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CLINICAL INVESTIGATION

Congenital Rotated Macula with Good Vision and Binocular Function

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Abstract

Background: Two patients presented with congenital rotated macula with good vision and binocular function.

Cases: Two patients had retinal folds with an extremely rotated macula OD as the result of peripheral fibrous proliferation on the retina. Each macula was substantially rotated to the nasal border of the disc.

Observations: A 3-year-old girl (case 1) with best-corrected visual acuity (VA) of 0.5 OD and 1.0 OS was treated for amblyopia, after which she successfully achieved 1.0 bilaterally as well as good stereopsis of 120 seconds of arc measured with the TNO test. A 6-year-old girl (case 2) obtained a VA of 0.7 OD and 1.0 OS, and her stereopsis was of 240 seconds of arc.

Conclusions: Good VA and stereopsis may be achieved by adaptation in the brain and an extremely large fusional potential at an early infantile age or a gradual shift of the macula. Appropriate treatment of amblyopia should be performed in patients with rotated macula if the macula appears normal. **Jpn J Ophthalmol** 2009;53:452–454 © Japanese Ophthalmological Society 2009

Keywords: amblyopia, binocular function, congenital rotated macula, familial exudative vitreoretinopathy, retinal fold

Introduction

Macular displacement is often observed in infantile vitreoretinal abnormalities, including retinopathy of prematurity (ROP), familial exudative vitreoretinopathy (FEVR), persistent fetal vasculature/persistent hyperplastic primary vitreous (PFV/PHPV), Coats disease, ocular infection with *Toxocara canis*, and trauma.[1,2] In eyes with PFV/PHPV and FEVR, the macula is dragged and dislocated to various locations where peripheral fibrous tissue is present, while in almost all eyes with ROP, it is dislocated temporally. Eyes with a dragged retina cannot develop good visual acuity (VA) if the macular formation is distorted.[2,3] Good VA and stereopsis cannot be usually achieved in eyes with a macula dislocated to a great distance.[3] Only one case has been reported of a 3-year-old girl who achieved relatively good stereopsis (600 seconds of arc), although her left macula was displaced nasally.[4] We report two patients with a macula extremely rotated around the disc as the result of peripheral fibrous proliferation, both of whom obtained good VA and stereopsis.

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Case Reports

Case 1

A 3-year-old girl was referred with a diagnosis of strabismus OD. There were no abnormalities in the anterior segments, but ophthalmoscopy of the right eye detected a retinal fold

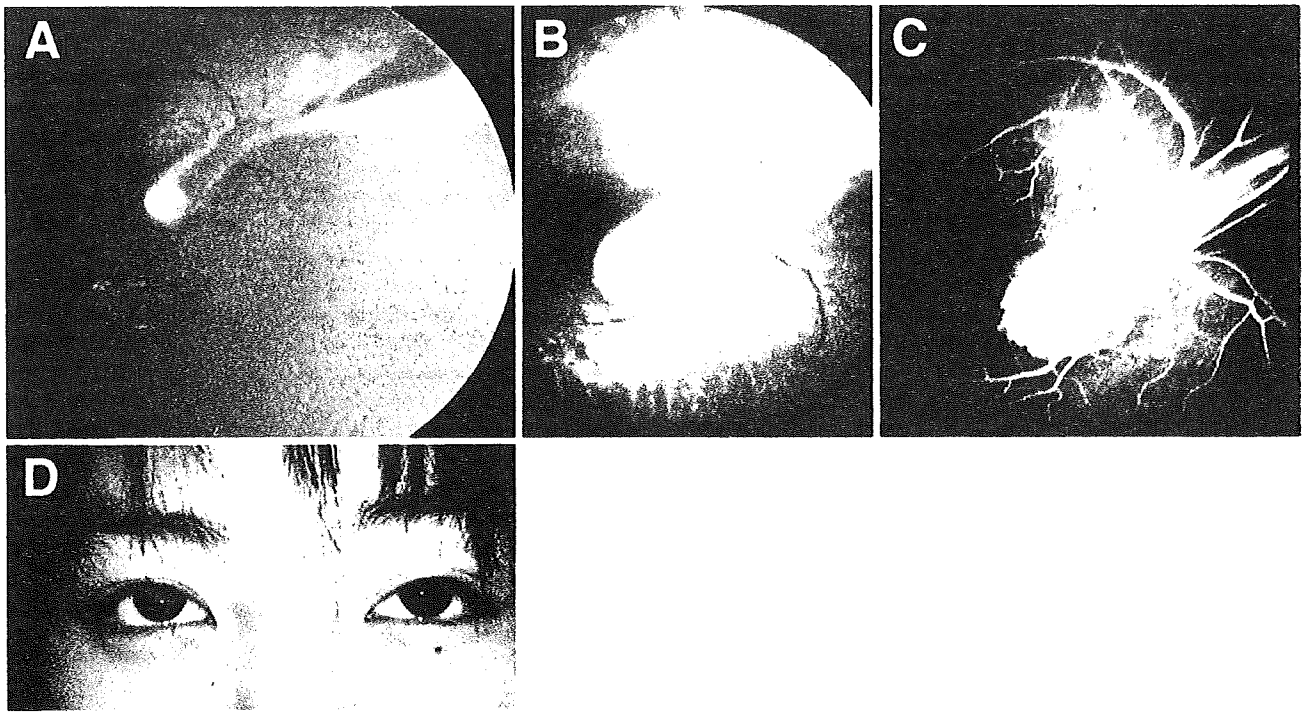

Figure 1A–D. Case 1. **A** A widefield fundus of the right eye taken with a RetCam-120 digital retinal camera (Massie Research Laboratories, Dublin, CA, USA). Retinal folds extending from the optic disc toward the fibrous tissue in the nasal superior periphery are shown. **B** Posterior fundus photograph. **C** Fluorescein angiogram. The macula is rotated to the nasal border of the disc in the right eye. **D** Photograph of eye position. Slight esodeviation and hyperdeviation of the right eye are detected.

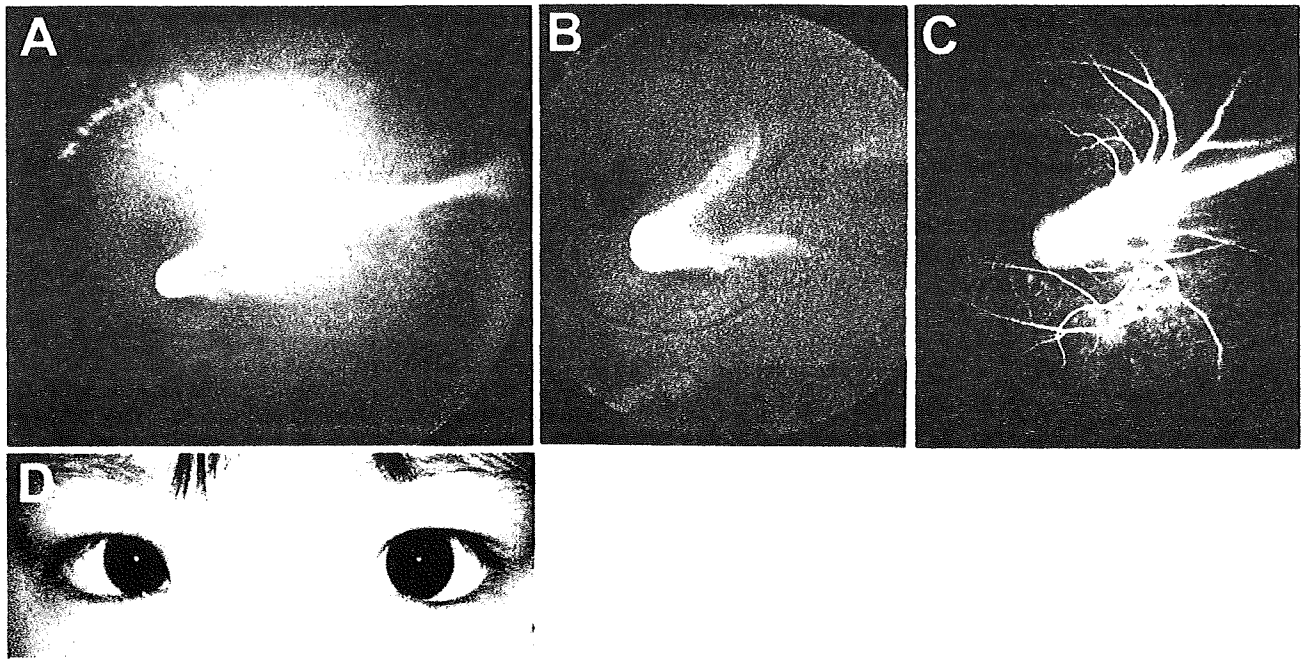

Figure 2A–D. Case 2. **A** A widefield fundus of the right eye taken with the RetCam-120 digital retinal camera. Retinal folds extending from the optic disc toward the fibrous tissue in the nasal superior periphery are shown. **B** Posterior fundus photograph. **C** Fluorescein angiogram showing rotated macula to the nasal border of the disc. **D** Photograph of eye position. Slight esodeviation and hyperdeviation of the right eye are detected.

Jpn J Ophthalmol
Vol 53: 452–454, 2009

extending from the optic disc toward the fibrous tissue on the nasal superior periphery. The temporal retina was rotated around the disc, and the macula was located at the superior-nasal border of the disc (Fig. 1). The left eye had an area of avascularity in the temporal periphery of the retina. She was diagnosed with insufficient retinal vascular development, possibly related to FEVR, although she had no family history of FEVR. The patient was the product of a full-term pregnancy and showed normal growth and no systemic abnormalities, and laboratory examinations showed no history of infectious diseases. A cover test indicated orthophoria, but a Krimsky test indicated 10 prism diopters of right pseudohypertropia and 10–20 prism diopters of right pseudoesotropia. The corrected VA was 0.5 OD and 1.0 OS using a Landolt ring chart at 5 m. Cycloplegic refraction was performed using 1% cyclopentolate and revealed +1.50 diopters cyl –3.50 diopters OD and cyl –0.50 diopter OS. Amblyopia in the right eye was treated using refractive correction and part-time occlusion (2–3 h/day) of the left eye. After 6 months of treatment, the best-corrected VA was 1.0 OD and 1.0 OS, and when the patient was 6 years of age a TNO test of binocular vision showed stereopsis of 120 seconds of arc.

Case 2

A 6-year-old girl was referred with a diagnosis of amblyopia in the right eye. There were no abnormalities in the anterior segments, but ophthalmoscopy of the right eye detected retinal folds extending from the optic disc toward the fibrous tissue on the nasal superior periphery. The temporal retina was rotated around the disc, and the macula was located at the superior-nasal border of the disc (Fig. 2). There were no abnormalities in the retina of the left eye. She had no family history of retinal disease. The patient was the product of a full-term pregnancy and had normal growth and no systemic abnormalities, and laboratory examination failed to identify a history of infectious disease. A cover test indicated orthophoria, but a Krimsky test indicated 10 prism diopters of right pseudohypertropia and 20 prism diopters of right pseudoesotropia. The VA was 0.7 OD and 1.0 OS using a Landolt ring chart at 5 m. Cycloplegic refraction was performed using 1% cyclopentolate and revealed +2.50 diopters cyl +2.00 diopters in the right eye and +3.00 diopters in the left eye. A TNO test of binocular vision showed stereopsis of 240 seconds of arc. Amblyopia in the right eye is now being treated with refractive correction and part-time occlusion (2–3 h/day) of the left eye.

Discussion

Ophthalmoscopy of the present cases showed good macular formation, and the patients obtained good VA and high-grade stereopsis, despite the macula's having rotated markedly to a position adjacent to the optic disc. Correction of refractive errors and orthoptics may be effective for obtaining good VA and excellent binocular vision if the macula is well formed. It is speculated that in the present cases, a large macular dislocation had already occurred when the patients were 3 to 4 months of age, when binocular vision begins to develop.[5] Another possibility is that the macula rotated gradually as a result of contraction of the fibrous tissue during the development of binocular function. It is suggested that if the macula becomes rotated during the early stages of visual development, the central nervous system may adapt to the anatomical disorder. Infants may have an extremely large fusional potential compared to that of adults.[6–8] The critical period and amplitude of infantile fusional potential remain uncertain; however, these cases may suggest that a positive prognosis is possible after macular rotation surgery in infants.

The affected eye of each patient showed relatively worse vision at first visit, probably because the affected eye in both had anisometropia and high astigmatism. Appropriate treatment of amblyopia should be performed in patients with a rotated macula if the macula appears normal.

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Pax6-5a Promotes Neuronal Differentiation of Murine Embryonic Stem Cells

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Pax6 genes are highly conserved and important for eye development. Vertebrates predominantly produce two alternatively spliced Pax6 isoforms, Pax6 and Pax6-5a. Pax6-5a differs from Pax6 by the presence of 14 additional amino acids encoded by exon 5a. These additional amino acids occur in the Pax6 paired domain and thus influence its DNA-binding properties. However, little is known about Pax6-5a's physiological functions. Here we establish murine embryonic stem (ES) cell lines in which expression of either the human Pax6 or Pax6-5a isoform is negatively controlled by tetracycline. We report that, in contrast to Pax6 expression, Pax6-5a expression strongly induces ES cells to differentiate into neurons. Moreover, using DNA microarray analysis, we have identified the transcription factor basic helix loop–helix domain containing, class b2 (bHLHb2) in Pax6-5a-expressing ES cells. Our Pax6 isoform-expressing ES cell lines may serve as useful models for identifying Pax6-regulated genes that are important for neurogenesis and/or eye development.

Key words Pax6; Pax6-5a; embryonic stem cell; neuron; DNA microarray; basic helix loop–helix domain containing, class b2

Pax6 is a transcription factor essential for the development of the eye, brain and pancreas.[1,2] Pax6 is defined by the presence of its paired domain, a highly conserved DNA-binding motif composed of two distinct DNA-binding subdomains called the N-terminal subdomain (NTS) and the C-terminal subdomain (CTS). The NTS and the CTS bind to distinct consensus DNA sequences.[3,4] Transcription of the human *Pax6* gene results in two alternatively spliced isoforms, Pax6 and Pax6-5a. Compared to Pax6 transcripts, Pax6-5a transcripts contain an additional exon 5a that encodes an additional 14 amino acids. These amino acids are inserted into the NTS, an event that abolishes the DNA-binding activity of this subdomain and unmasks the DNA-binding activity of the CTS. Thus, exon 5a transcription appears to function as a molecular switch that regulates the spectrum of Pax6 target genes that can be induced. Previously, we reported a missense mutation in the exon 5a region of the *Pax6* gene in patients with Peters anomaly. This mutation resulted in a Pax6 protein with impaired DNA-binding and transactivation activities.[5] Subsequently, we found that although overexpression of either Pax6 or Pax6-5a in chick embryos induced transdifferentiation of ectopic neural retina from primitive retinal pigment epithelium, Pax6-5a was a much stronger driver of this process than was Pax6.[6,7] However, little is known about how Pax6 and Pax6-5a actually function *in vivo*.

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of preimplantation mouse embryos. ES cells can be propagated stably in the undifferentiated state *in vitro* and, under the appropriate culture conditions, can be induced to differentiate into a variety of cell types. For example, forced transgenic expression of Pdx1 causes ES cells to differentiate into pancreatic cells (endoderm), whereas GATA2 expression promotes leukocyte differentiation (mesoderm), and Mash1 expression induces neuronal differentiation (ectoderm).[8,9] This plasticity makes ES cell culture a useful tool for elucidating the functions of transcription factors.

In this report, we have employed conditional expression of human Pax6-5a driven by a tetracycline-regulatable promoter and have found that forced Pax6-5a expression in murine ES cells enhances their differentiation into neuronal cells. Our system may be useful for uncovering the molecular mechanisms underlying Pax6-5a-dependent eye development.

MATERIALS AND METHODS

Establishment of Tet-Regulated ES Cell Clones The murine ES cell line E14tg2a was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum, 0.1% β-mercaptoethanol, and 1000 U/ml leukemia inhibitory factor (LIF). The tetracycline (Tet) regulatory system was used to obtain inducible Pax6- or Pax6-5a-expressing ES cell clones as described previously.[10] Briefly, Tet-regulatable Flag-Pax6 and Flag-Pax6-5a constructs were generated by inserting Flag-human Pax6 or Flag-human Pax6-5a cDNA into the NotI site of pUHD10-3.IRES-EGFP. Expression of these Tet-regulatable constructs in ES cells was completely suppressed by the addition of tetracycline to the culture medium. The ES cell clones whose enhanced green fluorescent protein (EGFP) expression was most tightly regulated by tetracycline were selected for further study, including examination of the expression of relevant genes by reverse transcription-polymerase chain reaction

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(RT-PCR) and Western blotting.

ES Cell Differentiation Embryoid bodies (EBs) were prepared as described previously.[11] Briefly, undifferentiated ES cells were dissociated into single-cell suspensions and cultured in hanging drops to induce embryoid body (EB) formation. Initial cell density was 2000 cells per drop (25 μl) of differentiation medium without LIF in the absence or presence of 1 μg/ml Tet (Day 0). After 2 days in a hanging drop culture (Day 2), the resulting EBs were transferred to non-coated culture dishes. On Day 3, bFGF (20 ng/ml, R&D Systems) was added to the culture medium. On Day 7, the EBs were plated in plastic gelatin-coated dishes.

Immunoprecipitation and Immunoblotting For detection of induced Flag-Pax6 and Flag-Pax6-5a proteins, ES cells were cultured for 48 h in the absence of Tet. ES cells (5×10^6) were then lysed and immunoprecipitated with anti-Flag M2 antibody (Ab) (Sigma, F3165). The immunoprecipitates were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with anti-Flag M2 Ab. Bands were visualized using the SuperSignal West Pico chemiluminescent substrate according to the manufacturer's instructions (PIERCE, IL, U.S.A.).

Confocal Microscopy Immunofluorescence studies were performed as described previously.[12] Briefly, EBs cultured on gelatin-coated dishes were washed three times with phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde. After permeabilization with 0.2% Triton X-100, the EBs were incubated with blocking solution [5% bovine serum albumin (BSA) in TBS], followed by anti-βIII-tubulin Ab (Covance, MMS-435P). The stained EBs were washed with PBS and incubated with Alexa-568-conjugated secondary Ab (Molecular Probes). After a last wash in PBS, EBs were viewed on a Carl Zeiss confocal microscope equipped with LSM510 software.

RT-PCR Analysis ES cells were lysed with Trizol reagent (Invitrogen) and first-strand cDNA was synthesized by using SuperScript III RNase H-reverse transcriptase (Invitrogen). Primers used were: Flag-Pax6, 5′-ATGGATTACAAGGATGACGACG-3′ and 5′-ATCTGTTGCTTTTCGCTAGCC-3′; Acetylcholine esterase (Ache), 5′-CCGATTTTCCTTCGTGCCTG-3′ and 5′-TGGAGGCACGGTGTTCAAAG-3′; Serotonin receptor 5a (5-HT5a), 5′-AGTCGGCCTTTTCTCTCAGC-3′ and 5′-GGTCCAGTGCTATTGCTGTC-3′; L1, 5′-AGGACACCATGTGCTAGAGC-3′ and 5′-GGGTTGCAAGGCAGAACTAC-3′; Elongation factor 1 (EF1), 5′-TCACACAGCCCACATAGCAT-3′ and 5′-CACCACTGATTAAGACTGGG-3′; Oct3/4, 5′-CTGAGGGCCAGGCAGGAGCACGAG-3′ and 5′-CTGTAGGGAGGGCTTCGGGCACTT-3′; bHLHb2, 5′-TTATTGCACAGCTAGACACGG-3′ and 5′-ACACTGTAACCTCGCCTCTC-3′.

DNA Microarray Total RNAs from EBs were extracted using TRIZOL (Invitrogen, Tokyo, Japan) and purified using an RNeasy mini kit (Qiagen, Tokyo, Japan). Purified RNA was labeled with biotin according to the manufacturer's protocol and hybridized to a mouse genome 430A 2.0 array (Affymetrix Japan, Tokyo, Japan). After washing, the array was scanned to measure fluorescence intensity (representing gene expression). The fluorescence intensity of each probe was further analyzed by dChip, a model-based expression analysis program that estimates gene expression levels. For the dChip analysis, a perfect match (PM)-only model was used. The estimated gene expression level values were applied to the Gene-Spring software program (Silicon Genetics, Redwood City, CA, U.S.A.).[13]

Construction of Plasmids A DNA fragment encompassing −90 to −1996 bp of the 5′ region of the murine *bHLHb2* gene was subjected to PCR amplification using the iProof High Fidelity DNA Polymerase system (BioRad). After digestion with *Mlu*I and *Xho*I, the PCR product was subcloned into the *Mlu*I/*Xho*I sites of the pTA-Luc vector (CLONTECH), resulting in pbHLHb2 (−90 – −1996). To construct the pTA-5aCON-Luc vector, which served as a positive control for the Pax6-5a reporter assay, the oligonucleotides 5′-AAATCTGAACATGCTCAGTGAATGTTCATTGACTCTCGAGGTC-3′ and 5′-GACCTCGAGAGTCAATGAACATTCACTGAGCATGTTCAGATTT-3′ were annealed, phosphorylated and ligated into the *Sma*I site of the pTA-Luc vector. Underlining indicates Pax6-5a binding sequences.

Transient DNA Transfections and Luciferase Reporter Assays DNA transfections were carried out using the Lipofectamine 2000 reagent (Invitrogen). Briefly, the human embryonic carcinoma cell line NTERA2 were plated in a 24-well plate. After 24 h transfection with the above reporter plasmids, the cells were harvested and firefly and sea pansy luciferase activities were determined using the dual-luciferase reporter assay system (Promega) and a Wallac ARVO.SX 1420 Multilabel Counter (Amersham Pharmacia Biotech). All experiments were repeated at least three times with different batches of transfected cells and the results were fully reproducible.

RESULTS

Tet-regulatable Pax6 and Pax6-5a Expression To investigate the biological functions of Pax6 and Pax6-5a, we established murine ES cell lines with tetracycline (Tet)-regulatable Flag-human Pax6 or Flag-human Pax6-5a expression. To this end, a Tet-regulatable bicistronic vector encoding Flag-Pax6 or Flag-Pax6-5a plus the EGFP was introduced into ES cells. ES cell lines were then established in the presence of Tet (Tet-on) to suppress the expression of the exogenous Flag-Pax6 or Flag-Pax6-5a. Conditional expression of Flag-Pax6 or Flag-Pax6-5a and EGFP in these ES cells was achieved by withdrawal of Tet (Tet-off). The successful expression of Flag-Pax6 and Flag-Pax6-5a proteins in Tet-off ES cell lines was confirmed by Western blot analysis using anti-Flag antibody (Fig. 1A), whereas EGFP expression was detected with confocal microscopy (Fig. 1B). These results confirm that our Pax6 isoform-expressing ES cell lines showed EGFP plus exogenous Pax6 or Pax6-5a protein expression that was fully regulated by Tet.

Pax6-5a Expression Enhances Neuronal Differentiation by ES Cells Cultured murine ES cells can be induced to differentiate *in vitro* into a variety of cell types, including cardiac cells and neurons. To separately examine the effects of Pax6 and Pax6-5a on ES cell differentiation, we cultured our Tet-regulatable ES cell lines without LIF in the presence or absence of Tet and allowed them to form embryoid bodies (EBs). Interestingly, Tet-regulatable Pax6-5a-expressing ES cells differentiated into neuron-like cells in absence, but not presence, of Tet (Fig. 2A). To confirm that the observed dif-

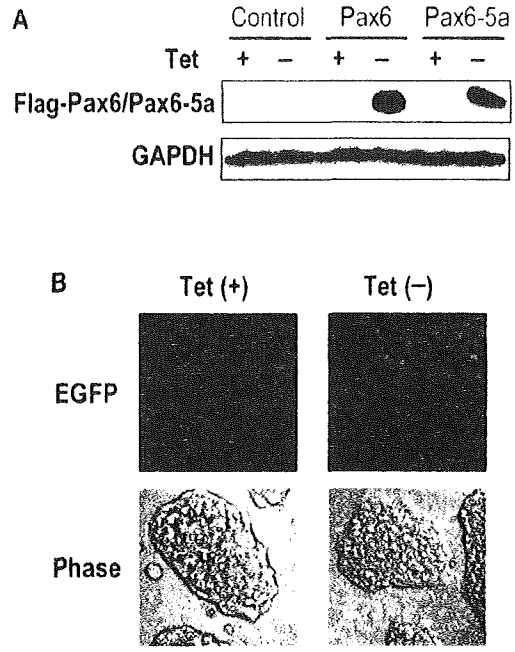

Fig. 1. Establishment of Murine ES Cell Lines with Tet-Regulatable Expression of Pax6 Isoforms

(A) Confirmation of Tet-regulated expression by Western blot. Murine ES cell lines expressing either EGFP vector alone (control) or EGFP plus either Flag-human Pax6 (Pax6) or Flag-human Pax6-5a (Pax6-5a) were cultured with (+) or without (−) Tet for 2 d and subjected to Western blotting using anti-Flag Ab. GADPH, loading control. (B) Confirmation of transfection. Detection of EGFP expression in monolayers of Tet-regulatable Pax6-5a-expressing ES cells in the presence or absence of Tet. Top, confocal microscopy; bottom, phase contrast microscopy. For all figures, results shown are one trial representative of at least three independent experiments.

ferentiating cells were in fact neurons, we immunostained them with anti-βIII-tubulin Ab and obtained positive results (Fig. 2A, right panel). On the other hand, this type of neuronal differentiation did not occur in Tet-off Pax6-expressing ES cells (data not shown). We then used RT-PCR to examine the expression by both of our Tet-regulatable ES cell lines of typical neuronal marker genes such as acetylcholinesterase (Ache; cholinergic neurons), serotonin receptor 5a (5-HT5a; serotonergic neurons), and L1 (neuronal cell adhesion molecule). Expression levels of Ache, 5-HT5a and L1 mRNAs were increased in Tet-off Pax6-5a-expressing ES cells, but not in Tet-on Pax6-5a-expressing ES cells, nor in Tet-off or Tet-on Pax6-expressing ES cells (Fig. 2B). These data indicate that expression of Pax6-5a by murine ES cells enhances their neuronal differentiation.

Pax6-5a Expression Enhances Neurite Development by Murine EBs To determine the timing of Pax6-5a-induced neuronal commitment, we examined neurite development in EB cultures of Pax6-5a-expressing ES cells from which Tet was removed for 0, 1, 2, 3, 4 or 11 d after Day 0 (Fig. 3A). When Tet was withdrawn for 11 d, more than 60% of EBs were found to be neurite-positive (Fig. 3B, row f). The most dramatic increase in the percentage of neurite-positive EBs observed occurred between 2 d (condition c) and 3 d (condition d) of Tet withdrawal, suggesting that Pax6-5a-induced neuronal commitment is established in this system during the first 3 d of culture.

Pax6-5a Expression Upregulates Neuron-Related Genes in Murine EBs To determine the identity of genes activated downstream of Pax6-5a, we performed a DNA mi-

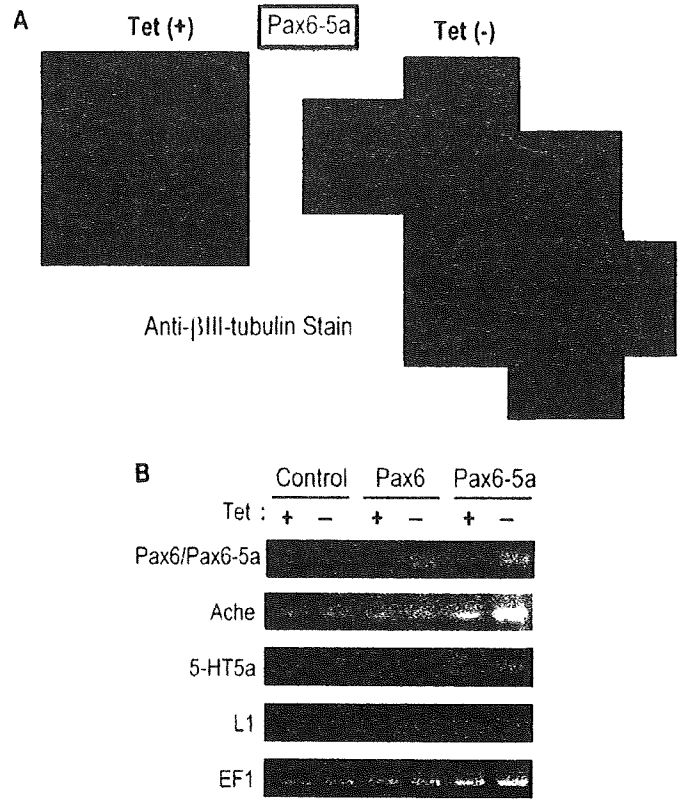

Fig. 2. Pax6-5a Enhances the Neuronal Differentiation of Murine ES Cells

(A) Neuron-like differentiation. Tet-regulatable Pax6-5a-expressing ES cells were allowed to form EBs in the presence or absence of Tet, followed by immunostaining with anti-βIII-tubulin Ab. (B) Neuronal marker expression. Control, Pax6- and Pax6-5a-expressing ES cell lines were cultured in the presence or absence of Tet for 11 d and expression levels of the neuronal marker genes Ache, 5-HT5a and L1 were determined by RT-PCR. EF1, loading control.

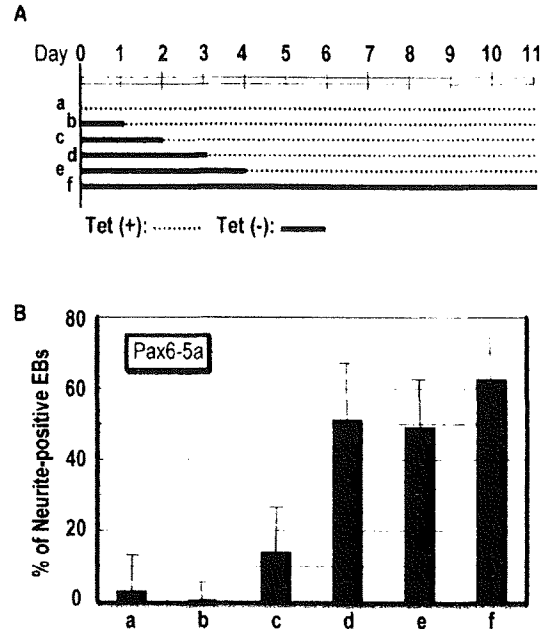

Fig. 3. Time Course of Pax6-5a-Induced Commitment to ES Cell Neuronal Differentiation

(A) Experimental scheme. Tet-regulatable Pax6-5a-expressing ES cells were cultured with Tet for 0–11 d as indicated (a–f). EB formation commenced on Day 0 and neuronal differentiation was assessed on Day 11. (B) Day 3 commitment. Percentages of neurite-positive EBs in the cultures in (A) were assessed by microscopy. A significant increase in neurite-positive EBs occurs between Day 2 and Day 3.

croarray analysis of Tet-regulatable Pax6-5a ES cells cultured for 3 d in the absence or presence of Tet. Among the many genes whose expression was increased in a Pax6-5a-dependent manner were the transcription factors basic helix-loop–helix domain containing, class b2 (bHLHb2) and Oct3/4 (data not shown). Both bHLHb2 and Oct3/4 are able to induce ES cells to differentiate into neuronal cells.[14,15] To confirm the effects of Pax6-5a on the expression of bHLHb2 and Oct3/4, we carried out an RT-PCR time course analysis of Tet-regulatable Pax6- or Pax6-5a-expressing ES cells cultured in the presence or absence of Tet for 1—5 d. bHLHb2 mRNA expression was constant in Tet-on and Tet-off Pax6-expressing ES cells regardless of the number of days of Tet withdrawal, whereas Oct3/4 expression declined in a time-dependent manner in both Tet-on and Tet-off Pax6-expressing ES cells (Fig. 4A, upper panel). In contrast, expression levels of both bHLHb2 and Oct3/4 mRNAs were increased in Tet-off Pax6-5a-expressing ES cells compared to Tet-on Pax6-5a-expressing ES cells (Fig. 4A, lower panel). These results suggest that *bHLHb2* and *Oct3/4* are downstream target genes of Pax6-5a.

To determine whether the *bHLHb2* gene was truly a transcriptional target of Pax6-5a, we carried out luciferase reporter assays examining *bHLHb2* promoter activity. A DNA fragment encompassing −90 to −1996 bp of the 5′ region of the murine *bHLHb2* gene was positioned upstream of the pTA-Luc firefly luciferase reporter gene to create the pTA-bHLHb2-Luc reporter. This reporter was cotransfected along with a plasmid expressing Pax6-5a into NTERA2 cells, a human embryonic carcinoma cell line. As a positive control, we generated the pTA-5aCON-Luc reporter plasmid that contains a Pax6-5a-binding sequence and responds to Pax6-5a. Interestingly, the degree of induction of pTA-bHLHb2-Luc was equivalent to that of pTA-5aCON-Luc, indicating that the *bHLHb2* gene is directly or indirectly regulated by Pax6-5a at least in NTERA2 cells.

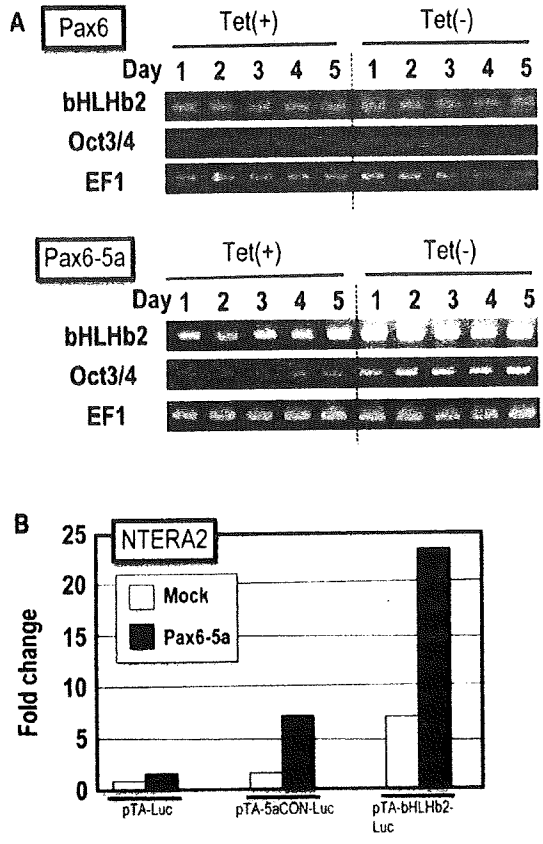

Fig. 4. Influence of Pax6-5a on *bHLHb2* and *Oct3/4* Gene Expression and *bHLHb2* Transcriptional Activity

(A) Increased *bHLHb2* and *Oct3/4* expression. Tet-regulatable Pax6- or Pax6-5a-expressing ES cells were cultured for the indicated times in the presence or absence of Tet and levels of bHLHb2 and Oct3/4 mRNAs were assessed by RT-PCR. (B) *bHLHb2* is a Pax6-5a target gene. NTERA2 cells were co-transfected with a plasmid expressing Pax6-5a or the vehicle alone (mock), plus the indicated luciferase reporter constructs: pTA-Luc (negative control), pTA-5aCON-Luc (positive control) or pTA-bHLHb2-Luc (5′ region of the *bHLHb2* gene). Results shown are the fold change in luciferase activity relative to negative control.

DISCUSSION

In this study, we have shed light on the function of the Pax6-5a isoform using a Tet-regulated conditional expression system in the context of EB-mediated ES cell differentiation into neuronal cells. Firstly, we established ES cell lines in which the expression of human Pax6 or Pax6-5a was regulated by the presence or absence of Tet. Secondly, we demonstrated that expression of Pax6-5a, but not Pax6, enhanced the differentiation of EBs, but not monolayer ES cells, into Ache$^+$ 5-HT5a$^+$ L1$^+$ neuron-like cells. Thirdly, we showed that Pax6-5a-induced neuronal commitment occurs within the first 3 d of EB formation. Finally, we provisionally identified *bHLHb2* and *Oct3/4* as downstream target genes of Pax6-5a.

We have previously described a missense mutation within *Pax6* exon 5a in four families with members suffering from Peters anomaly, congenital cataracts, Axenfeldt anomaly, and/or foveal hypoplasia.[5] Biochemical analysis showed that this mutation caused a decrease in CTS transactivation activity. Singh *et al.* have reported iris hypoplasia and defects in the cornea, lens and retina in mice lacking *Pax6* exon 5a.[16] On the other hand, overexpression of Pax6-5a induces a remarkably well-differentiated retina-like structure in chick embryos.[6] The collective results of these "loss of function" and "gain of function" studies suggest that the evolution of the Pax6-5a isoform has contributed to the advanced features of the vertebrate eye. Our data are consistent with this hypothesis, in that forced expression of Pax6-5a, but not Pax6, strongly induced the neuronal differentiation of ES cells.

As shown in Fig. 1, our established ES cell lines showed EGFP plus exogenous Pax6 or Pax6-5a protein expression that was strictly regulated by Tet in monolayer-culture conditions. However, the percentage of neuronal differentiation from EB culture, which is a kind of three-dimensional culture, were 23% in the presence of Tet and 97% in the absence of Tet, respectively (data not shown). We could observe the expression of neuronal marker genes such as L1 and Ache even in the presence of Tet by RT-PCR (Fig. 2B). We speculate that Tet is less effective for EB culture compared to monolayer culture, resulting in a leak of Pax6-5a expression and an induction of neuronal differentiation from EB culture.

As mentioned above, the paired domain of Pax6 is composed of two subdomains: NTS (also called PAI) and CTS (also called RED). Each subdomain recognizes a distinct half-site of the bipartite Pax6 binding site positioned in adjacent major grooves of the DNA. In Pax6-5a, the NTS domain is modified by the insertion of 14 amino acids encoded by

the additional exon 5a. This addition alters the DNA-binding specificity of the paired domain such that it recognizes a new consensus sequence, 5aCON. The 5aCON sequence consists of four interdigitated 5′ half-sites of the bipartite consensus sequence and is thus bound by four Pax6-5a molecules *via* the intact CTS domain. A previous study of transgenic mice overexpressing human Pax6-5a in the lens showed that the human α5 and β1 integrin promoters contain both Pax6 and Pax6-5a binding sites and maybe directly regulated by Pax6 isoforms.[17] Another report based on a genome database search and biochemical analysis showed that an enhancer present in the γE- and γF-crystallin genes is recognized by both Pax6 and Pax6-5a.[18] However, there is little physiological data on genes that are regulated by Pax6-5a, either directly or indirectly. Therefore, our Tet-regulatable Pax6 isoform-expressing ES cell lines should be useful for identifying target genes of Pax6 and/or Pax6-5a. Indeed, we found that expression levels of Oct3/4 and hHLHb2 were strikingly influenced by Pax6-5a.

In vivo, Oct3/4 plays a critical role in maintaining ES cell pluripotency. *In vitro*, sustained transgenic Oct3/4 expression in ES cells cultured in serum-free LIF-deficient medium causes accelerated differentiation to neuroectoderm-like cells, whereas suppression of Oct3/4 abolishes neuronal differentiation.[15] Thus, Oct3/4 promotes neuroectoderm formation and subsequent ES cell differentiation into neuronal cells. Our data indicate that the activation of Oct3/4 necessary for neuroectoderm formation depends on Pax6-5a activity. The other gene we found to be regulated by Pax6-5a, *bHLHb2*, encodes a basic helix–loop–helix protein with significant sequence similarities to the *Drosophila* hairy and enhancer-of-split proteins as well as mouse Hes proteins. Overexpression of bHLHb2 in monolayers of P19 embryonal carcinoma cells results in neuronal differentiation under conditions where P19 cells typically undergo only mesodermal/endodermal differentiation.[14] Thus, bHLHb2 is thought to be a repressor of several of the cell fate decisions that occur during cellular differentiation. Our data indicate that Pax6-5a may regulate bHLHb2 activation and thus determine neuronal differentiation during embryogenesis. With respect to adult vertebrates, Pax6 has been shown to be required for the production and maintenance of progenitor cells during postnatal hippocampal neurogenesis.[19] Thus, Pax6 plays important roles in both embryogenesis and adult body homeostasis. Our results suggest that Pax6-5a may be the Pax6 isoform most critical for influencing the neuronal differentiation necessary for these processes.

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乳幼児眼疾患の発見・受診経路と初診時期

The time and route for finding infants' eye disease

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【要約】 目的：乳幼児眼疾患の発見・受診経路と初診時期を調査した．

方法：2002年3月～2008年12月に初診した網膜芽細胞腫(Rb)群46例，早発型発達緑内障(Gla)群25例，先天白内障(Cat)群75例，網膜硝子体異常(Vit)群119例の患側，主訴，発見・受診経路，初診年齢を検討した．

結果：両眼性151例，片眼性114例，主訴はRb群白色瞳孔・猫眼57％，Gla群角膜混濁64％，Cat群瞳孔領白濁45％，Vit群斜視34％が最多であった．発見・受診経路は家族がRb群63％，Gla群32％，Cat群53％，Vit群58％を占め，Gla群は小児科，産科，Rb群は小児科，乳児健診が次に多かった．初診年齢の平均はRb群1歳6ヵ月，Gla群2.6ヵ月，Cat群3.5ヵ月，Vit群11ヵ月であった．

結論：乳幼児眼疾患の早期発見のため家族，小児科，保健所に知識を普及し早期に眼科受診を促す必要がある．

【キーワード】 乳幼児眼疾患，早期発見，網膜芽細胞腫，早発型発達緑内障，先天白内障

【Abstract】 Purpose : To survey the time and route for finding infants' severe eye disease.

Methods : Data were analyzed from 46 cases of retinoblastoma(Rb), 25 cases of congenital glaucoma (Gla), 75 cases of congenital cataract(Cat), 119 cases of vitreoretinal anomalies(Vit), referred to the National Center for Child Health and Development from March 2002 through December 2008. Affected eyes, chief complaint, route from finding to reference, age at the first consultation were investigated.

Results : There were 151 bilateral cases and 114 unilateral cases. The most complaint was leukocoria (57％) in Rb, corneal opacity(64％) in Gla, white pupil area(45％) in Cat, and strabismus(34％) in Vit. The direct route via family formed 63％ of Rb, 32％ of Gla, 53％ of Cat, and 58％ of Vit. The second route was reference via pediatrician or obstetrician in Gla, pediatrician or infants' screening in Rb. Mean age at the first consultation was 18 months in Rb, 2.6 months in Gla, 3.5 months in Cat, and 11 months in Vit.

Conclusion : To inform parents, health nurse, pediatrician of the enough knowledge of infants' eye disease is required for early detection.

【Keywords】 Infants' eye disease, Early detection, Retinoblastoma, Congenital Glaucoma, Congenital cataract

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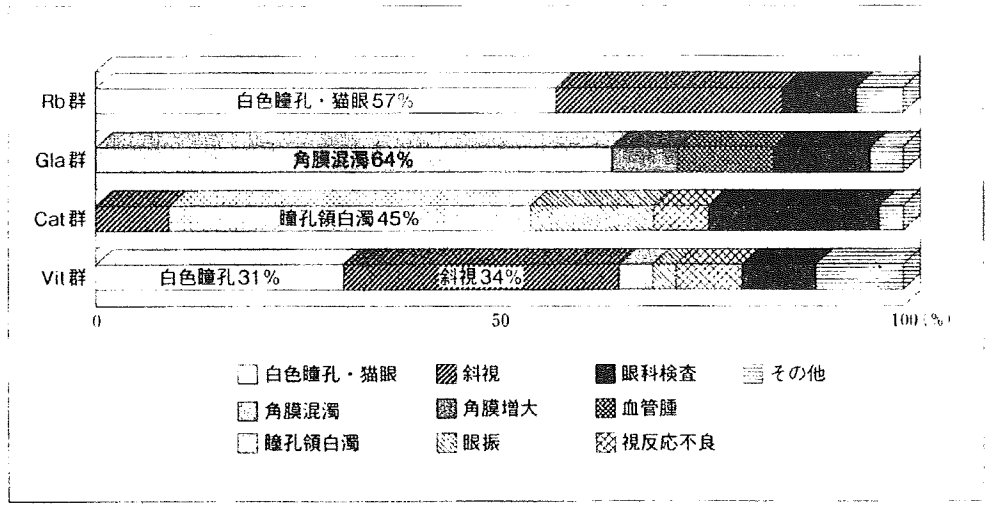
図1　主訴



緒言

乳幼児眼疾患のより良い治療のためには早期発見と的確な診断が必須である．しかし，乳児期に積極的に眼科検診を導入している地域は少なく，重症疾患を早期に発見するための対策は十分とはいえない．

今回我々は，国立成育医療センターに精査加療目的で紹介された乳幼児の重症眼疾患について，発見から受診までの経路，初診時期などの実態を調査し，早期発見のための課題を検討した．また10年前の調査[1]と比較し，近年の傾向についても検討を加えた．

対象および方法

対象は2002年3月～2008年12月に国立成育医療センター眼科へ精査加療目的で紹介され初診した網膜芽細胞腫(Rb群)46例，早発型発達緑内障(Gla群)25例，先天白内障(Cat群)75例，網膜硝子体異常(Vit群)119例，計265例の乳幼児である．Cat群には，生直後から高度の混濁をきたした例のみを含めた．Vit群の内訳は，第一次硝子体過形成遺残(PHPV)60例，家族性滲出性硝子体網膜症(FEVR)59例であった．初診に至る経緯の明確でない例やセカンドオピニオン目的で1回受診したのみの例は除外した．

各疾患群において患側，性別，家族歴，全身既往歴，主訴，発見から受診までの経路，初診年齢を調査し，初診時期の遅れについて検討を加えた．また過去の調査[1]と比較検討した．

結果

1．患側・性別

対象265例の患側は両眼性151例(57％)，片眼性114例(43％)，性別は男児148例，女児117例であった．各疾患群別にみると，患側はRb群：両眼性20例(43％)，片眼性26例(57％)，Gla群：両眼性15例(60％)，片眼性10例(40％)，Cat群：両眼性52例(69％)，片眼性23例(31％)，Vit群：両眼性64例(54％)，片眼性55例(46％)で両眼性の大部分はFEVR，片眼性はPHPVであった．性別はRb群：男児20例，女児26例，Gla群：男児15例，女児10例，Cat群：男児36例，女児39例，Vit群：男児77例，女児42例であった．

2．家族歴・既往歴

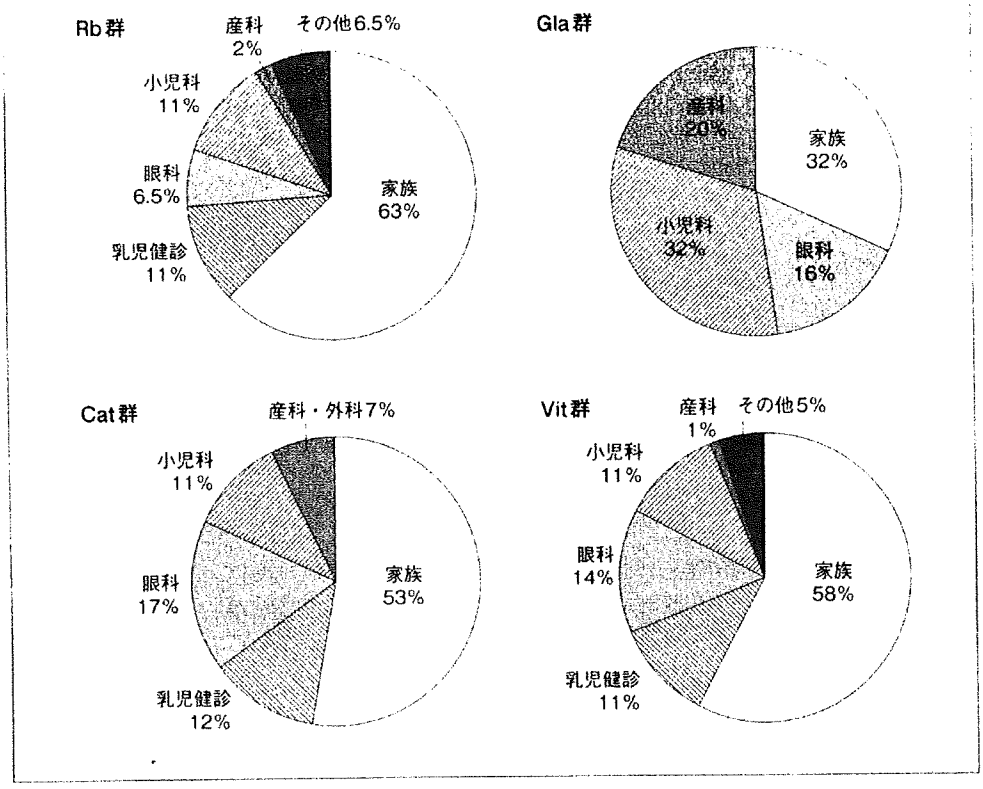
初診時に家族歴があると判明していた例はRb群：3例(6.5％)，Gla群：3例(12％)，Cat群：15例(20％)，Vit群：14例(12％)で，特にCat群に高率であった．

全身既往歴はRb群：4例(9％)，Gla群：9例(36％)，Cat群：22例(29％)，Vit群：10例(8％)で，特にGla群，Cat群に高率であった．主な全身疾患はRb群：多発奇形，ヘルニアなど，Gla群：顔面血管腫，Sturge-Weber症候群，染色体異常(21 trisomy，13 trisomy)，多発奇形，Cat群：染色体異常(21 trisomy)，全身症候群(Lowe，Hallermann-Streiff，Rubinstein-Taybi)，多発奇形，先天性心疾患，発達遅延，Vit群：染色体異常，先天性心疾患，川崎病であった．

3．主訴

眼異常の発見に至った主訴を図1に示す．Rb群では白

図2　発見・受診経路



色瞳孔・猫眼が26例(57％)を占め，斜視13例(28％)，視力低下2例(4％)，飛蚊症1例(2％)，眼底検査で発見された例が4例(9％)であった．Gla群では角膜混濁・白濁が16例(64％)を占め，角膜径増大2例(8％)，眼脂1例(4％)であった．また顔面血管腫により発見された例が3例(12％)，全身疾患のため眼科検査を依頼され発見された例が3例(12％)と高率にみられた．Cat群では瞳孔領白濁が34例(45％)を占め，眼振・異常眼球運動11例(15％)，斜視7例(9％)，視反応不良5例(7％)，小眼球・その他2例(3％)であった．また眼底検査を依頼され発見された例が16例(21％)と高率であった．Vit群では斜視が41例(34％)，白色瞳孔が37例(31％)と多く，視反応不良10例(8％)，角膜混濁5例(4％)，眼脂・充血4例(3％)，眼振3例，小眼球2例，異常頭位2例，眼瞼下垂・その他が4例であった．また家族歴等のため眼科検査を行い発見された例が11例(9％)であった．

4．発見・受診経路

眼異常が発見され成育医療センター眼科へ受診するに至った直接の契機を発見・受診経路として**図2**に示す．家族が異常に気づいて眼科受診した例(家族)，乳児健診で異常を指摘され眼科受診を促された例(乳児健診)，眼科医が発見して紹介された例(眼科)，小児科医が異常を疑い眼科へ紹介した例(小児科)，産科・小児外科医が眼科へ紹介した例(産科・外科)に大別した．

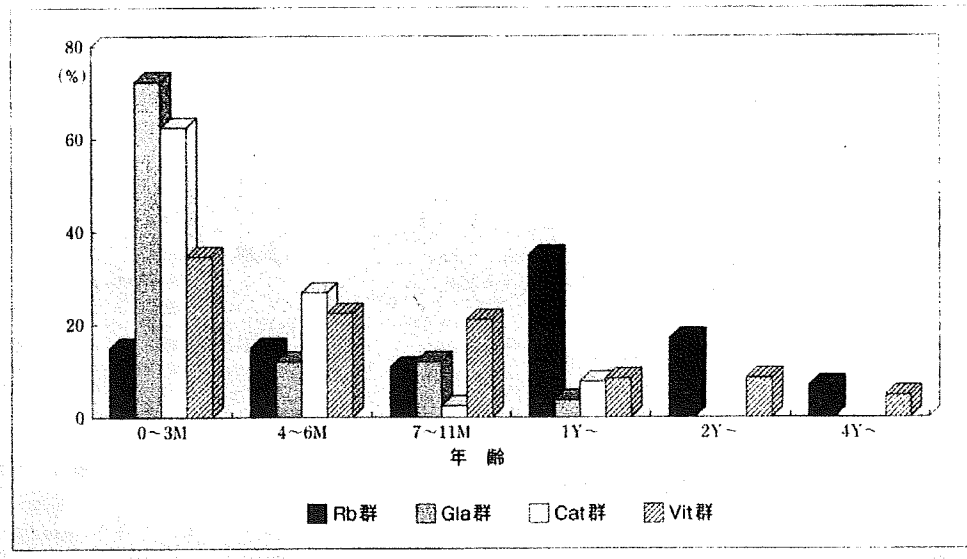
Rb群では家族が29例(63％)を占め，乳児健診，小児科が各5例(11％)，眼科が3例(6.5％)であった．Gla群では家族と小児科が各8例(63％)と多く，産科が5例(20％)，眼科が4例(16％)であった．Cat群では家族が40例(53％)を占め，眼科が13例(17％)，乳児健診が9例(12％)，小児科が8例(11％)，産科・外科が5例(7％)であった．Vit群では家族が69例(58％)を占め，眼科が17例(14％)，乳児健診，小児科が各13例(11％)，産科が1例であった．

その他に，Rb群では保育園で指摘された1例，視力低下・飛蚊症など自覚症状を契機に眼科受診した2例があった．Vit群では3歳児眼科健診で発見された4例，幼稚園で指摘された1例，就学前健診で発見された1例があった．

5．初診年齢

各疾患群の初診年齢の分布を**図3**に示す．初診年齢を0～3ヵ月，4～6ヵ月，7～11ヵ月，1歳，2～3歳，4歳以降に区分した．生後3ヵ月までに初診した例はRb

図3　初診年齢の分布



群：7例（15％），Gla群：18例（72％），Cat群：47例（62.5％），Vit群：41例（34.5％），生後6ヵ月までに初診した例はRb群：14例（30％），Gla群：21例（84％），Cat群：67例（89.5％），Vit群：68例（57％）であった．Rb群では1歳で初診した例が16例（35％）と最多であった．またVit群では初診年齢が遅い傾向があった．

各疾患群の初診年齢と両眼性，片眼性に区分した結果は以下のとおりである．Rb群：生後2週～6歳11ヵ月（平均1歳6ヵ月），両眼性；生後2週～2歳5ヵ月（平均10ヵ月），片眼性；生後2週～6歳11ヵ月（平均2歳1ヵ月），Gla群：生後2日～1歳5ヵ月（平均2.6ヵ月），両眼性；生後2日～9ヵ月（平均1.1ヵ月），片眼性；生後3週～1歳5ヵ月（平均4.8ヵ月），Cat群：生後4日～1歳7ヵ月（平均3.5ヵ月），両眼性；生後4日～6ヵ月（平均2.3ヵ月），片眼性；生後2週～1歳7ヵ月（平均6.2ヵ月），Vit群：生後6日～6歳1ヵ月（平均11ヵ月），両眼性；生後6日～6歳1ヵ月（平均1歳），片眼性；生後2週～4歳8ヵ月（平均10ヵ月）であった．Rb群，Gla群，Cat群ともに両眼性のほうが初診時期が早いが，Vit群では，両眼性（主にFEVR）のほうが軽症例を含むためか，初診時期が遅い傾向であった．

6．過去の調査との比較

初診年齢，発見・受診経路を1992年6月～1998年12月に国立小児病院を初診したRb群13例，Gla群20例，Cat群26例，Vit群58例，計117例の調査結果[1]と比較し，**表1,2**にまとめた．

初診年齢は，10年前と比較してGla群，Cat群では明らかに早期になっているが，Rb群では遅くなっていた．また発見・受診経路を10年前と比較検討すると，Gla群では家族，眼科の比率が低下しており，産科，小児科で早期に発見して眼科へ紹介される例が増えている．Cat群では乳児健診で斜視や眼振により異常が発見される前に，眼科，小児科で瞳孔領白濁や眼科検査依頼により早期発見される例が増えた．Vit群では乳児健診で指摘される例が減り，家族や眼科で直接異常を発見する例が増加している．しかし，Rb群では，家族や眼科で発見する比率が低下し，乳児健診や小児科で指摘を受けて発見される例が増加しており，発見・初診時期の遅れをきたしていた．

7．初診時期の遅れ

Rb群における初診時期の遅れを，Gla群，Cat群と比較検討した結果を**表3**に示す．異常に気づいてから国立成育医療センター眼科へ初診するまでに要した期間（主訴～初診まで）は，Gla群：平均0.7ヵ月，Cat群：平均1.4ヵ月に比べ，Rb群：平均2.6ヵ月と長く，Rb群には異常に気づいてから最長で1年5ヵ月の例があった．また主訴～初診までに3ヵ月以上を要した例（3ヵ月以上の遅れ）は，Gla群：1例（4％），Cat群：12例（16％）に対し，Rb群では15例（33％）と明らかに高率であった．乳児健診で異常なしと判断されて眼科受診に至らなかった例，健診で眼科受診を促されたが受診せず放置してしまった例が散見されたが，中には眼科受診歴があるが診断に至らなかった例があり，Gla群：3例（12％），Cat群：3例（4％）に対し，Rb群では11例（30％）と明らかに高率であった．このうちRb群には網膜剝離，硝子体混濁，硝子体出血，白内障，水晶体脱臼と診断された例のほか，

表1　過去の調査と比較：初診年齢

	1992～1998年	2002～2008年
Rb群	19日～2歳3ヵ月 （平均10.8ヵ月）	2週～6歳11ヵ月 （平均1歳6ヵ月）▲
Gla群	7日～3歳8ヵ月 （平均8.8ヵ月）	2日～1歳5ヵ月 （平均2.6ヵ月）▼
Cat群	25日～5歳1ヵ月 （平均9.5ヵ月）	4日～1歳7ヵ月 （平均3.5ヵ月）▼
Vit群	13日～10歳2ヵ月 （平均12.8ヵ月）	6日～6歳1ヵ月 （平均11.0ヵ月）

表2　過去の調査と比較：発見・受診経路

	1992～1998年		2002～2008年	
Rb群	家族	84％	家族	63％▼
	乳児健診	8％	乳児健診	11％▲
	眼科	8％▼	小児科	11％▲
Gla群	家族	40％	家族	32％▼
	小児科	30％	小児科	32％▲
	眼科	25％▼	産科	20％▲
Cat群	家族	50％	家族	40％▼
	乳児健診	27％▼	眼科	17％▲
	眼科	15％	小児科	11％▲
Vit群	家族	48％	家族	58％▲
	乳児健診	29％▼	眼科	14％▲

眼底検査を行わなかった例が含まれていた．

考按

乳幼児眼疾患の中で，特に網膜芽細胞腫，早発型発達緑内障，先天白内障，網膜硝子体異常（PHPV / FEVR）などの重症疾患は，発症頻度は少ないが，診断・治療時期が視機能や生命予後に直結するため，早期発見が重要な課題である．3歳児健診は，平成3年から眼科検診が全国で導入され，平成9年からは実施主体が都道府県から市町村に移管されて地域格差，検査精度・効率などの問題が指摘されているものの[2-4]，弱視や斜視の早期発見・治療に大きく寄与してきた．しかし，乳児健診は母子保健法のもと保健所・小児科医を中心に行われており，実施時期や方法は地域により異なっている．眼異常の検出は，問診によるスクリーニング（アンケート方式）[5]を主体としており，乳児（3～4ヵ月）や1歳6ヵ月児に眼科検診を導入する試み[6-10]は，依然として普及していない．

今回の調査結果では，眼異常の発見に至った主訴は，Rb群：白色瞳孔（57％），Gla群：角膜混濁（64％），Cat群：瞳孔領白濁（45％），Vit群：斜視（34％）が最多であった．Gla群，Cat群では生直後から外眼部を注意深く観察することによって早期発見が可能であるが，Rb群，Vit群では，かなり進行しないと外眼部から発見することは困難である．白色瞳孔や斜視に関しては，写真を撮ってみて気づいたという例が比較的多く，家族が早期に発見するためには，眼疾患に関する注意，知識の普及が不可欠であると思われた．

一方，各疾患群ともに家族歴のある例が6.5～20％と高率であり，Gla群，Cat群では全身疾患のある例がそれぞれ36％，29％と高率であった．各疾患群ともに家族歴・全身疾患があるため生後早期に眼科を受診もしくは眼科検査を依頼されて超早期発見に結びついた例がみられた．現状では，未熟児以外は眼科スクリーニング検査の基準や対策がないが，家族や他科への知識の普及と連携によって，リスクのある乳幼児が早期に眼科検査を受けられる体制をつくることが大切である．

表3　初診時期の遅れ

	Rb群（n=46）	Gla群（n=25）	Cat群（n=75）
主訴～初診まで	0～17ヵ月 （平均2.6ヵ月）	0～13ヵ月 （平均0.7ヵ月）	0～13ヵ月 （平均1.4ヵ月）
3ヵ月以上の遅れ	15例 33％	1例 4％	12例 16％
眼科受診歴あるが診断に至らず	11例 30％	3例 12％	3例 4％

発見・受診経路，初診年齢について，10年前と比較検討すると，依然として各疾患群ともに家族が異常に気づいて直接眼科に受診する例が最も多かった．Gla群，Cat群では，10年前と比較して初診時期が大幅に早くなっており，乳児健診において斜視や眼振で発見されるより前に，小児科，産科で異常を疑い眼科へ紹介される例も増加している．眼科のみならず小児科，産科においても生後早期の発見・治療が必要な重症眼疾患であるという知識が普及している成果と考えられる．これに対し，眼底疾患であるRb群，Vit群は，眼科医が積極的に散瞳して眼底検査を実施しないと早期発見が困難であるため，依然として初診年齢が遅いと考えられる．Vit群には軽症例も含まれているが，PHPV，FEVRともに乳幼児期の管理が重要であること，積極的に家族の眼底検査を実施する必要があることなど，さらに眼科医，小児科医，家族にも知識を普及させる必要がある．Rb群に関しては，

10年前と比較して，家族や眼科の発見よりも，乳児健診や小児科で指摘を受けて発見される比率が増え，初診年齢が遅くなっており非常に問題である．

Rb群における初診時期の遅れは，Gla群，Cat群に比べて明らかであり，異常に気づいてから初診までに3ヵ月以上を要した例が33％にものぼること，最長で1年5ヵ月も要した例があったことは，眼球外浸潤のリスクを高め，生命予後を左右する重大な問題である．白色瞳孔となっても外眼部からはわかりにくいが，網膜芽細胞腫について十分に情報を発信し，乳児健診で異常を指摘されたり，家族が異常に気づいたら放置せず，すぐに眼科受診をするように注意を喚起する必要がある．またGla群，Cat群に比べ，眼科受診歴があるにもかかわらず正確な診断に至らなかった例，眼底検査を行わなかった例が30％と高率であった点も，緊急度の高い疾患として問題である．特に眼底疾患に関しては，小児眼科を専門とする眼科医が少なくなり，以前よりも検出率が低下している可能性がある．乳幼児の眼底疾患の知識，眼底疾患の検出・診断法，眼底検査の重要性について，一般眼科医に対するさらなる啓蒙も必要であると思われた．

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液晶視力表システムチャートSC-2000によるロービジョン児のコントラスト視力測定と遮光レンズの効果

Evaluation of contrast visual acuity and effect of absorptive lens in children with low vision

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【要約】　目的：ロービジョン児のコントラスト視力を測定し，遮光レンズが低コントラスト視力に与える影響を検討した．

対象および方法：国立成育医療センターを受診した48例，(男児21例，女児27例，年齢3～16歳，平均6.4歳)を対象とし，液晶視力表システムチャートSC-2000を用いてコントラスト視力を測定した．48例中27例で遮光レンズ装用下での低コントラスト視力を測定した．

結果：コントラスト視力の平均値 (log MAR) は，コントラスト100％で0.73，25％で0.86，12.5％で0.92，6％で1.03で，視標のコントラストが低下すると有意に視力も低下した．遮光レンズ装用下での6％コントラスト視力の平均値は，遮光レンズ装用の前後で有意差はなかった．

結論：ロービジョン児では，視標の種類が多く低視力用の視標が表示できる液晶視力表は有用であった．

【キーワード】　ロービジョン児，液晶視力表，低コントラスト視力，遮光レンズ

【Abstract】　Purpose : To measure the contrast visual acuity and evaluate the effect of absorptive lenses in children with low vision.

Methods : The visual acuities of different contrast of 48 children aged from 3 to 16 with low vision, who visited National Center for Child Health and Development, were measured by liquid crystal displayed visual acuity chart, SC-2000. In these 48 cases, 27 cases could be assessed low contrast visual acuity with and without absorptive lenses.

Results : The average contrast visual acuity (log MAR) was 0.73 in contrast ratio 100％, 0.86 in 25％, 0.92 in 12.5％, 1.03 in 6％ respectively. The contrast visual acuities liner related with the chart contrasts. There was no significant difference of the 6％ contrast visual acuity with and without absorptive lenses.

Conclusion : The visual acuity chart displayed by liquid crystal was useful for measuring low contrast visual acuities in children with low vision.

【Keywords】　Low vision children, Liquid crystal displayed visual acuity chart, Low contrast visual acuity, Absorptive lens

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緒言

コントラスト視力は日常の視環境における視力を推定

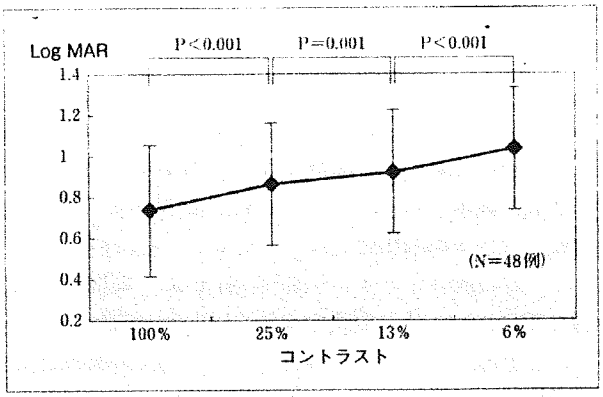

図1　コントラスト視力の平均値(log MAR)

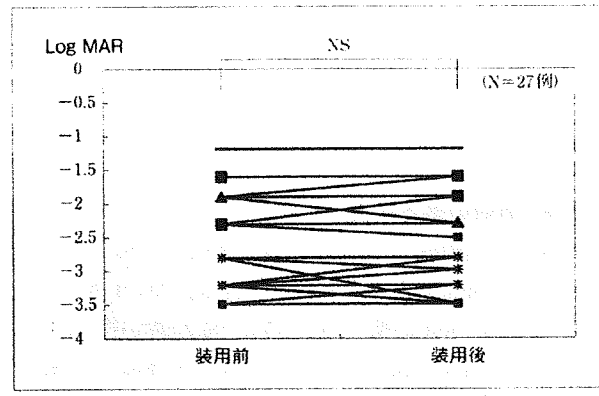

図2　遮光レンズ装用下での低コントラスト視力(平均値)

でき，視機能をより鋭敏に評価する方法として有用である[1-4]．しかし，コントラスト感度の測定は，低年齢のロービジョン児では，測定環境に左右されたり，注意力が低下するため，施行が困難である[3,4]．また，自覚的な訴えの少ないロービジョン児においても，遮光レンズの効果は大いに期待されるが，その装用効果を客観的に判定することは難しい[4,5]．

液晶視力表システムチャートSC-2000®（ニデック社製，以下：SC-2000）は，小児に用いられる絵視標やランドルト環などの視標を低視力者用に表示することができる[6]ため，年齢やロービジョンにより検査が難しいロービジョン児における低コントラスト条件での検査も可能と考えられる．

今回，我々はSC-2000を用いてロービジョン児のコントラスト視力を測定し，遮光レンズの低コントラスト視力に与える影響を検討した．

対象および方法

対象は，国立成育医療センターのロービジョン外来で2007年9月～2009年1月に診療した48例，性別は男児21例，女児27例，年齢は3～16歳(平均6.4歳)である．視覚障害の原因疾患は，先天異常が最も多く56％で，次いで錐体ジストロフィが15％，中枢性視覚障害が13％，未熟児網膜症が10％，網膜芽細胞腫が4％，その他が2％であった．先天異常では，小眼球，家族性滲出性硝子体網膜症，先天白内障，など多彩な疾患が視覚障害の原因であった．

コントラスト視力の測定条件として，検査距離3m，視標コントラストは100％，25％，12.5％，6％の4段階で，両眼開放・屈折矯正下で字ひとつ視力を測定した．児の発達の段階に応じて視標を選択したため，84％でランドルト環，16％で絵視標であった．コントラスト視力は，コントラスト100％，25％，12.5％，6％の順に各1回測定し，明らかに信頼性のない測定値については除外した．

全48例中27例で東海光学CCP®およびCCP400®シリーズの遮光レンズ装用下での6％コントラスト視力を測定し，遮光レンズ装用前の6％コントラスト視力と比較した．遮光レンズは，検査時間の関係上，トライアル回数に制限があるため，あらかじめ児や家族の好みや効果を予測して数種類を選定し，最も効果的であったレンズを装用したときの6％コントラスト視力を評価した．また，実際の遮光眼鏡の処方状況も検討した．統計解析は，paired-t検定を用い，有意水準は1％未満とした．

結果

1．コントラスト視力

コントラスト視力の平均値は，log MAR値で，視標コントラスト100％で0.73，25％で0.86，12.5％で0.92，6％で1.03で，視標のコントラストが低下すると有意に視力も低下した(図1)．

2．遮光レンズ装用下での6％コントラスト視力

トライアルに用いた遮光レンズの種類は，東海光学CCP®およびCCP400®シリーズの，AC 9例，OY 6例，BR 3例，LY 3例，YL 1例，RO 1例，FL 1例，YG 1例，SC 1例，NL 1例，であった．48例のうち最適の遮光レンズ装用下での6％コントラスト視力を測定した27例の平均値は，装用前が1.09であったのに対し，装用後では