NR layers was in a back match with that of the endogenous NR, corresponding to the direction of optic cup layers.

Compared with the Pax6-induced large-scale phenotypic changes and uniform expression of NR-specific markers in correct layers of the ectopic NR, GFP expression was restricted in a small number of cells (Figs 2C and 3C). It is likely that GFP faded out in cells that had rapidly proliferated and differentiated but still stayed in cells that had slowly proliferated. There is another possibility that Pax6 may induce the ectopic NR tissue in a cell non-autonomous manner. Pax6 may do so by activating the transcription of a diffusible factor that triggers NR tissue formation. The former idea is consistent with a study in *Xenopus larves*. The cell autonomous activity of Pax6 misexpressed in *Xenopus* is thought to cause ectopic eye formation and ectopic expression of genes that relate to eye development including *Rx*, *Otx2*, *Six3* and endogenous *Pax6* (11).

An opposite finding of the present study has been reported: combination of loss-of-function of Pax6 and Pax2 in the optic vesicle results in transdifferentiation of presumptive RPE to NR (46). The finding physiologically places Pax6 upstream of MITF and as a pro-RPE factor. This does not conflict with our data, however, because we transduced Pax6 into under-maturating RPE, in which endogenous Pax6 had been already downregulated. Probably, there may be differences in Pax6 function depending on timing.

Ectopic eye- or NR-like architecture is also induced by the misexpression of other homeobox transcription factors. Ectopic expression of Six3, a vertebrate functional orthologue of the *Drosophila* gene *sine oculis*, or Six6 that is closely related to *Six3* (47) induces the formation of ectopic optic vesicle-or NR-like architectures in the brains of the fish, *Xenopus* and mouse embryos (15–17). Ectopic Six6 expression in embryonic or mature chicken RPE cells also results in a neuronal morphology and expression of markers characteristic of developing NR (18). *Xenopus* embryos injected with synthetic *Rx* RNA develop ectopic retinal tissue (20). However, fully structured NR, as induced by Pax6 misexpression, has not been yet obtained. Expression of endogenous *Six3* and *Rx* in ectopic NR at the early phases post-*Pax6* transduction, as shown in Figure 1, suggests that Pax6 regulates Six3 and Rx in the field of NR transdifferentiation, as in Pax6-induced ectopic eye formation in *Xenopus* embryo (11). Pax6 may be critical to induce a set of transcription factors that form NR laminar structure because of very high incidence of fully structured NR induced by the gene transduction.

The Pax6 protein has two DNA-binding domains, PD and HD (48–50). In PD, two structurally distinct subdomains, NTS and CTS, bind respective consensus sequences (23,24), and an insertion of additional 14 amino acid residues encoded by exon 5a in the NTS abolishes the NTS function and enhances the transactivation activity via CTS (25,26). Thus, exon 5a probably functions as a molecular switch to select specific targets. Recently, we found functional differences of the two isoforms in NR development: Pax6(−5a) is expressed in the entire NR, whereas Pax6(+5a) is especially in the NR portion where visual cells accumulate during eye development. Pax6(+5a) promotes the NR growth and, when overexpressed, induces an excessive well-differentiated NR-like architecture, whereas Pax6(−5a) shows much weaker effect (51). In the present *in ovo* misexpression study, however, no difference was seen between two Pax6 isoforms with respect to their abilities to trigger NR transdifferentiation. One explanation for this is that the two isoforms may initiate the same genetic cascade via distinct pathways, possibly through control of partially overlapping target genes. Another explanation is based on the evidence of feedback regulation of *Pax6* expression. Transcription of the *Pax6* gene is intricately regulated via three promoters and a number of tissue-specific enhancers. Recently, several short sequences that closely match the Pax6 binding consensus (P6CON) were identified in *Drosophila* and vertebrate enhancers that drive *Pax6* expression in the nervous system and eye, and it was suggested that these evolutionarily conserved P6CON sites may mediate the auto-activation of *Pax6* by Pax6(-5a) (52). If so, both isoforms would be expressed after transduction of Pax6(-5a). Such a mechanism may account for similar phenotypic manifestation after transduction of Pax6(-5a) or Pax6(+5a). Although binding consensus sequences of the PD have been studied, little is known about its target genes, especially those recognized by CTS. This issue needs to be addressed to understand the mechanism of NR transdifferentiation by Pax6.

It has been considered that RPE is necessary for correct morphogenesis of NR in early stages and for organization of its layers by end of gestation, although signaling molecules emanating from RPE are not elucidated. Data obtained from organ culture suggest that RPE organizes the laminar structure of the differentiated NR (53). Transgenic mice expressing attenuated diphtheria toxin-A in RPE exhibit malformed RPE and disorganized NR (54). In contrast, our studies indicate that fully structured NR can be formed endogenously and ectopically, even though RPE is absent in areas of NR transdifferentiation from RPE. This suggests that RPE is not involved in the NR layers formation, but rather controls nutrition supply and/or cell proliferation at later stages. Compatible with this, ectopic NR is thinner than the normal NR, yet the laminar structure is clearly formed (Fig. 2).

Because primitive RPE and NR are contiguous in the optic vesicle, RPE cells has been considered as a possible candidate for a source of stem cells required for NR transdifferentiation (55). The retinogenic potential may be still preserved in RPE cells even in adult eyes, because RPE of chicken or other eye tissues, such as pigmented ciliary margin cells of mice and iris tissues of rats, generate immature NR-specific cells (3,4,18). In contrast, it has been thought that fully structured NR is generated from RPE only at early stages of development except for in amphibian eyes (5–8). However, our studies showed that RPE has the potential even at late stages. Pax6 induces the complete conversion from RPE to NR even at HH stage 40, whereas FGFs are able to transform RPE only before stage 24. As ectopic NR can be formed in broad and numerous spots at early stages (Fig. 2A), retinogenic RPE cells appear to be distributed widely throughout the RPE layer. In contrast, NR transdifferentiation was seen as small spotted areas at later stages, although expression of the exogenous gene monitored by GFP were detected in wider areas. This suggests that areas of NR transdifferentiation decrease not by inefficiency of gene transfer in late-stage-embryos. Retinogenic stem cells

may decrease in number as the RPE matures, as observed in mammalian brains (56), but be preserved widely even in late stages.

Transdifferentiation of NR from RPE by FGF treatment is a well-known phenomenon (5–8). Transcription factors or signaling cascade components that lie downstream of FGFs have been clarified recently. Switching of RPE to a neuronal fate by FGF8 is coupled with the induction of NR genes such as *Rx*, *Sgx-1* and *Fgf-8* itself (7). Switching of RPE to a neuronal fate by FGF9 is mediated by the Ras-Raf-MAPK pathway (8). It is very likely for several reasons that transdifferentiation of NR from RPE by FGFs is also mediated by increased expression of Pax6. First, Pax6 is strongly induced in RPE cells by FGF treatment (Fig. 5A and B). Secondly, transdifferentiation of NR from RPE by FGF8 is significantly disturbed by co-expression of dominant-negative Pax6 (Fig. 5E and F). Thirdly, *in vitro* assays using P19 cells demonstrate the upregulation of *Pax6* expression by FGFs (Fig. 6A and B). Finally, P6CON- and 5aCON-CAT reporters are activated by FGF treatment in a dose-dependent manner (Fig. 6C). Because CAT activities of P6CON- and 5aCON-CAT reporters did not significantly respond to even high concentrations of FGFs when a small amount of Pax6 was introduced exogenously (Fig. 6D), FGFs induce expression of the *Pax6* gene, but do not affect the transactivation potential of its gene product. Pax6 activity is also known to be controlled by FGF8 in somitogenesis (57). In this case, however, expression of Pax6 is suppressed by FGF signaling and is induced at the anterior limit of FGF expression that regresses caudally. Hence, regulatory relationship between Pax6 and FGF signaling may be different in these tissues.

The present study clarified roles of the *Pax6* gene in ectopic NR formation, by itself and under a control of FGFs signaling. Further investigation using the mouse and rat eyes is under way, and Pax6-dependent NR transdifferentiation from RPE cells also has been preliminarily detected (data not shown). Our studies provide a new cue to regenerate functional NR in the eye with congenital anomalies or acquired degenerations by transfer of the *Pax6* gene. Clinically, the RPE in the anterior portion of eye can be obtained easily by surgical procedures of peripheral iridectomy. NR reproduced from the retinogenic stem cells obtained from perinatal eyes would be a new therapeutic tool for reproduction and transplantation of functional NR tissues. Further steps to induce projection to a suitable portion in CNS are necessary to obtain useful vision. However, advanced surgical technique of experimental and clinical NR transplantation recently is achieving successful survival of the donor NR and visual improvement (58,59). Thus, reproduction of functional NR by use of Pax6 and RPE cells may be at least contribute to resurrect light sensation and visual field in patients who suffer from damaged NR and blindness.

MATERIALS AND METHODS

Expression and suppression plasmids

Expression plasmids ([pCAGGS–Pax6(-5a) and pCAGGS–Pax6(+5a)] to produce the entire human *Pax6* coding region with or without exon 5a, under the control of a cytomegalovirus enhancer and a chicken β-actin promoter, were previously described (25,26). The mutant forms of Pax6 expression plasmid were generated by PCR-based *in vitro* mutagenesis (25–27). To produce a Pax6 suppression plasmid, a fragment carrying *En* repression domain (40) was connected to the N-terminal fragment of mouse Pax6 cDNA (*Bam*HI–*Acc*II sites that contains 1–928 nucleotides) (41) and inserted into the *Bgl*II–*Xho*I sites of pCAGGS. Expression plasmid (pCAGGS–Fgf-8) to produce the entire *Fgf-8* coding region was generated by inserting chicken *Fgf-8* cDNA cloned by RT–PCR into pCAGGS.

In ovo electroporation

Each Pax6 expression or suppression plasmid cited above was electroporated into a chick embryo at HH stage 8–40 together with the pCAGGS–GFP plasmid to monitor incorporation of DNA (22,28). For electroporation, a CUY 21 electroporator (BEX) with platinum electrodes was used. A small window was opened on the stage 12 fertilized eggs for access, and embryos were allowed to develop in humidified incubators after sealing the window. At stage 12, 18, 24, 30, 35 and 40 (we used 100 embryos for each stage), the window of egg-shells was unsealed and phosphate buffered saline was poured over the embryo to obtain the appropriate resistance. After injecting DNA solution into the outer coat of the eye with a sharp glass pipette, the head of the embryo was placed between the electrodes and electric pulses were applied (25–40 V, 90 ms, 1–6 times). The egg-shells were sealed again and embryos were allowed to develop in humidified incubators. Eyes were incised 1–10 days after electroporation (stage 18–45) and fixed in 4% paraformaldehyde. Eight micrometer frozen sections were prepared for immunohistochemistry and *in situ* hybridization.

In ovo injection of FGFs

FGF2 and FGF8 recombinant proteins were purchased from Genzyme. Fertilized eggs were purchased from Nisseizai (Tokyo). A small window was opened for access, then phosphate buffered saline was poured over the embryo to preserve humidity. Each FGF at a concentration of 10–100 ng/ml was injected into the mesenchymes around the eyes of HH stage 12-40 chick embryos with a sharp glass pipette. The egg-shells were sealed and embryos were allowed to develop in humidified incubators.

In situ hybridization and immunohistochemistry

Section *in situ* hybridization was performed as described (60). Probes were prepared from plasmids containing chick *Musashi* (*EcoR*I, T7), *Notch1* (*Spe*I, T7 polymerase), *Six3* (*Hin*dIII, T3) and *Rx* (*Hin*dIII, T3). A monoclonal antibody against Pax6 protein was gifted by Dr Fujisawa (44). A monoclonal antibody against Islet1 protein was purchased from DSHB, that against Chx10 protein from Exalpha Biologicals, that against glutamate transporter 1 from Affinity BioReagents, that against parvalbumin from Sigma and that against glutamine synthetase from BD Transduction Laboratories. Tissues from chick embryo were fixed in 4% paraformaldehyde.

Eight micrometer of frozen sections were stained immunohistochemically using a method described previously (61).

Cell culture and RNA detection by RT–PCR

Mouse embryonic carcinoma P19 cells were maintained in MEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2. Cells at a density of 1×10^5 cells per 35 mm Petri dish were maintained in MEM supplemented with 5% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2. For each dish, 10, 30 or 100 ng/ml of either FGF2 or FGF8 recombinant protein (Genzyme) was added, and the medium was changed each other day. After 3 days, total RNA was isolated from cells in each dish using an RNA easy Mini Kit (Qiagen) and converted to cDNA by a standard procedure using SuperScript II RNase H- reverse transcriptase and adaptor primers (GibcoBRL) (62). DNA segments for mouse *Pax6* and β-*actin* were amplified in 30 and 19 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 2 min with the following primers: mouse *Pax6*-forward primer 5′-CACAGCGGAGTGAATCAGCTTG-3′ and reverse primer 5′-CCAGAATTTTACTCACACAACCGT-3′ [respective product size:160 bp for *Pax6*(−5*a*) and 202 bp for *Pax6*(+5*a*)]; β-*actin*-forward primer 5′-GTGGGCCGCCC TAGGCACCA and reverse primer 5′-CTCTTTGATGTC ACGCACGATTTC (product size:540 bp).

Reporter plasmid

To obtain clones carrying the promoter region of the *Pax6* gene, we first screened the human BAC Library (Research Genetics) and detected one clone (32H10). A *Hin*dIII–*Psh*AI fragment carrying ~2 kb *Pax6* promoter region (1285–3381 nucleotides in GenBank accession no. U63833) was excised and inserted into the *Hin*dIII–*Sal*I sites of pCAT Basic (Promega). The insert was verified by sequencing as having the reported sequence. CAT reporter constructs carrying six copies of P6CON or two copies of 5aCON were reported previously (23–25).

Transient transfection and CAT assay

P19 cells at a density of 5×10^5 cells per 60 mm petri dish were transfected with 0.5 μg of reporter plasmid (*Pax6* promoter, P6CON or 5aCON) and 0.05 μg of pSVβgal (Promega) as an internal control coated with polycationic liposome (Lipofectoamine Plus, Life Technology) according to the manufacturer's instruction. For each dish, 10, 30 or 100 ng/ml of FGF2 or FGF8 recombinant protein (Genzyme) was added, and the medium was changed each other day. Cell extracts were prepared after 72 h and assayed for CAT activities using FAST CAT Green Reagent (Molecular Probes) according to the standard procedure (62). The CAT activity was quantified by measurement with a phosphofluor-imager (Molecular Dynamics) and illustrated in a fold-activation compared with the condition without application of FGF.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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BRIEF COMMUNICATION

Infantile Case of Occlusive Microvascular Retinopathy After Bone Marrow Transplantation

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Abstract

Background: A variety of anterior and posterior segment ocular complications following bone marrow transplantation (BMT) have been well documented in adults and children, but retinal complications after BMT in infants have rarely been reported.

Case: A 6-month-old male infant developed occlusive microvascular retinopathy after BMT to treat acute lymphocytic leukemia.

Observations: Four months after the transplantation, retinal edema, hemorrhage, soft exudates, and neovascularization were found in the posterior pole fundus of the right eye and in the peripheral fundus of the left eye. After oral prednisolone was administered, the retinal lesions regressed and cicatrices with chorioretinal atrophy and fibrous tissue formed.

Conclusions: Neovascularization following occlusive microvascular retinopathy after BMT in infant eyes responds well to oral prednisolone. The visual prognosis depends on the foveal involvement of the retinopathy. **Jpn J Ophthalmol** 2005;49:318–320 © Japanese Ophthalmological Society 2005

Key Words: bone marrow transplantation, graft-versus-host disease, infant, occlusive microvascular retinopathy

Introduction

Bone marrow transplantation (BMT) has been used to successfully treat a variety of hematologic malignancies and severe aplastic anemia. However, anterior and posterior ocular complications associated with BMT have been reported both in adult and pediatric patients. The anterior complications, including keratoconjunctivitis sicca, corneal infections, and cataract, frequently occur. The use of high-dose anticancer agents, steroids, and total-body irradiation (TBI) are suggested to be the principal causative factors of the complications. Graft-versus-host disease (GVHD) also causes these complications. Following BMT, the patients are predisposed to develop GVHD, because the donor graft mounts an immunologic response against the immunocompromised host. The posterior segment is also affected by BMT-related complications such as retinal occlusive microvasculopathy;[1–4] however, this is very rare in pediatric patients.[4] We report a case of an infant with occlusive microvascular retinopathy and neovascularization after BMT.

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Case Report

A 1-month-old male infant was brought to our hospital with loss of appetite and anemia, found at a screening examination. Laboratory analysis of the peripheral blood showed that the white blood cell count was 27700/μl, the hemoglobin was 7.5 g/dl, and the blood platelet count was 50000/μl during the first examination at our Department of Pediatric Oncology. The infant was diagnosed with acute lymphocytic

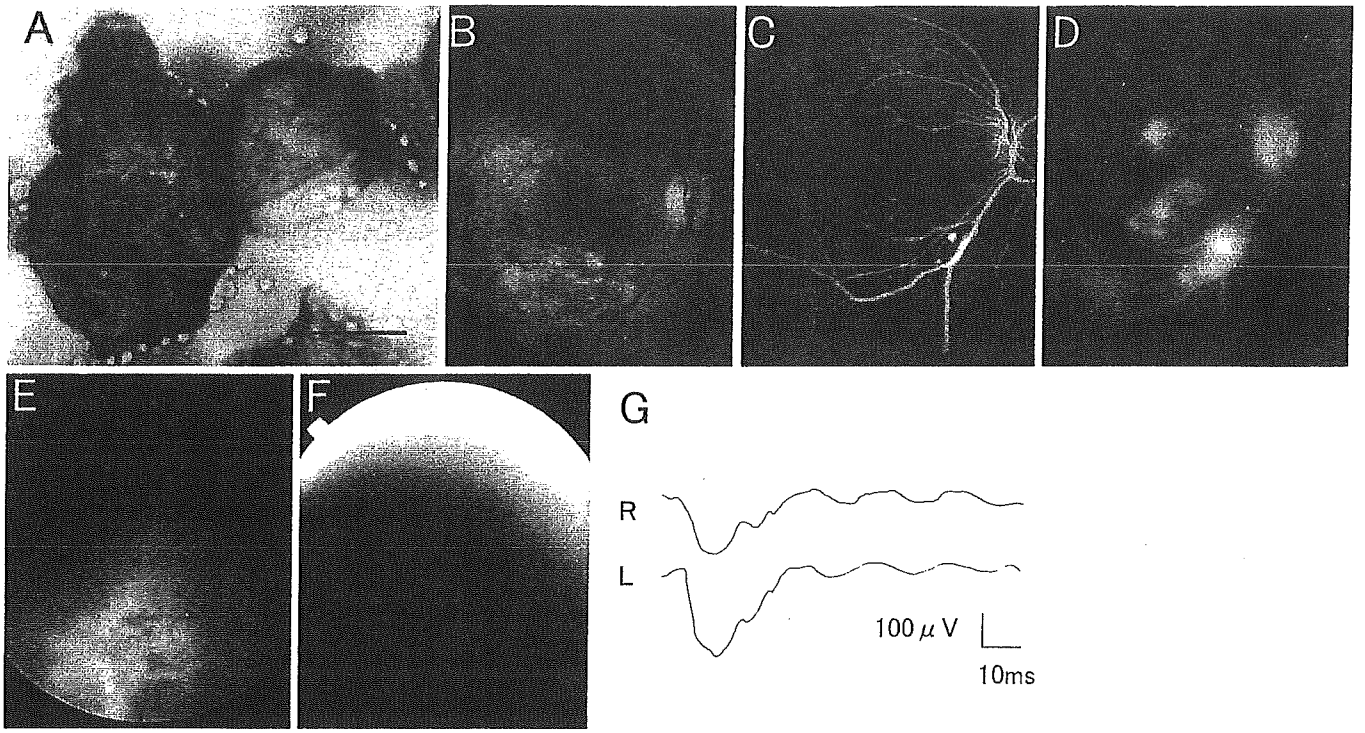

Figure 1. **A** Leukemic blasts obtained from bone marrow (May-Giemsa staining). Bar = 5μm. **B** Fundus photography of the right eye 4 months after BMT. Retinal edema, intraretinal hemorrhage, soft exudates, and lipid deposits are mainly in the central retina. **C** Early-stage fluorescein angiography of the right eye shows fluorescein dye leakage from the retinal vessels and microvascular abnormalities. **D** Late-stage fluorescein angiography of the right eye shows expansion of dye leakage indicating neovascularization. **E** Fundus photography of the left eye 4 months after BMT. The same findings as in the right eye are seen locally in the nasal periphery. **F** Fluorescein angiography of the left eye shows the same findings as in the right eye at the nasal periphery. **G** Electroretinography with a single flash stimulus of 25 J with a red filter shows normal responses from the left eye and reduced A and B wave amplitudes from the right eye. (100μV × 10ms).

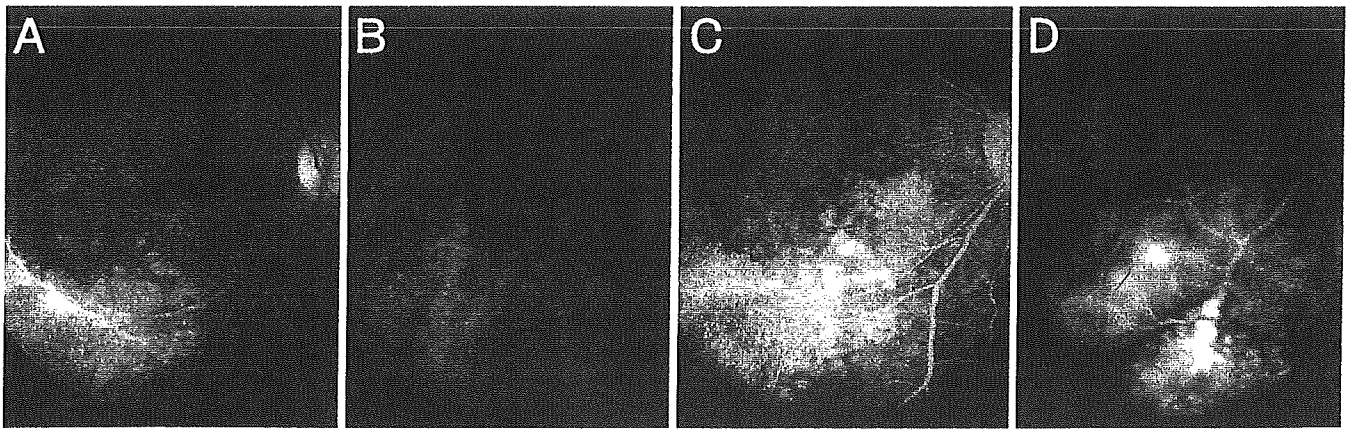

Figure 2. **A** Fundus photography of the right eye 3 months after prednisolone treatment. The chorioretinal atrophy remains in the posterior fundus and inferior branched arcade vessel sheathing. **B** Fundus photography of the left eye 3 months after prednisolone treatment shows chorioretinal atrophy is in the nasal periphery. **C** Fluorescein angiography of the right eye 3 months after treatment with oral prednisolone shows fluorescein staining in the chorioretinal atrophy area and branched vessel infarction. **D** Fluorescein angiography of the left eye 3 months after treatment with oral prednisolone shows fluorescein staining in the chorioretinal atrophy area and branched vessel infarction in the nasal periphery.

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leukemia (ALL). By bone marrow cytological analysis, the blast cells showed the rearrangement of the mixed lineage leukemia (*MLL*) gene (Fig. 1A). Immunohistochemical analysis showed that the blast cells were negative for CD10 but positive for CD19 and HLA-DR. Chromosomal analysis identified translocation of chromosomes 5 to 11 [t (5;11)(q31;q23)]. The patient initially underwent three sessions of chemotherapy with etoposide, cytosine arabinoside, and mitoxantrone, according to the protocol of the MLL98 study.[5] After pretreatment with whole-body irradiation (12 Gy) and administration of etoposide and cyclophosphamide, BMT was performed when the patient was 6 months old. Despite administration of tacrolimus and methotrexate to prevent GVHD, acute GVHD developed in the liver and skin 6 days after BMT. The GVHD gradually improved after administration of intravenous methylprednisolone. Careful examinations were performed by pediatricians, dermatologists, and ophthalmologists.

Four months after BMT, edema, hemorrhage, and soft exudates appeared widely in the posterior retina of the right eye (Fig. 1B) and locally in the periphery of the left eye (Fig. 1E); the anterior segment was normal. Fluorescein angiography showed some areas of nonperfusion of the capillary vessels and dye leakage, which indicated neovascularization of the retinal vessels (Fig. 1C, D, F). Electroretinography showed normal responses in the left eye and reduced A and B wave amplitudes in the right eye (Fig. 1G). Bone marrow aspiration indicated that the donor cells had survived and there was no recurrence of ALL; however, mild liver damage from the GVHD was still observed. Seven months after BMT, inflammation of the skin developed on the trunk, legs, arms, and face. Biopsy showed invasion of inflammatory cells into the subcutaneous tissue, suggesting chronic GVHD. Following administration of oral prednisolone (5 mg/kg per day), the retinopathy cicatrized to fibrous tissue and chorioretinal atrophy (Fig. 2A, B). The retinopathy gradually improved, and the dye leakage on fluorescein angiography stopped (Fig. 2C, D). The following fixation in the right eye was poor, and the visual evoked potential (obtained by flash stimulus of 1.2 J in a dark room) was also poor, while the responses of the right eye were normal. At 2 years of age, the patient's corrected visual acuity was 0.01 in the right eye and 0.5 in the left eye.

Discussion

Posterior segment complications have rarely been reported in pediatric patients after BMT. Suh et al.[4] surveyed ocular findings in 104 pediatric patients after BMT. In 14 of the 104 patients, the posterior segment had radiation retinopathy, disc edema, cytomegalovirus retinitis, and nocardia retinitis. Microvascular occlusive retinopathy developed in only four patients, aged 10 to 18 years, and there were no infants. In the 14 patients, telangiectasia, hemorrhages, cotton-wool spots, lipid exudates in the retina, macular edema, papillitis, and optic atrophy were seen, as is common in adults, but neovascularization, capillary nonperfusion, vitreous hemorrhage, tractional retinal detachment, and neovascular glaucoma did not develop.

Microvascular retinopathy after BMT occurs as the result of various mechanisms, including GVHD, total-body irradiation, immunosuppression, and administration of high-dose anticancer agents.[3] Our patient underwent total-body irradiation, received immunosuppressant and high-dose anticancer agents, and developed GVHD. Because the GVHD and occlusive microvascular retinopathy occurred simultaneously, the retinopathy in our patient was inferred to be mainly caused by the GVHD. Cyclosporine may worsen occlusive microvascular retinopathy because of its toxic effects, and total-body irradiation may modify the retinopathy, but it takes about 1 year for these changes to occur.[3] Thus, early occurrence of retinopathy in our patient also supports the theory that the retinopathy originated from the GVHD.

As a mechanism of chronic GVHD, the endothelia of the retinal vessels can be damaged by a lymphocyte-mediated immunologic attack that results in capillary occlusive retinopathy.[2,3] In our patient, occlusive microvascular retinopathy after BMT might have resulted from injury of the immature vessels in the neonatal and infantile eyes. Neovascularization was minimized not by the use of photocoagulation but by administration of oral prednisolone, because the occlusion of the capillary vessels occurred in a small area.

The visual prognosis after occlusive microvascular retinopathy after BMT is usually excellent, because lesions in the posterior retina are small and the fovea is rarely affected. However, the development of extensive neovascularization that covers the posterior retina is serious and vision threatening. As BMT has become an increasingly successful treatment and its indication is expanding in infantile patients, ophthalmologic evaluation followed by careful management is important for improving the visual prognosis after retinopathy.

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CASE REPORT

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Two patients with different features of congenital optic disc anomalies in the two eyes

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Abstract *Purpose:* Description of two patients, each with different features of congenital optic disc anomalies in the two eyes. *Methods:* Case report *Results:* Patient 1, a 3-month-old girl, showed retinochoroidal coloboma involving the optic nerve in the right eye and optic nerve hypoplasia in the left eye. Patient 2, a 5-month-old boy, showed retinal fold extending inferiorly in the right eye and optic disc coloboma in the left eye. *Conclusions:* Since in both cases coloboma was seen in one eye, the optic nerve hypoplasia or retinal fold in the fellow eye of these two patients may have been related to the timing of embryonic fissure opening or closing.

Keywords Congenital optic disc anomalies · Coloboma · Optic nerve hypoplasia · Retinal fold

Introduction

Numerous developmental events contribute to optic disc/nerve formation, including transient formation of embryonic fissure, hyaloid artery, and Bergmeister's papilla, and projection of nerve fibers. Thus, optic disc/nerve malformations occur when these developmental events transiently or spatially arrest and may present a variety of fundus features. Bilateral anomalies usually show the same phenotype, because of the same genetic background, intrauterine circumstances, or timing of a causative intervention. We report two patients with bilateral optic disc/nerve anomalies that were different clinical entities in the two eyes.

Case report

Patient 1, a 3-month-old girl, presented with nystagmus in both eyes. Ocular examinations showed normal anterior segments, large retinochoroidal coloboma involving the optic nerve in the right eye, and hypoplasia of the optic nerve with a small optic disc and surrounding depigmented ring (double-ring sign) in the left eye. Computed tomography (CT) identified hypoplasia of the cerebellar vermis, a callosal defect, ventricular enlargement, and extrusion of the posterior portion of the eyeball in the right eye and a thin optic nerve in the left eye (Fig. 1). The patient, now 3 years old and mentally challenged, has normal growth and no systemic abnormalities.

Patient 2, a 5-month-old boy, presented with nystagmus in both eyes. A retinal fold was seen extending from the optic disc and connected to fibrous tissue on the inferior portion of the posterior lens surface in the right eye. The left anterior segment was normal, although the fundus had a classic optic disc coloboma (Fig. 2). CT was normal, except for the retinal fold in the right eye and eye wall ectasia of the optic nerve region in the left eye. The patient, now 7 years old and mentally challenged, has normal growth and no systemic abnormalities. Other family members of each patient were apparently normal, thus indicating sporadic onset. Each patient was the product of a full-term pregnancy, and careful pediatric examination failed to identify any history of infectious disease.

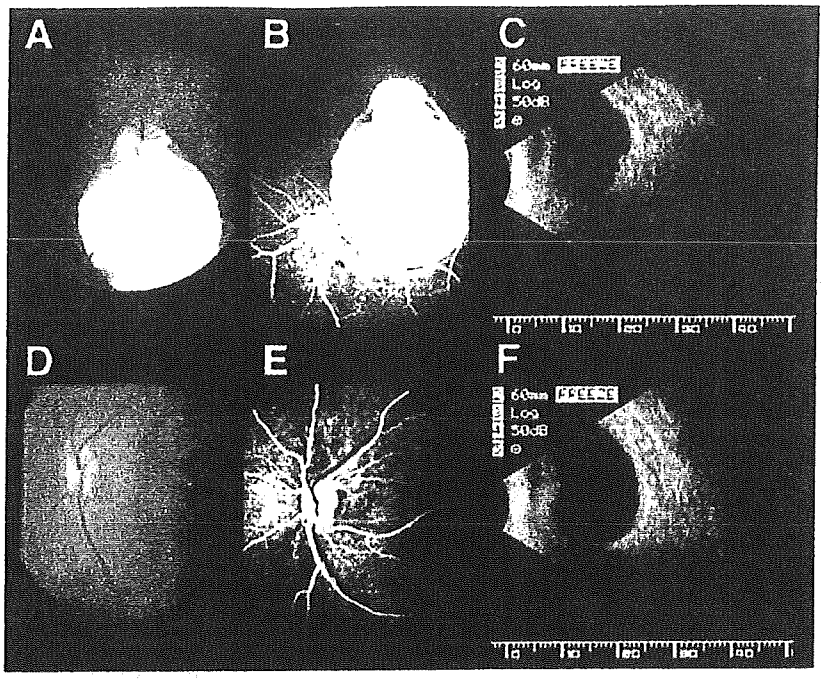

Fig. 1 Fundus photography (**a**, **d**), fluorescein angiography (**b**, **e**), and echography (**c**, **f**) of patient 1 show large retinochoroidal coloboma that involves the optic disc OD (**a–c**) and optic hypoplasia OS (**d–f**)

Discussion

Among events related to optic disc/nerve formation, opening and closing of the embryonic fissure at 5–6 weeks gestation, when transiently arrested, are the most common pathogenetic factors for malformations associated with peripapillary excavation, including coloboma, peripapillary staphyloma, and morning glory disc anomaly. Slightly different manifestations of optic disc/nerve anomalies (coloboma, optic disc pit) were reported bilaterally in the affected members of a pedigree showing inherited defects, suggesting that the difference depended on the degree of peripapillary excavation and that both anomalies are in the same spectrum [9].

In contrast, the fundus features in the two eyes of each of our patients markedly differed. Optic nerve hypoplasia is

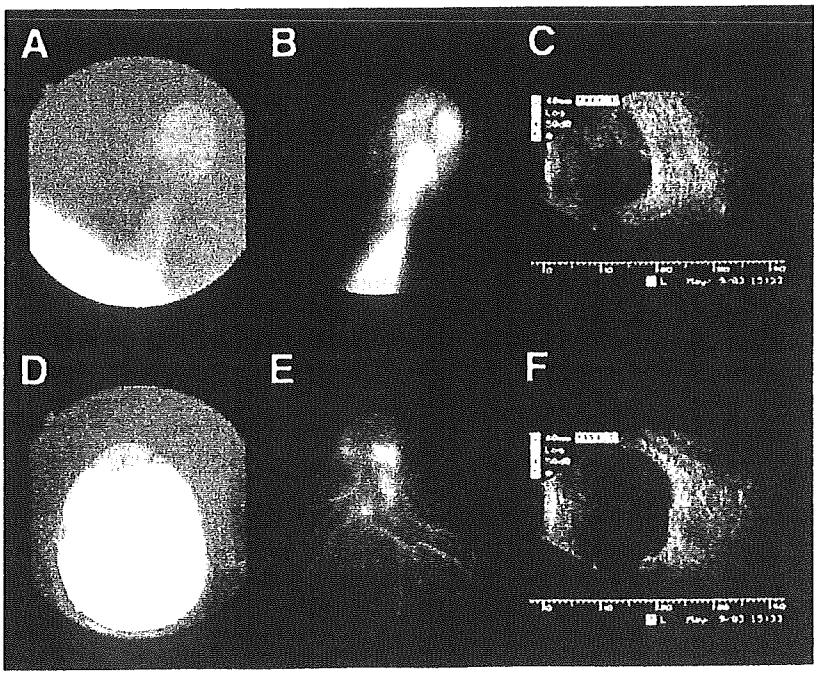

Fig. 2 Fundus photography (**a**, **d**), fluorescein angiography (**b**, **e**), and echography (**c**, **f**) of patient 2 show fibrous tissue on the inferior retinal periphery and retinal fold OD (**a–c**) and coloboma that involves the optic disc OS (**d–f**)

rarely associated with coloboma in the same patient [1, 3]. The case of a patient with hemifacial microsomia showing optic nerve hypoplasia in the ipsilateral eye and optic nerve coloboma in the contralateral eye has been reported [5]. Optic disc/nerve hypoplasia arises from insufficient growth of retinal ganglion cells and nerve fibers [4], or retrograde nerve fiber degeneration secondary to central nervous system abnormalities [6], while excessive closure of the embryonic fissure may disturb nerve fiber projections in the optic nerve, resulting in optic disc/nerve hypoplasia [3]. Retinal folds and tractional retinal detachments caused by vascular or mesenchymal proliferation in the developing vitreous and retina occur in eyes with persistent fetal vasculature (PFV), familial exudative vitreoretinopathy, and retinopathy of prematurity. Because fibrous proliferations in patient 2 were in the inferior peripheral vitreous cavity, which coincides with part of the embryonic fissure, the tissue may be PFV with excessive migration of mesenchymal cells through the fissure. Thus, each anomaly might result from abnormalities in closing of the embryonic fissure.

Mutations of the *PAX2* or *PAX6* gene have been identified in a variety of optic disc/nerve anomalies [2, 7]. *PAX2* plays a crucial role in the development of the optic stalk, and *PAX6* in that of the optic cup [8]. The affected members of a pedigree showed a variety of phenotypes when these genes were mutated, while there was not much difference in the phenotypes between the two eyes in each affected member, suggesting that downstream *PAX2* or *PAX6* genes modify phenotypic expression. However, differences in phenotypes between the two eyes also occurred, albeit in few cases [2]. Although no mutation of these genes was identified in our patients, stochastic effects on developmental events may modify ocular cell growth and differentiation, resulting in different phenotypic manifestations.

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Small Eye Phenotypes Observed in a Human *tau* Gene Transgenic Rat

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ABSTRACT We developed a rat line showing small eye from transgenic rats that were obtained by microinjection of a DNA segment containing the human (h)*tau* cDNA (GenBank: BC000558: 31-677,774-1180) expressed under control of CAG promoter, which is related to Alzheimer disease, into the pronuclei rat embryos. The rat line was established by selective brother-sister mating of rats showing small eyes. Of 11 offspring in the 11th generation, there were eight animals with microphthalmia and the transgene. The remaining three rats without transgene did not show the small eyes phenotype. The globes of affected rats were 1.2 mm in length compared with normal globes (3.5 mm), and all other ocular structures were normal. The expression of hTau protein was evident immunohistochemically in the ciliary body, extraocular muscle, lens epithelium, and pigment epithelium. Cytogenetic analysis suggested that the chromosome location of the transgene was chromosome 1 (1p12). This region may include genes related to lens development, such as *Cat5*.

KEYWORDS *Cat5*; cataract; rat; small eye; Tau

INTRODUCTION

It is reported that Parkinson disease is inherited as an autosomal dominant gene, and the gene is linked to chromosome 17q21-22.[1] Human (h)*tau* gene was located on the locus, and mutation of the gene was found in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). The neurofibrillary tangles composed of microtubule-associated protein Tau are related to not only the FTDP-17 but also to Alzheimer disease. The gene encoding microtubule-associated protein has been reported in rats as well, and the gene (Map1a) was linked to chromosome 3q36.[2] Many transgenic mouse lines that express hTau protein have been established to investigate the relationship between the hTau protein and tauopathy.[3] Recently, we generated three transgenic rats (founder) carrying the h*tau* gene, and they showed small eyes.

Mice and rats with ocular phenotypes, such as aniridia,[4] cataract,[5] microphthalmia,[6] and small eyes,[7] were reported, and a number of genes, including *Bld*,[8] *Cat*,[9] *Maf*,[10] and *Pax*,[11] related to these abnormalities have been reported as well. In this study, we characterized the phenotype of transgenic rats with small eyes, and the candidate gene causing the phenotype was predicted.

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MATERIALS AND METHODS

Transgenic Constructs and Animals

h*tau* cDNA (GenBank: BC000558: 31-677,774-1180) and rabbit β-globin polyA provided by Dr. Oyama (Department of Neuropathology, University of Tokyo)[12] were microinjected into the pronuclei of fertilized Jcl:SD rat (CLEA Japan Inc., Tokyo, Japan) embryos, and the embryos were transferred to the oviducts of pscudopregnant SD rats. Testing for the transgene in offspring was performed by polymerase chain reaction (PCR). The primers and conditions for the PCR are described below. In histopathological, immunochemical, and fluorescent *in situ* hybridization (FISH) analysis, four out of the 8 rats, aged 8 weeks, with small eyes were used. The rats were maintained in accordance with the Animal Care Guidelines of the Central Institute for Experimental Animals (Kanagawa, Japan).

PCR Analysis

To select rats carrying the h*tau* gene, PCR analysis was performed using the oligonucleotides, t1 (5′-AAG CTC GCA TGG TCA GTA AA-3′) and t2 (5′-GAC TTG ACA TTC TTC AGG TC-3′), and *Taq* polymerase (Takara Shuzo, Co., Ltd., Shiga, Japan) according to the manufacturer's protocol.

Histopathology

The formalin-fixed materials were embedded in paraffin, and 5-μm sections were stained by a standard method with hematoxylin and eosin (HE). The sections were examined under a light microscope to evaluate morphologic characteristics and pathologic changes. For detection of hTau protein in the tissues, all sections were stained by the dextran polymer–immunoperoxidase complex method (ENVISION kit, DakoCytomation, Kyoto, Japan) using anti-bovine Tau (mouse) serum (EMB Biosciences, Inc., San Diego, CA, USA) at 1:5000 dilution as the primary antibody and then counterstained with hematoxylin.

FISH Analysis

Determination of the chromosomal location of the h*tau* gene in the transgenic rats was undertaken by FISH analysis, and closely linked genes associated with ophthalmopathy were screened using the rat genome database (http://rgd.mcw.edu/). The chromosome samples were prepared from mitogen-stimulated splenocytes of transgenic rats. The biotin-16-dUTP-labelled *tau* cDNA clone in the pCXN2 vector[13] was used for hybridization. FISH analysis was performed essentially as described by Matsuda et al.[14] Observations were carried out with a Leica Q550 system (Leica Microsystems K.K., Tokyo, Japan), and chromosomes with fluorescent signals were identified according to G-banding standards.

RESULTS

Forty-five rats in total were obtained from the founder male rat carrying the h*tau* gene. Twenty-three out of the 45 rats had the h*tau* gene, and 3 of 23 (2 males and one female) rats showed small eyes. F2 rats were obtained by mating between a rat with small eyes and a Jcl:SD rat, and the animals were maintained by selective breeding of a small-eye line and brother-sister mating. At the 11th generation, 11 offspring were obtained, and 8 of the 11 offspring showed small eyes.

Histopathological Analysis

The globes of affected rats were 1.2 mm in length compared with normal globes (3.5 mm), and all other ocular structures were normal (Figs. 1 and 2A). Vacuolation was observed in lens of the rats, but no lesions were observed in other tissues such as the cornea and iris. These abnormalities were observed only in rats of this line bearing the h*tau* gene. On the other hand,

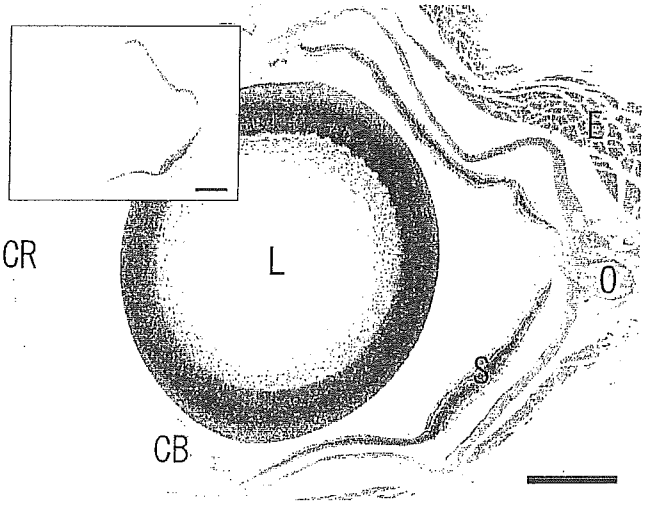
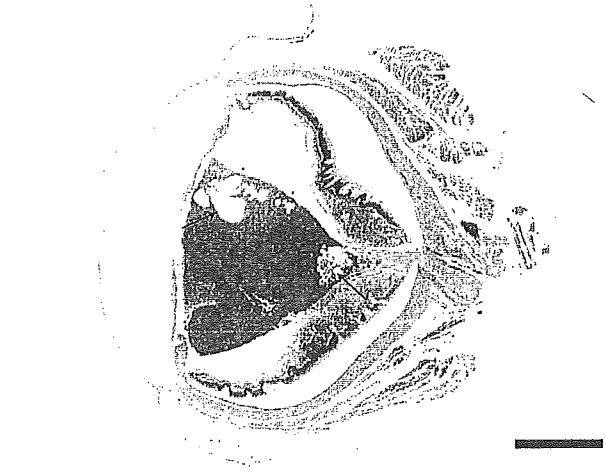
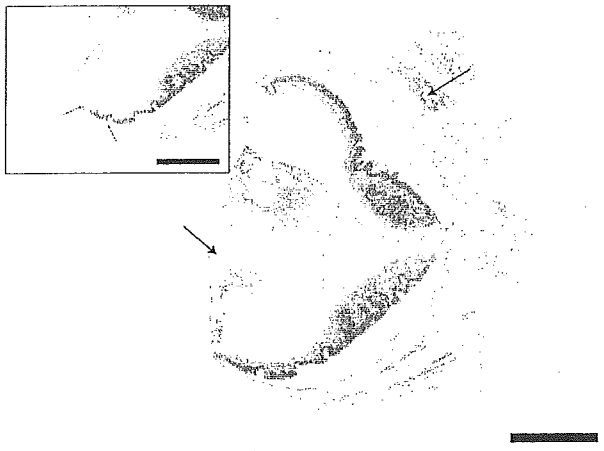


FIGURE 1 Ciliary body (CB), cornea (CR), extraocular muscle (E), lens (L), optic nerve (O), and sclera (S) from a rat not bearing the *tau* gene (H&E). Inset shows immunohistochemical stain, hematoxylin counterstain. Expression of *Tau* protein in the normal eye from a rat not bearing the *tau* gene. Bar = 1 mm.



(A)



(B)

FIGURE 2 (A) Small lens observed in human *tau* gene transgenic rats. Vacuolation is present in the lens (arrows) (H&E). Bar = 1 mm (B). Immunohistochemical stain, hematoxylin counterstain. Tau protein expression evident immunohistochemically in the ciliary body, extraocular muscle, lens epithelium, and pigment epithelium (arrow). Bar = 1 mm. Inset shows detail of ciliary body and pigment epithelium. Bar = 1 mm.

ophthalmic lesions were not observed in wild-type rats and rats from other rat lines carrying the gene (data not shown). Tau protein expression was evident immunohistochemically in the ciliary body, extraocular muscle, lens epithelium, and pigment epithelium (Fig. 2B).

Location of the h*tau* gene was analyzed by the FISH method, and the gene was found to be located on chromosome 1p12 (Figs. 3 and 4).

DISCUSSION

Tau protein is a microtubule-associated protein. In Alzheimer disease, Pick disease, and corticobasal degeneration, typical mutations were found in the gene.

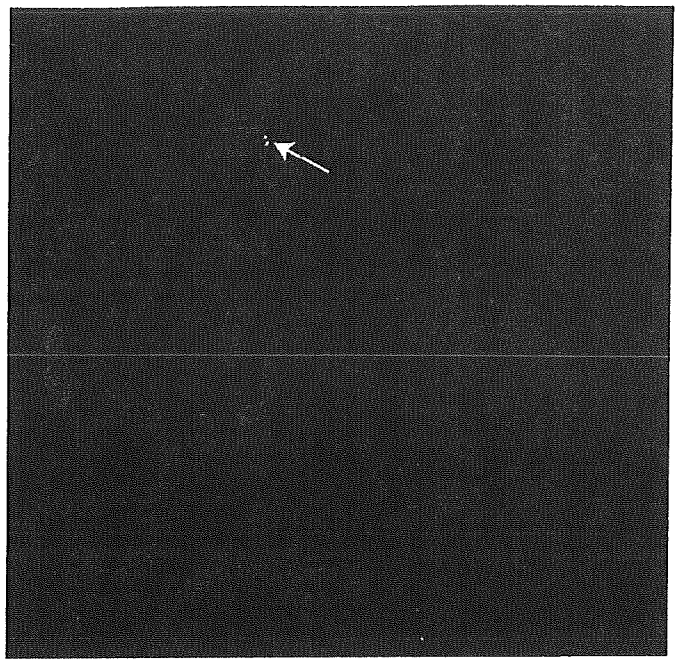


FIGURE 3 Chromosomal location of the human *tau* gene in a transgenic rat. The signal was visualized indirectly with FITC (arrow).

Mutations that affect exon 10 splicing cause frontotemporal dementia with parkinsonism.

In this study, we obtained 23 transgenic rats carrying the h*tau* gene from one founder transgenic rat, and in three out of the 23 rats small eyes appeared. Histopathologically, the lens of the rats was small in size with vacuolation. Lens development is regulated by a variety of genes, such as L-*Maf*, *Pax6*, and *Sox2*. Microphthalmic rats and mice caused by mutation of these genes were reported previously,[15,16] but all of them were not caused by the *tau* gene. Lewis et al. reported eye irritations in mice expressing mutant Tau protein but microphthalmia was not observed.[17] Only three (2 males and one female) of these 23 transgenic rats showed small eyes, suggesting that small eyes observed in this study were not caused by h*tau* gene. The human *Pax6* gene was first reported as a candidate gene for evolution of morphogenesis of the eye.[18,19] In rats, the *Pax6* gene is located on chromosome 3q32-3q36. Because the transgene in the rats with small eyes was mapped to chromosomal 1p12, it was suggested that the *Pax6* gene was not related to abnormalities in this study. Based on the database analysis of the transgene locus (1p12) in rats, several genes have been mapped in the locus. In the locus, *Cat5* was mapped as a cataract-related gene.

In conclusion, we established a rat line, that shows small eyes from transgenic rats carrying h*tau* gene.

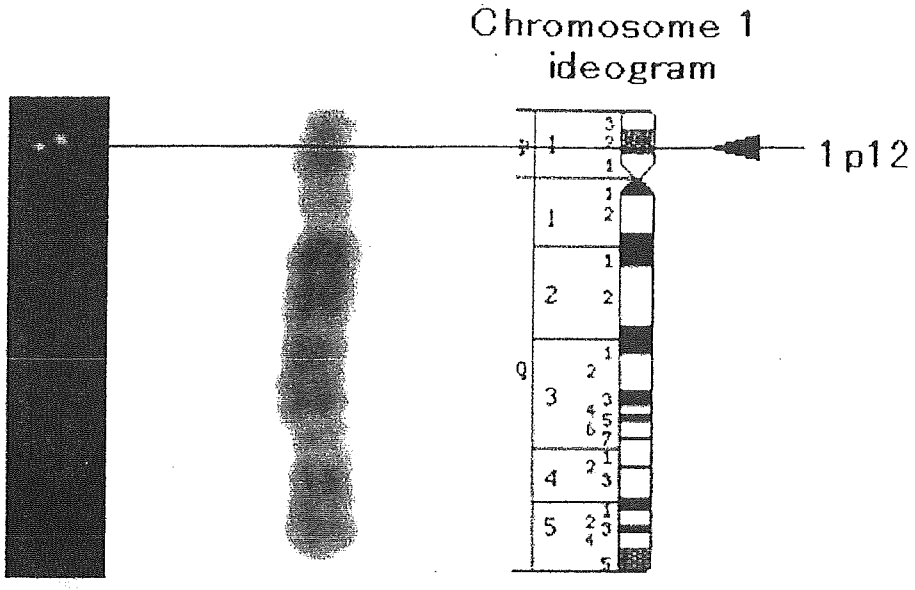


FIGURE 4 **Ideogram showing the cytogenetic location of *tau* in 1p12.**

Typical phenotypes were characterized by a small lens with vacuolations observed in the lens. The map location of the transgene suggested that the candidate gene causing small eye is located in 1p12.

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水平筋上方移動術施行例の検討

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Vertical transposition of horizontal rectus muscles for hypotropia

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I 緒 言

上転障害のある下斜視の矯正にはさまざまな手術方法が用いられるが，疾患の診断と術式の選択にはforced duction test（FDT）が必須である。FDT陽性の場合には，下直筋減弱（下直筋fibrosis，甲状腺眼症），上斜筋減弱（ブラウン症候群），眼窩底骨折整復術などがそれぞれ選択される。一方，FDT陰性または著明な抵抗のない場合，double elevator palsyや外眼筋形成不全などが原因疾患として考えられ，水平筋全筋上方移動術（Knapp法）[1~8]，水平筋部分移植術（Hummelsheim法）[9][10]，Foster法(水平筋全筋上方移動＋Faden手術)[11]，Jensen変法[12]などが選択される。このうち水平筋全筋上方移動術（Knapp法）は，double elevator palsyによる大角度の上下偏位の矯正に最も有効な術式として用いられてきた。しかし本術式の矯正効果は報告によりばらつきがあり[1~8]，適応となる症例数が少ないこと，症例により多様な臨床像を有すること，術式すなわち水平筋の移動量や縫着部位，後転・短縮の併施の有無が異なることなどが原因と考えられる。

今回我々は，異なった臨床的特徴をもつ下斜視3例に本術式を施行し，その治療効果について比較検討したので報告する。

II 症 例

3症例ともに上転障害のある下斜視で，全身麻酔下のFDTにて著明な制限はなかった。症例1，2はdouble elevator palsy，症例3は高度の両上直筋・上斜筋低形成の例である。術後経過観察期間は症例1が7ヵ月，症例2，3は4年である。各症例の所見と手術による治療結果を供覧する。

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症例1：3歳，女児，左眼double elevator palsy

現病歴：生後まもなくより斜視を認めており精査加療のため当院受診。

既往歴：特記すべきことなし。

現症：眼位は左眼下斜視，左眼の上転障害があり，偽眼瞼下垂を伴っていた（図1）。瞳孔正常，視力は右0.4（1.0×＋0.5D⊂cyl＋2.0D Ax80°），左0.4(1.0×＋1.0D⊂cyl－2.25D Ax180°），中心固視で前眼部・中間透光体・眼底には異常なかった。調節麻痺下屈折値をもとに屈折矯正を行った。遠見の交代プリズムカバーテスト（APCT），およびプリズム中和テスト（PAT）による最大斜視角は，第一眼位において左眼下斜視25Δ（右眼固視），左眼固視では右眼上斜視30Δであった。下方視では眼位正位となるが，頭位異常はみられず，両眼視機能は大型弱視鏡にて同時視（－），近見立体視（－）であった。MRIにて外眼筋に異常所見なく，FDTは陰性であった。

手術：3歳11ヵ月時に左眼水平筋全筋上方移動術を施行した。術式はKnapp法に準じて，内，外直筋を全筋上方移動して下縁を上直筋付着部に縫着した（図3）。最終診察時（術後7ヵ月）の眼位は左眼上斜位12Δとやや過矯正であるが，大型弱視鏡で融像および立体視が検出された。左眼上転は改善，偽眼瞼下垂も改善したが，左眼の軽度下転制限が生じ，下方視で左眼上斜視となっている（図2）。他に水平眼位の変化，左眼の内・外転制限，AV型の発症などの術後合併症は認めない。手術による遠見の第一眼位の矯正効果は37Δである。

症例2：4歳，男児，左眼double elevator palsy

現病歴：生直後からの左眼眼瞼下垂を主訴に精査加療のため当院受診。

既往歴：特記すべきことなし。

現症：眼位は左眼下斜視および内斜視，左眼の著明な上転制限があり，左眼の高度眼瞼下垂（挙筋機能0 mm）を伴い，Bell現象は陰性であった（図4）。瞳孔正常，視力は右0.8(0.9×＋0.5D)，左0.5(0.7×＋1.0D⊂cyl－

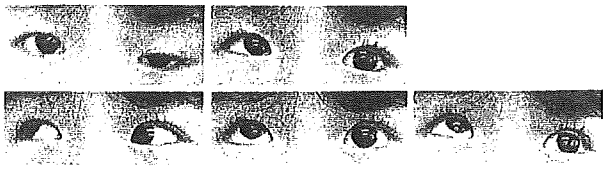

図1　症例1：術前眼位

上段：第一眼位（右眼固視と左眼固視）
左眼下斜視と偽眼瞼下垂を認める。左眼固視で下垂は消失する。
下段：上方視
左眼の上転制限を認める。

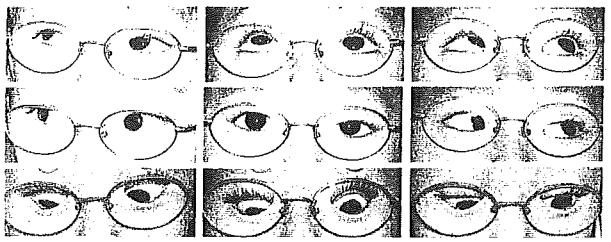

図2　症例1：左眼水平筋全筋上方移動術後7ヵ月の9方向眼位

第一眼位で左眼上斜位，左眼の上転と偽眼瞼下垂の改善を認める。下方視で左眼の下転制限を認め，左眼上斜視となっている。

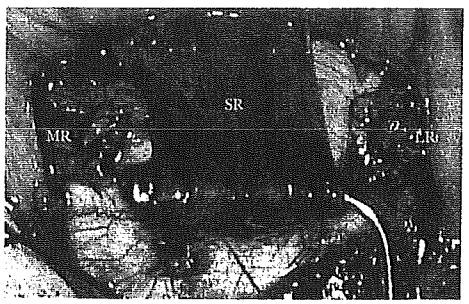

図3　術式：症例1の術中所見

内・外直筋を全筋上方移動して下縁を上直筋付着部位に縫着。

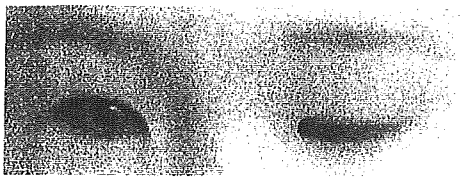

図4　症例2：術前所見（正面位）

左眼下斜視，内斜視に高度の眼瞼下垂を伴っている。

図5　症例2：左眼眼瞼挙筋短縮術後（水平筋上方移動術前）の第一眼位と上方視

左眼下斜視，内斜視および著明な上転制限を認める。

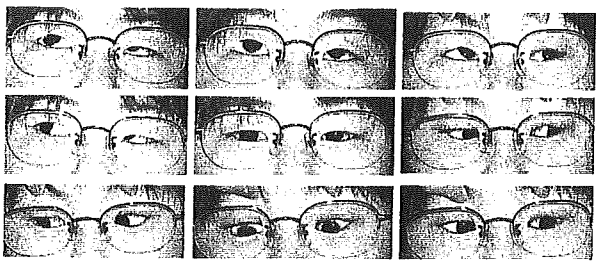

図6　症例2：左眼水平筋全筋上方移動術後4年（右眼内直筋後転＋外直筋短縮術後）の9方向眼位

第一眼位で左眼下斜位，内科位。左眼上転制限は著明に改善した。

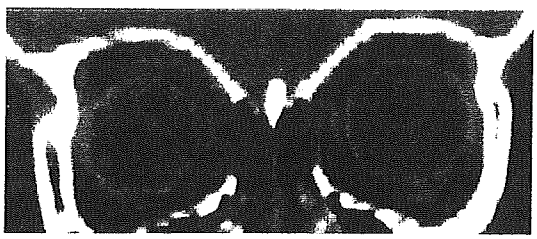

図7　症例2：CT所見

左眼上直筋低形成（または萎縮）を認める。

3.0D Ax15°），中心固視で前眼部・中間透光体・眼底には異常なかった。屈折矯正および健眼遮閉2時間/日による弱視治療を施行した。APCT（遠見）にて，第一眼位において左眼下斜視25Δ，内斜視40Δ（右眼固視），左眼固視では右眼上斜視50Δとなった。両眼視機能は大型弱視鏡にて同時視（－），近見立体視（－）であった。CTにて左眼上直筋低形成を認めた（図7）。FDTでは著明な制限はないものの，左眼上転と右眼外転に軽度の抵抗を認め，左眼下直筋と右眼内直筋の二次的拘縮を伴うと考えられた。

手術：4歳時に第1回手術として左眼眼瞼挙筋短縮術を施行した。初回手術後の眼位は左眼下斜視25Δ，内斜視40Δと変わりなく，左眼の上転制限も不変であった（図5）。これに対し第2回手術（4歳4ヵ月時）として症例1と同様の方法で左眼水平筋全筋上方移動術を施行，第3回手術（5歳10ヵ月時）として内斜視40Δに対する右眼内直筋後転6 mm＋外直筋短縮5 mmを施行した。最終診察時（水平筋移動術後4年）の眼位は左眼下斜位6Δ，内斜位12Δで，大型弱視鏡で融像を認めた。

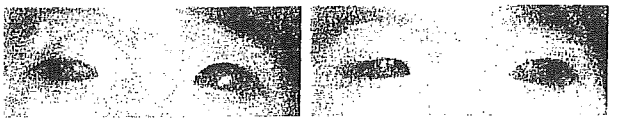

図8　症例3：術前所見（右方視，左方視）
両眼下斜筋過動，上斜筋遅動を認める。

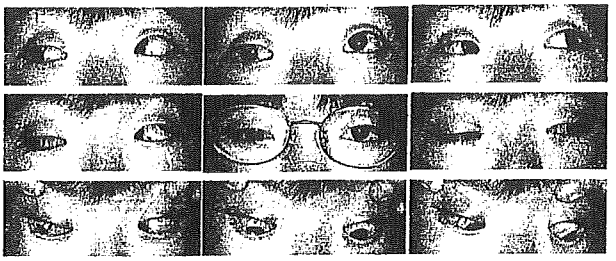

図9　症例3：両下斜筋減弱術後（水平筋上方移動術前）の9方向眼位
右眼下斜視，偽眼瞼下垂，両眼の高度上転制限を認める。

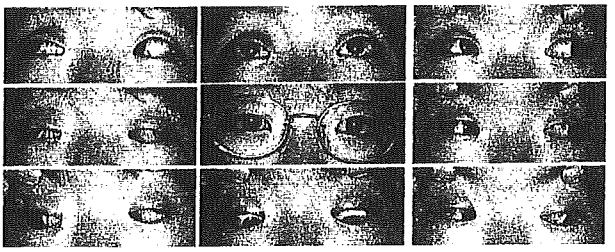

図10　症例3：右眼水平筋全筋上方移動術後1ヵ月の9方向眼位
第一眼位で正位となり偽眼瞼下垂は消失した。

左眼上転は著明に改善し，軽度の左眼下斜筋過動を生じたが，他に水平眼位の変化，左眼の内・外転制限，AV型の発症などの術後合併症は認めない（図6）。手術による遠見の第一眼位の矯正効果は19Δで，長期的に安定した眼位を保持している。

症例3：5歳，女児，両上直筋・上斜筋低形成

現病歴：生後まもなくより斜視と右眼瞼下垂を認めており精査加療のため当院受診。

既往歴：心房中隔欠損手術（3歳）

現症：眼位は右眼下斜視，潜伏眼振があり，両眼下斜筋過動および上斜筋遅動が著明で，両眼の外上転制限があった（図8）。瞳孔正常，視力は右0.3(0.5×＋3.5D○cyl－5.5D Ax180°)，左0.7(0.8×cyl＋1.0D Ax100°)，中心固視で前眼部・中間透光体に異常なく，眼底は右眼に外回旋所見を認めるほか異常なかった。APCT（遠見）にて第一眼位において右眼下斜視14Δ（左眼固視），右眼固視では左眼上斜視20Δ，両眼視機能は大型弱視鏡にて同時視（－），近見立体視（－）であった。随伴所見として右眼偽眼瞼下垂がありBell現象は陰性であった。FDTは陰性であった。

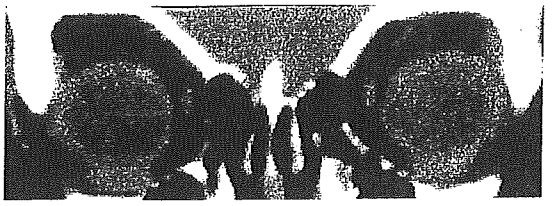

図11　症例3：CT所見
両眼の高度の上直筋・上斜筋低形成が疑われる。

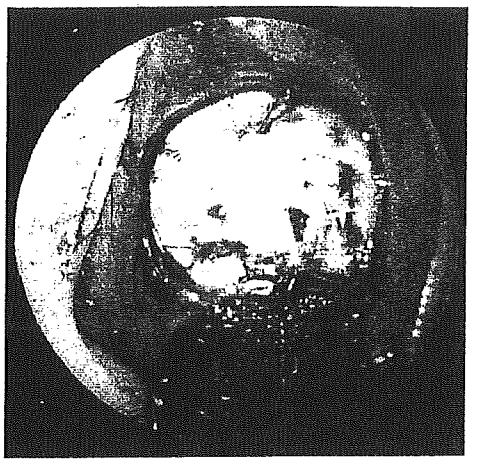

図12　症例3：術中所見
術野に上直筋，上斜筋は欠損していた。

手術：5歳9ヵ月時に第1回手術として両眼下斜筋減弱術を施行した。初回手術後の眼位は右眼下斜視25Δとなり，両眼の高度上転制限と右眼偽眼瞼下垂が著明となった（図9）。これに対し第2回手術（7歳6ヵ月時）として右眼水平筋全筋上方移動術を施行した。術前のCTにて両眼高度の上直筋・上斜筋低形成が疑われ（図11），術野に上直筋・上斜筋は欠損していた（図12）。そこで，正常の上直筋付着部位まで内・外直筋を上方移動して縫着した。最終診察時（水平筋移動術後4年）の眼位は正位で，大型弱視鏡で融像を認めた。右眼下斜視の改善とともに右眼偽眼瞼下垂も改善，残存する上転制限，Bell陰性のため角膜下方に軽度の上皮炎を認めるが，他に水平眼位の変化，左眼の内・外転制限，AV型の発症などの術後合併症は認めない（図10）。手術による遠見の第一眼位の矯正効果は25Δで，長期的に安定した眼位を保持している。

これら3症例の所見と治療結果をまとめて表1に示す。症例2では他に左眼眼瞼挙筋切除短縮術，右眼内直筋後転＋外直筋短縮術を施行したが，これらの手術前後で上下偏位に変化はなかった。また症例3は初回手術（両下斜筋減弱）の術後所見を水平筋全筋上方移動術の

表1　3症例の所見と治療結果

症例	手術年齢	疾患	術前所見	術前眼位	術後眼位	上下偏位矯正効果	両眼視獲得	術後所見
1	3歳	(L) double elevator palsy	上転制限 FDT（—） 偽眼瞼下垂	R/L 25 Δ	L/R 12 Δ	37 Δ	融像（＋） 立体視（＋）	上転改善 下転制限 過矯正傾向
2	4歳	(L) double elevator palsy	上転制限 FDT（＋） 眼瞼下垂 Bell（－） SR hypoplasia (CT)	R/L 25 Δ ET 40 Δ	R/L 6 Δ EP 12 Δ	19 Δ	融像（＋）	上転改善 下斜筋過動
3	7歳	(Bil) SR&SO hypoplasia	上転制限 FDT（－） 偽眼瞼下垂 Bell（－） SR&SO hypoplasia (CT)	L/R 25 Δ	ortho	25 Δ	融像（＋）	上転改善 角膜上皮炎

術前所見として表1に記載した。水平筋全筋上方移動術前後の第一眼位における矯正効果をみると，術前下斜視角は全例25 Δであるが，画像にて上直筋低形成を認めない症例1では手術による矯正効果が37 Δと最も大きく，症例2では19 Δと小さい結果となった。全例術後第一眼位にて正位または斜位を保持し両眼視を獲得している。また，いずれの症例も水平偏位の変化，内外転制限，A, V型の発症などの術後合併症を認めていない。症例2, 3では術後4年にわたり安定した眼位を維持している。

Ⅲ　考　　按

Knappの原法は，内・外直筋全筋を上方移動して上直筋付着部両縁に縫着する方法で，1歳～48歳（平均15歳）のdouble elevator palsy 15例に施行した結果，第一眼位における下斜視が21～55 Δ（平均38 Δ）矯正できたと報告されている[1]。同様にdouble elevator palsyに本術式を施行した結果，下斜視の矯正効果は，Watson 2例平均30 Δ[2]，Cooper 6例平均25 Δ（11～38 Δ）[3]，Dunlap 2例平均35 Δ[4]，Barsoum-Homsy 2例平均29 Δ[5]，Caldeira 10例平均36 Δ（19～50 Δ）[6]と報告されており，大角度（≧25 Δ）の下斜視の矯正に本術式が有効であることが示されているが，矯正効果には症例による差が大きいことがわかる。一方，Burke 13例平均21 Δ（5～39 Δ）[7]，Cadera 2例平均18 Δ[8]のように，矯正効果の比較的小さい報告もある。

今回の術式は，症例1, 2では内・外直筋の下縁が上直筋付着部両縁の位置に一致するように全筋を上方移動して縫着（図2），症例3では正常の上直筋付着部位の両端の位置を基準に計測して施行したが，表1のごとく術前の下斜視角はいずれも25 Δと同等であっても下斜視の矯正効果は19～37 Δと症例間で差がみられた。術前所見より症例1, 2ともにdouble elevator palsyと診断したが，臨床像には異なった特徴があり，随伴所見が偽眼瞼下垂のみの症例1に対し，症例2では高度眼瞼下垂，内斜視，患眼下直筋と健眼内直筋の二次的拘縮を伴っており，CTでは患眼上直筋の低形成を認めた。また症例3は高度の両眼上直筋・上斜筋低形成が特徴で，術野には上直筋・上斜筋が欠損していた。手術による矯正効果は，画像にて上直筋低形成を認めない症例1で最も大きく術後過矯正傾向となったが，下直筋の二次的拘縮を伴う症例2では小さい結果となった。症例2では第1回手術として左眼眼瞼挙筋短縮術，第3回手術として健眼の内直筋後転＋外直筋短縮術を行ったが，これらの手術前後で上下偏位に差は生じなかった。症例2, 3と同じように，矯正効果の小さいBurkeの報告[7]には下直筋の二次的拘縮例，Caderaの報告[8]にはMRIで上直筋低形成を呈する例が含まれており，臨床像，特に外眼筋の二次的変化の有無によって手術効果に差が出ると考えられる。しかし，他の報告と同様に，本術式によって水平眼位の変化，内外転制限，AV型の発症などの術後合併症は認めず，高度の下斜視の矯正に安全で有効な術式と考えられる。今回の3症例では，手術による矯正効果に差はみられたものの，全例で第一眼位で正位または斜位となり，術後に融像を獲得している。症例2, 3では術後4年安定した眼位を維持しており重篤な術後合併症を認めない。したがって整容面のみならず，眼球運動や両眼視機能の改善を目的に低年齢のうちに本術式を用いた手術を検討する価値があると思われる。

本術式には水平筋の上方移動量を増減した変法の報告もあり，原法[1]どおり水平筋を上直筋付着部位まで移動して付着部両縁に縫着した前述のWatson, Cooper, Barsoum-Homsy, Burke, Cadera, Caldeiraの報告[2][3][5]～[8]に対して，Dunlap[4]は10 mm以上の上方移動にて平均35 Δ

の矯正効果ありと報告し，術前斜視角と上転障害の重症度に従って上方移動量3 mm（筋腹の1/2幅）～10 mmの定量を試みた。またBarsoum-Homsyも25 Δ未満の下斜視に対して原法は過矯正となるため，5～10 mmの上方移動が適切であると述べている。

一方，double elevator palsyにおいて下直筋の二次的拘縮のある場合には，初回手術として患眼下直筋後転術が行われることがある。Burke[7]は下直筋後転術後に水平筋全筋上方移動術（Knapp法）を施行した結果をKnapp法のみ施行した例と比較した。Knapp法のみの眼位矯正効果は平均21 Δ（5～39 Δ）と他報告に比べて小さいが，下直筋後転術後のKnapp法による矯正効果は平均38 Δ（27～46 Δ）と大きいため過矯正となりやすいこと，長期経過によって矯正効果がさらに増大する傾向があること，下直筋後転術後では特に術前斜視角による定量が困難であることを指摘している。同様にKnapp[1]も下直筋後転術施行例に本術式を施行すると下転制限が生じ下方視で過矯正となることを指摘している。したがって，今回の症例2のような二次的拘縮を伴うdouble elevator palsyの例においても，初回手術術式として水平筋全筋上方移動術を選択するのが妥当と思われる。

内・外斜視が合併している場合，Dunlap[4]は水平筋の後転・短縮を上方移動術と同時に施行しているが，水平眼位の矯正に一定した効果が得られない。今回の症例2ではCooper[3]の見解と同様，水平筋上方移動術施行後に内斜視の矯正を他眼に行ったところ，良好な結果が得られた。特に高度の上下偏位と水平偏位の矯正を要する場合には，患眼の水平筋上方移動術後に水平偏位を正確に測定しなおし，健眼手術により水平眼位を矯正するのが望ましいと思われる。また今回の結果にもみられるように，偽眼瞼下垂は十分な下斜視の矯正により改善するため，随伴する眼瞼下垂の適切な鑑別が必須であり，真の下垂であっても軽度～中等度であれば眼位矯正後に手術を検討するのが妥当と思われる。症例2では高度の眼瞼下垂を合併し眼位の評価が困難であったため初回手術として眼瞼挙筋短縮術を行ったが，続いて眼位も良好に矯正され，術後問題となる所見はなかった。

近年Caderaはdouble elevator palsy 2例にMRIを施行し，患眼上直筋のvolumeの減少を認めたと報告している[7]。今回の症例2においてもCTにて同様の所見を認めた。Double elevator palsyは視蓋前域の障害による核上性眼球運動異常と一般に考えられているが，他に上直筋麻痺・低形成や下直筋運動制限を一次的な原因とする型の存在も示唆されている。Caderaの報告例や今回の症例2における画像所見が，患眼の上直筋低形成を示すものか，麻痺による萎縮すなわち二次的変化を捉えているのかは判別できないが，これらの症例では水平筋上方移動術による矯正効果が小さいことに注意すべきである。

今回の検討より，水平筋全筋上方移動術の矯正効果を左右する要因として上下直筋の形成異常や二次的変化の有無が重要であることが示された。したがって，今後も本術式の適応に際し，術前に症例の特徴をよく観察し，術前斜視角，上転障害の程度，第一偏位と第二偏位の差，FDT（二次的拘縮の有無）を正確に評価する必要があるが，さらに術式と術量の選択には外眼筋の画像所見を十分に検討することが有用であると考えられる。上直筋低形成と下直筋の二次的拘縮を随伴した症例2では最も矯正効果が小さかったものの，水平筋下縁を上直筋付着部まで上方移動して縫着した今回の術式によって斜位に矯正することが可能で上転障害も軽減し，安定した眼位を長期に保持している。しかし，外眼筋に変化を認めなかった症例1では，同様の術式によって術後7ヵ月の時点で大きな矯正効果を生じ，上転制限は残存するものの下転制限が新たに生じて下方視で過矯正となった。長期経過によってさらに過矯正となる可能性があり慎重に経過観察したい。症例1のように外眼筋に変化のない軽症例では，第一眼位や下方眼位を優先して上方移動量を少なく定量，あるいは水平筋部分移植術の適応を考慮すべきであると思われた。

Ⅳ 要　　約

1. 水平筋全筋上方移動術は高度の上転障害のあるdouble elevator palsy，上直筋低形成の下斜視の矯正に有効で，異なった特徴をもつ3症例に施行した結果，全例が第一眼位で正位または斜位となり両眼視が改善した。

2. 本術式によって大角度（約25 Δ）の上下偏位の矯正が可能であり，術後に水平眼位の変化，内・外転制限，AV型の発症を認めなかった。

3. 本術式による矯正効果は症例により差があり，画像にて外眼筋に変化のない軽症例では過矯正傾向となった。症例の特徴や画像所見により術量や術式を選択する必要がある。

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キーワード：水平筋全筋上方移動術，Knapp procedure，double elevator palsy，上直筋低形成，CT

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質問，三村　治（兵医大）　症例2眼球牽引試験陽性で画像上上直筋低形成とありましたが，下直筋線維症でも画像上で上直筋の菲薄化をみることがしばしばです。症例2ではそのような場合と同じようにまず下直筋の後転でも良かったのではないでしょうか。

答　症例2に関しては，下直筋 fibrosis のような高度の他動抵抗をみとめなかった。IR 後転のみでは低矯正になると思われ，また水平筋上方移動術と下直筋後転を同時施行すると過矯正となるおそれがあったためまず効果の高いと考えられる水平筋上方移動術のみを施行した。

質問，横山　連（大阪市立総合医療センター）　水平直筋を縫着した位置は，上直筋の付着部と説明しておられましたが，スライドでは上直筋の付着部よりもさらに後方に縫着されているように見えました。縫着位置は上転障害の程度等の臨床症状に応じて変えておられるのですか。

答　上方移動部位は上直筋付着部に水平筋の下縁を縫着した。直筋を結ぶラインよりは上方に付着したことになる。