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Case Report

Autopsy Case of Bilateral Optic Nerve Aplasia with Microphthalmia: Neural Retina Formation Is Required for the Coordinated Development of Ocular Tissues

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Human congenital anomalies provide information that contributes to the understanding of developmental mechanisms. Here we report bilateral optic nerve aplasia (ONA) with microphthalmia in the autopsy of the cadaver of a 70-year-old Japanese female. The gross anatomical inspection of the brain showed a cotton thread-like cord in the presumed location of the optic nerve tract or chiasm. Histologically, no neural retina, optic nerve bundle or retinal central vessels were formed in the eye globe, and the retinal pigment cells formed rosettes. The cornea, iris, and lens were also histologically abnormal. Immunohistochemically, no retinal cells expressed beta III tubulin, and Pax6-immunoreactive cells were present in the ciliary non-pigmented epithelial cells. This case of ONA could be attributed to the agenesis of retinal projection neurons as a sequel to the disruption of neural retina development. The neural retina formation would coordinate the proper development of ocular tissues.

Key words: eye development, human congenital anomalies, optic nerve aplasia, microphthalmia, neural retina

The eye is formed from 3 developmental tissues: ectoderm, mesoderm, and neural crest cells. Neural ectoderm gives rise to the retina, optic nerve, and smooth muscle of the iris, whereas surface ectoderm forms the corneal and conjunctival epithelium, lens, and ocular glands. Neural crest cells form the corneal endothelium and stroma, sclera, choroid, iris stroma, ciliary musculature, and part of the vitreous. Mesodermal cells between the 2 ectodermal tissues are also intermingled with the cornea, sclera, choroid and vitreous tissues [1, 2].

Given that these different derivatives constitute the elaborate structure of the eye, it is conceivable that the induction of one ocular tissue by another is crucial during development. The sensory component of the eye, *i.e.*, the retina, originates from a bilateral evagination of the forebrain, *i.e.*, the optic vesicle, by which the lens is induced to develop. As the lens vesicle separates from the surface ectoderm, the distal part of the optic vesicle invaginates to form a double-layered optic cup comprised of the outer retinal pigment epithelium (RPE) and the inner neural retina. The RPE is melanin-containing simple cuboidal epithelium, whereas the inner neural retina proliferates and

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differentiates into 6 major types of neurons and one type of glial cells. The retinal neurons include photoreceptors, modulating interneurons and ganglion cells that project to the brain via the optic nerve.

These intricate events during eye development are coordinated by controlled gene expression. Numerous master control genes during eye development have been identified using animal models [2, 3]. Gene analysis by animal manipulation or forward genetics techniques is a powerful tool to investigate developmental mechanisms. Studies of human congenital anomalies are also fundamental to the understanding of how precise tissue interactions are indispensible to the building-up of mature structures.

Here we report an autopsy case of bilateral optic nerve aplasia (ONA), the absence of the optic nerve, found in a cadaver that was donated for educational dissection and research. This human eye anomaly is a rare case, in which the complete absence of neural retina formation led to the ONA.

Materials and Methods

The present study was performed according to the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/). After a transarterial perfusion of formalin into a cadaver (a 70-year-old Japanese female) to be used for anatomy practice at the University of Toyama Medical School, the brain was dissected out. By observing the base of the brain, we found that the optic nerve was absent from this cadaver. The eyes were therefore cut apart and the eyeball was histologically examined. After Bouin fixation according to standard procedures, hematoxylin-eosin or azan staining of the eye was performed on paraffin sections (5μm thick).

For immunostaining, the primary antibodies used in this study were rabbit IgG anti-paired box gene 6 (Pax6; diluted 1 : 200; Covance, Princeton, NJ, USA), mouse IgG anti-beta III tubulin (TUJ1; diluted 1 : 500; Covance), rat IgG anti-myelin basic protein (MBP; diluted 1 : 100; Millipore, Bedford, MA, USA) and rat IgG anti-proteolipid protein (PLP) (AA3; diluted 1 : 250; kindly provided by Dr. Marjorie B. Lees through Dr. Kazuhiro Ikenaka at the National Institute for Physiological Sciences, Okazaki, Japan) [4, 5].

Immunohistochemistry on deparaffinized sections was performed with a Dextran polymer signal amplification system (EnVision HRP; Dako, Glostrup, Denmark) for anti-Pax6 and TUJ1 and by an avidin/biotin complex (ABC) system (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA) for anti-MBP and AA3 according to the manufacturers' protocols. All primary antibodies except for AA3 were used after antigen retrieval by 0.1 M citrate buffer (pH 6.0, Dako). Control immunostaining using AA3 was performed on frozen 20-μm-thick retinal sections obtained from 8-week-old male C57BL/6 mice (Ourgenic, Tokushima, Japan) without a peroxidase inactivation step. Immunoreactive signals were visualized by a peroxidase reaction using 3,3'-diaminobenzidine (DAB) as the chromogen. Micrograph images were taken with a Nikon digital camera (DS-Ri1; Nikon, Tokyo) and processed using Adobe Photoshop CS 5.1 (Adobe Systems, San Jose, CA, USA).

Case Presentation

In the cadaver dissection, cotton thread-like cords were found in the presumed location of the optic nerve on both sides in the brain. Gross anatomical observation showed no optic tract or optic chiasma at the base of the brain (Fig. 1A). The computed tomography (CT) of the head region showed no obvious abnormalities in other areas of the brain (data not shown). The extraocular muscles and their respective cranial nerves were normal. A pair of normally sized oculomotor nerves originated from the mesencephalon (black arrows in Fig. 1A, B). In contrast, the appearance of the cotton thread-like cords was quite different from that of a normal cranial nerve.

The dissected eyeballs were small with white chaotic tissue occupying the vitreous cavity of both eyes (Fig. 2A–C). The cross-sectional diameter of the right eye was approx. 17 mm (Fig. 2A), while that of the left eye was 15 mm (Fig. 2B). The histology revealed no typical optic nerve bundle or retinal central artery and vein, although small nerve-like tissues and vessels were found in the posterior sclera (Fig. 2D, E and data not shown).

Immunohistochemically, the nerve-like tissues contained beta III tubulin-positive areas, which was localized to the center of each cross-section of the tissue, indicating that they were axons (Fig. 2F).

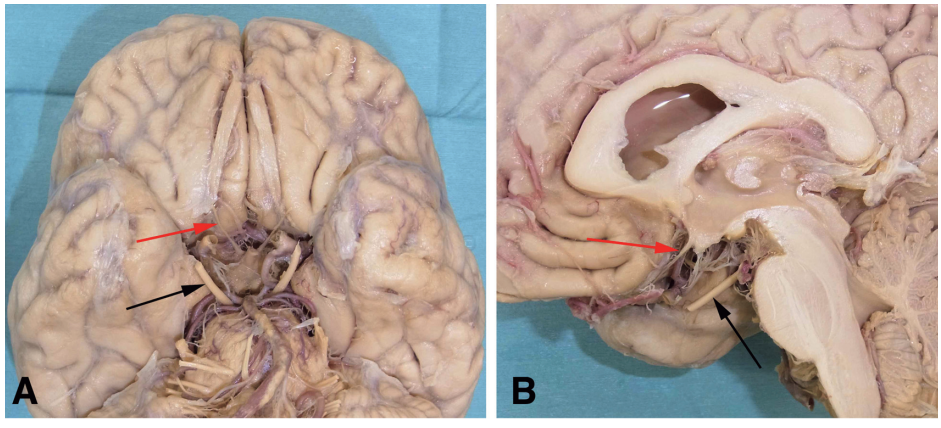

Fig. 1 Gross anatomy of the brain of the cadaver, a 70-yr-old female. **A**, Inferior view of the brain; **B**, Medial view of the brain. In (**A**, **B**), the red arrow indicates the cotton thread-like cord in the place of the optic nerve, and the black arrow indicates a normal oculomotor nerve.

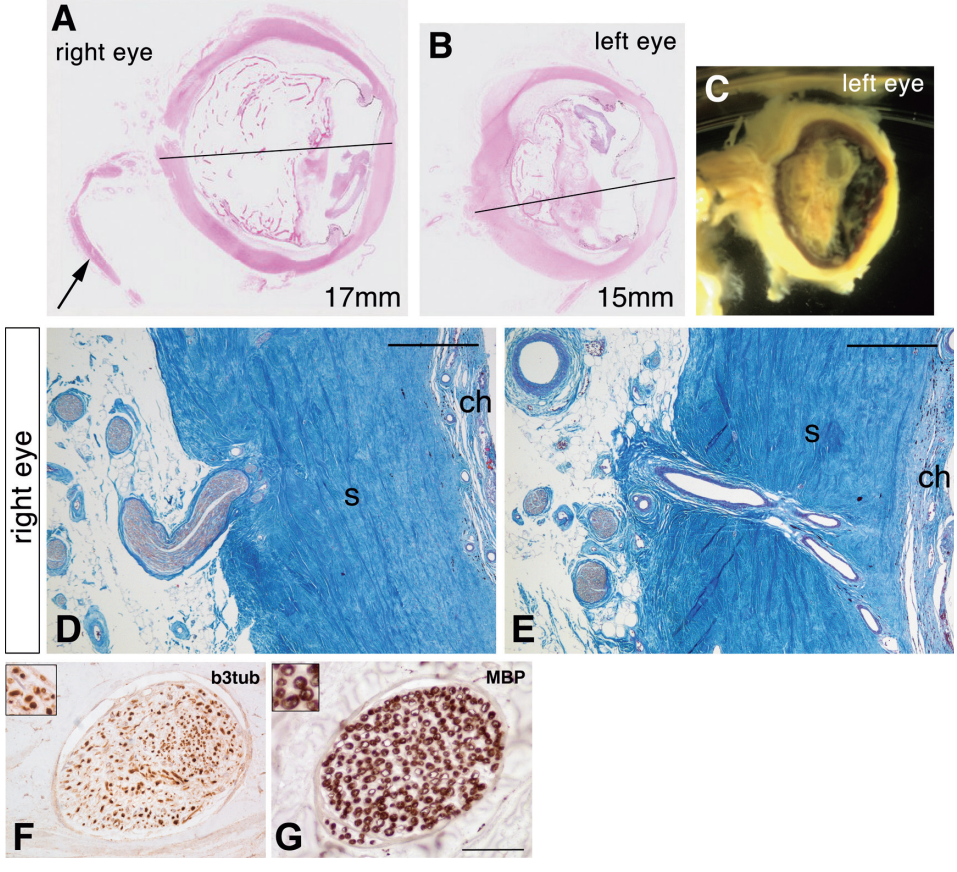

Fig. 2 Histology of the eyes. **A**, **B**, Hematoxylin-eosin (HE) staining; **C**, Dissecting macrograph showing half of the left eye; **D**, **E**, Azan staining revealed connective tissues as blue. Cross sections of the right (**A**) and left (**B**) eyes with diameters of 17 mm and 15 mm, respectively. Cotton-threadlike fibrous tissue was observed at the posterior pole of the eye (arrow in **A**); **C**, Whitish chaotic tissue was observed inside the eye; **D**, **E**, Small nerve-like tissue (**D**) and vessels (**E**) entered the scleral wall (s), but no optic nerve bundle was observed; **F**, Localization of beta III tubulin (brown) to the axons; **G**, Localization of myelin basic protein to the myelin sheath surrounding the axons. Insets in (**F**, **G**) are high-magnification. A negative control without primary antibodies gave no signals (not shown). Scale bar: 500 μm in (**D**, **E**); 100 μm in (**F**, **G**). ch, choroid.

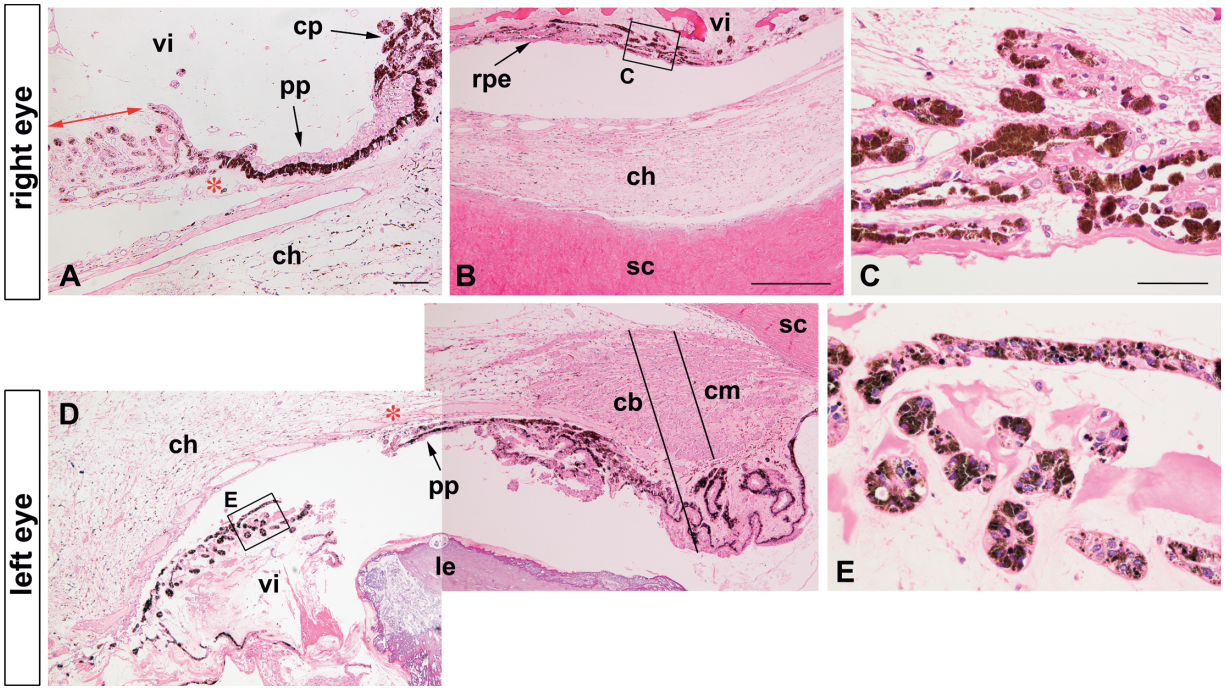

Fig. 3 Retinal dysplasia (no neural retina formation) of the ONA (HE staining). **A**, Anterior region of the right eye. *Pars plana* (pp) of the ciliary body. The left-right arrow indicates retinal dysplasia located posteriorly from the putative *ora serrata* (asterisk); **B**, Middle region of the right eye. The boxed area is enlarged in (**C**). The abnormal retina with proliferated RPE-like cells (rpe) was continuous with the vitreous (vi); **C**, Ingrowth of RPE-like pigmented cells; **D**, Retinal dysplasia in the left eye. The boxed area is enlarged in (**E**); **E**, The RPE-like cells formed rosettes. Scale bars: 100 μm in (**A**); 500 μm in (**B**, **D**); 50 μm in (**C**, **E**). cb, ciliary body; ch, choroid; cp, ciliary processes; vi, vitreous; sc, sclera; cm, ciliary muscle; le, lens.

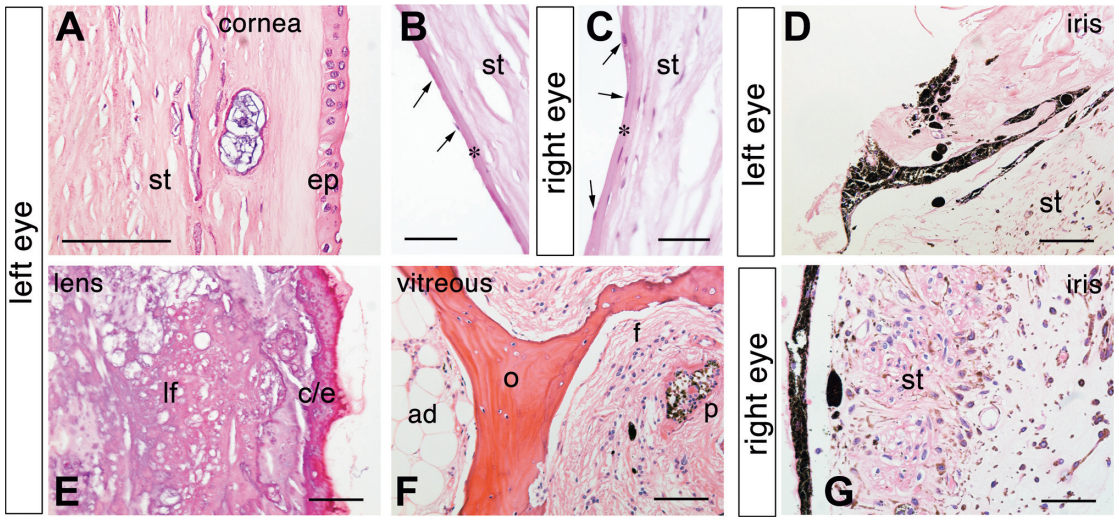

Fig. 4 Other ocular abnormalities associated with the ONA (HE staining). **A**, Corneal epithelium (ep) and stroma (st). An abnormal capillary was observed in the stroma; **B**, Corneal endothelial cells (arrows) and stroma (st); **C**, In the right eye, the endothelial cells were more preserved. Asterisks in (**B**, **C**) show Descemet membrane; **D**, Proliferation of pigmented cells of the iris; **G**, In the right eye, the iris was more preserved; **E**, Dysplasia of the lens; **F**, Ossification (o) in the vitreous. Adipose tissue (ad) and fibrous connective tissue (f), partly pigmented (p), are included. Scale bars: 100 μm in (**A**, **D**–**G**), 50 μm in (**B**, **C**). c/e, putative capsule and epithelium region; lf, putative lens fiber region.

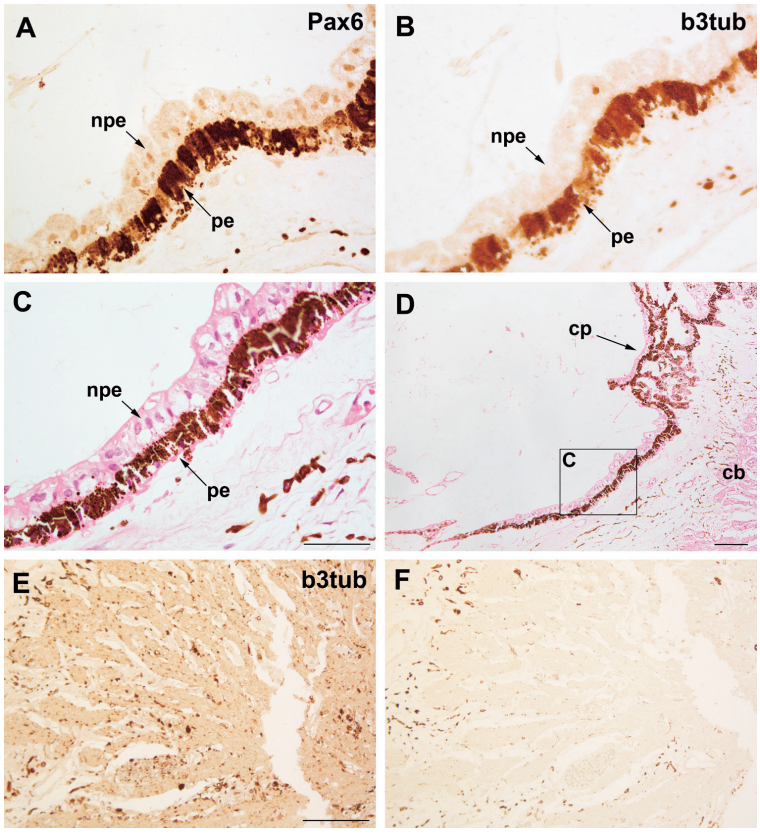

Fig. 5 Neuronal cells expressing beta III tubulin were not present, whereas Pax6-expressing cells were present in the *pars plana*. Immunohistochemistry of Pax6 (**A**, brown nuclei in non-pigmented epithelial cells and beta III tubulin (**B**, not present). For histology, H-E staining is shown: **C**, high magnification of boxed area shown in (**D**); **E**, Immunostaining of beta III tubulin in the ciliary body (positive control); **F**, No primary antibody in the ciliary body (negative control). Scale bars: 50 μm in (**A**–**C**, **E**, **F**), 100 μm in (**D**). cb, ciliary body with smooth muscle fibers; cp, ciliary processes; npe, non-pigmented epithelium in the *pars plana* of the ciliary body; pe, pigmented epithelium of the *pars plana*.

MBP and PLP were localized to the circular region surrounding to the axon (Fig. 2G and data not shown for PLP). These immunohistological data indicated that the small nerve-like tissues were myelinated nerves, but they were morphologically far from the optic nerve bundle.

Another noticeable finding in the histology was the lack of any neural retina formation posteriorly from the putative *ora serrata*, the anterior edge of the retina (asterisk in Fig. 3A, D). Instead, RPE-like pigmented cells proliferated and formed rosettes (Fig. 3B, C, E). These pigmented cells and surrounding fibrous tissues were continuous with abnormal vitreous tissue (Fig. 3B, D). The choroid and sclera were observed outside of the proliferated RPE tissues (Fig. 3B).

The development of the anterior structures such as the cornea, iris, and lens was also disrupted (Fig. 4A–E, G). The corneal epithelium was thin and comprised of only 2 cell layers, and vascularization was observed in the stroma (Fig. 4A). The corneal endothelial cells were observed but their numbers appeared to be reduced (Fig. 4B, C). Proliferation of pigmented cells was observed in the iris (Fig. 4D). In the right eye, the morphology of the iris was more preserved, as a line of pigmented epithelial cells was observed with stromal cells accumulated in the place of sphincter muscles (Fig. 4G). In the vitreous cavity, disorganized mesenchymal structures, such as bones, adipose tissue, and fibrous tissue were observed (Fig. 4F).

To determine the presence or absence of any retinal progenitors or neuronal cells within the eye, we performed immunohistochemistry. We found that

Pax6 protein was localized to the nuclei of non-pigmented epithelial cells in the *pars plana* (Fig. 5A, C, D). In contrast, beta III tubulin was not present (Fig. 5B). An internal control showed that beta III tubulin was localized to the ciliary nerve components (Fig. 5E) [1].

Discussion

We have described a human autopsy case of bilateral optic nerve aplasia (ONA). The gross anatomical observation showed a cotton thread-like cord in the presumed location of the optic nerve tract or chiasm. The histological analysis showed no neural retina formation and dysplasia of the cornea, iris, lens, and vitreous. In the normal eye, the *ora serrata*, which contains a single columnar epithelium of non-receptive retina and a single layer of pigment epithelium, is continuous with 2 layers of ciliary epithelium, the *pars plana*. Retinal progenitor-like cells that expressed Pax6 were found in the non-pigmented cells of this cadaver's *pars plana*, where retinal stem cells reside [6]. However, neither Pax6-expressing retinal cells [6, 7] nor beta III-expressing neuronal cells [8] were detected or intermingled among the RPE-rosettes. This is the first report presenting the gross anatomy and histology of bilateral ONA with other ocular complications.

ONA (Online Mendelian Inheritance in Man [OMIM] database #165550) is a rare congenital anomaly that can be bilateral or unilateral as well as syndromic or nonsyndromic. ONA is clinically diagnosed as a blind eye lacking an optic disc and retinal vessels in the ocular fundus [9, 10]. In contrast, optic nerve hypoplasia (ONH) is an anomaly of the optic disc, which is distinct from ONA and is thought to be a nonspecific anomaly resulting from an insult to any part of the developing visual system [11]. Histologically, the definition of aplasia includes total absence of the optic nerve, retinal ganglion cells, or nerve fiber layer in the retina [12, 13]. In the present case, there was no optic nerve bundle inserting and continuing to the retina within the eyeball, no ganglion cells and no neural retina formation. This case therefore meets the histological criteria for optic nerve aplasia.

ONA is usually associated with other ocular anomalies such as microphthalmia, sclerocornea, cataracts, and retinal dysplasia [10]. Normally, the anteroposterior diameter of the eye measures approx. 24 mm [1]. Microphthalmia is defined as a globe with a total axial length < 21 mm in the adult eye [http://www.ncbi.nlm.nih.gov/books/NBK1378/] (October, 2015). Although histological sections shrink after fixation and dehydration, both eyes (17 mm, right; 15 mm, left) in this case are considered microphthalmic. This ONA case was thus diagnosed as being associated with microphthalmia.

Bilateral syndromic ONA may have related brain, cardiac, and other abnormalities, whereas unilateral ONA is typically nonsyndromic [11]. Bilateral ONA cases often occur in association with congenital anomalies of the brain, but in the present case the CT did not reveal any brain abnormalities. It is therefore most likely that this instance of ONA was caused by a developmental arrest specific to the eye. This case also demonstrates that bilateral ONA with microphthalmia can occur without brain anomalies.

Several hypotheses concerning the etiology and pathogenesis of ONA have been postulated [10]; it may be due to developmental problems of retinal ganglion cells and/or related to abnormalities of the development of mesenchyme around the optic stalk. In the present case, in addition to a failure of neural retina formation, the chaotic vitreous tissues suggested the mesenchyme's failure to develop normally. Since calcification or ossification can occur in the *phthisis bulbi* [14], pathological changes seem to have been added after the subject's long survival (70 years old).

During early eye development, the optic vesicle begins to invaginate shortly after its formation and the double-layered optic cup develops [15]. Invagination is not restricted to the distal portion of the cup; it also involves a part of the inferior surface at the proximal optic stalk. The hyaloid artery, *i.e.*, the future central artery, reaches inside the eye from this fissure. Since a vitreous cavity filled with mesenchymal derivatives formed in this ONA case, the development of the neural retina was arrested after the invagination and arrival of the hyaloid artery at the inner chamber of the eye. It is thus conceivable that the central artery, *i.e.*, the proximal portion of the hyaloid artery, disappeared—possibly due to a lack of anti-apoptotic factors from the optic nerve.

Both environmental and genetic factors are thought

to contribute to ONA [11]. Since the family pedigrees were not known and no genetic materials were available for this case, we could not examine gene mutations relating to the ocular anomaly. However, it is intriguing to discuss possible genetic factors deduced from the phenotypes. A *Pax6* mutation was reported in a patient with bilateral ONA, and the overexpression of *Pax6* gave rise to extra neural retina formation from the RPE [16, 17]. In the present case, since the immunohistochemistry showed that Pax6 protein was present only in the ciliary region (Fig. 5A), it seems likely that a dysfunction of Pax6 was responsible for this case of ONA.

A homeobox gene, Chx10, is known for *ocular retardation* (*or*) mice, in which *Chx10* is spontaneously mutated. Given that *or* mice exhibit transdifferentiation of the neural retina into pigmented cells [18], a loss-of-function in *Chx10* may arrest neural retina formation. Likewise, the expression of *Sox2*, *fibroblast growth factor 8*, or its receptor genes might be disrupted in the present case, because they are all known to contribute to neural retina formation [19–22].

In conclusion, because the family history and genetic information were not available for this case, it is tempting to speculate that certain mutations or epigenetic alterations of gene expression by environmental factors underlie the absence of neural retina formation, ONA and the other ocular complications of this case. We suggest that neural retina formation is required for the coordinated development of ocular tissues.

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