Special Article

Neuropathology of Joubert Syndrome

Anthony T. Yachnis, MD; Lucy B. Rorke, MD

ABSTRACT

Very little documentation of the neuropathologic changes in Joubert syndrome exists. This paper presents a detailed postmortem neuropathologic study of a clinically and radiographically well-documented case of Joubert syndrome. In addition to aplasia of the cerebellar vermis and fragmentation of the dentate nuclei, there was marked dysplasia of structures at the pontomesencephalic junction and caudal medulla. There was abnormal decussation of the superior cerebellar peduncles and an enlarged iter (rostral 4th ventricle) with elongated tegmental nuclei (including the locus coeruleus). Neurons of the basis pontis and reticular formation appeared reduced. Extensive malformations of the medulla included hypoplasia of the inferior olivary nuclei, solitary nuclei and tracts, and the nucleus and spinal tracts of trigeminal nerve (cranial nerve V). Even more striking was dysplasia of the caudal medulla at the cervicomedullary junction, which was characterized by the absence of a posterior median sulcus, neuronal swelling and axonal spheroids in the region of malformed nuclei gracilis and cuneatus, and absence of pyramidal decussation. This study suggests that, in addition to vermal agenesis, Joubert syndrome is characterized by malformation of multiple brainstem structures. The latter could explain certain clinical features of the syndrome, including episodic hyperpnea and oculomotor apraxia. (*J Child Neurol* 1999;14:655–659).

Most of what is currently known about Joubert syndrome is based on clinical and radiographic studies of affected individuals.[1–5] The clinical spectrum includes episodic hyperpnea, abnormal eye movements (oculomotor apraxia), ataxia, and mental retardation with associated agenesis (or hypoplasia) of the cerebellar vermis.[1,2] The recently reported "molar tooth sign" seen on magnetic resonance imaging (MRI) is a consistent characteristic of Joubert syndrome.[6]

Autopsy findings of a 2-year-old girl with classic clinical manifestations of Joubert syndrome have been reported by Friede and Boltshauser.[7] The initial description of this syndrome by Joubert et al[1] contains only a brief account of autopsy findings. While it is known that the full clinical syndrome is not expressed by all patients,[2] what remains unknown is the spectrum of gross and microscopic abnormalities of Joubert syndrome and the disorder's possible relationships to other syndromes that have cerebellar vermian agenesis as a component. The purpose of this paper is to document autopsy findings of a second patient who had Joubert syndrome and to compare these observations with those of Friede and Boltshauser.[7]

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From the Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine and UF Brain Institute (Dr Yachnis), Gainesville, FL, and the Department of Pathology, Children's Hospital of Philadelphia (Dr Rorke), Philadelphia, PA.

Address correspondence to Dr Anthony T. Yachnis, Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine, PO Box 100275, Gainesville, FL 32610-0275. Tel 352-395-0238; fax: 352-395-0437; e-mail: yachnis.pathology@mail.health.ufl.edu.

CASE HISTORY

The patient was a 31-year-old white man who died after being discovered submerged in a swimming pool. He was a hypotonic newborn, but no hyperpnea or apnea was reported. He had congenital nystagmus and was later noted to have oculomotor apraxia. He rolled at age 12 months, sat at 18 months, walked at 42 months, and combined words at 5 years. He was born with a partially cleft palate and protruded his tongue as a young child. He began having seizures at age 1 year, but at the time of his death had been seizure free for 6 years. His gait had always been wide-based and ataxic. An MRI scan obtained 6 years before death confirmed cerebellar vermal agenesis and the "molar tooth sign." The neuropathologic findings have been previously reported in part by Maria and colleagues.[6]

MATERIALS AND METHODS

Tissues were embedded in paraffin wax and stained with hematoxylin and eosin, Luxol fast blue and periodic acid-Schiff, and cresyl violet. Selected sections were immunostained for glial fibrillary acidic protein (GFAP; Dako Corp, Carpinteria, CA), neurofilament protein (RMdO20; Zymed, San Francisco), neuron-specific class III β-tubulin (Research Diagnostics, Flanders, NJ), and microtubule-associated protein 2 (MAP2; Zymed) using microwave antigen retrieval.[8]

RESULTS

Cerebral Hemispheres and Deep Nuclei

The unfixed brain weighed 1340 g. The cerebral hemispheres were symmetric and displayed a subtle "simplified" gyral and sulcal pattern. There was focal interdigitation of the fron-

tomedial gyri. The corpus callosum and olfactory bulbs and tracts were present. The basal vasculature was normally formed, and there was no significant atherosclerosis. Examination of serial coronal sections of the cerebral hemispheres revealed good demarcation of gray- and white-matter structures. The deep cerebral nuclei appeared to be grossly normal. The left hippocampus was slightly smaller than the right, but there were no grossly apparent architectural abnormalities.

Microscopic study of the cerebrum showed a six-layered neocortex without apparent dysplasia as determined by routine and immunohistochemical study. The basal ganglia and hippocampus also were normally formed. However, notable abnormalities of the deep nuclei included apparent absence of the fields of Forel, substantia innominata, and subthalamic nuclei. The posterior thalamic nuclei consisted of fragmented groups of neurons.

Microscopic study of the amygdala revealed an abundance of glioneuronal hamartias[9,10] consisting of immature-looking, small round cells with perinuclear halos that often were intermixed with mature neurons. The immature cells occurred diffusely or in small clusters, were distributed along subependymal areas of the amygdala, and appeared to extend focally into deep layers of the adjacent entorhinal cortex.

Midbrain and Pons

The rostral midbrain appeared grossly normal with a well-pigmented substantia nigra. However, the caudal midbrain showed an apparent elongation of the iter (rostral extent of the 4th ventricle) and of tegmental structures (Figure 1A and B). This apparent elongation of the caudal midbrain tegmentum seemed to be due in part to a reduction in volume of the decussation of the superior cerebellar peduncles.

Microscopic study confirmed the dispersal of neurons of the locus coeruleus along the elongated iter. There was only a rudimentary decussation of the superior cerebellar peduncles, but despite this, the peduncles were identified in their normal position in the upper-outer quadrants of the midbrain and were not significantly reduced in size. There was an apparent reduction in oculomotor nerve (cranial nerve III) nucleus neurons and in neurons of the raphe nuclei. The red nucleus, substantia nigra, and cerebral peduncles appeared normal.

The basis pontis was small and firm, and contained a few large, coarse, obliquely oriented fiber bundles corresponding to aberrant crossing pontine fibers (Figure 1C). There was an apparent decrease in the volume of the medial lemnisci and in neurons of the pontine base without significant glial reaction. The middle cerebellar peduncles were reduced in size. Neurons of pontine cranial nerve nuclei and the reticular formation appeared to be reduced in number. The trapezoid body was not identified.

Cerebellum

There was almost complete aplasia of the cerebellar vermis, with only a rudiment of the rostral-most portion of the superior vermis remaining (Figure 1C). The folia of the lateral cerebellar hemispheres, especially the cerebellar cortex adjacent to the enlarged 4th ventricle, were whitish and firm, with poor demarcation of cortex and white matter. The dentate nuclei were indistinct and separated into small islands of gray matter (Figure 1D). No other cerebellar dysplasias were identified. There was marked atrophy of the cerebellar cortex with Purkinje cell loss and proliferation of Bergmann glia. A few apparently normal areas of cerebellar cortex remained.

Medulla

The 4th ventricle was widened and had the shape of an inverted umbrella (Figure 1C). The usual bulges corresponding to the medullary pyramids and inferior olives were not observed grossly (Figure 1E). Because of this, cranial nerves XI and XII appeared to exit the medulla in a rather haphazard fashion. Otherwise, cranial nerves were present and appeared grossly intact.

The inferior olivary nuclei were markedly hypoplastic (Figure 1F). Neurons of the reticular formation were apparently reduced in number, while there was a relative increase in myelin staining in the tegmentum (Figure 1F). Periventricular tegmental nuclei, such as the dorsal motor nucleus of the vagus, vestibular nuclei, and external arcuate nuclei, were splayed out. The hypoglossal (cranial nerve III) nucleus was apparently normal. Clumps of large neurons were present in the tegmentum—possibly representing dysplastic inferior olivary nuclei. It was impossible to determine whether accessory olives were present. There was focal cytoplasmic basophilia and hyperchromasia of scattered neurons within the ventral arcuate nucleus. The medial lemnisci appeared small. Circumferential subpial gliosis was demonstrated by GFAP immunostaining.

There was marked dysplasia of the caudal medulla near the cervicomedullary junction (Figure 2). The posterior median sulcus was absent, and there was poor demarcation of the posterior columns and nuclei gracilis and cuneatus (Figure 2A and B). The fasciculi gracilis and cuneatus were not separated into distinct tracts; instead they formed peculiar C-shaped structures ending from a medial to mediolateral position in the medullary tegmentum. The nuclei gracilis and cuneatus were dispersed in haphazard fashion in most caudal levels, but displayed a more normal arrangement rostrally. Neurons of these two nuclei were swollen and contained abnormal accumulations of neuronal intermediate filaments seen by immunohistochemistry (Figure 2C). Some neurons had an odd club or tennis racquet shape, a configuration reminiscent of dysplastic-looking neurons in cortical dysplasias. There were many axonal spheroids (Figure 2D) associated with the disorganized tegmental gray matter, the latter representing residual gracile and cuneate nuclei.

The nucleus of the spinal tract of the trigeminal nerve (cranial nerve V) was fragmented and hypoplastic (especially the pars gelatinosa). The solitary nucleus and tracts were hypoplastic. A peculiar decussation of the pyramidal tract

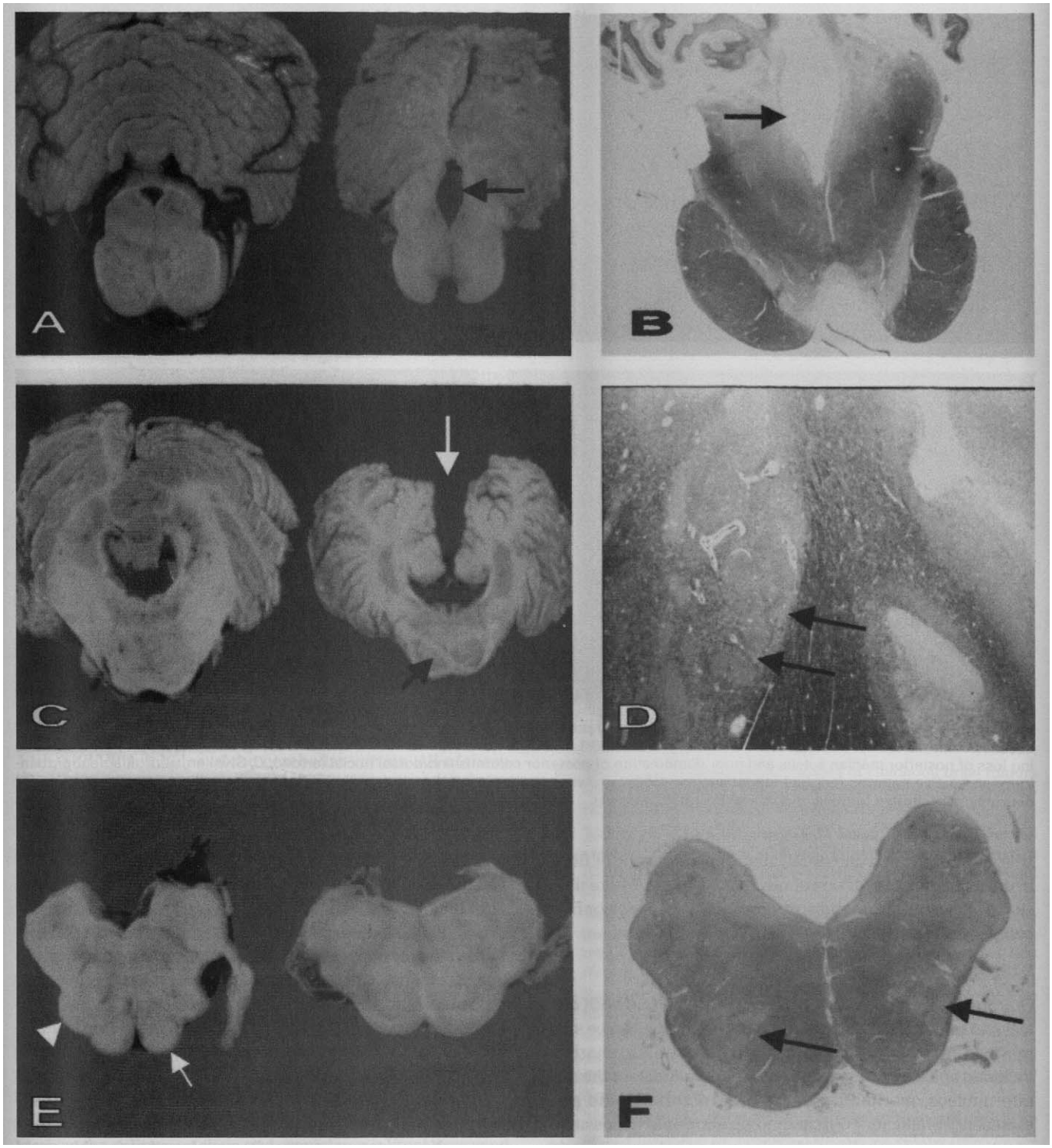

Figure 1. *A*, Pontomedullary junction. The case of Joubert syndrome (right side of figure) shows widening of the iter with tegemental elongation (arrow) compared to normal, age-matched control brain (left side of figure); *B*, Luxol fast blue and periodic acid-Schiff-stained section from caudal midbrain showing widened iter and tegmental elongation (arrow); *C*, Midpons at level of trigeminal nerve (cranial nerve V) exit. Striking vermal aplasia has resulted in 4th ventricular enlargement and gives the latter structure the appearance of an inverted umbrella (white arrow). Obliquely oriented crossing fibers of basis pontis are also indicated (black arrow); *D*, Luxol fast blue and periodic acid-Schiff-stained section of cerebellum showing fragmentation of dentate nucleus into multiple islands of gray matter (original magnification ×100) (arrows); *E*, Section at level of rostral medulla showing loss of normal demarcation of inferior olives and pyramids in Joubert syndrome (right of figure). Arrows indicate normal locations of these structures in an age-matched control (left of figure); *F*, Luxol fast blue and periodic acid-Schiff-stained section showing hypoplasia and fragmentation of inferior olivary nuclei.

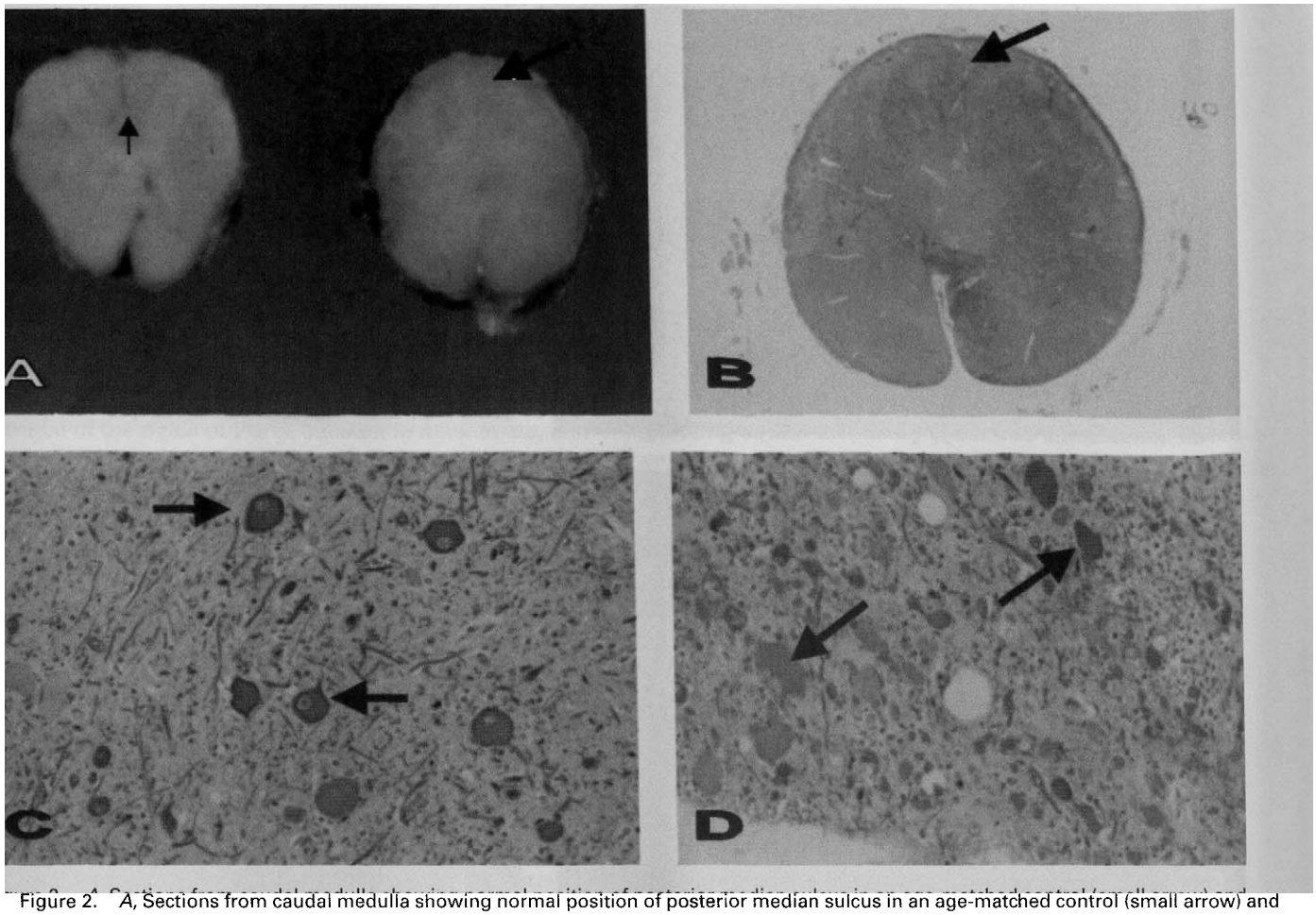

Figure 2. *A,* Sections from caudal medulla showing normal position of posterior median sulcus in an age-matched control (small arrow) and absence of this structure in Joubert syndrome (large arrow); *B,* Luxol fast blue and periodic acid-Schiff-stained whole-mount preparation showing loss of posterior median sulcus and poor demarcation of posterior columns and dorsal nuclei (arrow); *C,* Swollen, neurofilament protein-immunoreactive neurons (original magnification ×800) (arrows) in tegmentum of caudal medulla; *D,* Axonal swellings immunoreactive for neurofilament protein (original magnification ×800) (arrows).

was apparent with a unilateral, abnormal scattering of fibers in the most caudal sections of medulla at a point where there should have been a lateral corticospinal tract. Anterior horn cells appeared normal at this level.

DISCUSSION

Our postmortem study of the brain from a patient with clinical and radiologic features of Joubert syndrome can be summarized as follows (Table 1): cerebellar malformations included aplasia of the vermis with fragmentation of the dentate nucleus; marked dysplasia of structures at the pontomesencephalic junction, including abnormal decussation of the superior cerebellar peduncles and an enlarged iter with elongated tegmental nuclei (including the locus coeruleus); reduction in reticular formation neurons and neurons of the basis pontis; extensive hypoplasia of the inferior olivary nuclei, solitary nuclei and tracts, and the nucleus and spinal tracts of cranial nerve V; marked dysplasia of the caudal medulla characterized by the absence of a posterior median sulcus, neuronal swelling, and axonal spheroids in the region of malformed nuclei gracilis and cuneatus, and the absence of pyramidal decussation.

The neuropathologic findings reported here share some striking similarities with the brain of another patient with Joubert syndrome described by Friede and Boltshauser (Table 1).[7] Specifically, both cases share aplasia of the cerebellar vermis and sequestration of the dentate nucleus into islands. Other common features include elongation of the locus coeruleus; anomalies of the descending tract of cranial nerve V, the solitary nucleus and tract, the dorsal column nuclei, and inferior olivary nuclei; and absence of all but minor pyramidal decussation.

One significant difference is that Friede and Boltshauser reported the cerebellar hemispheres as enlarged, but they are small in the present case. One explanation for this could be that the cerebellar cortical changes we observed (Purkinje cell loss and Bergmann glia proliferation) were the result of secondary damage caused by seizure activity or hypoxia in our patient.

A definite morphologic explanation for our patient's seizure disorder was not found. The subependymal glioneuronal hamartias found in the amygdala suggest an abnormality in cell migration. However, while such hamartias have been observed in temporal lobes resected from patients

Table 1. Joubert Syndrome: Neuropathology

Friede and Boltshauser's Case[7]	*Current Case*
Patient age: 2 Years	*Patient age: 31 Years*
Aplasia of vermis	Aplasia of vermis
Large cerebellar hemispheres	Small cerebellar hemispheres
Cortical cytoarchitecture normal	Neuronal loss and gliosis of Purkinje cell layer
Fragmented dentate nucleus	Fragmented dentate nucleus
Heterotopias of roof nuclei	No apparent heterotopias
Abnormal elongation of locus coeruleus	Abnormal elongation of locus coeruleus
	Abnormal decussation of superior cerebellar peduncles
Dysplasia of inferior olives	Hypoplasia of inferior olives
Absence of pyramidal decussation	Absence of pyramidal decussation
Anomalies of dorsal column nuclei, solitary tract, and cranial nerve V tract	Anomalies of dorsal column nuclei, solitary tract, and cranial nerve V tract
	Apparent reduction in reticular formation neurons

with intractable epilepsy,[10,11] similar collections of immature-appearing cells can be found in the caudal amygdala region of those without epilepsy.[12] Therefore, the role of these interesting cells in the adult brain remains unknown.

Clinicopathologic correlation is difficult in Joubert syndrome. Although complex malformations are present, a particular structure that appears absent or malformed could still be able to serve certain functions. In this case, for example, apparent absence of the subthalamic nuclei was not associated with hemiballismus clinically.

With the above caveat, it is reasonable to assume that extensive brainstem malformation could explain two classic clinical features of Joubert syndrome: oculomotor apraxia and episodic hyperpnea. The fine tuning of saccadic eye movements involves the tecto-PPRF (parapontine reticular formation) pathway, nuclei of the basis pontis and the IVth, VIth, and VIIth lobules of the cerebellar vermis. All of these structures are at least partly affected in the Joubert malformation. Friede and Boltshauser suggested that anomalies of the gracile nuclei and solitary tract could contribute to an abnormal respiratory pattern because these centers receive afferent respiratory impulses.[7] Also, since the ventral arcuate nucleus might play a role in cardiorespiratory function,[13,14] alterations of it or its associated tracts and connections (including the raphe nuclei) might contribute to the abnormal respiratory pattern seen in Joubert syndrome.

Hypoplasia of the inferior olives, accessory olives, neurons of the basis pontis, and possibly the ventral arcuate nuclei suggest an abnormality in migration of young neurons from the lateral corpus pontobulbare of the developing brain stem.[15,16] (Also see Yachnis and Rorke this issue.)

Since the positions of cranial nerve nuclei and tracts, such as the solitary nucleus and cranial nerve V, are governed by the paths of neurons migrating from the corpus pontobulbare, it can be hypothesized that the defect occurs between 6 and 8 weeks' gestation—the time when these processes are thought to occur.[15]

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