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Precapsular layer of the anterior lens capsule in early pseudoexfoliation syndrome *

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Abstract. We examined a possible correlation between clinical signs of early pseudoexfoliation (PSX) syndrome related to pigment dispersion and iris stroma atrophy and morphological alterations of the lens capsule. 63 anterior lens capsules (30 PSX suspects, 3 pre-PSX, 10 PSX, 20 controls) were studied by transmission and immuno-electron microscopy (TEM). In 20 PSX suspect and 3 pre-PSX capsulotomy specimens, TEM revealed a precapsular layer (0.1–11 μm in thickness) composed of microfibrils, amorphous material, and granular inclusions. The incidence of this fibrillar layer was significantly higher ($p=0.001$) in PSX suspect and pre-PSX eyes than in controls (5 positive). Ultrastructural and immunohistochemical similarities of the fibrillar surface network in PSX suspect and typical PSX specimens indicate that the precapsular layer may represent a precursor of PSX. The beginning PSX process in the eye is obviously indicated by certain clinical signs.

Introduction

The clinical diagnosis of pseudoexfoliation (PSX) syndrome is commonly made by slit-lamp detection of grayish-white material in the anterior ocular segment, predominantly on the anterior lens capsule and at the pupillary margin of the iris. However, there are several additional clinical signs related to loss and dispersion of pigment from the iris pigment epithelium that are usually present in eyes with PSX syndrome [1, 6, 8, 10, 12, 13, 16, 20, 21] and in uninvolved fellow eyes in unilateral cases [14]. These signs include peripupillary ruff atrophy, iris transillumination, pigment deposition on the iris surface, corneal endothelium, and trabecular meshwork, and anterior chamber pigment dispersion after pupillary dilation. Even in the absence of biomicroscopically evident PSX material in either eye, these pigment-related signs correlated with ultrastructural findings of PSX fibers in the conjunctiva of PSX suspect patients [14]. Another study demonstrated PSX material in the skin of the eyelids of PSX suspect patients revealing iris stroma atrophy, poor mydriasis, loss of pigment from the peripupillary pigment epithelium with subsequent pigment dispersion, but no evidence of PSX material deposition in either eye [19]. These findings obviously suggest certain clinical signs to be an early manifestation of PSX syndrome preceding the biomicroscopically evident accumulation of typical PSX material on the anterior lens surface. In the present study, we examined a possible correlation between clinical signs of early stages of PSX syndrome and more subtle morphological alterations of the anterior lens capsule. Although a number of histologic and ultrastructural studies relate to the fully developed PSX syndrome in the anterior ocular segment, little information is available regarding the early stages of this condition. Early alterations of the anterior lens capsule include the presence of peripheral grey striations [2] and a layer of diffuse, subtle opacification termed precapsular film [4]. This surface layer of the lens capsule has been considered a common finding in many older patients without any signs of pigment dispersion and a possible precursor of PSX.

To determine the possible relationship between clinical signs indicating early PSX and alterations of the lens surface, we investigated anterior lens capsules of different groups of patients by transmission electron microscopy and immunohistochemistry.

Materials and methods

Four groups of patients were categorized on the basis of the following clinical signs:

1. *Patients with typical PSX syndrome* as revealed by deposition of PSX material on the anterior lens surface and pupillary margin.
2. *PSX suspect patients* (Fig. 1A, B) who exhibited at least two

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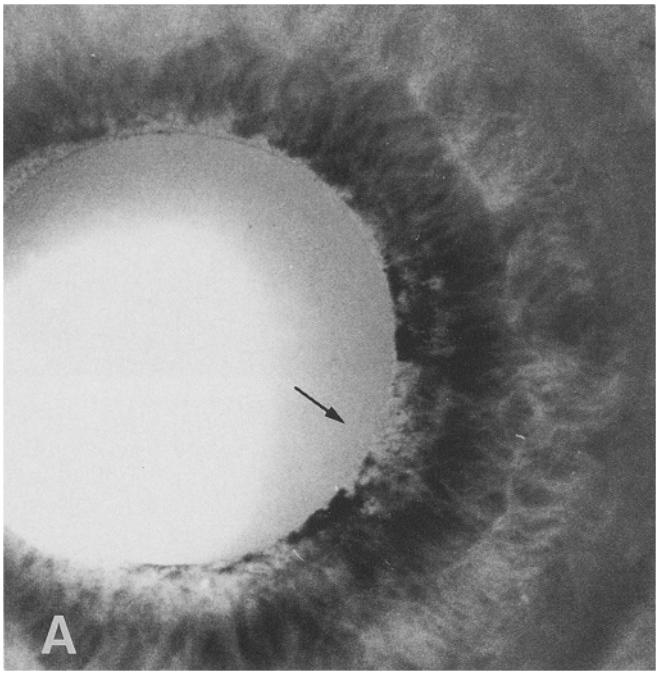

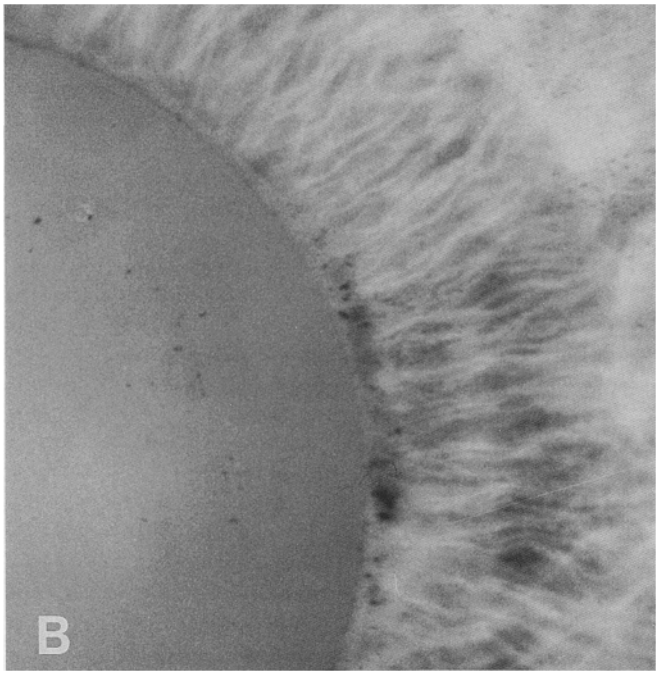

Fig. 1 A, B. Slit-lamp view of PSX suspect eyes. **A** Iris stroma atrophy and incomplete mydriasis after topical application of mydriatics. Loss of melanin from peripupillary iris pigment epithelium and pupillary ruff atrophy (*arrow*). **B** Pigment dispersion with deposition of melanin granules on iris surface and lens

of the following clinical signs, but no PSX material on lens capsule and pupillary rim in either eye:
- iris stroma atrophy and incomplete mydriasis after topical application of mydriatics;
- loss of melanin from the peripupillary iris pigment epithelium (pupillary ruff atrophy);
- anterior chamber pigment dispersion after mydriasis;
- deposition of melanin granules on iris surface, anterior lens capsule, trabecular meshwork, and corneal endothelium;

3. *Pre-PSX patients* (Fig. 4B) were defined by revealing the clinical signs of PSX suspects and additionally a diffuse opacified surface layer on the anterior lens capsule; focal defects in this surface layer usually occurred in the upper nasal area.
4. *Control patients* showed none of the above mentioned clinical signs.

After informed consent was obtained from the patients, assessment of clinical signs was performed by two of the authors (K.T. and M.K.) by slit-lamp examination in mydriasis before cataract surgery.

Anterior lens capsules were obtained during extracapsular cataract surgery from 30 eyes of 30 patients (male: 11, female: 19, age 52–85 years, mean age 75.6 years) diagnosed as PSX suspects, from 3 eyes of 3 patients (male: 1, female: 2, age 71–80 years, mean age 76.3 years) diagnosed as pre-PSX, and from 10 eyes of 10 patients with PSX syndrome (male: 4, female: 6, age 64–85 years, mean age 74.4 years) and were processed for standard transmission electron microscopy. Patients with a history or findings of trauma, uveitis, pigmentary dispersion syndrome, other forms of primary or secondary glaucoma and previous laser or surgical treatment were excluded. As a control, 20 age-matched anterior lens capsules of cataract patients with otherwise normal eyes and without any of these clinical signs were studied (age 64–89 years, mean age 75.7 years).

Transmission electron microscopy (TEM)

Sixty-three anterior lens capsules (PSX suspects: 30, pre-PSX: 3, PSX: 10, control: 20) were fixed immediately after anterior capsulotomy in 2.5% glutaraldehyde in 0.15 mol/L of Sörensens phosphate buffer (pH 7.2) for 24 h at 4° C. After postfixation in 2.0% buffered osmium tetroxide for 1 h, the specimens were dehydrated and embedded in epoxy resin (Epon). Ultrathin sections were cut on a Reichert-Ultracut (Cambridge Instruments, Nussloch, Federal Republic of Germany), stained with uranyl acetate-lead citrate, and examined with a Zeiss EM 9A electron microscope (Zeiss, Oberkochen, Federal Republic of Germany). If TEM did not reveal a precapsular layer as described in the result section, the block was further step sectioned at approximately 200 µm intervals for a maximum of 3 steps before dismissing the specimen as negative. A portion of the lens capsules (PSX: 5, PSX suspects: 10, control: 5) were bisected prior to fixation and separately processed for standard and immuno-electron microscopy.

Immunohistochemistry

Twenty lens capsules (PSX: 5, PSX suspects: 10, control: 5) were processed for immuno-electron microscopy applying the protein A-gold postembedding technique as described elsewhere in detail [18]. In brief, specimens were fixed immediately after anterior capsulotomy in a freshly made solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) for 1 h at 4° C. After rinsing in buffer, the lens capsules were dehydrated and embedded in LR White resin. Ultrathin sections were mounted on uncoated nickel grids, incubated in drops of the primary antibody (rat anti-laminin monoclonal antibody, Chemicon, Temecula, CA; diluted 1:500 in TBS) for 2 h, then incubated in a second antiserum (rabbit anti-rat immunoglobulins, Dakopatts, Glostrup, Denmark; diluted 1:50 in TBS) for 1 h, and then reacted with 10 nm protein A-gold solution (BioCell, Cardiff, U.K.; diluted 1:30 in TBS) for 1 h at room temperature. Non-specific binding was blocked by TBS containing 0.1% ovalbumin and 0.5% cold water fish gelatin between the incubation steps. The sections were finally rinsed in distilled water, stained with uranyl acetate and examined with a Zeiss EM 9A electron microscope. Controls included omission of the primary antibody.

Table 1. Evidence of precapsular layer (PCL) on the anterior lens capsule

	n	mean age	PCL
PSX suspect eyes	30	75.6	20+(66.7%)
Pre-PSX eyes	3	76.3	3+(100%)
Control eyes	20	75.7	5+(25%)

Results

In the 30 eyes categorized as PSX suspects, incidence of each clinical sign was as follows: pupillary ruff atrophy, 94.4%; trabecular meshwork pigmentation, 94.4%; retrocorneal pigment, 61.1%; pigment particles on iris surface, 50%; anterior chamber pigment dispersion after mydriasis, 22.2%; pigment particles on lens, 16.7%.

1. *Patients with typical PSX syndrome*

The surfaces of all 10 PSX capsule specimens revealed distinct, bush-like deposits of PSX material in varying quantities. The PSX materials consisted of the characteristic electron-dense fibers 20–35 nm in diameter with a major cross banding at about 50 nm and intermingled with microfibrils (3–6 nm and 8–10 nm in diameter) (Fig. 3B). A thin layer of microfibrils was frequently interposed between mature PSX fiber aggregates and the lens capsular surface; a comparable fibrillar layer containing scattered PSX fibers usually covered the surface area of the capsules not occupied by PSX deposits.

Gold labeling for laminin was predominantly observed on the microfibrillar component of the PSX materials and the fibrillar surface layer (Fig. 3D).

2. *PSX suspect patients*

In 20 eyes of 30 PSX suspect capsules, TEM revealed a discontinuous precapsular layer (PCL) on the anterior lens capsular surface composed of two types of microfibrils (3–6 nm and 8–10 nm in diameter), amorphous material, and granular inclusions (Figs. 2B, 3A). Mature PSX fibers could not be identified in any of these cases. This PCL showed considerable interindividual variations in thickness (0.1–3.8 μm) and extension, focal interruptions and fragmentation, peeling off, and, occasionally, intermingling of pigment granules and cellular debris with the microfibrils. The microfibrils were largely arranged parallel to the capsular surface and appeared to merge into the superficial capsule. The remaining ten capsules mainly disclosed an irregular surface outline with occasional peeling of the superficial portions, but failed to reveal any precapsular deposits. Immunohistochemically, the PCL showed a similar staining pattern with anti-laminin antibodies as the fibrillar layer of PSX capsules; the gold label was predominantly localized to the microfibrils of the PCL (Fig. 3C).

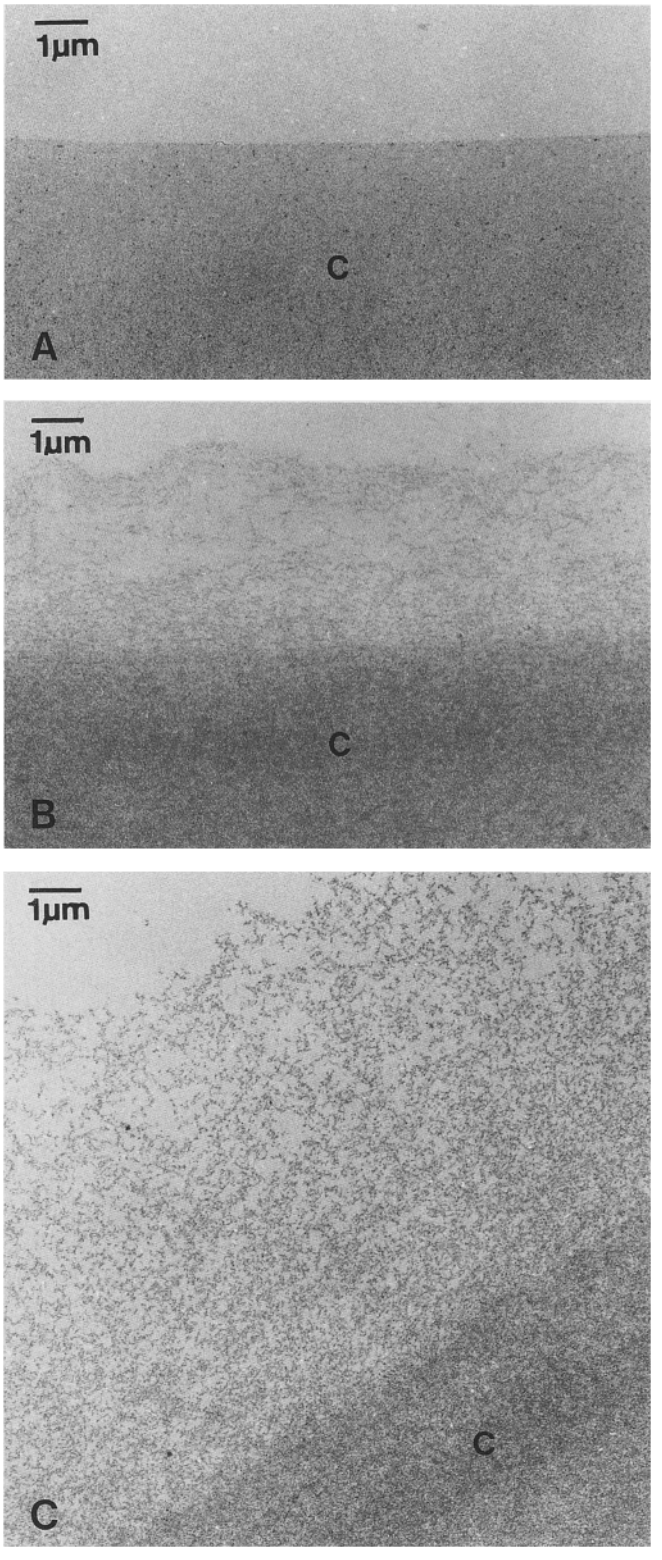

Fig. 2A–C. Transmission electron microscopy of anterior lens capsule (c). **A** Control anterior lens capsule, showing no precapsular depositions. **B** Anterior lens capsule of a PSX suspect patient, showing PCL. **C** Anterior lens capsule of a pre-PSX patient, showing thicker PCL

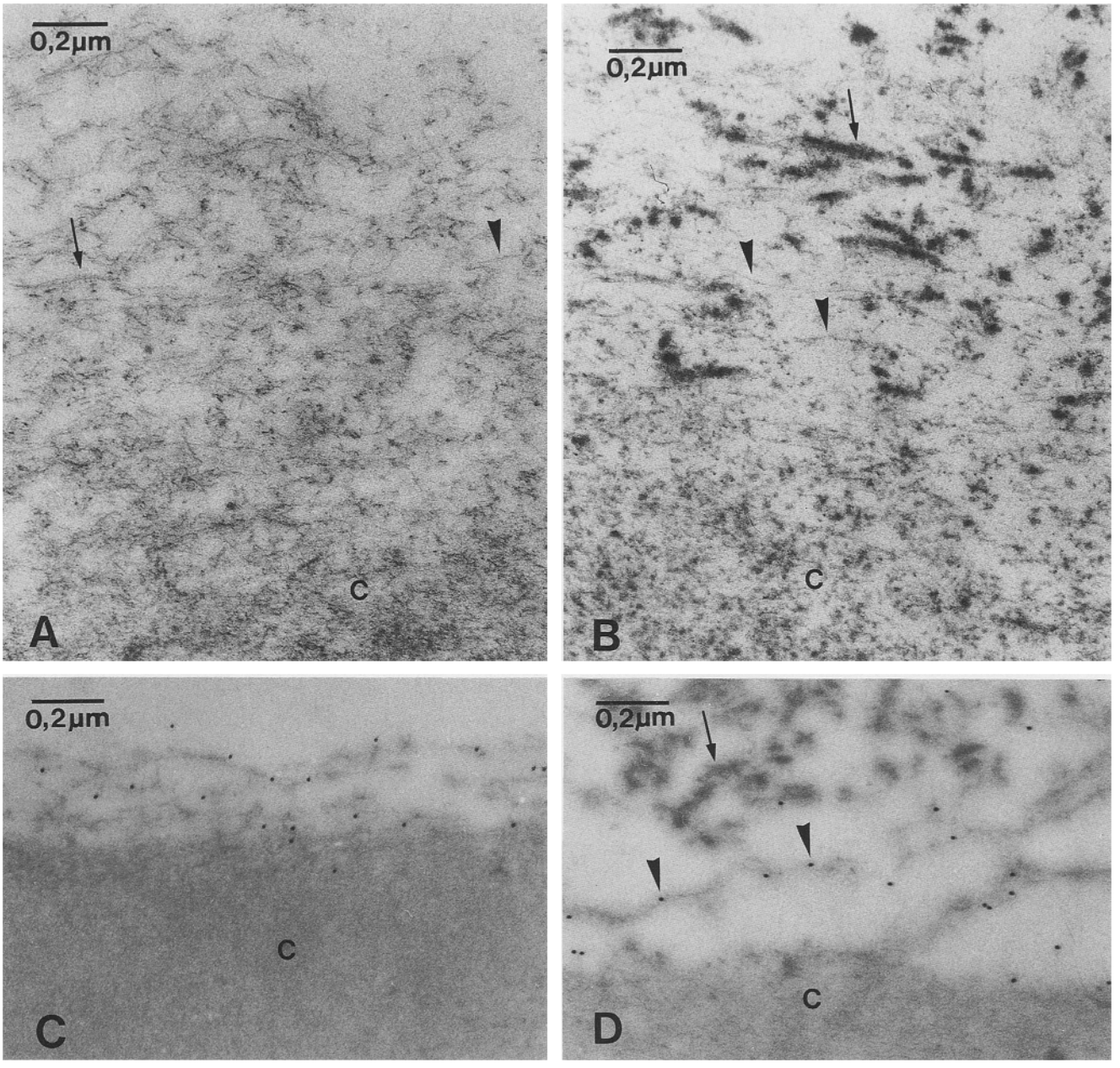

Fig. 3A–D. Comparison of fibrillar components between PCL and typical PSX material on anterior lens capsule (c). **A, B** Transmission electron micrographs. **A** Higher magnification of PCL in a PSX suspect patient. Note microfibrils of 3–6 nm (*arrow head*) and 8–10 nm (*arrow*) in diameter, amorphous material, and granular inclusions. **B** The subunits of typical PSX fibers in a PSX patient, consisting of 3–6 nm microfibrils (*arrow head*), 8–10 nm microfibrils, and thick PSX fibers up to 35 nm in diameter (*arrow*). **C, D** Immunogold localization of laminin. **C** Anterior lens capsule of a PSX suspect patient. The microfibrils of PCL stained positively for laminin. **D** Anterior lens capsule of a typical PSX patient. Gold particles are primarily localized to microfibrils (*arrow head*). Typical PSX fibers (arrow) are largely unmarked

3. *Pre-PSX patients*

The three capsulotomy specimens of pre-PSX patients were characterized by a thick, largely continuous PCL comprising the same structural components as in PSX suspects. A special feature of the pre-PSX cases was an increase in thickness (0.3–11 µm) and homogeneity of the PCL (Fig. 2C); peeling off and focal interruptions were, however, also noticed.

4. *Control patients*

In 5 of 20 control specimens, TEM revealed some evidence of a rather patchy PCL with the same ultrastructural characteristics of PSX suspect and pre-PSX patients. The remaining 15 capsules showed a relatively smooth surface without any precapsular deposits (Fig. 2A). All control capsules contained granular and fibrillar inclusions similar to those noted by Cohen [3]

and Dark et al. [5]. The incidence of a PCL in each patient group is summarized in table 1. The frequency of occurrence of a PCL was significantly higher in PSX suspect and pre-PSX eyes than in the control eyes ($p = 0.001$, chi-square analysis).

Discussion

PSX syndrome is of considerable clinical importance since it is frequently associated with open-angle glaucoma [20] and with a higher incidence of complications in extracapsular cataract surgery [9, 11].

In order to detect rapidly progressing glaucoma and to pay attention to surgical procedures, an early diagnosis of PSX syndrome is required. To date, very little information is, however, available regarding early stages of PSX syndrome.

Bartholomew [2] considered radial, peripheral, grey striations of the anterior lens capsule to be the earliest biomicroscopic changes in PSX syndrome and termed the condition "pre-granular stage". Dark and Streeten [4] reported the presence of a subtle opacified surface layer of the anterior lens capsule in many older patients and suggested this "precapsular film" to be a precursor of PSX syndrome.

In the present study, we observed definitive ultrastructural changes in 23 of 33 (69.7%) anterior lens capsules in PSX suspect and pre-PSX patients exhibiting a number of clinical signs related to iris stroma atrophy and loss and dispersion of pigment from the iris pigment epithelium. Although 5 of 20 (25%) control capsules revealed comparable alterations, the frequency of occurrence was significantly higher in PSX suspect and pre-PSX patients than in control patients, suggesting a clear correlation between these clinical signs and lens capsular alterations. Of the clinical signs evaluated, pupillary ruff atrophy and trabecular meshwork pigmentation showed the highest incidence, followed by deposition of pigment on the corneal endothelium, on the iris surface, in the anterior chamber, and on the lens surface. PSX suspect and pre-PSX patients differed in the additional presence of a biomicroscopically evident film with focal defects on the anterior lens surface in the latter group. The structural changes of the anterior capsulotomy specimens consisted in the formation of a precapsular fibrillar layer of varying thickness and continuity. In the pre-PSX eyes, this PCL was markedly thicker and more continuous as compared to PSX suspect eyes; it obviously corresponded to the clinically visible, opacified film on the lens surface.

A comparable fibrillar surface network could also be identified on lens capsules of established PSX eyes in addition to deposits of characteristic PSX material; in these cases, the fibrillar layer also contained scattered mature PSX fibers, whereas PSX suspect and pre-PSX specimens failed to reveal any typical PSX material. The microfibrils of the PCL in PSX suspect and pre-PSX patients closely resembled the fibrillar components of the surface layer in PSX capsules and also the filamentous subunits of typical PSX material in size and morphology. Moreover, the microfibrils of the PCL in PSX suspect and pre-PSX eyes on the one hand and the microfibrils of PSX material deposits on the other showed a similar staining pattern with monoclonal antibodies against laminin. The presence of laminin in typical PSX material has already been demonstrated by Konstas et al. with an immunogold method [7]. Therefore, ultrastructural and immunohistochemical similarities of the microfibrillar constituents both in the PCL of PSX suspect and pre-PSX eyes and in PSX material deposits of established PSX eyes suggest that the PCL represents a precursor of PSX syndrome.

The PCL observed in the present study obviously corresponds to the precapsular film demonstrated by Dark and Streeten [4]. Both studies describe the surface layers to vary considerably in thickness and continuity, and to be composed of 3–6 nm microfibrils arranged in parallel to the capsular surface. 8–10 nm microfibrils, amorphous material, and granular inclusions supplemented the composition of the PCL in our specimens. While the precapsular film in Dark and Streeten's study was biomicroscopically evident as a ground glass film on the lens, in our study clinical evidence of a PCL was only present in the pre-PSX, but not in the PSX suspect patients. The formation of a PCL, therefore, obviously precedes its clinically evident appearance on the anterior lens surface. While our study demonstrated a clear correlation between the "preclinical" presence of a PCL and certain clinical signs, Dark and Streeten did not find a regular pattern of pigment deposition in their patients. It is, in fact, conceivable that the clinical signs evaluated might be a common phenomenon of aging unrelated to the development of a PCL and a beginning PSX syndrome. PSX material has, however, been identified in conjunctival specimens of 8 (34.8%) of 23 PSX suspect eyes [14] and in eyelid skin specimens of 8 (80%) of 10 PSX suspect eyes [19]. Both studies strongly suggest a correlation between the presence of pigment-related clinical signs and the occurrence of PSX material in peribulbar tissues. Further, in a previous study, we identified typical PSX material in some additional extrabulbar tissues of clinically unaffected fellow eyes in unilateral PSX syndrome. By TEM, PSX material was found in extraocular muscles, orbital connective tissue septa, optic nerve sheaths, walls of vortex veins, and posterior ciliary arteries, but not in intrabulbar locations, suggesting that PSX fiber formation outside the globe may precede its intraocular manifestation [17]. We therefore assume that certain clinical signs may not only indicate PSX manifestations outside the globe but also the beginning PSX process inside the eye, as revealed by the formation of a PCL on the lens surface.

From the clinical and ultrastructural findings of the present study, we propose the following sequence of morphological changes of the anterior lens capsule during the development of PSX syndrome (Fig. 4): A precapsular layer develops in the course of the aging process, either produced by the lens epithelial cells or secondarily deposited from the aqueous humor during a "preclinical stage". In this earliest stage, the PCL cannot be detected by slit-lamp examination, but might possibly

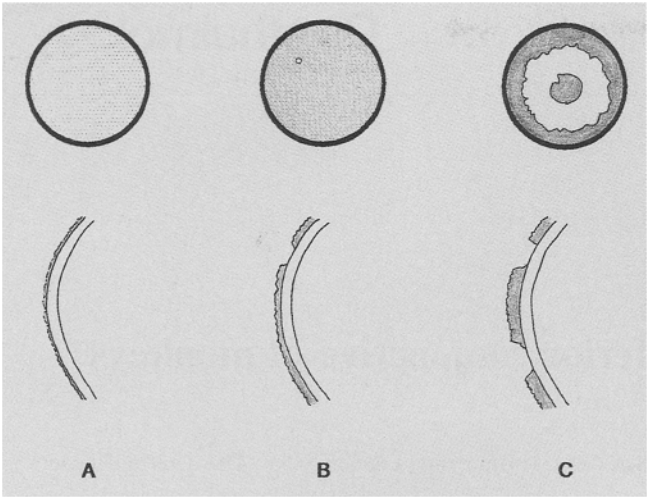

Fig. 4A–C. Proposed stages of the development of PSX syndrome. **A** Preclinical stage with pigment dispersion signs and a thin precapsular layer that may not be clinically detected: PSX suspect. **B** Diffuse film on the anterior lens capsule with a focal defect: pre-PSX. **C** Typical bull's eye pattern, consisting of a central disc, an intermediate clear zone, and a peripheral band of PSX material: PSX

cause increasingly poor mydriasis and signs of pigment dispersion by increased friction between the iris pigment epithelium and the PCL.

The immunohistochemical evidence of laminin, an adhesion-promoting glycoprotein, in the fibrillar component of the PCL supports this assumption. This conception could explain the involvement of the iris early in the course of PSX syndrome. Later, as the PCL increases in thickness and extension, it becomes not only clinically detectable but develops focal defects, which possibly might be caused by mechanical abrasion by the iris pigment epithelium. Accordingly, such defects are usually located nasally, where the iris-lens contact is more intense. Finally, the defects of the PCL coalesce, thus developing into the typical bull's-eye pattern of PSX syndrome with central and peripheral PSX depositions and an intermediate clear zone.

Whether the typical PSX fibers directly arise from the microfibrils of the PCL or whether they are produced at multiple sites and are simply trapped on and within the microfibrillar network, remains obscure. Roh et al. [15] demonstrated immature, intermediate, and mature forms of PSX material in the conjunctiva of patients with PSX syndrome and suggested the development of abnormally produced microfibrils into typical PSX fibers. Maybe the PCL observed in our specimens also corresponds to an immature form of PSX material. PCL formation might result from a disordered synthesis of microfibrillar extracellular matrix components of unclear origin which might represent the initial stage of PSX syndrome. In future, more comprehensive studies will further elucidate the relationship between early and fully established PSX syndrome.

References

1. Aasved H (1973) Incidence of defects in the pigmented pupillary ruff in eyes with and without fibrillopathia epitheliocapsularis. Acta Ophthalmol 51:710–715
2. Bartholomew RS (1971) Pseudo-capsular exfoliation in the Bantu of South Africa I. Early or pre-granular clinical stage. Br J Ophthalmol 55:693–699
3. Cohen AI (1965) The electron microscopy of the normal human lens. Invest Ophthalmol 4:433–446
4. Dark AJ, Streeten BW (1990) Precapsular film on the aging human lens: precursor of pseudoexfoliation? Br J Ophthalmol 74:717–722
5. Dark AJ, Streeten BW, Jones D (1969) Accumulation of fibrillar protein in the aging human lens capsule. Arch Ophthalmol 82:815–821
6. Jerndal T (1985) The initial stage of the exfoliation syndrome. Acta Ophthalmol 63 [Suppl 73]:65–66
7. Konstas AG, Marshall GE, Lee WR (1990) Immunogold localisation of laminin in normal and exfoliative iris. Br J Ophthalmol 74:450–457
8. Krause U, Helve J, Forsius H (1973) Pseudoexfoliation of the lens capsule and liberation of iris pigment. Acta Ophthalmol 51:39–46
9. Küchle M, Schönherr U, Dieckmann U, the "Erlanger Augenblätter-Group" (1989) Risk factors for capsular breaks and vitreous loss in extracapsular cataract surgery. Fortschr Ophthalmol 86:417–421
10. Mapstone R (1981) Pigment release. Br J Ophthalmol 65:258–263
11. Naumann GOH, Küchle M, Schönherr U, the "Erlanger Augenblätter-Group" (1989) Pseudoexfoliation syndrome as a risk factor for vitreous loss in extracapsular cataract surgery. Fortschr Ophthalmol 86:543–545
12. Norn MS (1971) Iris pigment defects in normals. Acta Ophthalmol 49:887–894
13. Prince AM, Ritch R (1986) Clinical signs of the pseudoexfoliation syndrome. Ophthalmology 93:803–807
14. Prince AM, Streeten BW, Ritch R, Dark AJ, Sperling M (1987) Preclinical diagnosis of pseudoexfoliation syndrome. Arch Ophthalmol 105:1076–1082
15. Roh YB, Ishibashi T, Ito N, Inomata H (1987) Alteration of microfibrils in the conjunctiva of patients with exfoliation syndrome. Arch Ophthalmol 105:978–982
16. Roth M, Epstein DL (1980) Exfoliation syndrome. Am J Ophthalmol 89:477–481
17. Schlötzer-Schrehardt U, Küchle M, Naumann GOH (1991) Electron microscopic identification of pseudoexfoliation material in extrabulbar tissue. Arch Ophthalmol 109:565–570
18. Schlötzer-Schrehardt U, Dörfler S, Naumann GOH (1992) Immunogold localization of basement membrane components in pseudoexfoliation material. Curr Eye Res (in press)
19. Schlötzer-Schrehardt U, Küchle M, Dörfler S, Naumann GOH (1991) Ultrastructural findings of pseudoexfoliation (PSX) material in eyelid-skin of PSX and PSX suspect patients. Invest Ophthalmol Vis Sci 32 (Suppl):857
20. Tarkkanen A (1962) Pseudoexfoliation of the lens capsule. Acta Ophthalmol [Suppl 71]:9–98
21. Wishart PK, Spaeth GL, Poryzees EM (1985) Anterior chamber angle in the exfoliation syndrome. Br J Ophthalmol 69:103–107