Morphological Alterations Within the Peripheral Fixation of the Iris Dilator Muscle in Eyes With Pigmentary Glaucoma

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PURPOSE. To analyze the peripheral fixation of the iris dilator muscle in normal eyes and in eyes with pigmentary glaucoma (PG).

METHODS. Using 63 control eyes (age 18 months–99 years), the peripheral iris dilator was investigated by light microscopy, immunohistochemistry, and electron microscopy. Development was studied using 18 differently aged fetal eyes stained immunohistochemically against α -smooth muscle (SM) actin. The peripheral iris dilator muscle in PG was analyzed using semithin and ultrathin sections of six glutaraldehyde-fixed eyes from three donors aged 38, 62, and 74 years.

RESULTS. In normal eyes, the peripheral end of the iris dilator muscle is arranged in a sphincter-like manner. Arcade-shaped tendinous connections associated with myofibroblasts (iridial strands) anchor the iris dilator within the elastic–fibromuscular ciliary meshwork that also serves as fixation area for the elastic tendons of the inner ciliary muscle portions. The iridial strands are innervated and can adapt their length during accommodation. The PG eyes show incomplete circular bundles and iridial strands that are mainly anchored to the iris stroma and the flexible uveal parts of the trabecular meshwork.

CONCLUSIONS. The normal anchorage of the peripheral iris dilator and its presumably neuronally regulated length adaptation stabilize the peripheral iris during accommodation. Insufficient fixation in PG could promote posterior bowing of the iris with rubbing against the zonular fibers and pigment liberation from the iris pigmented epithelium.

Keywords: iris dilator muscle, anchorage, pigmentary glaucoma, morphology, immunohistochemistry

In pigment dispersion syndrome (PDS), accommodation causes rubbing of the pigmented iris epithelium against the zonular fibers, resulting in accumulation of pigment granules within the aqueous humor and the outflow tissue.^{1–5} If the aqueous outflow is sufficiently obstructed and intraocular pressure is elevated, secondary open-angle glaucoma (pigmentary glaucoma, PG) with optic neuropathy may occur.^{6–8}

The reason for the iridionzular contact and the increased pigment dispersion is unknown. A genetic etiology with multifactorial pattern of inheritance or a possible autosomal-dominant inheritance pattern with incomplete penetrance and expressivity has been suggested.^{9–11} It has been proposed that the iris is too large for the eye,^{1,12,13} and ultrasound biomicroscopy (UBM) measurements have shown a deeper anterior chamber and a larger distance between iris insertion and trabecular meshwork in PDS/PG eyes compared to normal age-matched control eyes.^{14–16} Moreover, UBM studies evaluating iridionzular architecture in the relaxed and stimulated accommodative state have found that the iridionzular angle and iris concavity are the most discriminatory and statistically different parameters between PDS/PG and controls. These differences were especially prominent in the accommodative

state, suggesting PDS/PG-related structural changes in this area.¹⁷

A major influence on the morphology of the iridionzular angle during accommodation could be exerted by the peripheral fixation of the iris dilator muscle, which appears to have connections to the ciliary muscle.^{18,19} Studies on the peripheral fixation of the iris dilator muscle in PDS/PG are lacking, and it is not even described in detail in normal eyes. The most comprehensive review article dates back to 1926.²⁰ The existence of filaments from the dilator muscle extending up to the venous vascular walls, pectinate ligament, ciliary muscle, or connective tissue of the ciliary body was described, but affirmation or further evaluations could not be made due to the lack of more specific investigative methods.

In this study we had a unique collection of six well-preserved postmortem eyes of human donors aged 38, 62, and 74 years who had suffered from PG. The morphological changes of the peripheral iris dilator and its fixation in the ciliary body of these eyes were compared with the normal morphology of these structures. The latter were studied with immunohistochemical and ultrastructural methods in a large group of control eyes and included the development of the

TABLE. Source and Concentration of the Primary Antibodies

Antibody	Host	Dilution	Source
α -Smooth muscle actin	Mouse	1:300	Sigma-Aldrich, St. Louis, MO, USA
Collagen type VI	Rabbit	1:200	Rockland, Gilbertsville, PA, USA
Collagen type IV	Mouse	1:200	Dako, Hamburg, Germany
Elastin	Rabbit	1:400	Chemicon (Millipore), Billerica, MA, USA
Pan neurofilament	Mouse	1:200	Zymed, San Francisco, CA, USA
Protein gene product (PGP) 9.5	Rabbit	1:100	Biotrend, Cologne, Germany
Synaptophysin	Mouse	1:20	Dako, Glostrup, Denmark
Calretinin	Rabbit	1:1000	Swant, Bellinzona, Switzerland
Neuropeptide Y (NPY)	Rat	1:1000	Biotrend, Cologne, Germany
Tyrosine hydroxylase (TH)	Rabbit	1:40	Chemicon, Hofheim, Germany
Substance P (SP)	Rabbit	1:200	Peninsula Laboratories, San Carlos, CA, USA
Vesicular acetylcholine transporter (VACHT)	Goat	1:1000	Bioscience, Heidelberg, Germany
Calcitonin gene-related peptide (CGRP)	Rabbit	1:600	Biotrend, Cologne, Germany

peripheral iris dilator and its adaptation during accommodation and disaccommodation.

The results point to an altered peripheral iris fixation in PG versus normal control eyes that could predispose to the disease.

MATERIALS AND METHODS

Control Eyes

To investigate the peripheral iris dilator fixation in normal eyes, 63 eyes from 45 human donors with an age range from 18 months to 99 years were investigated. The eyes had been obtained from the Department of Anatomy, University of Erlangen-Nürnberg, and the Cornea Eye Banks of Amsterdam, The Netherlands, and of Madison, Wisconsin, United States. All eyes were intact globes. No donor had a known history of eye disease, and informed consent had been obtained from every donor or family. All investigations were done in accordance with the provisions of the Declaration of Helsinki for research involving human tissue.

The eyes had been enucleated 3 to 20 hours post mortem. Two slits were cut anteriorly into the cornea and posteriorly into the posterior half of the globe before the eyes were transferred into fixative. For electron microscopy the eyes were placed and sent in Ito's solution²¹; for immunohistochemistry, the eyes were fixed in 4% paraformaldehyde (PFA) for 24 hours and sent in phosphate-buffered saline (PBS).

Light Microscopy and Immunohistochemistry. Forty-one eyes of donors aged 28 to 99 years were investigated with light microscopy or immunohistochemistry. The PFA-fixed eyes were washed in PBS and cut equatorially into two halves. The anterior halves were carefully freed from the lens and further divided into four quadrants. From each quadrant, 2- to 3- and 4- to 5-mm-wide segments were prepared that included the ciliary body with the iris root. Following immersion in graded

alcohols, the specimens were embedded in paraffin and serial 7- μ m thin sections were cut in sagittal, tangential (parallel to the iris surface), frontal, and oblique-frontal planes.

The sections were placed on 0.1% poly-L-lysine-coated glass slides, deparaffinized, and stained with hematoxylin-eosin (HE), Azan, Crossmon, or Weigert's stain for elastin or for immunohistochemistry as described below.

Additional PFA-fixed and rinsed specimens from each quadrant were deep frozen in isopentane precooled with liquid nitrogen, and series of 10- μ m thin sections were cut in midsagittal, tangential, and oblique-frontal planes. The sections were also placed on slides that had been coated with 0.1% poly-L-lysine and were stained immunohistochemically.

Immunohistochemistry.

Staining of Paraffin and Frozen Sections. Deparaffinized sections as well as frozen sections that had been allowed to dry for several hours were preincubated for 15 minutes in 1% dry milk solution to prevent nonspecific staining, then incubated overnight at room temperature with the primary antibodies (Table) diluted according to the manufacturer's recommendations. Following rinsing the sections were incubated with Alexa Fluor 488- or Alexa Fluor 555-labeled immunoglobulin G secondary antibody (MoBiTec, Goettingen, Germany). For several sections, various double stainings were also performed, for example, combining α -smooth muscle (SM) actin antibody with anti-type VI collagen, anti-calretinin, anti-tyrosine hydroxylase, anti-vesicular acetylcholine transporter (VACHT), or calretinin with anti-neurofilament. The sections were rinsed again, mounted in Kaiser's glycerine (Merck, Darmstadt, Germany), and viewed with a fluorescence microscope (Aristoplan; Ernst Leitz, Wetzlar, Germany).

Whole Mounts. As all serial sections through different planes could not reveal a complete picture of the connection between iris and ciliary body, whole mounts from this area were prepared that were stained in a free-floating manner following immunohistochemical staining protocols as described above, but with longer periods of incubation and rinsing time.

For this purpose, 10 eyes ranging in age from 38 to 91 years were chosen. From each quadrant 0.5- to 1.0-cm-wide specimens were prepared containing parts of the ciliary body and peripheral iris. In order to reduce the thickness of the whole mounts, the tips of the ciliary processes and parts of the outermost layers of the ciliary muscle were removed, taking care to preserve the intactness of the transitional zone of iris and ciliary body. In some whole mounts, iris pigment was scraped away with utmost caution using scissors or blades. Other whole mounts were bleached using 1% hydrogen peroxide for a period of 4 to 6 hours depending on the grade of iridial pigmentation.

Semithin Sections and Ultrastructural Analysis. To obtain 1- μ m thin sections and ultrathin sections for electron microscopic evaluation, 12 eyes from donors aged 16, 42, 52, 68, 71, and 73 years were fixed in Ito's solution.²¹ After rinsing for 12 hours at 4°C in cacodylate buffer, the globes were bisected equatorially; the lens was removed from the anterior halves; and small 2-mm-thick specimens of iris, ciliary body, and cornea/sclera were prepared and placed into fixative again. These pieces were postfixed in 1% osmium tetroxide (OsO₄), dehydrated with graded alcohols, and embedded in Epon (Roth, Karlsruhe, Germany). Semithin sections (1 μ m) were cut with a Reichert's microtome (Reichert Jung, Wien, Austria) and stained with Richardson's stain. Ultrathin sections were made with an Ultracut E (Reichert-Jung), treated with lead citrate and uranyl acetate, and viewed using a Zeiss EM 902 (Zeiss, Oberkochen, Germany).

Pretreatment With Pilocarpine or Atropine. An additional 10 pairs of control eyes from donors aged 18 months to 88 years were obtained at autopsy from the Lions Eye Bank,

FIGURE 1. (A) Sagittal section of the anterior segment of a 52-year-old donor (Richardson's stain). The peripheral end of the iris dilator muscle (arrowheads) does not show a marked delineation toward the neighboring pigmented epithelium. A connective iridial strand (arrows) takes its origin from the dilator muscle end and inserts into the ciliary meshwork (asterisk) lying anterior to the inner portions of the ciliary muscle (CM). There is also a connection with a bundle of the circular ciliary muscle portion (white arrow). I, iris. (B) Sagittal section from a 99-year-old donor eye (Weigert's stain for elastic fibers). The pigmented peripheral iris dilator cells form an extended long and thin spur-like end (arrowheads). A bundle of connective strands (arrows) connects the iris dilator cells and the ciliary meshwork (asterisk). Within the iridial strands no elastin-stained fibers are present, whereas the ciliary meshwork shows numerous dark-violet-stained cross-sectioned fibers indicating the presence of a circularly oriented net of elastic fibers. CM, ciliary muscle; I, iris.

Madison, Wisconsin, United States. One eye was immersed in a solution containing 1 mM atropine/saline, the other eye in 1 mM pilocarpine/saline for 15 minutes. The eyes were subsequently transferred to 4% PFA containing either atropine or pilocarpine as above, and were sent to Erlangen. Here the eyes were bisected sagittally through the optic nerve to sustain the three-dimensional structure of the ciliary muscle and its tendinous insertion. The halves were postfixed in either PFA or Ito's fixative enriched with atropine or pilocarpine. Additionally, one eye each of a 28- and a 30-year-old donor from the Department of Anatomy in Erlangen contributed to each group. These two eyes were also bisected in the anterior-posterior direction through the center of the cornea and the optic nerve; one half was then immersed in atropine, the other in pilocarpine solution for 15 minutes following fixation in either 4% PFA or Ito's fixative, enriched with either atropine or pilocarpine. The fixed eye halves were rinsed and dissected into smaller segments. Subsequently, the prepared specimens were processed for embedding in paraffin or Epon. Paraffin sections were stained with Azan, Crossmon, or Weigert's stain, and semithin sections were stained with Richardson's stain.

Developmental Investigations. To investigate the developmental changes in the iris dilator and ciliary muscle transition zone, paraffin-embedded eyes from 18 different fetal humans that had been provided by the Department of Pathology and the Department of Ophthalmology of the University of Erlangen-Nürnberg were studied. The material contained fetal stages from the 15th to the 40th week of gestation, as well as the eyes of one stillbirth. Thin sections (7 µm) were cut midsagittally and processed immunohistochemically for SM actin as described above.

PG Eyes

Six eyes from three female patients with a known history of PG were investigated. The eyes had been sent to us by courtesy of the late Douglas Johnson. They had been obtained at autopsy through the Mayo Clinic Eye Bank (Rochester, MN, USA) after informed consent of the donors. All enucleated eyes had been immersed in Ito's solution to enhance rapid fixation and provide intact morphology.

The eyes were sent to Erlangen in fixative. Enclosed were detailed clinical data concerning the course and treatment of the eye disease.⁸ The axon loss of the optic nerve as well as

changes within the trabecular meshwork had been analyzed in a previous study.⁸ Case 1, aged 38 years, had a 5-year duration of PG; case 2, aged 62 years, suffered from PG for a period of 12 years; and 74-year-old case 3 was affected with PG for 15 years. The eyes were washed in cacodylate buffer, further dissected, and embedded in Epon as described above. Semithin and ultrathin sections were cut from all quadrants. Due to glutaraldehyde fixation, the investigations on these PG eyes included light and electron microscopy but no immunohistochemical studies.

RESULTS

Control Eyes

Peripheral Iris Dilator Muscle. In sagittal sections, either the peripheral end of the iris dilator muscle is a rather inconspicuous transition toward the ciliary epithelium (Fig. 1A) or it appears in the shape of various spur-like configurations that protrude into the underlying stromal tissue (Fig. 1B). The complex architecture of the peripheral dilator is best seen in whole mounts that are freed from the pigmented epithelium and stained for α -smooth muscle (SM) actin or through serial tangential sections cut obliquely to the epithelial surface of the iris. These studies show that the muscular processes of the radially oriented iris dilator change their arrangement at the peripheral end and merge into proximate groups that divide in half to cross each other and bend into a transversal-circular course. Thus, at the peripheral end of the dilator, a distal sphincter-like bundle is formed (Fig. 2A). Between these circularly running groups, there are still radially oriented small bundles of processes that form tendon-like structures connecting the peripheral iris dilator with the ciliary body. Due to this regular arrangement of circularly running bundles and radially oriented processes, an arcade-shaped appearance is achieved (Fig. 2B).

Iridial Strands. The tendon-like structures deriving from the radially oriented dilator bundles (here termed iridial strands) consist of extracellular material and flat lining cells (Figs. 1A, 1B). Ultrastructurally, the fine fibrillar material shows no periodicity (Fig. 2C) and can be stained immunohistochemically for type VI collagen (Fig. 3A). At the peripheral dilator muscle cells, these fibrils are connected to the basal membrane of the dilator cells and their cytoplasmic invaginations (Fig.

FIGURE 2. (A) Iris whole mount of a 48-year-old donor eye stained immunohistochemically for α -SM actin and viewed from the posterior (inner) side of the iris (direction of pupillary border, *top*; ciliary body, *bottom*). The posterior pigmented epithelium of the iris had been removed. The radially oriented iris dilator muscle cells (DL) turn at the peripheral end of the dilator muscle at right angles to form a circularly arranged sphincter-like bundle (*asterisks*). At places, arterioles can be seen that approach the sphincter-like bundles to be enclosed by single muscular processes (*arrows*). Magnification $\times 20$. (B) Whole mount of a 64-year-old donor iris ciliary body specimen stained immunohistochemically for α -SM actin and viewed from its anterior side. The iridial strands can be recognized as actin-labeled cellular bundles (*arrows*) that are anchored within the stained ciliary meshwork (*asterisks*) adjacent to the ciliary muscle (CM). Magnification $\times 20$. (C) Electron micrograph of an iridial strand of a 52-year-old donor eye. A bundle of fine fibrillar material (F) without any periodicity is connected to the basal membrane of the iris dilator cell and its cytoplasmic invaginations (*arrows*). A fibroblast-like cell (*asterisk*) running in parallel to the fibrillar bundle is also connected to the iris dilator cell processes. Adjacent, nerve terminals can be seen (*arrowhead*).

2C). The cells of the iridial strands stain for α -SM actin (Fig. 2B). Ultrastructurally they show bundles of 6- to 8-nm-thick filaments, dense bodies and dense bands within long cytoplasmic processes, and numerous surfaces of rough endoplasmic reticulum (Fig. 2C). At places, an incomplete basement membrane can be seen, indicating that these cells are myofibroblasts. The myofibroblasts are connected with each other by gap and intermediate junctions. Following the course of the fibrillar strands, the myofibroblasts are at places attached to them by focal adhesions.

Insertion of the Iridial Strands at the Ciliary Meshwork. Most of the iridial strands reach the ciliary meshwork (ciliary body band) adjacent to the transition between the circular and reticular ciliary muscle portion (Figs. 1A, 1B). In this region the ciliary meshwork consists of an elastic fiber network (Figs. 1B, 3B, 3C) intermingled with few collagen fibers. This net is supported by numerous mainly circularly oriented α -SM actin-stained cells that can be differentiated from the adjacent ciliary muscle cells by their less intense staining (Fig. 2B). Ultrastructurally they do not show the typical morphology of ciliary muscle cells,²² but they contain irregularly arranged 6-nm-thick filaments and are incompletely surrounded by basement membrane indicating that they are also myofibroblasts. This region of the ciliary meshwork is the fixation area not only for the iridial strands but also for the

elastic tendons of the circular and reticular portions of the ciliary muscle. Often small bundles of ciliary muscle cells, at places in contact with the circular muscle, are present between the elastic fibers. Outwardly the elastic net of the ciliary meshwork is continuous with the elastic fibers of the uveal trabecular lamellae. Therefore, the ciliary meshwork is composed of an inner portion containing nearly parallel-arranged circular running elastic fibers (tendons of the circular ciliary muscle) and an outer portion with a coherent elastic net of irregular interlacing fibers containing the tendons of the reticular ciliary muscle and the elastic fiber connections of the uveal trabecular lamellae (Fig. 3C).

At the sites of insertion the fibrillar material of the iridial strands blends into the elastic net of the ciliary meshwork (Fig. 3C), and the myofibroblasts form focal adhesions with the elastic fibers and with the myofibroblasts of the ciliary meshwork.

At places, single iridial strands reach the circular ciliary muscle portion directly (Fig. 1A), or radiate into the outer region of the ciliary meshwork.

Innervation of Iridial Strands. Ultrastructurally, abundant nerve endings can be found adjacent to the most peripheral dilator cells, but there are also nerve fibers and terminals in the vicinity of the cells of the iridial strands (Figs. 2C, 3D). Tangential histological sections through the plane of

FIGURE 3. (A) Sagittal sections of a 52-year-old donor eye with immunocytochemical double staining for α -SM actin (red) and type VI collagen (green). The iridial strand (arrow), which connects the α -SM actin-labeled and type VI collagen-surrounded peripheral iris dilator cells (arrowhead) with the ciliary muscle (CM), shows staining for type VI collagen and single α -SM actin-labeled cells. The iridial strand inserts into the ciliary meshwork (asterisk), which shows also the presence of scattered α -SM actin-labeled cells and type VI collagen. (B) Sagittal sections of a 49-year-old donor eye stained immunohistochemically for elastin. Note the absence of elastin immunoreactivity within the ciliary strands (arrow) contrasting to the labeled elastin fibers within the ciliary meshwork (asterisk) and vessels. CM, ciliary muscle. Arrowhead: iris dilator muscle end. (C) Tangential section through the iris ciliary body connection of a 64-year-old donor eye (Weigert's stain for elastic fibers). The elastic fibers of the ciliary meshwork are seen as a coherent elastic net of more inner circular and parallel bundles (white asterisk) as well as irregularly oriented fibers (black asterisks). Elastic tendons (arrowheads) from the reticular ciliary muscle portion (CM) merge into this elastic net, as well as the two iridial strands indicated by arrows. The short arrow shows the connection of an iridial strand to the wall of an iridial vein (V). (D) Electron micrograph of an iridial strand of a 52-year-old donor eye. A fibroblast-like cell (asterisk) as well as nerve terminals (arrows) can be seen in the vicinity of the fibrillar bundle (F) of an iridial strand.

the iridial strands that are stained with neurofilament antibodies reveal the abundance of nerve fibers within this region (Fig. 4A). Some of these fibers and varicosities stain for tyrosine hydroxylase (Fig. 4B) and VACHT. Substance P (SP)- and calcitonin gene-related peptide (CGRP)-immunoreactive terminals are present within the ciliary meshwork, but there are only few labeled terminals in the region of the iridial strands. Most of the nerves and terminals at the iridial strands and their insertion in the ciliary meshwork stain for calretinin (Figs. 4A, 4C) and are presumably afferent, sensory nerves. In fact, by electron microscopy, in the region of the peripheral iris dilator and in the vicinity of the iridial strands, there are terminals that show numerous mitochondria, similar to afferent endings recently described in the ground plate of the anterior ciliary muscle.²³ In addition, at the insertion of the iridial strands within the ciliary meshwork, there are large nerve endings in contact with the elastic fibers showing abundant filaments, differently sized granular and agranular

vesicles, and lysosome-like lamellated structures (Fig. 4D), a morphology typical for mechanosensory receptors.²³

Connections Between Iridial Strands and Iridial Vasculature. The arcuate arrangement of the iridial strands allows branches of the iridial vessels arising from or draining into vessels at the iris root to enter or leave the iris.

At places, iridial strands are closely associated with iris veins localized close to the place of insertion of the iridial strands (Fig. 3C). Here, thin bundles of strands split to reach the vascular wall and are attached to it via cellular adhesions. Small arterial vessels are regularly found approaching the most peripheral dilator processes, which take part in the circularly oriented sphincter-like formation. At these places the arterioles become nearly embraced and covered in a loop-like fashion by the peripheral iridial dilator cells before the vessels continue their further course within the iris stroma (Fig. 2A).

FIGURE 4. (A) Oblique-tangential frozen section through the plane of the iridial strands (arrows) of a 38-year-old donor eye with immunohistochemical double staining for neurofilament (red) and calretinin (green). The dense net of nerve fibers (red) within the region of the connecting strands points to its dense innervation. The green-labeled staining for calretinin suggests also the presence of numerous afferent nerve fibers. CM, ciliary muscle; I, iris. (B) Sagittal frozen section of a 52-year-old donor eye stained immunohistochemically for tyrosine hydroxylase. Within the iris (I), labeling is seen along the dilator cells (DL) up to its peripheral end (arrowhead) and the vascular walls (asterisks). Additional weak staining is also seen within the region of the iridial strands (arrow). CM, ciliary muscle. (C) Frozen section of a 38-year-old donor eye stained immunohistochemically for calretinin. Between iris (I) and ciliary muscle (CM), numerous fibers with green fluorescent immunoreactivity for calretinin are seen. The location of an iridial strand that connects the iris dilator end (arrowhead) with the ciliary muscle (CM) is indicated by the arrow. (D) Electron micrograph through the ciliary meshwork anterior to the reticular ciliary muscle portion of a 42-year-old donor eye. A myofibroblast (M), numerous elastic fibers (arrowheads), and a nerve with two larger terminal endings (asterisks) are seen. These endings show differently sized vesicles and lysosome-like lamellated structures and are adjacent to elastic fibers, which approach those areas of the terminals that lack basement membrane (arrows).

Changes in Pilocarpine- and Atropine-Treated Eyes.

Sagittal sections of eyes treated with pilocarpine (Fig. 5A) show the characteristic image of a contracted ciliary muscle with its circular portion moving anterior-inwardly. The ciliary meshwork follows this anterior movement of the muscle only slightly. In the eyes of the young donors 18 months and 6 years old, the ciliary meshwork reaches the level of the scleral spur. In the older eyes, the ciliary meshwork remains farther posteriorly. Due to the contraction of the pupillary sphincter, the peripheral end of the iris is shifted so far anteriorly that the distance between the peripheral dilator end and its tendinous insertion at the ciliary meshwork is increased. Because of the more posterior position of the ciliary meshwork in older eyes, this distance is longer than in young eyes. In both young and old eyes, the iridial strands are straight and elongated.

The atropine-treated eyes (Fig. 5B) reveal a relaxed ciliary muscle with the circular ciliary muscle portion drawn posteriorly. In the young eyes, the ciliary meshwork is also

located slightly farther posteriorly than in the pilocarpine-treated eyes. In the older eyes the position of the ciliary meshwork is similar to that of the pilocarpine-treated eyes. Contraction of the dilator muscle changes the position of the peripheral iris, which comes in close proximity to the ciliary meshwork. Thus the distance between peripheral iris dilator and ciliary muscle is shortened. The iridial strands appear shortened and straight especially in the young eyes, which show several rows of myofibroblasts along the iridial strands.

Embryonic Eyes and Further Stages of Development.

From the 15th week to the 26th week of gestation, there is no α -SM actin staining in the region of the peripheral dilator muscle or the iridial strands. The staining reveals only a clear labeling of the pupillary sphincter, but no staining for the dilator muscle. Thin labeled cells can be seen first from the 29th week of gestation. The stained region occupies one-third of the iris length, however, without reaching the peripheral

FIGURE 5. Sagittal sections of the anterior eye segments of a 30-year-old donor eye pretreated prior to fixation with pilocarpine (A) or atropine (B). Within the pilocarpine-treated eye (A), the contracted ciliary muscle (CM) shows an anteriorly shifted circular portion, and the peripheral iris dilator ends show a small spur that contrasts to the large formation of dilator ends seen in the atropine-pretreated eye (B). Note the different lengths of the iridial strands in both eyes, marked by arrows from their beginning at the dilator ends to their insertion at the ciliary meshwork (asterisks). The iridial strands are longer in the pilocarpine-treated (A) than in the atropine-treated (B) eye. I, iris; CM, ciliary muscle; TM, trabecular meshwork.

end of the iris dilator, which is distant to the ciliary muscle. From the 35th week onward, the peripheral dilator end, which is still distant to the ciliary muscle, is clearly stained for α -SM actin (Fig. 6A). Although the distance between the peripheral end of the dilator muscle and the inner portions of the ciliary muscle is reduced during the following developmental stages (up to birth), α -SM actin-labeled cells of iridial strands are not found, and there are also no labeled cells in the ciliary meshwork. The same was true for an eye of a stillbirth. In eyes from 1-year-old (Fig. 6B) and 3-year-old donors there are labeled iridial strands.

Eyes From Donors With PG

Peripheral Iris Dilator Muscle. In all six donor eyes, the principal changes were the same in both eyes of the three cases. In all eyes the peripheral ends of the dilator muscle and their tendons (the iridial strains) show distinct changes, and

FIGURE 6. Sagittal section through a fetal eye at 36 gestational weeks (A) and an 18-month-old donor eye (B) stained immunohistochemically for α -SM actin. (A) Within the eye at 36 gestational weeks, the iris dilator cells and their peripheral processes (arrowhead) are labeled, whereas no staining is seen within the iridial strands (arrow) and ciliary meshwork (asterisk). CM ciliary muscle, I iris. (B) Within the eye of the 18-month-old donor, the peripheral dilator ends (arrowheads) are connected to the α -SM actin-stained ciliary meshwork (asterisk) by strands that are also labeled by α -SM actin-positive cells (arrows). CM, ciliary muscle; I, iris.

most of the iridial strands do not insert into the fixation area of the ciliary meshwork as seen in normal eyes.

These changes are most prominent in both eyes of case 1, the youngest donor, 38 years old with a 4-year duration of glaucoma.

Here, the peripheral ends of the dilator muscle in most parts of the circumference form brush-like processes that protrude into the iris stroma or form other irregular formations (Figs. 7A, 7B). Iridial strand-like structures run outward toward the iris stroma and/or to the lamellae of the uveal trabecular meshwork (Figs. 7A, 7B). In contrast to their normal more elongated shape, the lining iridial strand cells have a more star-like appearance. The thin cellular processes are connected to the processes of neighboring cells, thereby forming a loose and delicate cellular reticular net in the region of their fixation in the iris stroma or the uveal meshwork (Fig. 7A).

In comparison with the control eye, the inner ciliary meshwork appears light microscopically loose and rarified (Figs. 7A, 7B). Also ultrastructurally, the densely packed elastic fibers that are attached to numerous myofibroblasts of the age-

FIGURE 7. Sagittal semithin section of the left (A) and right (B) 38-year-old donor eye with a 4-year history of PG. (A) In the left eye the peripheral dilator ends show brush-like extensions (*arrowheads*) that are separated from each other by areas of very short cells. The iridial strands (*arrows*) take their origin from a distant dilator cell accumulation and insert into the uveal meshwork. (B) In the right eye, the peripheral dilator muscle (*arrowheads*) shows two larger spur-like extensions. A bundle of strands (*arrows*) can be followed running outward toward the outer uveal meshwork. (C) Electron micrograph through the ciliary meshwork anterior to the reticular portion of the ciliary muscle of a 42-year-old control donor eye. A ciliary muscle cell (CM) covered by a complete basement membrane can be differentiated from the numerous myofibroblasts (M), which show cytoplasmic filaments with dense bands and bodies but lack of a complete basement membrane. The extracellular material consists of few collagen fibrils and abundant elastic fibers (*arrows*) that are in close contact to the myofibroblasts. A small axon (*arrowhead*) is also seen. (D) Electron micrograph of the right 38-year-old donor eye with a 4-year history of PG cut through the ciliary meshwork anterior to the reticular ciliary muscle portion. A muscle cell (CM) from this portion is seen on the left border. The adjacent lying ciliary meshwork shows few collagen and elastic fibers (*arrows*). Scattered cells consist of a myofibroblast (M), macrophage (*asterisk*), and pigment-loaded cells. Two axons are marked by *arrowheads*. In comparison with the ciliary meshwork of the control eye (C), the ciliary meshwork of the PG eye appears loose and rarified.

matched control eye (Fig. 7C) contrast with the only few myofibroblasts and elastic fibers of the PG eye (Fig. 7D).

Shape and location of the dilator cells are inconsistent with the state of contraction of the ciliary muscle. The relaxed ciliary muscle that predominates in both eyes is not combined with a prominent dilator end in the vicinity of the ciliary muscle as in age-matched controls. In contrast, the various ends of the dilator are located farther anteriorly, increasing the distance to the ciliary meshwork, and the chamber angle appears widened (Figs. 7A, 7B).

The changes seen in both eyes of case 2, the 62-year-old donor (with a 12 year-duration of PG, Figs. 8A, 8B), and of case 3, the 74-year-old donor (with PG for a period of 15 years and most pronounced loss of axons of the optic nerve,⁸ Figs. 8C, 8D), are very similar and therefore described together. The peripheral end of the dilator shows various shapes, and a sphincter-like spur is missing in most parts of the circumference. At places, delicate and acute tapering ends of the dilator protrude slightly into the iris stroma (Figs. 8B, 8C). Iridial strands appear very delicate, some taking a course toward the uveal meshwork, some radiating into the iris stroma (Figs. 8A–D). Single iridial strands reach the ciliary meshwork but here they do not enter the meshwork at the fixation point of the reticular and circular ciliary muscle

tendons; rather they follow the most anterior portion of the loosely arranged elastic network toward the movable uveal lamellae (Fig. 8A).

All four eyes show a slightly contracted ciliary muscle. The position of the ciliary meshwork appears unchanged, similar to that of age-matched normal contracted muscles, but the peripheral dilator end is located often more anteriorly so that in most parts of the circumference the distance between dilator end and ciliary meshwork is increased. Iridial tendon-like strands connecting the two regions are nearly absent, and in most sections of the circumference the width of the chamber angle is increased.

Within the iris root of all six eyes, iridial arterioles are often seen in the vicinity of the peripheral dilator cell processes, but they are rarely enclosed by them.

The pigmented epithelial cells that are connected to the peripheral dilator muscle cells and those of the regions directly anterior to the iris root appear normal in all six eyes. Only farther anteriorly and distant to the iris root, small areas are seen in which the epithelial layer and at places also the underlying dilator muscle cells are damaged or even absent. The ultrastructure of the neighboring cells is not different from that of the control eyes.

FIGURE 8. Sagittal semithin section through the left (A) and right (B) 62-year-old donor eye with a 12-year history of PG. In both eyes the iridial strands (arrows), which take their origin from the peripheral dilator end (arrowhead), are not anchored within the ciliary meshwork (asterisk) but run toward the uveal meshwork (A) and iris stroma (B). Within the ciliary meshwork the orientation of the extracellular fibers appears less reticular, but more longitudinal toward the uveal meshwork. CM, ciliary muscle. Note the wide chamber angle in both eyes. Sagittal semithin section through the left (C) and right (D) 74-year-old donor eye with a 15-year history of PG. The iridial strands (arrows) do not reach the ciliary meshwork (asterisk). In (C) they are restricted to the iris stroma that is attached to the outer ciliary and uveal meshwork, whereas in (D) a delicate bundle is directed to the outer ciliary meshwork that radiates into the outer uveal lamellae. CM, ciliary muscle.

DISCUSSION

Normal Eyes

The question of a “punctum fixum” of the iris dilator muscle as formulated by Berner²⁰ in fact is not only a matter of anatomical interest but is also of clinical relevance. Our present studies show that in normal eyes the sphincter-like peripheral iris dilator is anchored within the muscular-elastic tissue anterior to the ciliary muscle (termed ciliary meshwork) via numerous tendinous structures (iridial strands). Their complex three-dimensional architecture and composition are described in this study for the first time and summarized in the schematic drawing of Figure 9A. The anchoring area of the iridial strands in the ciliary meshwork is also the fixation area of the circular and reticular ciliary muscle portions. Their elastic tendons and a circularly arranged ring of myofibroblasts stabilize this region and counteract the force that is exerted by the iridial strands and the iris dilator.

In contrast to the rather fixed position of the ciliary meshwork, the iris dilator ends undergo positional changes during accommodation and disaccommodation, leading to changes in length of the iridial strands. These are able to adapt to the changes due to their type VI collagen composition and their connections to myofibroblasts.

The presence of afferent and efferent nerve terminals in the vicinity of the iridial strands suggests underlying nervous control mechanisms. In a previous study²³ we could verify

proprioceptive nerve terminals in the ciliary muscle and ground plate of the ciliary body. The presence of similar mechanoreceptor-like nerve terminals at the elastic fibers of the ciliary meshwork and the innervated myofibroblasts of the iris strands presumably allows neural adjustment and flexibility according to the accommodation-induced changes.

Contraction and relaxation of the iris muscles also influence the iris vasculature; for example, straightening of the iris straightens the iridial vessels, whereas the specific connective tissue sheath surrounding the iridial vessels prevents their collapse during shortening of the iris. In the region of the iris root, the vessels lose their specific sheath. Here the structural embedding of the vasculature within the peripheral dilator ends, and their tendinous strands could be of importance by regulating the blood flow through reduction of blood flow into the iris and support of venous drainage during contraction of the iris dilator.

As the tension and tonus of the iris tissue are influenced only by the iris muscles and vessels, their right structure and function is necessary to ensure tightening of the iris during accommodation and following a pressure rise within the anterior chamber. Therefore, the distal fixation of the iris dilator that not only counteracts the central fixation of the iris dilator at the pupillary sphincter, but also presumably serves for the special vascular embedding of the iridial vessels, is of particular importance.

FIGURE 9. Summarizing diagram of the morphological characteristics of the peripheral iris dilator and its anchorage within the ciliary body in the normal control (A) and PG eyes (B). For better demonstration of the iris dilator muscle, the pigmented epithelium of the iris (PE) is not completely shown. In control eyes the peripheral iris dilator (DL) and its bordering sphincter bundle (*asterisk*) are tent-like fixed by arcade-shaped iridial connection strands (*arrows*) to the elastic-fibromuscular ciliary meshwork lying anterior to the inner portions of the ciliary muscle (CM). The PG eyes show structural irregularities (1) at the peripheral border of the iris indicating an insufficient sphincter bundle (*asterisk*) and (2) at the iridial strand connections (*arrows*), which are anchored to the iris stroma and outer flexible uveal parts of the trabecular meshwork (TM). These changes could promote posterior bowing of the iris and cause rubbing of the zonular fibers (ZF) against the underlying pigmented epithelium of the iris, leading to damage of the cells and loss of pigment in these locations (*short arrows*). L, lens; CP, ciliary processes.

Pigmentary Glaucoma

The complicated anchoring system of the peripheral iris shows marked changes in all investigated eyes with PG, which are summarized in Figure 9B.

The striking variability of the peripheral dilator ends (Fig. 9B) suggests the lack of the sphincter-like morphology in many parts of the circumference that excludes also the embedding of iridial arterioles in these places. Iridial strand-like structures deriving from several processes of the peripheral dilator and terminating in the iris stroma or uveal meshwork cannot participate in a proper outward fixation of the peripheral iris. Even those strands that radiate toward the ciliary body lack fixation at the inner portions of the ciliary meshwork. Instead, many strands are connected to the outer lamellar portions of the flexible uveal trabecular meshwork (Fig. 9B). Thus instead of the rather fixed tent-like anchorage that provides stability and counterforce when the iris is pulled anteriorly (Fig. 9A), our findings indicate that there is a weakness of the iris dilator

muscle fixation in many parts of the circumference. This preferential insertion into the iris stroma or flexible uveal meshwork could allow the peripheral iris to be pulled inwardly when the pressure in the anterior chamber rises during accommodation. The classical picture seen by ultrasound biomicroscopy in eyes of patients with PDS/PG is in fact a posterior bowing of the peripheral iris.²⁴ Our findings of an insufficient fixation of the peripheral dilator in major parts of the circumference could explain the observed changes in the tone and position of the iris that abet zonular rubbing and pigment release.

Structural changes within iris dilator cells and the connected pigmented epithelium that contribute to the observed church window phenomenon are restricted only to spare and small regions of the midperipheral iris, leaving the epithelium and dilator muscle cells at the peripheral end unchanged. They contrast to the more frequently seen fixation defects of the peripheral iris dilator and have therefore to be considered as secondary changes. That all investigated eyes showed similar structural peculiarities regarding the peripheral fixation of the iris, with the most obvious changes in the youngest eye with the shortest period of glaucoma, argues strongly for this explanation.

To exclude the possibility that glaucoma with its increased intraocular pressure might be causative of the changes, we reinvestigated donor eyes suffering from primary open-angle glaucoma that have been published previously.²⁵ The findings show no alterations within the peripheral iris fixation indicating that the described structural changes in PG cannot be generally associated with glaucoma.

Our results of actin staining in fetal eyes confirm the findings of Mann²⁶ about the rather late development of the iris dilator occurring after the development of the iris pupillary sphincter muscle. The formation of iridial connective strands and their attachment within the ciliary meshwork takes place even later and occurs after birth. Whether this rather long developmental period contributes to various gene mutation-induced interferences that eventually impede the proper fixation of the iris dilator and lead to PDS/PG is not known.

In summary, our investigations indicate that structural changes within the peripheral iris fixation might be causative of the development of PDS or PG.

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