表1 無治療経過観察後24か月時以降に腫瘍摘出がなされた例

No. 文献	手術時年齢（月）	部位	腫瘍マーカー	腫瘍サイズ（最大径 mm）	組織型
1．20)	28	後腹膜	上昇	増大（59）	GNB, int
2．14)	27	副腎	低下	増大（53）	GNB, int
3．14)	27	縦隔	不変	増大（60）	GNB, int
4．14)	44	後腹膜	正常化	増大（72）	GN, mat
5．14)	48	副腎	正常化	増大（39）	GN, mat
6．14)	53	副腎	正常化	増大（34）	GN, mat
7．自験例	40	副腎	不変	増大（70）	GNB, int

GNB, int：GNB, intermixed. GN, mat：GN, maturing.

さて，安全に行われているとされる無治療経過観察であるが，症例によりその経過は異なることが示されている．Nishihiraら[11]は26例の検討から4群に分けられるとし，A群（4例）：VMA，HVAの正常化と共に腫瘍退縮が著明で画像上検出されなくなるもの，B群（14例）：VMA，HVAは正常化し腫瘍も縮小するが消失には至らぬもの，C群（4例）：VMA，HVAは50 μg/mgCre以下の範囲で種々の値を示すと共に腫瘍サイズが増大するもの，D群（4例）：VMA，HVAが40～110 μg/mgCre程度まで上昇すると共に腫瘍サイズが増大するもの，が存在したと述べている．AおよびB群については，腫瘍マーカー値は18か月時までに正常化し，また完全な自然退縮や明らかな腫瘍サイズの縮小を示す例であり，両親からの外科治療の希望がなければ，無治療経過観察を継続することが妥当な対応となり得る症例と考えられる．CおよびD群の症例はいずれも腫瘍摘出が行われる結果となっているが，その経緯には明らかな違いがある．C群は12か月から27か月時の年齢まで経過が見られており，特に18か月以降まで経過したものではGNやGNB，intermixedの所見を呈し，Schwann細胞の増加を反映した成熟分化傾向が著しい．一方，D群は経過観察開始早期より腫瘍マーカー値と腫瘍サイズに増大があり，12か月時までに腫瘍摘出がなされ，NBの組織型を示したという．

組織所見における同様の観察は，2002年に日本小児がん学会が報告[14]した無治療経過観察を行った神経芽腫82例においても指摘されている．経過観察中に腫瘍摘出（23例）あるいは生検（2例）がなされた症例は25例（30.5%）であり，このうち24例について組織学的に検討されているが，12か月時までに腫瘍が摘出された9例では，1例のみGNB，nodularの所見で他の例は全てNBの組織型であった．一方，1歳を越える15例については，15か月頃からGNB，intermixed，GN，maturingなどへの成熟傾向を示し，18か月以降になると7例中6例がこれらの組織型を示したことが報告されている．

さて，こうした無治療観察症例の群分けを背景に自験例を比較すると，我々の症例は腫瘍マーカー値が正常化せずに3歳4か月（40か月）時の摘出標本ではGNB，intermixedの所見を示し，Nishihiraら[11]の言うところのC群や日本小児がん学会報告[14]の18か月以降に顕著に見られる成熟傾向を示す神経芽腫に関連すると思われる．ここで，腫瘍摘出が24か月時以降である症例[14)20)]を他報告[11)21)]と重複の恐れのあるものを除外して抽出してみると（表1），自験例以外に6例が該当し腫瘍サイズは不変の1例を除き5例で増大していた．また腫瘍マーカーについても正常化したものが3例であったが，他の3例は低下，不変，上昇を示していた．自験例では，腫瘍サイズが70 mmまで増大し，またHVA値はおおよそ30 μg/mgCre弱で不変であり，これらの6例とほぼ同様の所見を示していると解釈される．しかし，組織型に注目すると，28か月時までに摘出された3例がGNB，intermixedで悪性腫瘍の範疇であるのに対し，他の44か月以降のものはGN，maturingと良性腫瘍にまで成熟した所見を示している．これを鑑みると，自験例が40か月時で未だ完全には分化成熟せず腫瘍マーカー上昇の源と思われる神経芽細胞成分を維持していたことは，本例における特異な所見である可能性がある．すなわち，自験例は分化成熟傾向を示す一群の中でも，その過程が完全には進まず，長期にわたり悪性の神経芽腫成分が維持される例であるとも考えられる．仮にGNBのままさらに長期に経過したとすれば，その予後にも影響を及ぼしたかも知れない．

一方，自験例でさらに待機して腫瘍を摘出すれば，完全なGN，maturingまで成熟が進んでいたか否かは不明であり，むしろ40か月時までHVAが横ばいで正常

化しなかった経過を考えるならば，さらに1年程を経過してもマーカー値の変化は期待できなかった可能性がある．また，2歳時点から著明となってきた腫瘍増大傾向には，表1に挙げた例も同様であるが，Schwann 様細胞すなわち GN 成分の増生によるところが大きいと思われる．この増生は 40 か月以降も続いたと想像され，自験例ではこの時点を越えて無治療経過観察を継続することは手術を困難にする可能性が高く妥当な選択ではないと判断された．

他方，IVS 期の症例を含む乳児神経芽腫においては完全切除が行われずとも自然退縮の過程が加わり完全緩解に至ることがしばしば経験されるが，その後年余を経過して悪性度の高い腫瘍として再発する例[22][23]も，以前より報告されている．また，乳児症例以外でも GN からの悪性転化を示唆する報告[23-25]も見られている．こうした事実は，腫瘍が摘出されず無治療経過観察が継続された症例においても，その一部に将来再発を来すことを無視し得ない一群が含まれる可能性を示唆している．

自験例の取り扱いに関しては，結果的には3歳4か月より以前に腫瘍摘出が適切である可能性も考えられた．特に生検については GN の部分にしか操作が及ばず正診に至らなかったが，こうした不確実性も考慮して，少なくともこの時点では生検に代えて腫瘍摘出を施行しておくべきであったと思われた．

自験例における腫瘍の生物学的因子については，*MYCN*，染色体，TrkA，嶋田分類などにおいて，いずれも予後不良因子は認めていなかった．本例のごとく分化成熟傾向が明らかで 18 か月以降に摘出された腫瘍では生物学的予後因子は良好な所見を示すようであるが，これ以前に摘出に至った腫瘍にはしばしば予後不良因子を有することを報告するもの[10]もある．ただし無治療経過観察後に摘出された腫瘍で生物学的予後因子を検討された例は多くはなく，実際の状況は未だ明確にはなっていないと思われる．

これまでに自然退縮する例のほとんどは月齢 18 か月程度までに尿中 VMA，HVA は正常化していることが示されている[10][11][14]．2004 年3月で全国的な MS は廃止されたが，現在いくつかの自治体では自然退縮しない症例のみを有効に検出することを期待して，18 か月など施行時期を変えて MS が行われている[17]．自験例なども，18 か月時にスクリーニングを受診していても陽性例として検出される例に当てはまることになる．こうした試みが有効なスクリーニング事業となり得るかどうか今後の成果が期待される．

（本症例の病理組織所見について，国立成育医療センター・秦順一先生にご教示いただきました．深謝致します．）

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A Case of Intermixed Ganglioneuroblastoma Detected by Mass Screening and Observed Without Treatment for 2 Years and 9 Months

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A female infant with a mass screening (MS)-detected neuroblastoma originating from the right adrenal gland is presented. The tumor was 38×21 mm in size and classified as stage I, which led us to adopt a wait-and-see policy without any treatment. Tumor markers, however, did not decrease to the normal levels, and the tumor size increased after the 1st year of age. Histological finding of open biopsy performed at 2 years and 10 months old was ganglioneuroma (GN), maturing, and the observation policy was continued. After biopsy, the tumor size still kept increasing, and resection of the tumor was finally performed when the patient was 3 years and 4 months old. The tumor was 70×50×50 mm in size and histologically diagnosed as ganglioneuroblastoma (GNB), intermixed. After surgery, tumor marker levels were promptly normalized. Although this case was found by MS, it neither spontaneously regressed nor completely matured into GN during the observation period of 2 years and 9 months, but rather kept growing in size while maintaining malignant neuroblastic components. Such findings and natural course of the tumor in our case are likely to represent the unusual character of MS-detected neuroblastoma.

Key words: ganglioneuroma, ganglioneuroblastoma, neuroblastoma, mass screening, observation policy

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the probe may be subjected to thermal effects during lengthy surgical procedures.

Caution should be applied regarding light intensity in lengthy surgical procedures using this illumination system, and preventive methods must be further investigated.

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Prolonged exposure to topical cyclosporine A does not seem to promote conjunctival malignancy

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Editor,

Yarosh et al. (2005) recently reported an inhibitory effect on cyclobutane pyrimidine dimer repair in normal epithelial keratinocytes by calcineurin inhibitors such as cyclosporine A (CsA) and the ascomcin derivatives tacrolimus and pimecrolimus. They suggest an increased susceptibility of sun-exposed skin and conjunctiva towards malignant transformation from mutations induced by ultraviolet light (UV). Accordingly, conjunctival carcinomas have been observed after systemic administration of CsA in organ transplantation (Macarez et al. 1999); the US Food and Drug Administration (FDA) issued a cancerogenicity alert for topical tacrolimus by way of precaution.

We prescribed CsA eye drops 1% and 2% for Thygeson's superficial punctate keratitis and adenoviral opacities in a series of 76 eyes to avoid cataract and glaucoma from protracted application of topical corticosteroids (Reinhard & Sundmacher 1999; Hillenkamp et al. 2001). Treatment period was 2.2 ± 2.1 [standard deviation (SD)] years; follow-up averaged 5.9 ± 1.9 years. Because these patients could be at increased risk of conjunctival carcinoma from UV-induced DNA damage, slit-lamp examinations and brush impression cytology were performed at the end of follow-up.

Signs of malignant transformation were found neither in slit-lamp examinations nor in brush cytology specimens from conjunctival epithelium. In case of pronounced cancerogenicity from CsA eye drops 1–2%, slit-lamp examinations should detect advanced carcinoma during follow-up whereas brush cytology is expected to detect even early dysplastic cellular changes. Our negative finding in 76 eyes renders a major risk of conjunctival malignancy from CsA eye drops unlikely. The superficial layers of conjunctival epithelium might protect the vulnerable basal stem cells by absorbing a sufficient amount of mutagenic UV. Superficial epithelial cells are most likely shed before DNA damage can culminate into malignoma. However, caution should be exercised in cases with atopic dermatitis (Heinz et al. 2003).

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Glial extrusion from regressed retinoblastoma after conservative treatment

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Editor,

Eyes with a retinoblastoma are preserved by conservative treatment, including chemotherapy, radiotherapy, photocoagulation, cryotherapy, transpupillary thermotherapy, and episcleral plaque brachytherapy, or a combination of these. Once retinoblastoma recurs and is uncontrolled by additional conservative treatments, enucleation is the only option. Although regrowth or

the presence of subretinal seeds or retinal infiltrates from the regressed retinoblastoma suggest a recurrence (Judisch & Folberg 1987), we report a case of recurrence-like large glial proliferation from conservatively treated retinoblastoma, identified by transcorneal biopsy using a vitreous surgery system.

A 6-month-old girl had bilateral leucocoria. The tumour (Reese-Ellsworth group Vb) occupied almost the entire vitreous cavity in the left eye. Two bulky masses (group IVa) of 10–15 disc diameters were observed in the right eye (Fig. 1A). Computed tomography failed to detect systemic anomalies except for the intraocular tumours. The left eye was enucleated; the right eye underwent three cycles of chemotherapy (carboplatin, vincristine and etoposide) and 30-Gray of external beam irradiation. Twelve months later, a dome-like proliferation gradually developed from the margin of one regressed tumour, and a rhegmatogenous retinal detachment developed with the break at the edge of the other regressed tumour (Fig. 1B). A biopsy was performed using a three-port vitreous surgery system inserted through the peripheral cornea. The lens was removed, and the proliferative tissue was resected using endo-ocular scissors and forceps. Rapid frozen-section examination failed to identify any signs of malignancy. The retinal detachment was treated by scleral buckling, vitrectomy, fluid–gas exchange and endophotocoagulation. The retina was reattached (Fig. 1C). Corrected vision in the right eye was 20/40 at the last examination. Immunohistochemistry disclosed that the proliferative tissue was widely and strongly positive for glial fibrillary acidic protein (GFAP), locally and weakly positive for vimentin, and negative for Ki67, suggesting benign glial proliferation (Fig. 2).

Immunohistochemistry has shown that Müller cells and immature astrocytes are positive for vimentin, and astrocytes and activated Müller cells are positive for GFAP. Because of the types of staining identified, the glial proliferation in this case may be of astrocytic origin. When the central nervous system is damaged by trauma, neurodegeneration, inflammatory disease or ischaemia, the astrocytes commonly proliferate to protect and repair neurons (Dayer & Cepko 2000). In the retina, reactive astrocytic proliferation is identified when retinal detachment, proliferative vitreoretinopathy or diabetic retinopathy develop (Nork et al. 1987). Because the glial cells survive in the regressed retinoblastoma (Demirci et al. 2003), they may represent another source of proliferation after conservative treatment. One pathology report showed benign

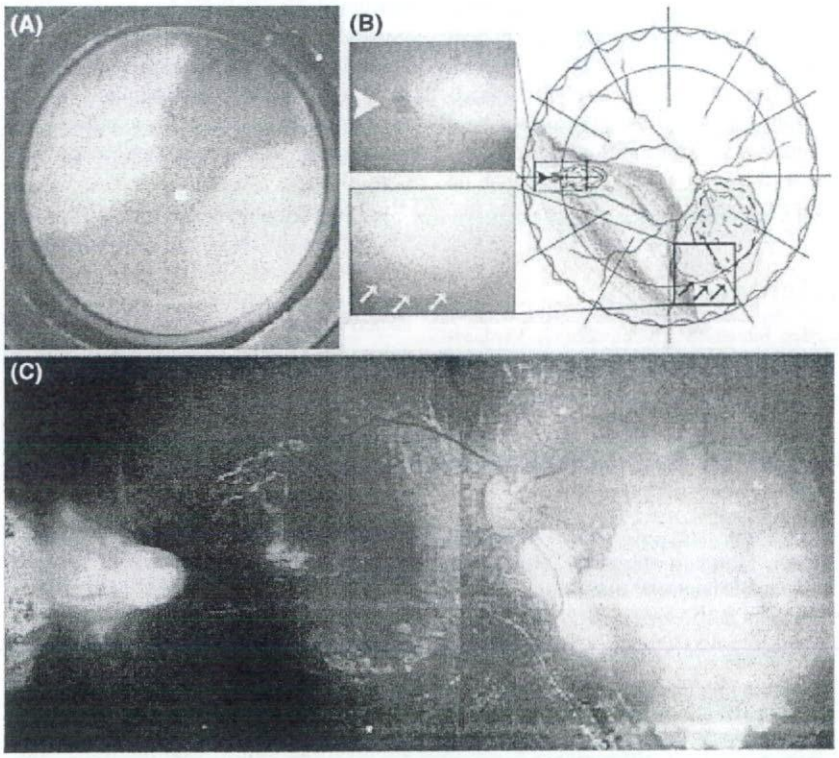

Fig. 1. Fundus photographs and chart of the preserved right eye. (A) The right eye showed two bulky masses. (B) A dome-like proliferation (arrows) from the regressed tumour and a retinal break (arrowhead) are seen associated with the second regressed tumour. (C) The detached retina is reattached after biopsy, scleral buckling and vitrectomy.

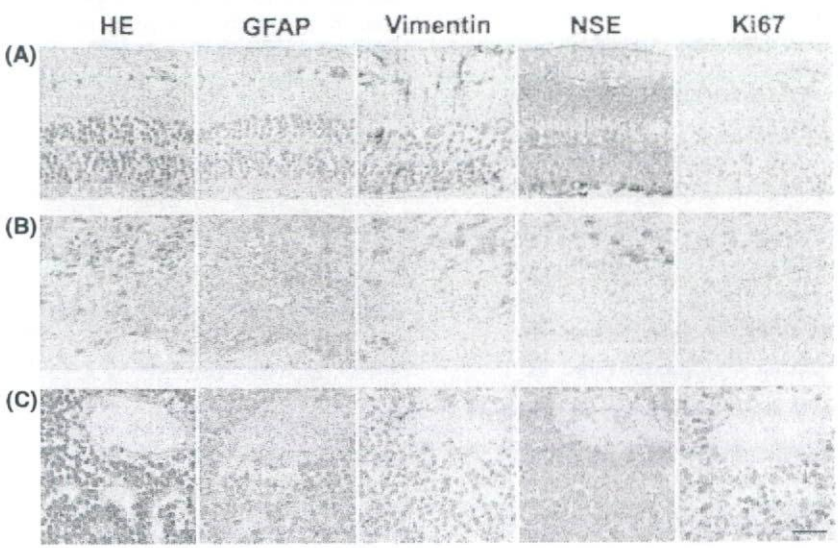

Fig. 2. Immunohistochemistry of the proliferative tissue. (A) Control retina, (B) proliferative tissue, and (C) retinoblastoma in the left eye. The tissue is strongly positive for GFAP, weakly positive for vimentin, and negative for Ki67, while the retinoblastoma is strongly positive for Ki67, suggesting high mitotic activity (HE = haematoxylin-eosin; GFAP = glial fibrillary acidic protein; Scalebar = 50 μm).

retinal infiltration from the regressed retinoblastoma that probably originated from the retinal pigment epithelium (Judisch & Folberg 1987). Thus, a biopsy may be helpful to determine whether the proliferation is malignant or benign, especially in subjects with only one seeing eye.

Fine-needle aspiration biopsy is recommended when the diagnosis of primary retinoblastoma is difficult to confirm with other examinations (Shields et al. 1993). The needle should be inserted through the peripheral cornea and the zonules of Zinn to prevent malignant cell metastasis (Shields et al. 1993). In the case of a solid tumour, which is difficult to aspirate by fine-needle biopsy, tissue resection using endo-ocular scissors and forceps via the transcorneal approach may be a safe and reliable way of establishing a diagnosis, although removal of the lens is necessary.

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A new diagnostic approach in patients with type 2 macular telangiectasia: confocal reflectance imaging

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Editor,

Type 2 idiopathic macular telangiectasia (type 2 IMT) usually presents with a slow decrease in visual acuity in the fifth to seventh decade. This typically bilateral disease is characterized by occult, parafoveolar telangiectasis and minimal exudation that may be visible on fluorescein angiography only (Gass & Blodi 1993; Charbel Issa et al. 2007a). Typical clinical findings are located predominantly in the temporal parafovea and include a reduction of parafoveal retinal transparency, superficial retinal crystalline deposits, dilated blunted venules. Functional deterioration is predominantly due to foveal atrophy, intraretinal pigment proliferation or development of neovascular membranes (Charbel Issa et al. 2007b). Diagnosis is usually confirmed by invasive fluorescein angiography.

We present findings in two patients with type 2 IMT using non-invasive blue (488 nm) and infrared (820 nm) reflectance imaging with a confocal scanning laser ophthalmoscope (cSLO; HRA2, Heidelberg Engineering, Heidelberg, Germany). The images are compared to findings of fluorescein angiography and digital fundus photography.

Patient 1 (female, 65 years) had a visual acuity of 20/80 in the right eye and 20/63 in the left eye. There was slight parafoveal greying bilaterally and a dilated blunted venule in the upper temporal quadrant of the macula in the left eye.

Patient 2 (male, 54 years) presented with a visual acuity of 20/63 in the right eye and 20/200 in the left eye. He had an advanced disease with intraretinal pigment proliferation in both eyes and a secondary vascular membrane in the left eye.

In both patients, red-free imaging (Zeiss FF450 fundus camera; Zeiss, Oberkochen, Germany; filter with a transmission range of 515–575 nm) highlighted the dilated right-angled venules and enabled the visualization of small ectatic parafoveal capillaries (Fig. 1D,J). Retinal crystals in patient 2 appeared to follow the topography of retinal nerve fibres, suggesting a superficial location of these deposits.

In both patients, confocal blue reflectance imaging (Fig. 1E,K) using the cSLO disclosed a well-defined area of increased reflectance topographically related to the leakage in late-phase angiography (Fig. 1B,H).

Infrared reflectance (Fig. 1F,L) showed a uniform reflectance in patient 1 and a decreased reflectance in patient 2. The latter again coincided with the area of leakage in late-phase angiography.

Spectral narrowing of the fundus illumination by using filters may reduce chromatic aberrations and enhance the contrast and resolution of details. Small retinal vessels are best imaged at wavelengths of 510–570nm (Delori et al. 1977). The use of an appropriate filter enhanced the visualization of the parafoveal ectatic vascular changes in type 2 IMT.

Macular imaging by cSLO technique allows the detection of light from a conjugate plane and decreases artefacts by stray light (Jorzik et al. 2005). Increased confocal reflectance at 488 nm may be explained by decreased light absorption and/or increased reflection in the retina. These retinal areas are related to the area of leakage in late-phase fluorescein angiography and hence may provide information as to the extent of the macular area involved. Since alterations in type 2 IMT can be detected in an early disease stage (patient 1) using confocal blue reflectance imaging, this imaging modality might be helpful in the early detection of the disease. Because confocal infrared reflectance imaging showed major changes only in patient 2 (who represents a later disease stage), this imaging mode may rather be suitable for follow-up in patients with type 2 IMT.

In summary, spectral narrowing in fundus photography using red filters

Case of Aggressive Posterior Retinopathy of Prematurity with Atypical Neovascular Growth

Fibrovascular proliferation in eyes with retinopathy of prematurity (ROP) usually, but not always, appears at the junction of the vascularized and nonvascularized retina.[1] Aggressive posterior ROP (AP-ROP) occurs in the posterior retina and progresses rapidly to total retinal detachment.[2] We report an atypical case of AP-ROP in which the neovascularization developed in the posterior retina around the optic disc.

Case Report

A female infant was born at 30 weeks' gestation (birth weight, 1670 g) with severe persistent pulmonary hypertension from prolonged premature rupture of the membranes and oligohydramnios. She was treated with nitric oxide (NO) inhalation for 28 days. At 33 weeks postmenstrual age, an ophthalmoscopic examination identified initial signs of zone I AP-ROP bilaterally, including marked dilation and tortuosity of the posterior pole vessels (zone I, stage 1 ROP with plus disease).

Argon laser photocoagulation was performed (duration, 300–400 ms; power, 300–400 mW; 3751 shots OD, 3658 shots OS) under intravenous sedation (fentanyl) with topical anesthesia. However, fibrovascular proliferation and retinal detachment developed bilaterally in the posterior retina around the optic disc 1 week postoperatively (Fig. 1a, b). The patient underwent vitrectomy with lensectomy as a secondary treatment at 35 weeks postmenstrual age. The retina was reattached and the ROP stabilized in the left eye, but the fibrovascular tissue regrew from the posterior retina of the right eye (Fig. 1c). A second vitrectomy stabilized the ROP in that eye (Fig. 1d).

Immunohistochemistry of the fibrovascular tissue collected during vitrectomy was strongly positive for factor VIII over a wide area and locally positive for vimentin but negative for glial fibrillary acidic protein. These findings suggested that the tissue consisted mainly of vascular endothelial cells (Fig. 2).

Comments

We report the successful surgical results of early vitrectomy for AP-ROP.[3] Our findings suggest that when neovascularization develops only at the peripheral end of the developing vessels, the retina can be reattached by removing the vitreous framework around the fibrovascular tissue and the vitreous base. These procedures reduce the tractional forces of the fibrovascular tissue and suppress neovascular growth. Residual vitreous gel did not affect the retinal reattachment, and a regrowth of neovascularization was not observed in a previous study.[3]

In our case, the neovascularization that developed in the posterior retina could have grown along the residual vitreous gel on the retinal surface and around the optic disc. This tissue could not be completely removed during the initial vitrectomy. In cases such as this, another vitrectomy to peel the residual vitreous gel can lead to retinal reattachment, which worked well in our case.

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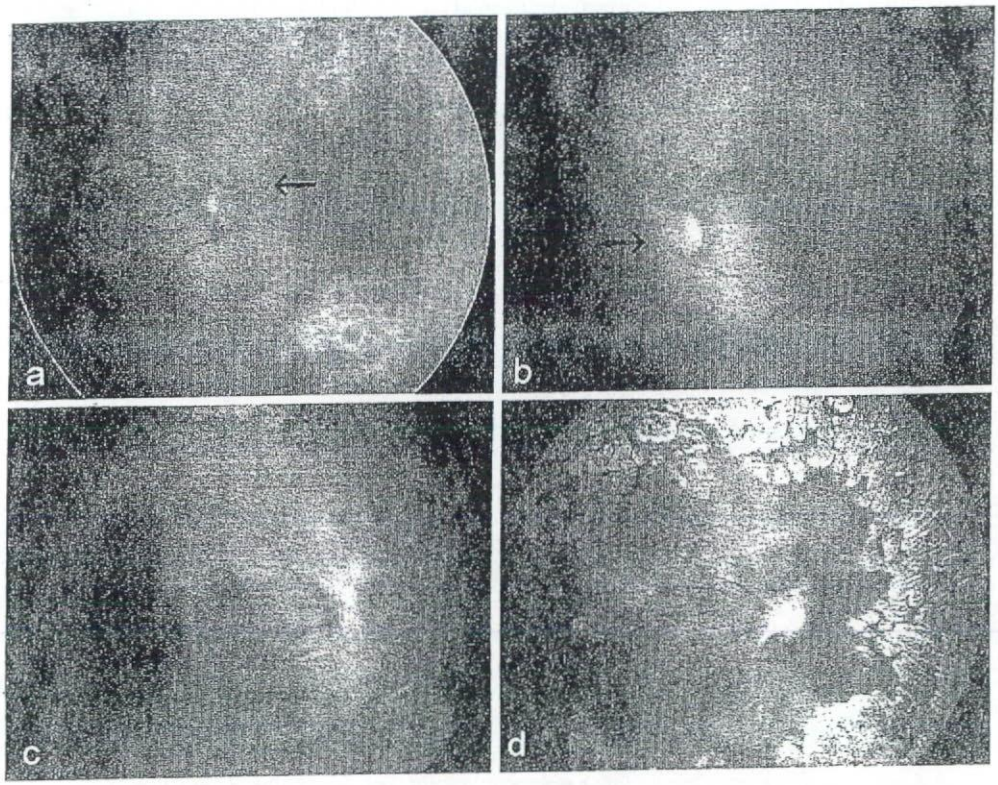

Figure 1a–d. Preoperative ar postoperative fundus phot graphs of eyes with aggressiv posterior retinopathy of prem turity (ROP). **a** Preoperativ fundus image of the right eye. Preoperative fundus image of th left eye. Fibrovascular prolifer tion and tractional retinal detacl ment (*arrows*) are present nasal in the posterior retina of bot eyes. **c** Two weeks after the initi vitrectomy, the neovasculariza tion has regrown and formed fibrous membrane and traction retinal detachment in the rigl eye. **d** The retina is reattache and the ROP is stabilized afte additional vitrectomy of the rigl eye.

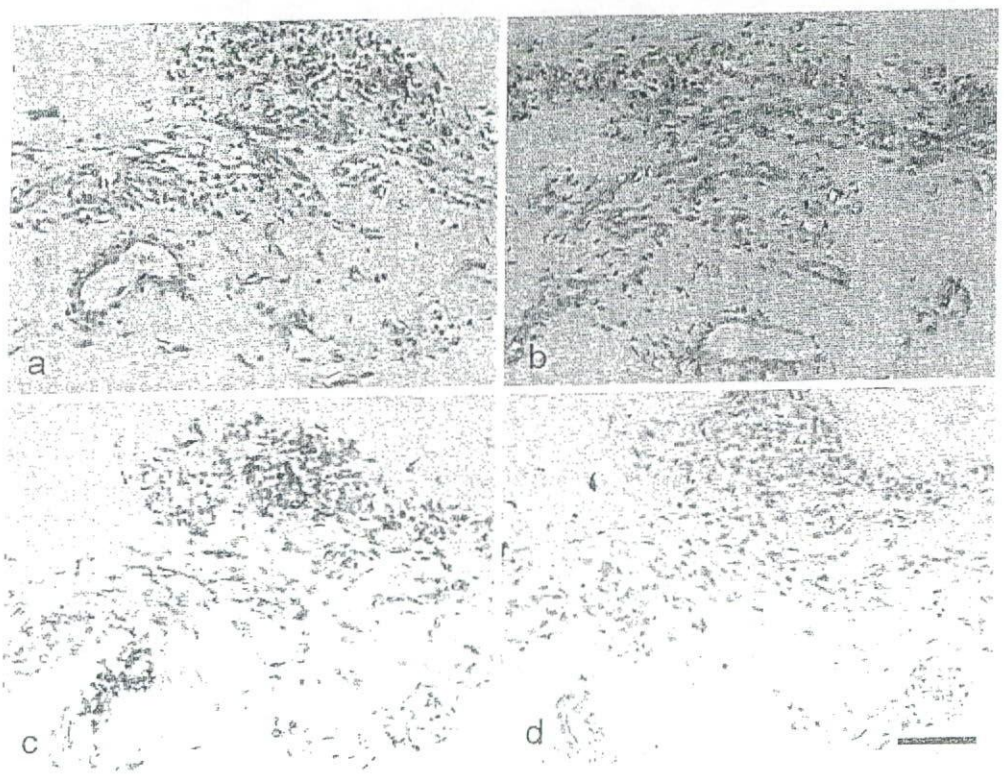

Figure 2a–d. Pathology an immunohistochemistry of th fibrovascular tissue obtaine during the second vitrectomy H&E staining (**a**) and immuno histochemistry with antibodie against factor VIII (**b**), vimentii (**c**), and glial fibrillary acidi protein (**d**). Immunohistochemis try showed that the fibrovascula tissue was strongly positive fo factor VIII over a wide area (b) and was locally positive fo vimentin (**c**) but negative for glia fibrillary acidic protein (**d**). These findings suggest that the tissu was composed of vascular endo thelial cells (scale bar = 50 μm).

In eyes with AP-ROP, a flat network of neovascularization arises from the peripheral terminals of the developing vessels as in classical ROP, even though vascular shunts occur in the vascularized retina. However, in our patient, the fibrovascular proliferation developed atypically in the posterior retina around the optic disc. Except for the prolonged NO inhalation, systemic therapies including oxyger administration and laser application might not contribute

to the atypical growth of the neovascularization. While NO is known to derive vasodilatation and up-regulates regional basal blood flow,[4] it also activates angiogenic cell migration and proliferation-inducing factors, including fibroblast growth factor 2 and vascular endothelial growth factor.[5] Because retinal angiogenesis is ongoing in premature infants, NO might have contributed to the atypical neovascularization near the optic disc in our patient.

In animal models of oxygen-induced retinopathy, neovascularization induced by obliteration of the immature capillaries also develops from the optic disc and posterior retina.[6] Because AP-ROP develops in the posterior retinal area, including zone I, this suggests that immature capillaries may be widely present, and neovascularization arises from the retina near the optic disc. Capillary nonperfusion in vascularized retinas has been identified in eyes with threshold ROP.[7] Thus, there might be a much wider area of nonperfusion in the posterior retina in eyes with AP-ROP, which should be studied using fluorescein angiography.

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Key Words: aggressive posterior retinopathy of prematurity, fibrovascular proliferation, photocoagulation, regrowth, vitrectomy

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CASE REPORT

Exudative retinal detachment following cataract surgery in Hallermann-Streiff syndrome

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Abstract
Purpose To report two cases of Hallermann-Streiff syndrome with exudative retinal detachment after cataract surgery.
Methods Case report.
Results Four eyes of two patients with Hallermann-Streiff syndrome developed exudative retinal detachments after lensectomy and anterior vitrectomy at 2 and 4 months of age. Both patients had extreme microphthalmia. The exudative retinal detachment regressed spontaneously in three of the four eyes; however, one eye required subscleral sclerectomy. In one patient, the best-corrected visual acuity was 20/200 at 3 years of age; the other patient had good fixation and following behavior in each eye at 1 year of age.
Conclusions Early surgery to treat congenital cataracts in extremely microphthalmic eyes associated with the Hallermann-Streiff syndrome may induce exudative retinal detachment. However, the retinal detachments tend to regress and may not cause severe visual impairment.

Keywords Hallermann-Streiff syndrome · Exudative retinal detachment · Cataract surgery · Microphthalmos

Introduction

The Hallermann-Streiff syndrome is a rare complex of developmental abnormalities characterized by dyscephaly with bird face, beak nose and micrognathia, dental anomalies, hypotrichosis, skin atrophy, microphthalmia, congenital cataracts, and proportionate dwarfism [1]. Most cases are sporadic, and the etiology is unknown. Various ocular findings and fundus anomalies have been reported, including vitreous degeneration, retinal folds, coloboma, and Coats' disease; however, a few reports have described detailed fundus changes after cataract surgery [2–4]. To our knowledge, this is the first report on the development of exudative retinal detachments after cataract surgery in four microphthalmic eyes of two patients with the Hallermann-Streiff syndrome.

Case report

Patient 1, a 1-month-old Japanese male infant, referred with a diagnosis of bilateral congenital cataracts and microphthalmia. He had the typical features of the Hallermann-Streiff syndrome, including dyscephaly with beak nose and micrognathia, dental anomalies, hypotrichosis, skin atrophy, and proportionate dwarfism (Fig. 1a). A slit-lamp examination revealed total cataracts, a microcornea (corneal diameter, 7×7.5 mm OD and 8×8.5 mm OS), a shallow anterior chamber, posterior synechiae, and poor pupil dilation in both eyes. Ultrasonography showed bilateral microphthalmia (axial length, 13 mm OD and 14 mm OS) but no other posterior segment anomalies. Lensectomy and anterior vitrectomy via the limbal approach using a 25-gauge surgical system was performed in both eyes at 2 months of age. No intraoperative or postoperative complications developed except for transient corneal edema. Ophthalmoscopy identified small retinal folds between the disc and fovea in both eyes. The aphakic eyes were corrected with glasses, and both eyes developed fixation and following behavior.

The authors have no proprietary interest in any aspect of this report.

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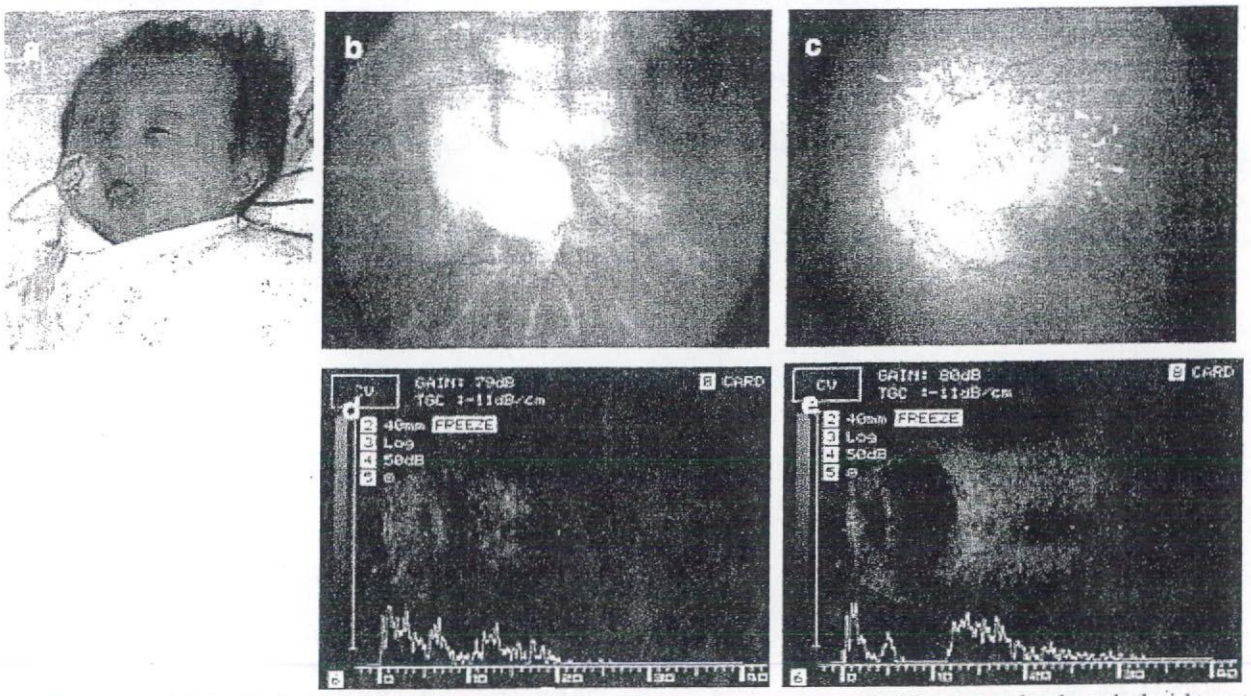

Fig. 1 Patient 1. (**a**) Facial characteristics at 2 months of age. Bilateral exudative retinal detachments at 7 months of age. The right eye (**b**) has the more severe retinal detachment and requires subscleral sclerectomy. The left eye (**c**) also has an exudative retinal detachment that regressed spontaneously. Ultrasonography shows both right eye (**d**) and left eye (**e**) have exudative retinal detachments with choroidal thickening

We examined the patient every month and then at 5 months after cataract surgery (7 months of age) when exudative retinal detachment developed in both eyes (Fig. 1b,c). Ultrasonography also showed bilateral exudative retinal detachment with choroidal thickening (Fig. 1d,e). The retinal detachment spontaneously regressed in the left eye, but progressed and did not regress in the right eye. Following behavior in the right eye deteriorated, and we performed a subscleral sclerectomy twice in that eye using 0.02% mitomycin C at 1 year 9 months and 2 years 4 months of age. The retinal detachment regressed, and at 3 years 5 months of age did not recur in either eye. The best-corrected visual acuity (BCVA) was 2/100 in the right eye and 20/200 in the left eye.

Patient 2, a 5-month-old Japanese female infant, referred with a diagnosis of bilateral retinal detachments after cataract surgery. She had undergone lensectomy and anterior vitrectomy at 4 months of age in another hospital. She exhibited the characteristic features of the Hallermann-Streiff syndrome, including dyscephaly with beak nose and micrognathia, dental anomalies, hypotrichosis, and proportionate dwarfism. A slit-lamp examination showed bilateral aphakia and a microcornea (corneal diameter, 7×7.5 mm OD and 8×8.5 mm OS). Ophthalmoscopy of both eyes showed exudative retinal detachments (Fig. 2). Ultrasonography showed bilateral microphthalmia (axial length, 13 mm OD and 14 mm OS) and retinal detachments with choroidal thickening. The retinal detachments spontaneous-

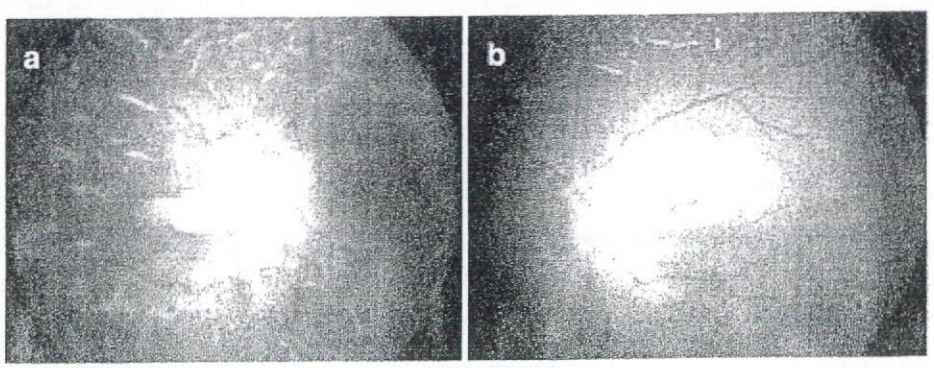

Fig. 2 Patient 2. Bilateral exudative retinal detachments at 1 years of age. The exudative retinal detachments regressed spontaneously in the right eye (**a**) and the left eye (**b**)

ly regressed in both eyes, and the patient had good fixation and following behavior with each eye at 1 year of age.

Discussion

These four eyes of two patients had severe microphthalmos and developed exudative retinal detachments after early surgery for congenital cataracts at 2 and 4 months of age. One of the four eyes required surgery; however, the retinal detachment regressed in three eyes, and the visual acuity was not severely impaired. This suggests that the extreme microphthalmic eye in the Hallermann-Streiff syndrome may have considerable scleral abnormalities that impede transscleral intraocular fluid outflow and result in congestion of the choroidal vein [5]. Early cataract surgery is supposed to induce hypotony, marked intraocular inflammation and transiently accelerate production of a protein-rich exudate. Intraocular fluid outflow may severely be resisted postoperatively and accumulated in choroid without venous drainage. It may possibly cause early onset of exudative retinal detachment in these eyes.

In this syndrome, spontaneous cataract absorption sometimes occurs, but results in deprivation amblyopia, iridocyclitis, and glaucoma [4, 6]. Although exudative retinal detachment tends to occur, it is preferable to perform early cataract surgery using less invasive procedures.

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Research Letter

SOX10 Mutation in Waardenburg Syndrome Type II

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To the Editor:

Waardenburg syndrome (WS) is a congenital developmental disorder characterized by sensorineural hearing loss and abnormal pigmentation of the eye, hair, and skin [Jones, 2006]. This condition is divided into four types [reviewed in Jones, 2006; Bondurand et al., 2007]. Type I WS (WS1) consists of dystopia canthorum and broad nasal root, and is almost exclusively caused by heterozygous mutations of *PAX3*. Type II WS (WS2) lacks the dystopia canthorum and results from heterozygous mutations of *MITF* (WS2A) in ~15% of patients and homozygous deletions of *SNAI2* (WS2D) in two patients. Type III WS (WS3) (Klein–Waardenburg syndrome), a severe form of WS1, is associated with upper limb defects, and is ascribed to heterozygous or homozygous mutations of *PAX3*. Type IV WS (WS4) (Shah–Waardenburg syndrome) is characterized by Hirschsprung disease, and is caused by heterozygous or homozygous mutations of *EDNRB* or its ligand *EDN3*, or by heterozygous mutations of *SOX10*.

Thus, the underlying causes remain to be clarified in most of the WS2 patients. While a WS2 locus is mapped to chromosome 1p (WS2B) [Lalwani et al., 1994] and chromosome 8q23 (WS2C) [Selicorni et al., 2002], a causative gene(s) has not been identified from these regions. In this regard, Bondurand et al. [2007] have recently identified *SOX10* deletions in patients with WS2, implying that *SOX10* abnormalities can cause WS2 (WS2E) as well as WS4. Here, we describe another case of WS2E caused by heterozygous *SOX10* mutation.

This Japanese girl was born to nonconsanguineous healthy parents at 41 weeks of gestation after an uncomplicated pregnancy and delivery. At birth, her length was 49.6 cm (+0.6 SD), and her weight 3.4 kg (+0.1 SD). She was found to have light blue eyes, and referred to us at 12 days of age. She manifested hypopigmented irides and a piece of white forelock, but lacked dystopia canthorum, broad nasal root, and Hirschsprung disease. Ophthalmologic examinations revealed bilateral ocular albinism with hypopigmented fundus and hypochromic iris. At 3.5 months of age, auditory brainstem response was performed because of poor responses to sounds, showing bilateral severe sensorineural deafness (hearing level, 90 dB bilaterally). Brain computed tomography showed no abnormal finding. On the basis of the above findings, she was diagnosed as having WS2.

After obtaining written informed consent, direct sequencing was performed for leukocyte genomic DNA of this patient, detecting no abnormality in the coding sequences of *PAX3*, *MITF*, and *SNAI2*. However, we identified a heterozygous *SOX10* frameshift mutation (c.506delC) on exon 4 that is predicted to result in a premature termination at the 284th amino acid (p.Pro169fsX284) (Fig. 1A). The primer sequences and the annealing temperature used were: exon 3, GTTGGACTCTTTGCGAGGAC and ATCCACCCGAAGCTAGAGG (58°C); exon 4, AGCCCCTCTGCTGTCCTCT and CACCCTCAGCTCTGTCATCA (60°C); and exon 5, CTAACCTGCTTCCCCCTTG and CAAGGAACAGGGCACACAG (58°C). This frameshift mutation located within the high mobility group (HMG) DNA-binding domain, and removed the C-terminal part of the HMG domain and the whole transactivation domain. This mutation is predicted to destroy an *Nci*I restriction site, and

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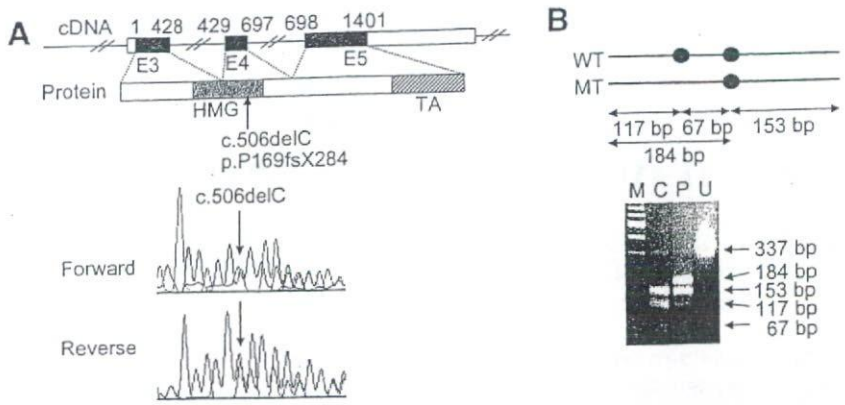

FIG. 1. Mutational analysis of *SOX10*. A: Direct sequencing of exon 4. Shown on the upper part is a schematic representation indicating the coding exons 3–5 (E3–E5) and the functional domains. For the *SOX10* cDNA, the black and white areas denote the coding regions and the untranslated regions, respectively, and the Arabic numbers indicate the cDNA sequence encoded by each exon. For the SOX10 protein, the gray and striped squares represent the high mobility group (HMG) DNA-binding domain and the transactivating (TA) domain. Electrochromatograms (forward and reverse) indicate a heterozygous c.506delC mutation on exon 4. B: Restriction enzyme analysis. The black circles represent *NciI* restriction sites. PCR products contain naturally occurring two *NciI* sites on the wild-type (WT) exon 4, and one of the two *NciI* sites is predicted to be destroyed on the mutant (MT) exon 4. After *NciI* digestion, WT sequence specific 117 and 67 bp bands only are found for a control subject (C), whereas WT specific 117 bp and 67 bp bands and a MT specific 184 bp band are shown for the patient (P). M: size marker; and U: undigested PCR product (337 bp).

this was confirmed by the *NciI* digestion of the corresponding PCR products (Fig. 1B). While the parents postponed the decision to have the genetic testing, this mutation was absent in 100 control subjects.

The results provide further support for the notion that WS2 can be caused by heterozygous abnormalities of *SOX10* (WS2E). In this regard, a *SOX10* frameshift mutation (c.1076–1077delGA, p.Thr360fs X399) has been identified not only in a patient with a typical WS4 but also in the mother with an apparently WS2-compatible deafness and white forelock only phenotype [Pingault et al., 1998]. In addition, another *SOX10* missense mutation (p.Ser135Thr) has also been detected in a patient with "Yemenite deaf-blind hypopigmentation syndrome" mimicking WS2 [Bondurand et al., 1999]. These findings, together with *SOX10* deletions in patients with WS2 [Bondurand et al., 2007], imply that heterozygous *SOX10* abnormalities lead to not only WS4 but also to the WS2 phenotype. Such phenotypic variability would not be unexpected, because it is known that heterozygous mutations of developmental genes are usually associated with wide range of expressivity and penetrance [Fisher and Scambler, 1994]. In addition, the position of the frameshift mutation on exon 4 may also be relevant to the lack of associated features, because *SOX10* mutations residing on the last exon frequently lead to more severe phenotypes such as chronic intestinal pseudo-obstruction and/or neurological features, probably due to escape from the nonsense mediated mRNA decay [Pingault et al., 2000, 2002; Inoue et al., 2004].

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黄斑を形成する遺伝子システムと再生医療への応用

Gene mechanism that relates to formation of the fovea and its contribution to reproducing medicine

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◎黄斑は中心視力を得るための高度な網膜構造である．Pax6 はすべての動物における眼形成の master control 遺伝子であるが，ヒトの黄斑低形成で Pax6 遺伝子の変異が発見されたことから，黄斑の形成に関与していると思われる．Pax6 は選択的スプライスのエクソン 5a を含むアイソフォーム Pax6(+5a)と含まない Pax6(−5a)があり，異なる転写因子の働きをもつが，黄斑の形成には Pax6(+5a)がかかわっていることが示唆された．このような黄斑の形成にかかわる遺伝子システムを応用すれば，網膜の再生において高度な視覚を獲得できることが期待される．

Key word：黄斑，形態形成遺伝子，Pax6，選択的スプライス

黄斑は網膜において高度な視覚である中心視力をつかさどるために細胞が密に集中する特殊な部位である．この形成機構には何らかの遺伝子が働いているはずであるが，これまでほとんど検討されていなかった．先天性の黄斑低形成において眼の形成遺伝子 Pax6 の変異がみつかったことが発端になって，この遺伝子の働きが *in vitro*，*in vivo* で検討され，網膜の高度構造をつくるシステムが明らかになりつつある．

眼形成のmaster control遺伝子Pax6

Pax 遺伝子群は paired box と homeobox を共通モチーフとしてもつ遺伝子ファミリーで，422 のアミノ酸をコードする．Pax 蛋白では paired box から翻訳される paired domain がおもに標的遺伝子に結合する(図 1)．この遺伝子群は最初にショウジョウバエで発見され，脊椎動物では 9 種みつかっており，Pax6 はその 6 番目にあたる．ヒトの Pax6 遺伝子は最初に先天無虹彩の原因遺伝子として染色体 11p13 領域の欠失部位から positional cloning によって発見された[1]．

その後，この遺伝子がマウスやラットで変異があると小眼球を起こす small eye(Sey)や，ショウジョウバエで複眼が形成されない eyeless と相同であることが判明した．さらに，ショウジョウバエ初期胚のさまざまな部位にこの遺伝子を導入すると(target expression)，触覚や翅，肢などに異所性に複眼が発生したことから，眼の器官全体をつくる強力な形態形成遺伝子であることが明らかになった[2]．器官の形態形成には全体的に支配する master control 遺伝子があると予測されていたが，下等動物とはいえ，眼というもっとも複雑な器官でその遺伝子がいきなりみつかったのである(図 2)．

その後，さまざまな動物で Pax6 遺伝子がみつかり，脊椎動物，軟体動物の眼や昆虫の複眼だけでなく，プラナリアの原始眼や線虫の光感受性細胞にも存在しており，塩基配列が高度に保存されていたことから，眼の起源に関する考えに大きな転換をもたらした．動物には，種によって複眼，鏡眼，カメラ眼などさまざまな形態の眼があり，従来は 40〜60 系統が別々に発生した(収斂進化)と考えられていた．しかし，Pax6 がすべての動物

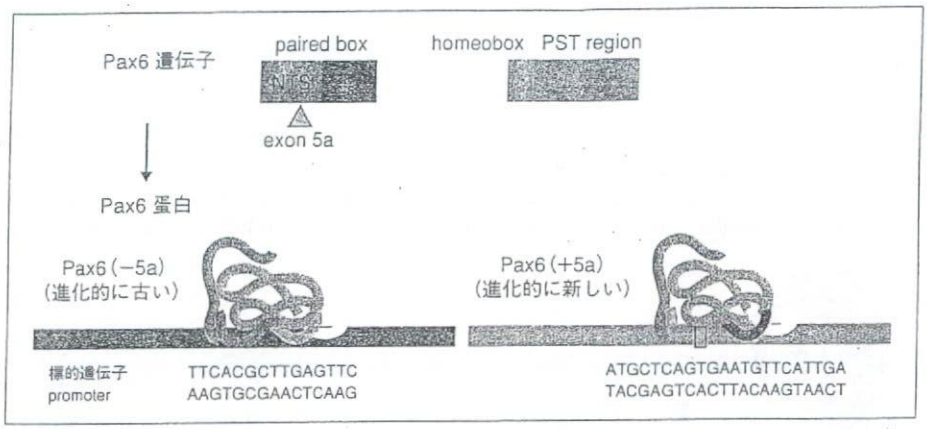

図 1　Pax6遺伝子と蛋白の構造

主要構造として，paired domain(標的 DNA に接触する部位，ここに相当する遺伝子配列を paired box という)，homeodomain(標的 DNA に接触するとともに形態形成遺伝子に特徴的な配列，遺伝子では homeobox)，末尾にプロリン，セリン，スレオニンを多く含む activating domain をもつ．エクソン 5 とエクソン 6 の間に 14 のアミノ酸をコードする選択的スプライスのエクソン 5a があり，2 種類のアイソフォームがつくられる．Paired domain はさらに N-terminal subdomain(NTS)と C-treminal subdomain(CTS)の 2 つに分かれ，標的 DNA が異なる．エクソン 5a による 14 アミノ酸が入れば CTS が，入らなければ NTS が働く．

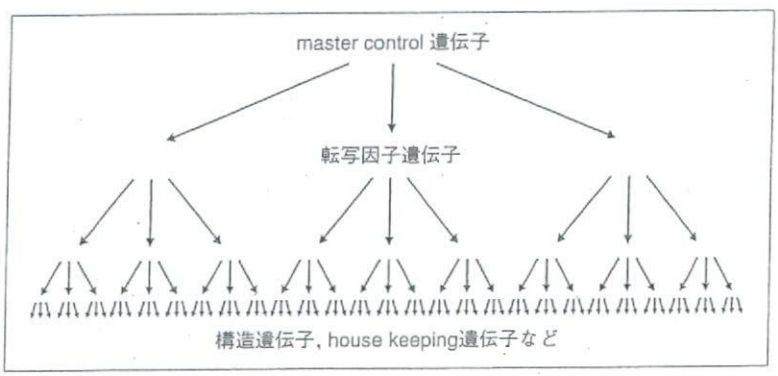

図 2　転写因子遺伝子カスケード

発生において組織ごとに上流遺伝子が下流を支配し，その頂点に器官形成全体を統合する master control 遺伝子が存在する．

の眼に存在することから，眼が原始の祖先動物で光を感じる細胞としてただ一度だけ出現し，進化とともに多彩な形態をとるようになったという単一起源説が支持されるようになった[2]．

Pax6遺伝子の変異によって起こるヒト眼形成異常

In situ hybridization や免疫染色によって Pax6 の発現を検討すると，発生初期は中枢神経や眼原基，中枢神経では前脳，後脳，神経管脳室復側，下垂体，嗅脳，眼ではまず視溝，ついで眼胞，表面外胚葉と水晶体板，網膜，角膜の順で，眼球ほぼ全体を網羅している(図 3)[3]．以上から，この遺伝子に変異が起こればきわめて多くの先天異常を起こすと推察された．

先天無虹彩では多くの変異が見出されてきたが，そのほかにも Peters 奇形のような前眼部形成不全，角膜ジストロフィー，瞳孔形成異常，先天白内障，黄斑低形成，視神経形成不全で変異がみつかり(図 4)[4-6]，Pax6 がヒトでも前眼部から眼底まで広い範囲で眼の形成を担っていることが分子遺伝学からも証明された．太古に光を感じる細胞

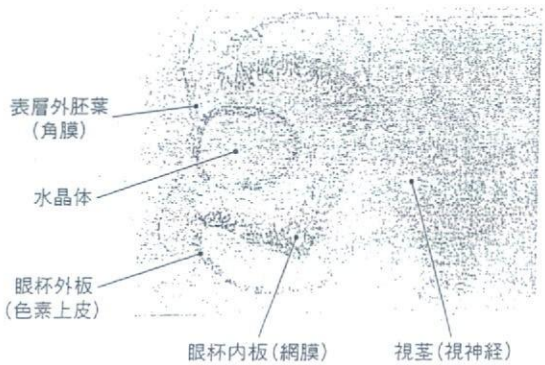

図 3　Pax6のモノクローナル抗体による発生ヒト眼(胎齢5週)の免疫染色[3]
発生初期では眼球のほぼ全体が染まる.

から出発した遺伝子が進化とともに眼形態形成の中心にいつづけて，角膜，虹彩，水晶体，網膜をつくるようになり，ついには視覚進化の頂点である黄斑を形成するに至ったことになる.

これまでにみつかった Pax6 の変異型と表現型には，遺伝子の変異が重篤なほど表現型も重症であるという法則がある．これは Pax6 に，①一対の対立遺伝子の両方が揃っていないと正常に機能しない(haploinsufficiency)，②遺伝子障害の程度と表現型が相関する(dose dependent)，という特徴があるためである．変異形式がストップコドン，フレームシフト，スプライシングエラーといったナンセンス変異では無虹彩のような眼球全体の形成不全を起こし，1 アミノ酸が置換した軽度なミスセンス変異では角膜，水晶体，網膜などで限局した形成不全を起こす．黄斑のみの形成不全がある孤立性黄斑低形成でみつかった変異はいずれもミスセンス変異である[4-6].

Pax6の選択的スプライスの働きと黄斑低形成の遺伝子変異

Pax6 遺伝子には，エクソン 5 とエクソン 6 の間に，14 のアミノ酸をコードする選択的スプライスのエクソン 5a が存在する．そして，これが読まれるか読まれないかによって，Pax6 蛋白は 14 アミノ酸が入るもの〔Pax6(+5a)〕と入らないもの〔Pax6(−5a)〕，2 種類のアイソフォームがつくられる[5]．Pax6 蛋白では，転写因子として標的 DNA に接着する部位の paired domain があるが，14 アミノ酸はこのなかに存在する．paired domain はさらに N-terminal subdomain と C-terminal subdomain の 2 つに分かれ，異なるタイプの binding consensus をもつ標的 DNA を支配する．しかも生化学的検討によれば両 subdomain はたがいの働きを抑制しあっている．そして，エクソン 5a による 14 アミノ酸が入れば C-terminal が，入らなければ N-terminal が働くので，エクソン 5a は molecular switch の働きをもっている(図 1)[5]．Pax6 の進化からみると，N-terminal subdomain は原始的動物にある基本的なもので，標的 DNA もいくつか判明している．一方，エクソン 5a は無脊椎動物では存在せず，脊椎動物に至って出現したので，C-terminal subdomain が働きはじめたのは進化的に比較的新しい．しかも，その機能はまったく不明で，標的遺伝子もみつかっていない.

これまでに発見された孤立性黄斑低形成の Pax6 ミスセンス変異はことごとく C-terminal subdomain あるいはエクソン 5a のなかに存在する[4,5]．したがって，黄斑の形成にはこの C-terminal subdomain が関与していると推測された.

黄斑発生領域におけるPax6アイソフォームの発現

発生期の動物で時期別，眼組織別に mRNA を採取して cDNA を作成し，Pax6 の 2 つのアイソフォームを RT-PCR で検討すると，Pax6(−5a)は発生期全般にわたって広範な組織に発現する．しかし，Pax6(+5a)は発生期後半に後方網膜に強く発現することが示された．さらに免疫染色では，Pax6(−5a)に対する抗体では網膜は後方から前方まで均一に染まるのに対して，エクソン 5a がコードする 14 アミノ酸に対する抗体では黄斑領域を中心とする後極のみに染色がみられ，Pax6(+5a)は黄斑領域に限局して発現することが判明した(図 5)[7].

Pax6アイソフォームの網膜形成・分化に関する機能

Pax6 が黄斑形成に関与するならば，発生期の網膜に Pax6 を過剰に導入すると網膜の形成が進むはずである．しかし，過去の研究報告は逆の結果

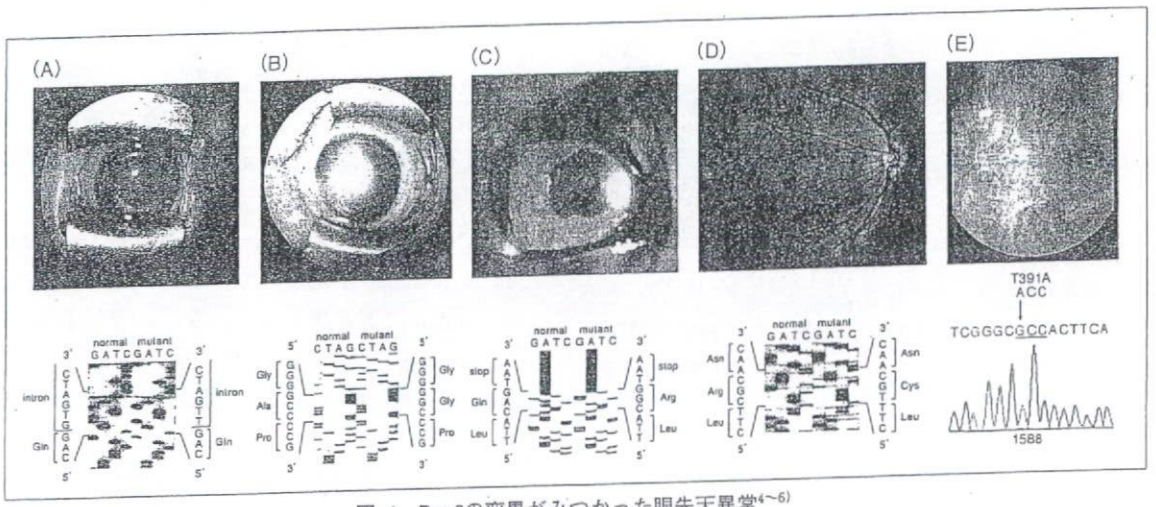

図 4　Pax6の変異がみつかった眼先天異常[4~6]

A：無虹彩，B：前眼部形成不全，C：瞳孔形成異常，D：黄斑低形成，E：視神経低形成．

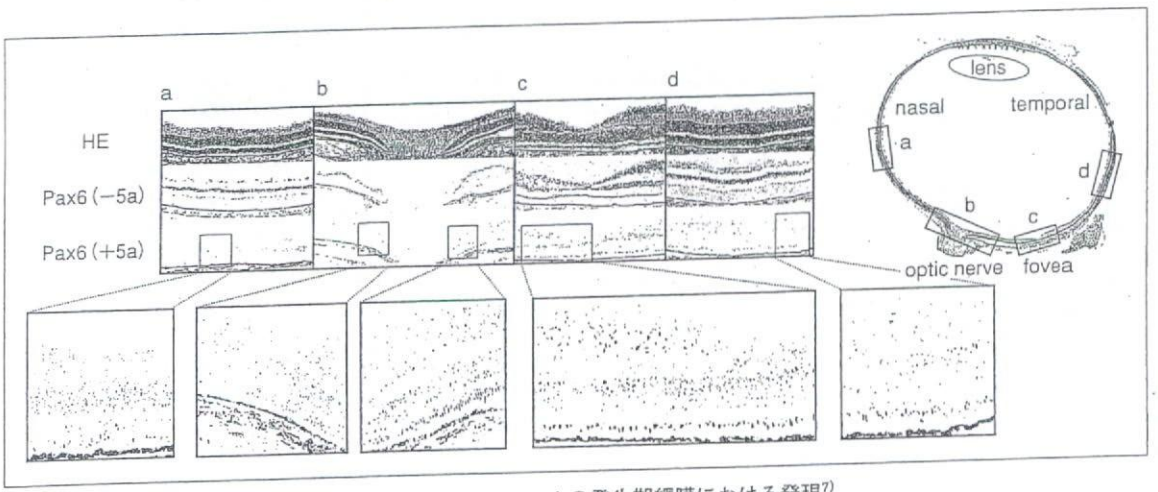

図 5　Pax6アイソフォームの発生期網膜における発現[7]

出生直後のマーモセット Pax6(−5a)に対する抗体では，網膜は後方から周辺部まで均一に染まるのに対して，エクソン 5a がコードする 14 アミノ酸に対する抗体では黄斑領域を中心とする後極のみに染色がみられる．Pax6(+5a)は黄斑領域に限局して発現することが示唆される．

を示していた．Pax6 の変異をもつマウスは小眼球になるが，一方で，Pax6 を過剰に導入したトランスジェニックマウスをつくっても小眼球が生ずる[8]．ここから Pax6 の発現量は少なくても多過ぎても正常に機能しないという考えが定着した．しかし，トランスジェニックマウスでは，導入した Pax6 が眼球だけでなく，中枢や視神経など多くの組織に発現する．小眼球は発生のわずかな均衡がくずれれば容易に起こるので，多くの組織に Pax6 が異常量発現すれば，組織間相互作用が障害され，結果として小眼球になることも考えられる．網膜への Pax6 の影響を知るためには網膜だけに遺伝子を導入しなければならない．そこでニワトリの発生期網膜に electroporation で Pax6 を直接導入した．Electroporation で導入した遺伝子は細胞質内で短期間発現するので，発生のような一時期に働く遺伝子の機能を観察する点では都合がよい．

発生初期(stage 12～16)の網膜に，エクソン 5a を含まない Pax6 のアイソフォーム Pax6(−5a)を導入すると網膜が厚くなり，神経節細胞が増加し(図 6-B)，神経線維が硝子体腔に向かって増加した(図 6-C)．導入直後では神経芽細胞の分裂が亢

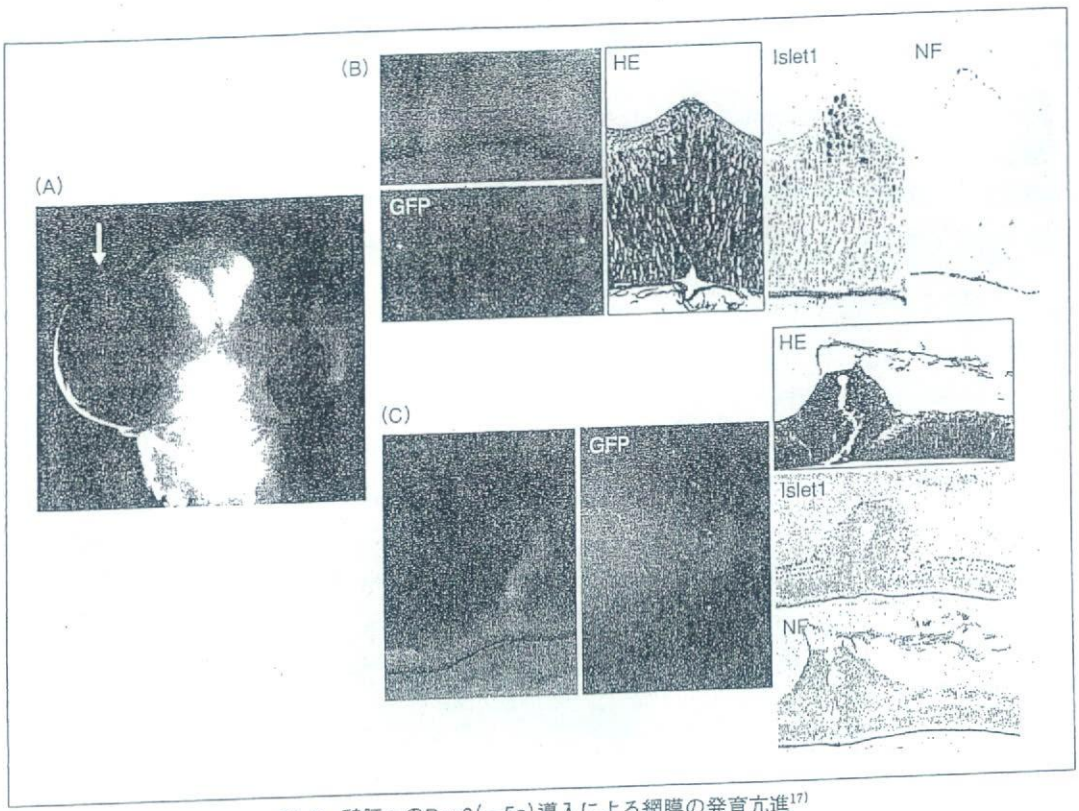

図 6　鶏胚へのPax6(−5a)導入による網膜の発育亢進[17]

2 日胚に導入，8 日胚の所見．
A：Pax6(−5a)を入れた右眼が大きくなる(矢印)．B：網膜が厚くなり，GFP で遺伝子の導入が確認され，組織所見では神経節細胞が増加している．C：網膜から硝子体腔へ線維構造が立ち上がり，組織所見では神経線維である．

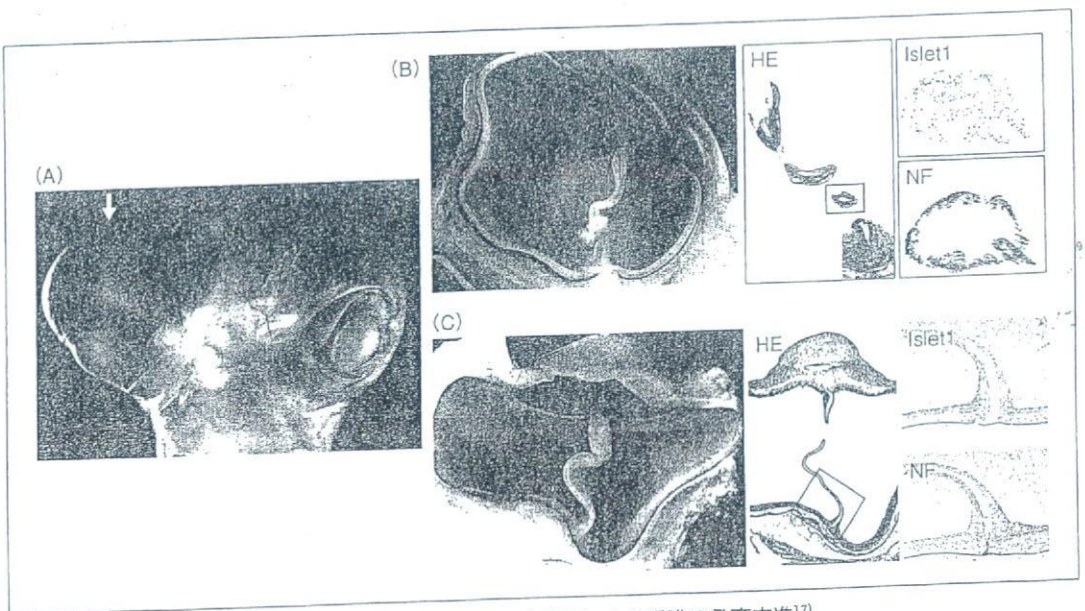

図 7　鶏胚へのPax6(+5a)導入による網膜の発育亢進[17]

2 日胚に導入，10 日胚の所見．
A：Pax6(+5a)を入れた右眼が極度に大きくなる(矢印)．B：網膜から茎状構造が立ち上がり，組織所見では管状の網膜で層構造はほぼ保たれている．C：網膜が水平に過剰発育して折りたたまれている．