

CASE REPORT

An autopsy case of cyclopia with 13 trisomy with special reference to histological abnormalities of the eyeball

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ABSTRACT We present an autopsy case of cyclopia and alobar holoprosencephaly and polydactyly with 13 trisomy. A 27 year-old Japanese female at the 27th gestational week was diagnosed as hydramnios and the fetus showed hydrocephalus and intrauterine growth retardation. The fetus was suspected to be cyclopic and holoprosencephalic by ultrasonograph and MRI images. The mother delivered a stillborn male baby at the 30th week of gestation. At autopsy, the baby showed true cyclopia having one eyeball and two irides in a single ocular opening, and one proboscis. On histological analysis of the eye, there was marked dysplastic hyperplasia of the retina with rosettes, focal degeneration of the retina with calcification, and prominent proliferation of glial cells beneath the hyperplastic retina. Multiple glomerular structures in the cerebral cortex and aplasia of the corticospinal tract were observed. In the spinal cord, a few neurons with pyknosis were observed in the ventral horn. Although no mutation was detected in the Sonic hedgehog in the present case, we reviewed recent studies concerning the molecular mechanisms of cyclopia and holoprosencephaly.

Key words: cyclopia, holoprosencephaly, 13 trisomy, synophthalmia

INTRODUCTION

Holoprosencephaly (HPE) is a common developmental defect of the forebrain and frequently associated with malformations of the midface in humans with both genetic and environmental causes. HPE has a prevalence of 1:250 during embryogenesis and 1:16,000 newborn infants (Matsunaga and Shiota, 1977), and involves incomplete development and sep-

tation of midline structures in the central nervous system with a broad spectrum of clinical severity (Cohen, 1989). The condition can be graded according to the degree of severity as alobar, semilobar, or lobar holoprosencephaly. Various gradations of facial dysmorphism are commonly associated with holoprosencephaly including cyclopia, ethmocephaly (ocular hypotelorism with a proboscis), cebocephaly (ocular hypotelorism and blind-ended, single-nostril nose), median cleft lip (Cohen and Sulik, 1992). Cyclopia is estimated to occur about once in 40,000 births (Barber and Muelling, 1950). Most fetuses with cyclopia have two eyeballs with varying degrees of fusion called synophthalmia. Extremely rare cases present only one eyeball, called true cyclopia (Torczynski *et al.*, 1977; Garzosi and Barkay, 1985). Here we present an autopsy case of true cyclopia with 13 trisomy with special attention focused on histological examination of the eyeball and a review of the molecular mechanisms of cyclopia and holoprosencephaly.

CASE REPORT

A 27 year-old Japanese female at the 27th gestational week was admitted to the Hamamatsu University Hospital in the diagnosis of hydramnios with suspicion of having a fetus with hydrocephalus and intrauterine growth retardation in 1998. She had no history of previous delivery and had no background of hereditary diseases or a consanguineous marriage. There had been no increase of titers of antiserum against microbes such as toxoplasma or cytomegalovirus. The fetus was suspected to be holoprosencephalic and cyclopic by ultrasonograph and MRI images (Fig. 1A and 1B, arrow shows cyclopia). Chromosomal pattern was 13 trisomy by amniocentesis (not shown). She delivered a stillborn male baby at the 30th week of gestation. Birth weight was 740 g and length was 40 cm. The placenta was normal but had a single umbilical artery (not shown). Laboratory tests revealed slight hypercholesterolemia (234 mg/dl). She was not under any medi-

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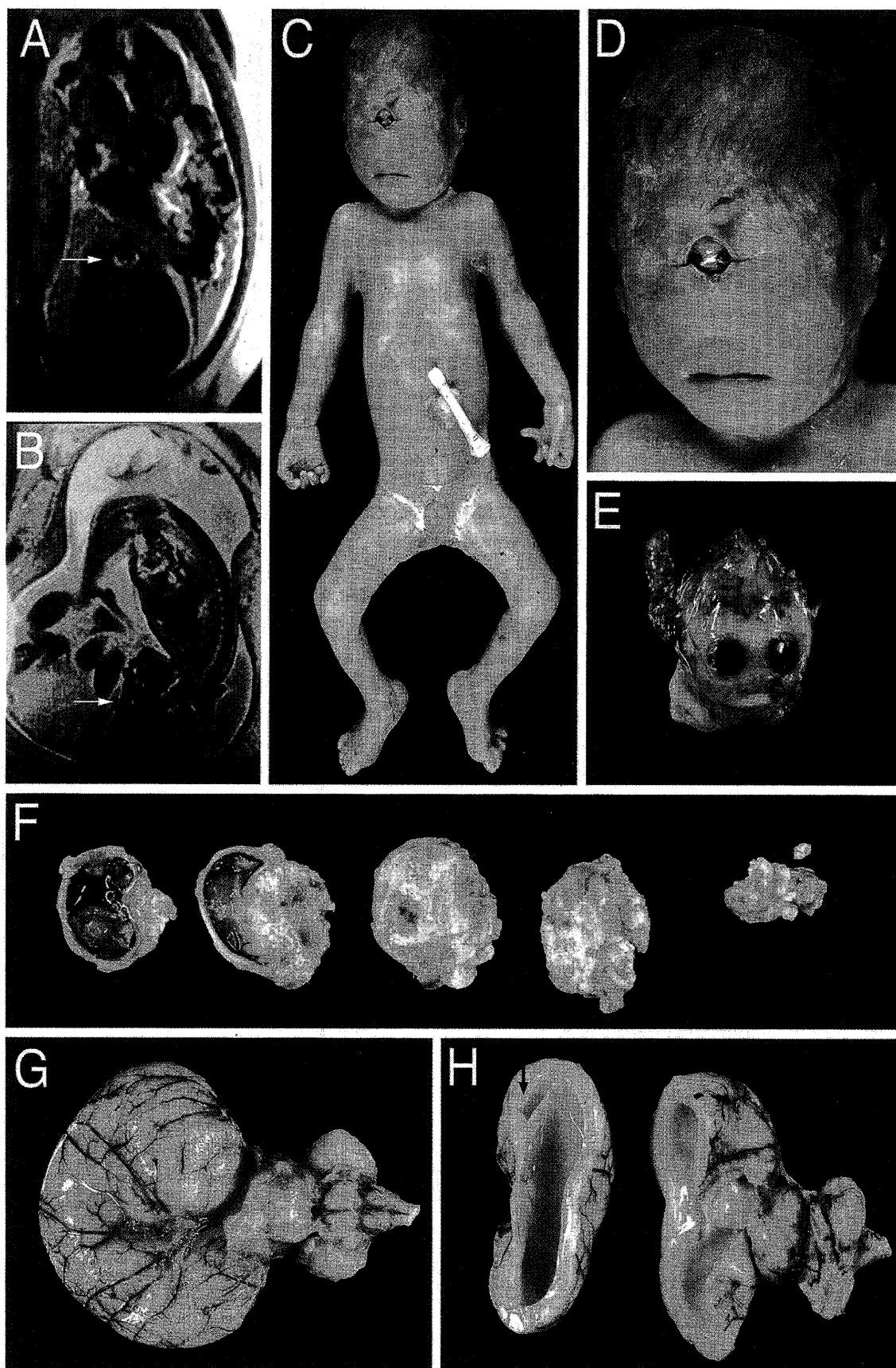


Fig. 1 Gross appearance of holoprosencephaly and cyclopia. **A and B:** MRI images of the fetus. The frontal section view (**A**) and the sagittal section view (**B**). The cyclopia is indicated by the arrow. **C and D:** Anterior view of the full-length figure (**C**) and the face (**D**). Fetus shows cyclopia and a proboscis in the face and hexadactyly of both hands and the left foot. **E and F:** The anterior view (**E**) and the serial coronal section view of the eye (**F**). Eye has one eyeball and two irides and one lens without septum. **G and H:** The brain shows no olfactory bulbs and shows a single central ventricle without corpus callosum. A periventricular cyst is observed in the cerebrum (**H**, arrow). The brain stem and the cerebellum are hypoplastic.

cation and had no history of alcohol abuse.

On external examination, the fetus showed marked malformations, such as cyclopia and a proboscis, hexadactyly of the both hands and the left foot (**Fig. 1C and 1D**). The craniofacial abnormalities consisted of a single ocular opening that had one eyeball and two irides (**Fig. 1D and 1E**), and a proboscis positioned in the midline directly superior to the ocular aperture (**Fig. 1C and 1D**).

Autopsy was performed 3 hours after the stillbirth. The brain had no olfactory bulbs and had a single central ventricle without corpus callosum and midline fissure, which was compatible with alobar holoprosencephaly. The gyri were poorly developed and the basal ganglia, thalamus and globus pallidus could not be identified (**Fig. 1G and 1H**). The brain stem and cerebellum were small in size (**Fig. 1G and 1H**).

For histological examination of the eye, serial coronal sections were made from the frontal portion of the eyeball to the optic nerve (**Fig. 1F**). There was only one eyeball and one lens without a septum (**Fig. 1F and 2A**), which is compatible with true cyclopia, although two irides were observed (**Fig. 1E**). The cornea, iris and lens were relatively well developed, but the ciliary body could not be identified (**Fig. 2A**). The sensory retina showed irregular hyperplasia retained behind the lens (**Fig. 2A and 2D**). These dysplastic hyperplasia of the retina often showed rosettes as described by Torczynski *et al.* (1977). Most parts of the retina could be stratified into several distinct layers similar to a normal retina (**Fig. 2E**). The retina showed some degeneration with heterotopic calcification (**Fig. 2F and 2H**). Beneath the retina, there was a thickened layer with glial cell proliferation, stained with antibodies to GFAP and S-100 (**Fig. 2G and 2H**). A single optic nerve entered the cranial cavity through a single optic canal at the sphenoidal bone (**Fig. 2B**). At the posterior portion, a single optic nerve divided into two hypoplastic optic nerves (**Fig. 2C**). The degenerated retina was entered into the optic nerve (**Fig. 2J, 2K and 2L**). Axonal hypoplasia or degeneration was observed by Bodian staining (**Fig. 2J**). The entire optic nerve was labeled with anti-GFAP and anti-tau (**Fig. 2K and 2L**), and also with anti-S100 (not shown). Tau is a microtubule-associated protein expressed in outgrowing axons. Tau positive neurons were observed along the hyperplastic and dysplastic retina (**Fig. 2I**).

The cytoarchitecture of the cerebral cortex was almost normal, although the brain mantle was hypoplastic (**Fig. 3A**). Multiple glomerular structures, as described in the brain of alobar holoprosencephaly by Mizuguchi and Morimatsu (1989), were observed between the internal granular layer and the external pyramidal layer (**Fig. 3C**). We could not find any evidence of neuronal migration disorder in this case. There were abnormal neuronal clusters in the brain basalis (**Fig. 3E and 3F**) and also in the ventral side of the pons (**Fig. 3G and 3H**). The arrangement of these neuronal cells was different among these portions (**Fig. 3E, 3F and 3H**). In the cerebel-

lum, the dentate nucleus showed no abnormalities (**Fig. 3I**) and the superficial granular layer persisted but appeared normal for this gestational age (**Fig. 3J**). Aplasia of the corticospinal tract was observed in the ventral portion of the pons (**Fig. 3G**) and in the ventral portion of the olivary nucleus in the medullar oblongata (**Fig. 3K and 3L**). The spinal cord showed marked hypoplasia of the ventral column (**Fig. 3M**) and only a few neuronal cells were observed with occasional pyknotic cells (**Fig. 3N**).

DISCUSSION

The present case was true cyclopia with two irides, combined with alobar holoprosencephaly and 13 trisomy with polydactyly. Holoprosencephaly has a prevalence of 1:250 during embryogenesis and 1:16,000 newborn infants (Matsunaga and Shiota, 1977). Chromosomal anomalies have been reported in 24-45% of live born infants with holoprosencephaly, the most common of which is trisomy 13 (Golden, 1999). Trisomy 13 is estimated to occur in 1 in 5,000 live births (Nielsen and Sillesen, 1975). Among trisomy 13, nearly 70% of the patients have holoprosencephaly (Cohen, 1989). Occurrence of cyclopia was estimated to be 4.9% among holoprosencephaly (Olsen *et al.*, 1997). Prevalence of polydactyly was estimated to be 16.8% among holoprosencephaly (Matsunaga and Shiota, 1977) and 46.2% among cyclopia (Torczynski *et al.*, 1977).

In the present case, we focused on the histology of the eye. Three prominent features were observed in our case. First, there was marked dysplastic hyperplasia of the retina with rosettes, although mild dysplasia with rosettes was reported previously (Torczynski *et al.*, 1977; Garzosi and Barkay, 1985). Second, focal degeneration of the retina with calcification was prominent. Mild degenerative change was reported previously (Torczynski *et al.*, 1977), however, calcification of the retina was not mentioned. Thirdly, prominent proliferation of glial cells was observed beneath the hyperplastic retina. The reason for the retinal hyperplasia with tau positive neurons along the retina is not known. However, degeneration with calcification of the retina was also observed, probably due to axonal degeneration of the optic nerve (Kakita *et al.*, 1997) or insufficient blood supply (Torczynski *et al.*, 1977). These degenerative processes may stimulate glial proliferation beneath the retina (Fulcrand and Privat, 1977; Privat *et al.*, 1981).

The optic nerve of the present case showed poor development of axonal fibers revealed by Bodian staining although GFAP and S100 and tau stainings were preserved. These findings are somewhat different from former case report in which tau immunoreactivity was restricted to the marginal zone and the central zone was labeled with anti-GFAP (Kakita *et al.*, 1997). Hypoplastic and degenerative changes of the optic nerve are in accordance with experimental studies in which Wallerian

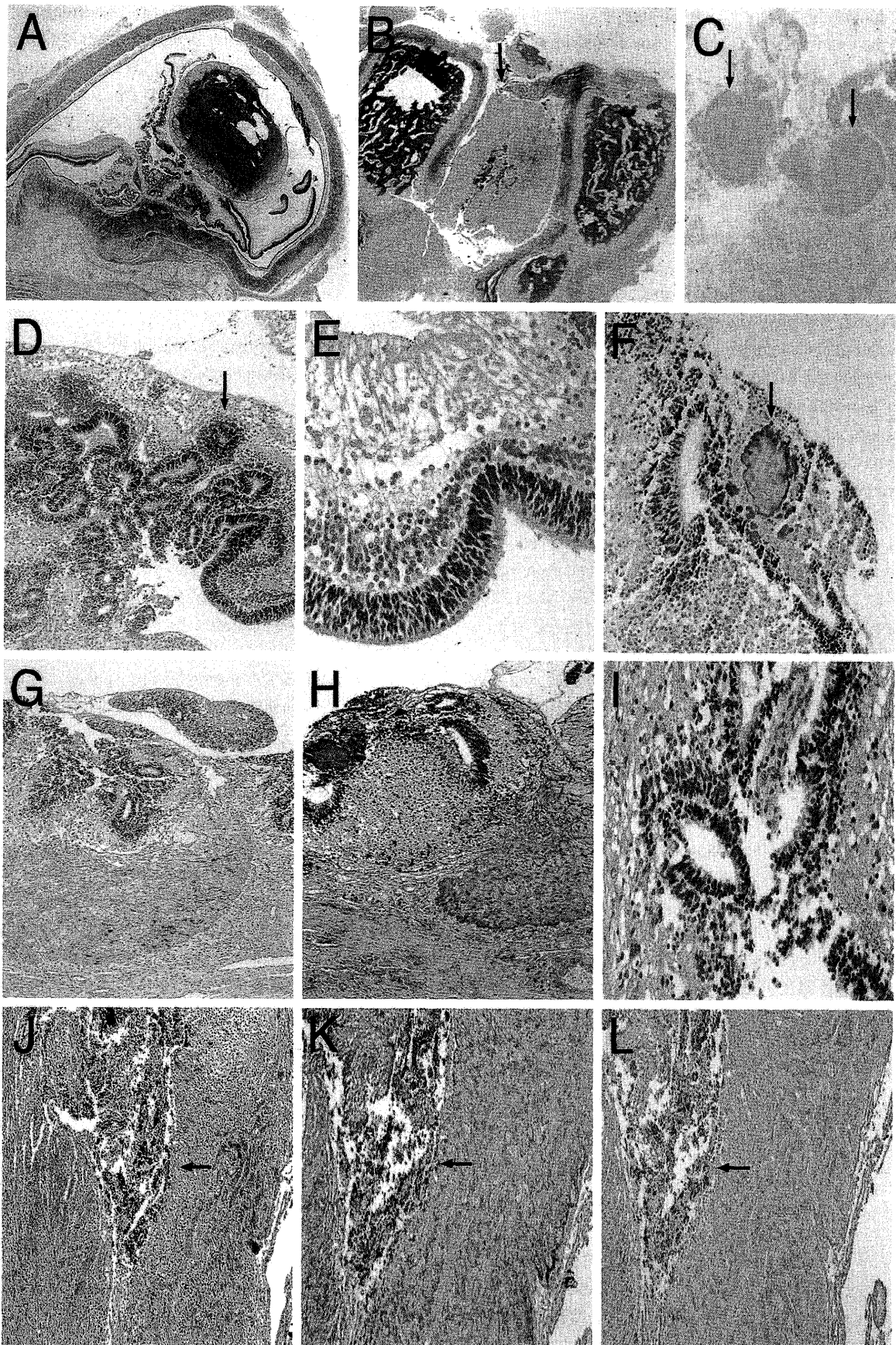


Fig. 2 Histopathology of the eyeball and optic nerve. **A-C:** Serial coronal sections of the eye and the optic nerve. **A:** Low power field, HE $\times 4$. **B and C:** Portion of the optic nerve. **B:** A single optic nerve, HE $\times 4$. **C:** Two hypoplastic optic nerves, HE $\times 10$. The optic nerves are indicated by the arrows. **D:** Dysplastic hyperplasia of the retina with rosette formation (arrow), HE $\times 40$. **E:** A portion of the retina showing relatively normal cytoarchitecture, HE $\times 120$. **F:** Degenerated retina with calcification (arrow), HE $\times 80$. **G-I:** Thickened layer with cell proliferation beneath the retina. **G:** GFAP $\times 10$. **H:** S-100 $\times 10$. **I:** Tau-positive neuronal cells along with the retina. tau $\times 80$. **J-L:** Hypoplastic axonal fibers. Degenerated retina is observed in the optic nerve (arrow). **J:** Bodian $\times 30$, **K:** GFAP $\times 30$, **L:** tau $\times 30$.

degeneration of neuroepithelial cells was induced as described above (Fulcrand and Privat, 1977).

In the cerebral cortex of the present case, multiple glomerular structures were observed between the internal granular layer and the external pyramidal layer in the cerebral cortex. Mizuguchi and Morimatsu (1989) reported that multiple glomerular structures were observed in 3 of 6 cases of alobar holoprosencephaly and these structures consisted of fine dendrites and axons. Glioneural heterotopia with irregular neural arrangement were observed in the brain basalis and ventral portion of the pons as described by Dimmick and Kalousek (1992). Mizuguchi and Morimatsu (1989) reported that marginal neuroglial heterotopia was also observed in all six autopsy cases of alobar holoprosencephaly.

Aplasia of the corticospinal tract observed in the present case is a consistent feature in holoprosencephaly and anencephaly. It also occurs in many cases of porencephaly and hydranencephaly (Friede, 1989). Severe hypoplasia of the ventral column and only a few neurons in the ventral horn were prominent findings in the present case. The pyknotic neurons were scattered in these regions, suggesting the presence of neurons in the early neuronal stage.

Until recently, the pathogenesis of holoprosencephaly and cyclopia remain to be fully clarified. However, recent findings of neural tube patterning and the identification of mutations in several genes have begun to clarify this mechanism (Wallis and Muenke, 1999). It has been known that holoprosencephaly and cyclopia may be caused by some environmental and maternal factors such as maternal ingestion of massive doses of salicylates, smoking, maternal alcohol consumption, or maternal diabetes (Croen *et al.*, 2000), and also maternal infection with microbes such as cytomegalovirus (Byrne *et al.*, 1987). In the experimental studies, exposure of vertebrate embryos to ethanol causes cyclopia and holoprosencephaly (Peiffer *et al.*, 1979; Bonnemann and Meinecke, 1990; Siebert *et al.*, 1991). The cyclopamine, a steroid isolated from the desert plant *Veratrum*, also produces cyclopia and holoprosencephaly when administered to sheep embryos (Bryden *et al.*, 1973). Cholesterol inhibitors, triparanol and AY 9944 are also causative agents for these anomalies (Repetto *et al.*, 1990; Golden, 1999). Both cyclopamine and cholesterol inhibitors block the Sonic hedgehog signal transduction pathways (Kelley *et al.*, 1996; Cooper *et al.*, 1998). In the present case, the mother was not suffering from diabetes but from slight hypercholesterolemia. She was not under any medications, nor an alcohol abuser, and showed no elevation of antiserum titer to cytomegalovirus. We do not think that the known environmental factors influenced the development of this baby to cause cyclopia.

Except for teratogens, genetical factors play a major role in the pathogenesis of this anomaly. Non-random cytogenic rearrangements revealed four loci, HPE1 (21q22.3), HPE2 (2p21), HPE3 (7q36), and HPE4 (18p) as the cause of this

anomaly (Ming and Muenke, 1998). HPE3 contains the Sonic hedgehog (SHH) gene (Belloni *et al.*, 1996). SHH is critical for the formation of several different organ systems, including the brain, eyes, somite, spinal cord, craniofacial structures, and limbs. SHH is expressed in Hensen's node, the notochord, and the ventral midline of the developing forebrain, and is important for induction of the developing ventral neural tube (Ericson *et al.*, 1995). Mutations in SHH have been found in familial holoprosencephaly cases and also in sporadic cases (Belloni *et al.*, 1996; Roessler *et al.*, 1996). Chiang *et al.* (1996) produced the SHH knockout mice that showed holoprosencephaly and cyclopia. In the present case, we examined exon1 and exon2 of the SHH genome sequence using the same primers described by Roessler *et al.* (1996). However, we could not find any mutations in these sequences (not shown), although the whole sequence of this gene was not examined.

Holoprosencephaly is seen in patients with deletions and duplications of chromosome 13. Mapping of the deletion sequence of patients with 13q32 deletion syndrome led to identification of the ZIC2 gene deletion. Haploinsufficiency for ZIC2 is likely to cause holoprosencephaly (Brown *et al.*, 1998). However, neither ZIC2 knockdown mouse nor ZIC2-deficient patients showed obvious abnormalities in the face, which is different from the SHH mutation syndrome (Brown *et al.*, 1998; Nagai *et al.*, 2000). Several findings suggest that ZIC2 is essential for the timely differentiation of the dorsal neural tube, the eye and the distal limb during development. Later expression is limited to the cerebellum (Nagai *et al.*, 1997). These findings suggest that the mechanism of the cause of holoprosencephaly by mutation of the ZIC2 may be different from that by mutation of the SHH.

It has been hypothesised that holoprosencephaly results from a primary defect of induction and patterning of the rostral neural tube which occurs in the first 4 weeks of gestation (Muller and O'Rahilly, 1989). Induction of the ventral neural tube is mediated by the secreted molecule coded from the SHH gene. On the other hand, the genetic pathways associated with dorsal induction and patterning are thought to involve bone morphogenic proteins (BMP) and the ZIC2 gene. However, detailed mechanisms are less well defined (Golden, 1999).

Patients with the 13 trisomy are highly associated with holoprosencephaly and also with cyclopia although the frequency is low (Cohen, 1989; Olsen *et al.*, 1997). The precise relationship between 13 trisomy and the mechanisms for these anomalies is not known. We are interested in gene ZIC2, because it is located on the chromosome 13 (Brown *et al.*, 1998). Both patients with deletion of chromosome 13 and with ZIC2 mutation showed holoprosencephaly but not facial abnormalities, but some cases with trisomy 13 showed both holoprosencephaly and cyclopia as shown in the present case. In 13 trisomy, it is suggested that expression of the ZIC2 protein should be higher than that of normal fetuses during

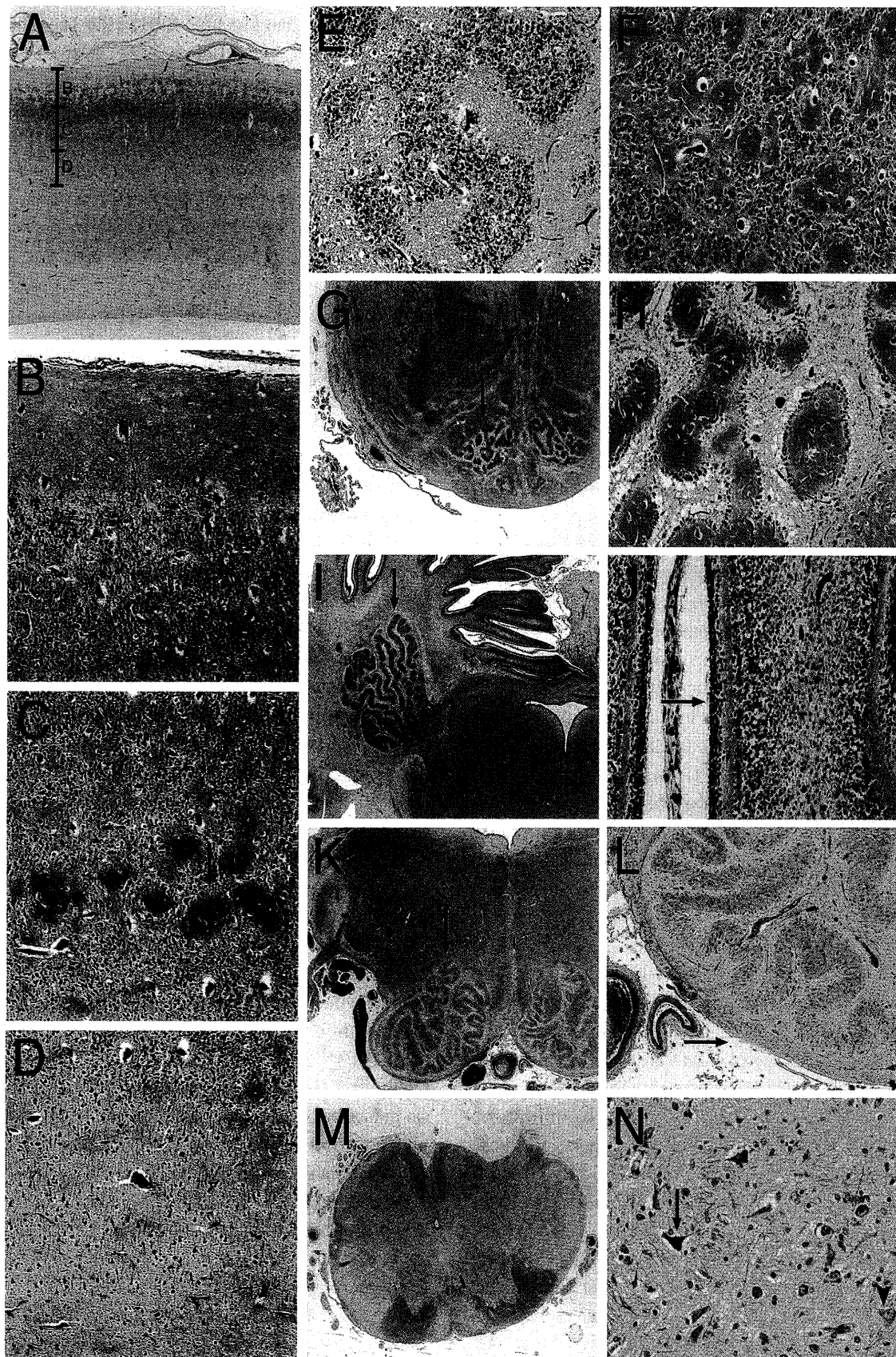


Fig. 3 Histopathology of the central nervous system. **A-D**: Cytoarchitecture of the cerebral cortex. **A**: Low power field, Bodian $\times 10$. **B-D**: The higher magnification fields of **A**. **C**: Multiple glomerular structure (arrow) are observed between the internal granular layer and the external pyramidal layer, Bodian $\times 70$. **E and F**: Abnormal neuronal clusters in the brain basalis, KB $\times 100$. **G and H**: Abnormal neuronal clusters in the ventral side of the pons (**G**, arrow). Agenesis of the pyramidal tracts. KB $\times 4$. **H**: High power field of the abnormal neural clusters. KB $\times 40$. **I and J**: Cerebellum. **I**: The dentate nucleus is indicated by an arrow. KB $\times 4$. **J**: Cerebellar cortex. The superficial granular layer is still present (arrow). KB $\times 100$. **K and L**: The olivary nucleus (arrow) in the medullar oblongata. Corticospinal tract cannot be observed, **K**: KB $\times 4$. **L**: KB $\times 20$. **M and N**: Spinal cord. Hypoplasia of the ventral column. **M**: Low power field, KB $\times 7$. **N**: The ventral column. Only a few neuronal cells (arrow head) are observed with occasional pyknotic cells (arrow), KB $\times 200$.

neurogenesis. Therefore, we focused on Gli, a zinc finger transcription factor that regulates the transcription of SHH target genes by both repressing and activating target gene expression (Alexandre *et al.*, 1996). Gli1 is expressed in the ventral neural tube (Ruiz, 1997). Interestingly, in *Xenopus*, Gli1 mediates SHH signaling to form floor plate cells and ventral neurons (Lee *et al.*, 1997). Brewster *et al.* (1998) reported in *Xenopus* ZIC2 inhibited neurogenesis and induced neural crest differentiation. In contrast, Gli proteins induced neurogenesis and inhibited neural crest differentiation and their functions counteract each other (Brewster *et al.* 1998). Human ZIC2 is a dosage-sensitive gene in which heterozygous mutations can lead to holoprosencephaly. It appears to be important to know whether overexpression of ZIC2 induces holoprosencephaly and cyclopia.

Golden *et al.* (1999) reported that ectopic expression of BMP4 or 5 in the chick prosencephalon results in holoprosencephaly, cyclopia, and a proboscis. Holoprosencephaly may be induced by disrupting the dorsal-ventral patterning of the neuronal tube, either by loss of ventralizing factors, such as SHH, or an excess of dorsalizing factors, such as BMPs. Similarly, if a 13 trisomy fetus shows over expression of ZIC2 protein more than a normal fetus, it might disrupt the dorsal-ventral patterning through inhibition of the Gli functions. Since SHH mediates Gli in normal neural development, the indirect persistent inhibition of SHH may cause holoprosencephaly and cyclopia. There may be a pathway other than the Gli/ZIC interaction that causes these anomalies, and this is also the possibility that the overexpression of unknown genes located on chromosome 13 might contribute to this malformation. The ZIC2 transgenic mice may be necessary for further examination and clarify this hypothesis.

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