molecule before deposition into the extracellular matrix occurs. In addition to the abnormal accumulation of fibrillin-containing PEX material, the normal fibrillin-positive fibrogranular inclusions in the lens capsule appeared to be larger and more numerous in PEX lenses. Moreover, increased amounts of fibrillin-positive microfibrils could be observed on the posterior surface of peripheral Descemet’s membrane and within fissures of the preequatorial lens capsule in some PEX specimens. Abnormal cellular protrusions of lens epithelial cells into the lens capsule also have been reported in a patient with Marfan’s syndrome, together with an altered zonular ultrastructure and a weakened immunoreactivity for fibrillin. Although the zonular fibers of the PEX specimens studied showed pathologic changes, such as coating and infiltration with PEX material, disorganization, and fragmentation, their fibrillin immunoreactivity appeared similar to that of normal eyes.

The ocular tissues and structures mainly involved in the PEX process correspond largely to the fibrillin distribution in normal anterior segment tissues. Similarly, the distribution of fibrillin in various connective tissue matrices throughout the body (e.g., skin, lung, kidney, muscle, tendon, vasculature) includes most of the organ systems affected in PEX. The apparent absence of PEX material in certain fibrillin-containing tissues of the anterior segment (e.g., the ciliary body stroma) and all posterior segment tissues may depend on metabolic differences in these tissues or lacking access to factors related to the deposition of PEX material.

The excessive production of abnormal fibrillin-containing PEX material may involve important functional implications. Because of the progressive accumulation of the abnormal material in the cell periphery and the associated extensive basement membrane defects, the normal structural integrity of the basement membrane-elastic microfibrils-elastic fiber-system in the connective tissues should be compromised considerably. This impairment particularly becomes evident in the instability of the zonular fiber system, which could be attributed partially to a loosened attachment of the zonules into the damaged basement membranes of ciliary body and lens by intercalating PEX accumulations. In the trabecular meshwork, the buildup of locally produced PEX material in the juxtagoninal canalicular connective tissue is associated with a concomitant decrease in normal fibrillin-containing microfibrillar bundles (formerly termed “type I plaques”) underneath Schlemm’s canal endothelium. Thus, the normal fibrillin-containing microfibrils, providing anchorage for the endothelial cells of Schlemm’s canal, are replaced gradually by PEX deposits. These structural alterations of the subendothelial microfibril population ultimately may lead
to a disorganization of the juxtaocular area and ab-
normalities in Schlemm’s canal architecture and finally
result in aqueous outflow obstruction and glaucoma de-
velopment in PEX syndrome.

The known constituents of PEX material com-
prise, however, not only components of the elastic
system, but also basement membrane proteins, such
as heparan sulfate proteoglycan and entactin,4 and
large quantities of carbohydrate moieties,51,52 charac-
terizing a complex glycoprotein–proteoglycan struc-
ture. The identification of different, apparently unre-
lated, epitopes in PEX material has led to controver-
sies about its nature and pathogenesis and to the
suggestion of an alternative theory of PEX syndrome
as a basement membrane disorder.8

Both the elastic microfibril- and the basement
membrane-theory appear to describe part of the
pathologic process only. Although the data of this and
previous studies suggest an abnormal aggregation of
fibrillin-containing microfibrils to PEX fibers, other
extracellular matrix components may interact and be-
come incorporated equally into the PEX fibers. Where
PEX fibers are produced by cells that normally form
a basement membrane, the PEX fibers are deposited
within or adjacent to the basement membrane inter-
ruping its continuity, and basement membrane com-
ponents may well contribute to the disordered assem-
blage of PEX fibers. It is still unknown which of the
various constituents represent primary products of a
disordered cell metabolism or which are incorporated
secondarily into the abnormal matrix aggregates.

In conclusion, the current immunohistochemical
findings are indicative of an excessive production and
abnormal aggregation of fibrillin-containing microfi-
brils in the extracellular matrix, suggesting the possi-
bility that defects in this family of microfibrillar glyco-
proteins may be involved in the pathogenesis of PEX
syndrome. Thereby, the findings support the abnor-
mal elastic microfibril theory that has been put for-
ward by Streeten and coworkers5,0,42 for many years. This
pathologic process of the extracellular matrix
involves various, potentially elastogenic cell types, in-
cluding epithelial cells, endothelial cells, pericytes,
smooth and striated muscle cells, fibrocytes, and melano-
cytes,1,52 which may be activated by an appropriate
stimulus. Fibrillin also can be synthesized by fibro-
blasts, smooth muscle cells, vascular endothelial cells,
and probably other cell types in vitro.11,12 Possible
pathogenetic mechanisms in PEX syndrome include
an abnormal stimulus (e.g., growth factors) toward
an enhanced expression of fibrillin or other matrix
molecules or both, abnormal glycosylation processes,
abnormal enzymatic processes,24 or even a possible
genetic defect in any of the involved matrix compo-
nents. Whether a defective metabolism of fibrillin-1 is
in fact the primary abnormality of the PEX process
has to be substantiated in the future by in situ hybrid-
ization experiments or by cell culture models using
metabolic-labeling strategies.

Key Words
electron microscopy, fibrillin, immunocytochemistry, mi-
crofibrils, pseudoexfoliation syndrome

Acknowledgment
The authors thank Katharina Endress for excellent technical
assistance.

References
1. Schlotzer-Schrehardt U, Koch MR, Naumann GOH, Vol-
kohltz H. Pseudoexfoliation syndrome: Ocular
manifestation of a systemic disorder? Arch Ophthal-
2. Streeten BW, Li Z-Y, Wallace RN, Eagle RC, Keshg-
ian AA. Pseudoexfoliative fibrillopathy in visceral or-
gans of a patient with pseudoexfoliation syndrome.
3. Forsius H. Exfoliation syndrome in various ethnic pop-
184:71–85.
4. Schlotzer-Schrehardt U, Dörrler S, Naumann GOH.
Immunohistochiecial localization of basement mem-
brane components in pseudoexfoliation material of the
5. Streeten BW, Gibson SA, Dark AJ. Pseudoexfoliative
material contains an elastic microfibrill-associated
320.
with pseudoexfoliative material: An immunoelectron
7. Li Z-Y, Streeten BW, Yohani A. Amyloid P protein in
515.
AJ. Pseudoexfoliative fibrillopathy in the conjunctiva:
A relation to elastic fibers and elastosis. Ophthalmology.
1987;94:1439–1449.
JA. Pseudoexfoliative fibrillopathy in the skin of pa-
tients with ocular pseudoexfoliation. Am J Ophthal-
11. Cleary EG. The microfibrillar component of the elas-
tic fibers: Morphology and biochemistry. In: Uitto J,
Pocjda AJ, eds. Connective Tissue Disease: The Molecular
Pathology of the Extracellular Matrix. New York: Marcel
12. Mecham RP, Heuser JE. The elastic fiber. In: Hay ED,
ed. Cell Biology of the Extracellular Matrix. New York:
13. Kiely CM, Shuttleworth CA. Fibrillin-containing mi-
crofibrils: Structure and function in health and dis-
14. Sakai IY, Keene DR, Engvall E. Fibrillin, a new 350-
Fibrillin in Pseudoexfoliation Syndrome


Announcement

**JIN H. KINOSHITA LECTURESHIP**

**Call for Nominations**

The National Foundation for Eye Research, an organization dedicated to the support of cataract research, is pleased to announce a call for nominations for the 4th Jin H. Kinoshita Lectureship. The Trustees of the Foundation have approved the following statement concerning the Lectureship and the qualifications of nominees:

The Kinoshita Lectureship was established in 1989 by the National Foundation for Eye Research to honor Dr. Jin H. Kinoshita for his outstanding contributions to vision research. It is given for meritorious research which contributes to the understanding of the etiology and prevention of cataract development. The recipient presents a lecture of his or her research at the biennial international U.S.–Japan Cooperative Cataract Research Meeting and is awarded an honorarium of $15,000 and a plaque.

A committee has been established to evaluate all nominees and to recommend two top candidates to the Trustees of the Foundation, who will make the final selection. The committee consists of Leo T. Chylack, MD, James F. Heimann, MD, PhD, and J. Samuel Zigler, Jr, PhD (Chair) with the President of the Foundation, Venkat N. Reddy, PhD, as an ex officio member without a vote. To nominate someone for this award, send a letter outlining that person’s relevant research accomplishments and a copy of his or her curriculum vitae to Samuel Zigler, 6 Center Drive MSC-2735, National Eye Institute, Bethesda, MD 20892-2735. The deadline for receipt of nominations is June 15, 1997.