Anterior Chamber Pathology in Alagille Syndrome

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Key Words

Alagille syndrome · Posterior embryotoxon · Iris clefts · Lacy vacuolization · JAG1 · Ocular pathology · Anterior segment, pathology · Genetics · Pediatric ophthalmology

Abstract

Background: Alagille syndrome is an autosomal dominant disorder characterized by neonatal cholestasis, characteristic facies, and cardiac abnormalities. Ocular abnormalities include posterior embryotoxon, mosaic pattern of iris stromal hypoplasia, microcornea, optic disc drusen, and pigmentary retinopathy. We present the second report of ocular pathology in two cases of Alagille syndrome. **Methods:** Gross and histologic preparations of four eyes of two patients. **Results:** Posterior embryotoxon is seen in both cases, with iris processes extending to the embryotoxon in case 1. Case 1 exhibited distinctly abnormal iris stroma with a prominent cleft separating the anterior and posterior stroma. Lacy vacuolization of the iris pigment epithelium was seen in case 2. **Conclusions:** Alagille syndrome is primarily a hepatic disorder but presents with several distinct ocular pathologic features, most specifically posterior embryotoxon. This and the unusual iris stroma may be caused by improper migration of neural crest cells due to mutation in the *Jagged 1* gene that

causes Alagille syndrome. Patients with Alagille syndrome rarely present to ocular autopsy. Pathology findings may help us better understand the pathophysiology of the ocular abnormalities in this disorder.

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Introduction

Alagille syndrome, also known as arteriohepatic dysplasia, is a systemic autosomal dominant disorder characterized by neonatal cholestasis due to intrahepatic biliary hypoplasia. It is caused by mutation in the *Jagged 1* (*JAG1*) gene of the NOTCH pathway [1, 2]. In 1975, Alagille and associates [3] first described the systemic abnormalities of Alagille syndrome, including pulmonary artery hypoplasia, pale skin and hair, characteristic triangular facies, butterfly vertebral arches, intellectual disability, and growth retardation. The ocular abnormalities of Alagille syndrome were discovered later and have aided in the diagnosis of Alagille syndrome. The most common ocular finding is posterior embryotoxon [4]. Other ocular anomalies include a mosaic pattern of iris stromal hypoplasia, microcornea, optic disc drusen, and pigmentary retinopathy [5]. We are aware of only one prior report of

Fig. 1. Posterior embryotoxon. **a, c** Posterior embryotoxon is seen as a translucent ridge (arrow) on the posterior surface of the peripheral cornea in cases 1 and 2, respectively. Several delicate iris processes bridge the angle and adhere to the embryotoxon in case 1. **b, d** Posterior embryotoxon (arrow) appears as an eosinophilic nodule of paucicellular collagen at the junction between the trabecular meshwork and Descemet's membrane. Embryotoxon is seen at higher magnification in the insets. **b** H&E, $\times 50$, inset $\times 400$. **d** H&E, $\times 50$, inset $\times 400$.

ocular pathologic features of Alagille syndrome [6]. We present two additional cases of Alagille syndrome with distinct iris and ciliary abnormalities in addition to posterior embryotoxon.

Materials and Methods

Four eyes from two patients were obtained at autopsy. Pathology preparations were made using hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and PAS after diastase digestion. IRB/Ethics Committee approval was not required for this study.

Results

Case 1

This boy was born at 37 weeks' gestation and was well until 3 months of age when he was found to have bilateral branch pulmonary artery stenosis and an anomalous left coronary artery, which was repaired. He was subsequently noted to have jaundice and elevated serum aminotransferases. An intraoperative cholangiogram was normal, but liver biopsy demonstrated cholestasis and bile duct paucity. A small intragenic deletion in *JAG1* confirmed the diagnosis of Alagille syndrome. No vertebral anomalies were present. Renal tubular acidosis and

systemic hypertension developed, and his cholestasis progressed with total bilirubin levels >13 mg/dl (normal 0.6–1.4) and cholesterol levels $>1,100$ mg/dl (normal 45–182). He developed progressive portal hypertension and splenomegaly in the third year of life. At 32 months of age, bilirubin markedly increased, anemia developed, portal hypertension worsened with hypersplenism, and thrombocytopenia developed. Ultrasound demonstrated evidence for hepatic cirrhosis. He died at 40 months of age as a result of intra-abdominal bleeding complicated by cardiac and hepatic failure.

At 14 months of age, he was noted to have central, steady, maintained equal vision in both eyes. He had bilateral scleral icterus and bilateral posterior embryotoxon. Intraocular pressures were normal. Refraction was $-1.50 + 2.25 \times 180$ in the right eye and $-1.00 + 2.00 \times 15$ in the left eye. The eye examination remained stable until the time of his death.

The right and left globes measured $22 \times 22 \times 23$ mm in diameter, and the iris and choroid were darkly pigmented. Relucent ridges consistent with posterior embryotoxon were evident grossly on the posterior corneal surface anterior to the angle in both eyes (fig. 1). A few delicate iris processes bridged the angle and inserted onto the embryotoxon. In routine histopathologic sections, the embryotoxon appeared as nodules of paucicellular extracellular matrix material at the junction between the trabecu-

Fig. 2. Case 1. Abnormal iris stroma (**a-d**). **b** Note the disorderly array of thick bands of iris stroma on the anterior surface of the iris peripheral to a discontinuous and indistinct collarette. **c** Thinner, radially oriented strands of stroma traverse the peripupillary iris. **d** Posterior embryotoxon is seen as thin irregular white arc (arrow) over the periphery of the iris after mechanical removal from the cornea and sclera.

Fig. 3. Case 1. Histopathology of iris. **b** Radial iris strands of peripupillary iris, shown in **a** and fig. 2d, contain vessels enveloped by melanocytes. **c, d** Prominent clefts in the mid-iris (arrow) separate the thick anterior lamellae of the stroma from the thinner layer of the posterior stroma on the anterior surface of the IPE. IPE contains many clear oval spaces. **b-d** H&E, $\times 100$.

lar meshwork and Descemet's membrane. The stroma of both heavily pigmented irides appeared strikingly abnormal macroscopically (fig. 2). This was particularly evident in the right iris, which was viewed en face after it was mechanically detached from the sclera. Peripheral to the poorly formed collarette, the anterior surface of the iris

was marked by a disorderly array of thick bands and sheets of stroma. Central to the collarette, the pupillary part of the iris was traversed by thinner, radially oriented strands of stroma. Histopathologically, much of the iris stroma was comprised of a thick lamella of anterior stroma with a prominent anterior border layer which was

Fig. 4. Case 2. Lacy vacuolization of IPE. **a** IPE shows a lacy pattern of vacuolation. Mid-stromal cleft separates the anterior and posterior lamellae of the lightly pigmented stroma. **b** IPE appears hypopigmented, atrophic, and lacking circumferential ridges. **c, d** Vacuoles in the IPE appear empty in H&E section (**c**), but contain PAS-positive material (**d**). PAS staining is abolished by diastase digestion consistent with glycogen (**e**). **a** H&E, $\times 100$. **c** H&E, original magnification $\times 400$. **d** PAS, original magnification $\times 400$. **e** PAS after diastase digestion, original magnification $\times 400$.

separated from a thinner posterior lamella of stroma on the anterior surface of the iris pigment epithelium (IPE) by a prominent mid-stromal cleft (fig. 3). Some vacuoles were present in the IPE. Macroscopically, the ciliary processes were delicate and relatively smooth. The zonular fibers were more easily visible compared to normal eyes, but appeared normal by light microscopy. There were no abnormalities of the retina, retinal pigment epithelium (RPE), or optic nerve macroscopically or microscopically.

Case 2

This Caucasian boy was born at 38 weeks' gestation after a prenatal fetal ultrasound demonstrated tetralogy of Fallot with pulmonary atresia. Jaundice developed at 2 months of age with a conjugated hyperbilirubinemia and elevated serum aminotransferases confirmed biochemically. Liver biopsy demonstrated cholestasis, portal fibrosis, evidence of large duct obstruction, and bile plugs. A Roux-en-Y Kasai hepatoportoenterostomy was performed. There were no vertebral abnormalities. A missense mutation in *JAG1* was identified confirming the diagnosis of Alagille syndrome. At 7 months of age, he developed severe hypoglycemia, acidosis, leukocytosis, and hyponatremia in the setting of a rhinovirus infection and suffered a cardiopulmonary arrest.

At 2 months of age, he was noted to be light averse. Anterior segment examination showed bilateral temporal

posterior embryotoxon. The remainder of the eye examination, including dilated retinal examination, was normal.

The right and left globes measured $19 \times 20 \times 19.5$ mm and transilluminated normally. Posterior embryotoxon was evident grossly as relucent ridges on the posterior cornea in the periphery of both anterior chambers macroscopically, and histopathologically as nodules of extracellular matrix material at the junction between the trabecular meshwork and Descemet's membrane (fig. 1). No iris processes were noted. In contrast to case 1, iris stromal abnormalities were not evident macroscopically, perhaps reflecting stromal transparency of the lightly pigmented irides. Macroscopically, the IPE of both irides appeared atrophic, hypopigmented and lacking normal circumferential ridges. Mid-stromal clefting was present but was less prominent histopathologically (fig. 3). The IPE showed lacy vacuolization (fig. 4). The IPE vacuoles appeared empty in H&E sections but contained PAS-positive material presumed to be glycogen that was digested by diastase. The RPE appeared hypopigmented grossly with speckled hyperpigmentation scattered throughout, especially in the periphery, and was thin and atrophic (fig. 5). The retina showed good preservation of its normal lamellar architecture. The optic nerve was unremarkable with no drusen.

Fig. 5. Case 2. Note atrophy of RPE. **a** RPE shows varying degrees of hypopigmentation. **b** RPE cells are flattened and atrophic with a diminished amount of apical melanin. **a** Flat prep macrophoto, $\times 25$. **b** H&E, $\times 400$.

Discussion

Alagille syndrome, a familial autosomal dominant disorder, most commonly presents with neonatal cholestasis caused by intrahepatic biliary hypoplasia [1, 7]. Hepatic and cardiac abnormalities are the most common causes of death, as seen in our cases. Many children with Alagille syndrome, however, can do well with long-term survival following appropriate intervention, in particular surgery for the liver disorder [7].

The major ocular pathologic feature found in Alagille syndrome is posterior embryotoxon, a thickening and anteriorization of Schwalbe's line, which designates the termination of Descemet's membrane. Glaucoma is not a manifestation of posterior embryotoxon in Alagille syndrome. Posterior embryotoxon occurs in 95% of patients with Alagille syndrome, far greater than the general population (8–15%) [7]. Both patients in this report had posterior embryotoxon, but only one had iris processes to the embryotoxon (fig. 1). Iris processes to the posterior embryotoxon are seen in 77% of patients with Alagille syndrome [6]. This finding is also seen in Axenfeld-Rieger spectrum, but our patient had no other features such as dental or umbilical anomalies.

Unusual iris morphology was present in case 1. The iris stroma was characterized by abnormal interweaving bands of disorderly stroma (fig. 2). There were thick

bands and sheets peripheral to the collarette and thinner, radially oriented strands of stroma central to the collarette. Prior reports of Alagille syndrome also indicate iris abnormalities, including iris hypoplasia, iris nodules, and corectopia [7]. One report noted a peculiar pattern of iris stromal hypoplasia in 6 of 6 patients, with radial strands extending from the iris root to the mid-iris or collarette [5]. The iris strands in their patients were elevated from the iris surface and often converged as they approached the pupil. These findings are similar to the iris morphology present in our patient. Typical iris stroma is characterized by interlacing fibers that either radiate towards the pupil or form a circumference around the iris perimeter. Blood vessels are interspersed and scattered among the iris stroma, without any direct relation between the vessels and the stroma. The irregular iris stroma seen in our case is patterned in a way that seems to follow the vessels of the iris, with melanocytes enveloping the central vessels. This may be explained by an irregular migration of the neural crest cells. *JAG1* codes for a transmembrane protein that is a ligand for the Notch-1 receptor of the Notch signaling pathway, which plays an important role in neural crest cell differentiation and cell fate determination [2, 8]. A defect in this pathway may have resulted in abnormal migration of neural crest cells. *JAG1* is essential for normal neural crest migration during craniofacial development [9]. Disruption of *JAG1* function is felt to be

the cause of the characteristic triangular facies in patients with Alagille syndrome [9].

In addition, there were noticeable clefts between the anterior and posterior parts of the stroma in both patients (fig. 3). Although normal irides have clefts in this position, the cleft seen in our patients was much larger than normal.

The lacy vacuolization of the IPE present in the second patient (fig. 4) is typical of diabetic patients with blood sugars above 200 mg/dl during the 72-hour period before death [10]. The cysts in the IPE are filled with glycogen, as was seen in our case [11]. Our patient was hypoglycemic, but he may have had unstable blood glucose levels, and could have been hyperglycemic at the time of death.

Abnormalities in the posterior segment were noted in the second patient (fig. 5). The retina was diffusely hypopigmented with scattered pigmentary speckling throughout, especially in the periphery. This is consistent with a case series in which 57% of 22 patients with Alagille syndrome had diffuse fundus hypopigmentation and 33% had pigmentary speckling of the RPE [7]. Both of our patients had no optic disc drusen or other optic disc abnormalities. This is atypical for patients with Alagille syndrome, in which 76% have been reported to have abnormalities in the optic nerve head [7].

The ocular features of Alagille syndrome help to characterize the disorder. Several of these findings, specifi-

cally posterior embryotoxon and iris abnormalities, may be visible on clinical slit-lamp examinations, potentially aiding in recognition of the syndrome. It is unusual for patients with Alagille syndrome to come to ocular autopsy. We present the second published report. The pathology findings may shed light on our understanding of the pathophysiology of the ocular anomalies that are characteristic for this syndrome.

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Statement of Ethics

IRB/Ethics Committee approval was not required for this study.

Disclosure Statement

None of the authors has any conflicts of interest to disclose.

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