Neuropathological Findings in Eight Children with Cerebro-oculo-facio-skeletal (COFS) Syndrome

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Abstract. Cerebro-oculo-facio-skeletal (COFS) syndrome is a rare autosomal recessive disorder with microcephaly, severe mental retardation, and death in early childhood. The pathogenesis is unknown. Neuropathological features of 8 children with COFS syndrome are presented. Seven of the children, ranging in age from 36 weeks gestation to 5 years 8 months, are of North American aboriginal background from Manitoba, Canada. The eighth child is a 3-year-old Caucasian male. In all children there was severe microencephaly and mild ventriculomegaly. Cerebral myelination appeared to be delayed in one infantile case. Swollen ubiquitinated granular cells appeared in the white matter shortly after birth. Older children displayed cortical neuron loss, patchy or diffuse absence of myelin and gliosis in the white matter, and pericapillary and parenchymal mineralization in the globus pallidus and to a lesser extent the putamen and cerebral cortex. The cerebellum of older children exhibited severe degenerative changes involving the internal granular layer and Purkinje cell layer. The neuropathological changes, previously not well documented, suggest that COFS syndrome is associated with a degenerative process that begins in utero and affects many brain cell types. Similarities to Cockayne syndrome are discussed.

Key Words: Brain calcification; Brain degeneration; Cockayne syndrome; Familial; Leukodystrophy.

INTRODUCTION

Cerebro-oculo-facio-skeletal (COFS) syndrome is a rare autosomal recessive disorder with microcephaly, severe psychomotor retardation, and death in early childhood. The molecular/biochemical pathogenesis is not known; therefore, the diagnosis is made clinically and is defined by a cluster of abnormalities. The major diagnostic criteria are microcephaly, ocular anomalies including cataracts and microphthalmia, dysmorphic facies with high and broad nasal bridge, large ears, overhanging upper lip, and micrognathia, dwarfism, flexion contractures of the limbs, and skeletal abnormalities including scoliosis, hip dysplasia or dislocation, narrow pelvis, and rocker-bottom feet with proximal displacement of the second metatarsals and longitudinal grooves in the soles along the second metatarsal. Affected children are usually small at birth, at which time the phenotype is evident. Infants may also have a short neck, hirsutism, widely spaced nipples, simian creases, hypotonia, renal anomalies, and osteoporosis. They suffer from feeding difficulties and repeated respiratory infections that lead to death in infancy or early childhood (1). Peña and Shokeir, in 1974 and 1978, first described COFS syndrome in 10 children, 9 of whom were from 2 families of North American aboriginal background (2, 3). Four of these children underwent autopsy. The authors made only brief mention of subcortical gliosis, decreased myelin content, and focal mineralization in gray and white matter. Since then little else has appeared in the literature concerning the brains of children with COFS syndrome. Herein we describe in detail the neuropathological findings in the brains and spinal cords of 8 children with COFS syndrome, 7 of whom were of North American aboriginal background, including 2 of the children described in the original reports (2, 3).

MATERIALS AND METHODS

This is a retrospective neuropathological study of patients diagnosed with COFS syndrome. This syndrome is overrepresented in the province of Manitoba, Canada, where approximately 20 affected children have been identified since 1970. Two known patients are currently alive. Patients 1 to 8 described in this report (Table 1) were all examined by clinical geneticists in the Section of Genetics and Metabolism at the Children’s Hospital in Winnipeg, Canada. All of these children were of North American aboriginal background and all had phenotypes considered typical of COFS syndrome. The age of death ranged from 3 weeks to 6 years. Complete autopsies have been performed on 7 children with the diagnosis of COFS syndrome. In the majority, the cause of death was respiratory failure. Four of the 7 children are from the original large pedigree and were discussed in the original publications (2, 3); these include patients #4 and #6, who are siblings; patient #5, who is a first cousin; and patient #3, who is a distant cousin. This family has proven consanguinous matings. Patient #2 was from a nearby community. The other 2 children (patients #1 and #7) are from apparently unrelated families. Patient #8, the dizygotic twin of patient #7, is still alive. Despite our failure to prove connections between the families or consanguinity within the last 2 families, it should be noted that this aboriginal population is from a restricted geographic region and in general is somewhat inbred. Historical and clinical features of 5 of the 7 deceased children have been presented in other publications (2-4). Interested readers are referred to Table 1 for relevant citations. Autopsies on 2 other children aged 1 and 2.5 years.
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at death</th>
<th>Height (cm)</th>
<th>Brain weight (expected for age/height)</th>
<th>Published details and pedigree (a)</th>
<th>Demyelination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>33 weeks gestation + 20 days</td>
<td>42 (&lt;3rd %ile)</td>
<td>155 g (308/247)</td>
<td>See ref. (4)</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>6 weeks</td>
<td>NA</td>
<td>176 g (500—)</td>
<td>? delay in optic pathways</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>6 months</td>
<td>NA</td>
<td>NA</td>
<td>See ref. (2, 3); case VI–11</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>f</td>
<td>4 years 2 months</td>
<td>NA</td>
<td>NA</td>
<td>See ref. (3, 4); case VII–17</td>
<td>Patchy in cerebrum and hindbrain</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>4 years 7 months</td>
<td>NA</td>
<td>NA</td>
<td>See ref. (2, 3); case VII–2</td>
<td>Patchy in cerebrum and hindbrain</td>
</tr>
<tr>
<td>6</td>
<td>f</td>
<td>5 years</td>
<td>63 (&lt;3rd %ile)</td>
<td>220 g (1260/677)</td>
<td>See ref. (2, 3); case VII–16</td>
<td>Diffuse severe; multinucleate astrocytes; rare sparing calcareous Patchy in cerebrum and hindbrain</td>
</tr>
<tr>
<td>7</td>
<td>f</td>
<td>5 years 8 months</td>
<td>84 (&lt;3rd %ile)</td>
<td>404 g (1263/1090)</td>
<td>This report</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>m</td>
<td>alive at 8 years 3 months</td>
<td>—</td>
<td>—</td>
<td>This report</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>m</td>
<td>3 years 2 months</td>
<td>75 (&lt;3rd %ile)</td>
<td>560 g (1141/875)</td>
<td>This report</td>
<td>Diffuse severe</td>
</tr>
</tbody>
</table>

* The pedigrees codes for cases 3, 4, 5, and 6 refer to those documented in the original description of COFS syndrome by Peña et al (2, 3). NA = not available.

are alluded to in one of the original publications, but no official records could be found in our institution.

The entire brain was available for examination in Cases 2, 6, and 7. Archived paraffin blocks and hospital records, some with incomplete details, were to be found for the other 4 cases. These 7 cases were all examined by one neuropathologist (MRD). The brains had been reasonably well sampled, with 10 to 22 tissue blocks available for microscopic examination. Sections from all blocks were stained with hematoxylin and eosin, and either Luxol fast blue or solochrome cyanine methods for myelin. The extent of myelinization in specific anatomic sites has been compared to that described by Brody and coworkers (5). Selected sections were stained by other methods including periodic acid Schiff (PAS), phosphotungstic acid hematoxylin (PTAH), cresyl violet, modified Bielschowsky, Perl's Prussian blue method for iron, and von Kossa's method for calcium. Centrum semiovale white matter samples from the 3 available brains were processed by the Marchi method to identify myelin debris. On selected paraffin-embedded tissue sections, immunohistochemical staining was performed to detect glial fibrillary acidic protein (polyclonal anti-GFAP) (1/1000 dilution; Biogenex) and neurofilament (1/500 dilution; Dako). To characterize the PAS-positive granular cells, antibodies to ubiquitin (1/100 dilution; Dako), HLA-DR (1/100 dilution; Dako), and muraminidase (1/1000 dilution; Dako), common leukocyte antigen (CD45) (1/200 dilution; Dako), CD68 (1/200 dilution; Dako), alpha-1 chymotrypsin (1/1000 dilution; Dako), and alpha-1 anti-chymotrypsin (1/5000 dilution; Dako) were used to label one section from each affected case. From one brain (Case #7), frozen sections were stained with oil red O, or examined by fluorescence microscopy to detect autofluorescent material. Samples of white and gray matter were also processed for electron microscopy. For all cases the frontal cortex thickness at the depths of sulci, the width of terminal cerebellar folia, and the thickness of the internal granular layer were measured using a calibrated ocular graticule. At each of the 3 sites, 4 sample measurements were taken. Age-matched control cases were assessed in a similar manner. Data are presented as means.

Patient #9 is a child from another geographic and racial background with a clinical diagnosis of COFS syndrome. The brain and spinal cord were examined by one neuropathologist (LBR). Tissue sampling was extensive and sections were stained by hematoxylin and eosin for microscopic examination.

**CLINICAL REPORTS**

**Cases #7 and #8**

Male and female dizygotic twins were born at term to parents of North American aboriginal background with
TABLE 1  (Continued)

<table>
<thead>
<tr>
<th>PAS-positive granular cells in white matter</th>
<th>Neuron loss cerebral cortex</th>
<th>Mineralization</th>
<th>Optic nerve/retinal degeneration</th>
<th>Cerebellar degeneration</th>
<th>Other neuropathological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NA</td>
<td>None</td>
<td>Rare binucleate Purkinje neurons</td>
</tr>
<tr>
<td>Scattered, cerebrum and brainstem</td>
<td>None</td>
<td>White matter</td>
<td>Optic nerve atrophy</td>
<td>None</td>
<td>Bifid central canal, lumbar spinal cord</td>
</tr>
<tr>
<td>Abundant all sites</td>
<td>None</td>
<td>Putamen</td>
<td>Mild pigmentary retinopathy</td>
<td>Early</td>
<td>Bifid central canal, lumbar spinal cord</td>
</tr>
<tr>
<td>Rare</td>
<td>Yes</td>
<td>Globus pallidus &gt; putamen &gt; cortex</td>
<td>NA</td>
<td>Severe</td>
<td>Atrophy of pyramidal tracts</td>
</tr>
<tr>
<td>Rare, brainstem</td>
<td>Yes</td>
<td>Globus pallidus &gt; putamen &gt; cortex</td>
<td>NA</td>
<td>Severe</td>
<td>Rare binucleate Purkinje neurons; spinal cord gliosis</td>
</tr>
<tr>
<td>Rare, cerebellum</td>
<td>Yes</td>
<td>Globus pallidus &gt; putamen &gt; cortex and white matter</td>
<td>Severe optic nerve atrophy</td>
<td>Severe</td>
<td>Rare binucleate Purkinje neurons; swollen axons thoracic spinal cord</td>
</tr>
<tr>
<td>Rare</td>
<td>Yes</td>
<td>See Case 4</td>
<td>NA</td>
<td>Severe</td>
<td>NA</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>None</td>
<td>Yes</td>
<td>Basal ganglia &gt; cortex</td>
<td>Optic nerve atrophy; choriotinal degeneration</td>
<td>Moderate</td>
<td>Neuronal loss spinal cord</td>
</tr>
</tbody>
</table>

no history of consanguinity. The birth details are vague, but both children were noted to be small and microcephalic with unusual facies. At age 4 months they were hospitalized for failure to thrive. At 7 months the girl (Case 7) developed pneumonia. At 1 year, both were referred for genetic assessment. The girl was microcephalic (head 36.8 cm, ≈ 2nd percentile) and small (5.3 kg, ≈ 3rd percentile) with microphthalmia, bilateral cataracts, overhanging upper lip, prominent nose, axial hypoplasia, limb hypoplasia, and minimal attentiveness to her surroundings. The brother had a similar appearance but was more interactive. Both were diagnosed as having typical COFS syndrome. The following year, the girl had cataracts surgically removed. Hearing deficits were documented in both children and both required placement of feeding gastrostomy tubes. At age 4 years 3 months the girl was still unable to sit or talk and was inattentive, although she would place toys in her mouth. Her head was 41.5 cm (≈ 2nd percentile) and weight was 11.5 kg (≈ 3rd percentile). At age 5 years 8 months she was admitted with dehydration, metabolic acidosis, and seizures. A CT scan of the head showed intracerebral calcification, particularly in the frontal white matter and basal ganglia, and enlargement of the lateral ventricles and subarachnoid spaces (Fig. 1a). She died shortly thereafter.

Her head size was unchanged, her height was 84 cm (≈ 3rd percentile), and she had lost considerable weight (5.3 kg). The brain weight (404 g) was less than the 2nd percentile as determined for age and for height.

At age 8 years 3 months, the time of manuscript submission, the male sibling (Case 8) remains alive. On most recent examination he was hypotonic and unable to sit, and severely microcephalic, with a head circumference of 39 cm (≈ 2nd percentile), and weighed 14.5 kg (≈ 3rd percentile). He responded to faces and large objects, often with grunting sounds. There were no cutaneous manifestations of freckling, actinic keratoses, photosensitivity, or neoplastic skin lesions. A CT scan showed ventriculomegaly with atrophy and basal ganglia calcification almost identical to that of his sister: EEG showed no seizure activity. He has developed mild liver failure, several episodes of pneumonia, and non–insulin-dependent diabetes mellitus.

Case #9

This white male weighed 3520 gm at birth (40th percentile), following an uncomplicated 41.5 week gestation. The mother and father were both 26 years old with no known consanguinity, and there were 2 normal older
male siblings. Examination at age 5 weeks revealed dysmorphic facies with long philtrum, micrognathia and enophthalmus, a small cataract in the left eye, a vertical tarsal deformity of both feet, hand contractures, a head circumference of 35.5 cm (10th percentile), and a length of 49.5 cm (< 3rd percentile). Muscle tone and reflexes appeared normal. He had a normal electromyography at 2 months. At 3 months he underwent corrective orthopedic procedures. At age 4 months he developed difficulty swallowing and at age 6–7 months he manifested poor weight gain and mild elevation of transaminase; a liver biopsy was unremarkable. A CT scan done at 10 months of age showed mild widening of the sulci and foci of calcification in the frontal white matter, right putamen, and, questionably, the left thalamus. An EEG at that time was normal. Developmental assessment at 14 months showed a developmental age of 6 months and bilateral sensorineural hearing loss. Muscle tone was markedly increased in the lower extremities. He had a fundoplication with gastrostomy at age 2 years 5 months. At age 30 months, his head circumference remained at 41 cm (< 2nd percentile), his weight was 8.6 kg (< 3rd percentile), and his developmental age remained at approximately 6 months. Repeat CT scan revealed worse cortical atrophy and ventricular enlargement, severe calcification in the subcortical and deep frontal white matter, basal ganglia (Fig. 1b), and dentate nuclei, and minimal calcification in the occipital white matter. The diagnosis of COFS syndrome was made at this time. He died at age 3 years 2 months of pneumonia. General autopsy findings included bilateral cryptorchidism and a small focus of skeletal muscle within the thyroid parenchyma.

RESULTS

The age of children with COFS syndrome ranged from a corrected age of 36 weeks gestation to 5 years and 8 months. In the 5 cases with documented brain weight, the brains were much smaller than expected for age; the other 3 brains were described as having been severely microencephalic. These data as well as the main neuropathological features are summarized in Table 1.

The gross neuropathological findings for all included normal or slightly fibrotic meninges, preservation of the basic gyral pattern, and gyral atrophy with sulcal widening. All had mild to moderate ventriculomegaly, which was more severe in the older children and involved the frontal and occipital horns (Fig. 2). Accompanying the ventriculomegaly was diminished white matter volume with atrophy of the corpus callosum. Cerebellar atrophy was prominent in the 5 older children. A detailed description of the microscopic findings follows.
COFS SYNDROME NEUROPATHOLOGY


table 2
cortex and cerebellar measurements

<table>
<thead>
<tr>
<th>Case #</th>
<th>Frontal cortex thickness (mm)</th>
<th>Cerebellar folia width (mm)</th>
<th>Internal granular layer thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5 (1.5)</td>
<td>0.72 (0.50)</td>
<td>0.16 (0.18)</td>
</tr>
<tr>
<td>2</td>
<td>1.6 (1.7)</td>
<td>0.76 (0.96)</td>
<td>0.14 (0.16)</td>
</tr>
<tr>
<td>3</td>
<td>2.0 (2.0)</td>
<td>0.68 (0.88)</td>
<td>0.10 (0.12)</td>
</tr>
<tr>
<td>4</td>
<td>2.1 (2.6)</td>
<td>0.62 (1.30)</td>
<td>0.05 (0.16)</td>
</tr>
<tr>
<td>5</td>
<td>1.6 (2.6)</td>
<td>0.6 (1.30)</td>
<td>0.12 (0.16)</td>
</tr>
<tr>
<td>6</td>
<td>2.0 (2.2)</td>
<td>0.56 (1.28)</td>
<td>0.05 (0.18)</td>
</tr>
<tr>
<td>7</td>
<td>1.4 (2.3)</td>
<td>0.6 (1.24)</td>
<td>0.05 (0.14)</td>
</tr>
</tbody>
</table>

* All values represent the mean of 4 measurement sites (see Methods for details). In parentheses are values obtained from age- and sex-matched control brains; one control case was matched to each patient.

**Case 1 (Corrected Age, 36 Weeks Gestation):** The cortical architecture was normal. No cerebral myelin was evident. Glia with cytoplasmic GFAP-immunoreactivity along one pole and scant processes, indicative of myelination glia and not reactive astroglia, were evenly distributed in the white matter. Along the frontal horns there was minimal residual periventricular germinal tissue, often surrounding abundant ependyma-lined channels. In the adjacent white matter there were dozens of 0.3 to 0.8 mm diameter round clusters of mature neuron-like cells, probably representing heterotopia. The cerebellum had a normal laminar pattern with a prominent external granular layer. Very rare binucleate Purkinje cells were seen. Myelin staining in white matter tracts of the brainstem and spinal cord was appropriate for the age. Cortical and cerebellar measurements were similar to control values (Table 2).

**Case 2 (Age, 6 Weeks):** Whisht flecks of calcium were grossly evident in the frontal and occipital poles of the white matter. On microscopic examination, the cortical and hippocampal architecture was normal. In the deep white matter, including the calcarine region, no myelinated axons were evident. Rare plump ovoid cells with granular, PAS-positive cytoplasm were seen in the deep frontal cortex and white matter (Fig. 3). These exhibited granular immunoreactivity for ubiquitin and muramidase, and diffuse strong cytoplasmic immunoreactivity for antitrypsin and antichymotrypsin, but no immunoreactivity for GFAP, CD68, HLA-DR, or CD45. However, cells that were clearly reactive astrocytes with strong GFAP immunoreactivity were also moderately immunoreactive for antitrypsin and antichymotrypsin and also exhibited small cytoplasmic granules that were immunoreactive for muramidase. The same description applies to the PAS-positive granular cells in all cases.

Fig. 2. Photograph of a coronal slice through the brain of Case #7 at age 5 years 8 months. The brain is small, the lateral ventricles are enlarged, and the periventricular white matter is thin.

Fig. 3. Photomicrograph showing a cell with granular cytoplasm (arrowhead) in the white matter of Case #2. In all cases with such cells, the appearance was similar. (PAS stain) Bar = 25 μm.
Marchi stain showed no typical myelin debris in the periventricular white matter. No axonal spheroids were identified by immunohistochemical staining for neurofilament. GFAP immunohistochemistry revealed scattered reactive astrocytes, some of which were binucleate, in the deep cortex and white matter, and plump myelination glia throughout the white matter. Rare small mineral deposits were present alongside capillaries of the white matter and deep calcarine cortex. The ventricle wall was covered by ependyma in only small patches. The cerebellum had a normal laminar pattern, although the white matter layer and external granular layer were subjectively thin (Fig. 4 and Table 2) with scattered PAS-positive granular cells. Rare reactive astrocytes were identified in the internal granular layer. Myelin in white matter tracts of the brainstem and spinal cord was appropriate for the age. However, scattered PAS-positive, ubiquitin-immunoreactive granular cells were found in the superior cerebellar peduncle, medial lemniscus, basis pontis, and pontine tegmentum. The optic nerves were small with no myelinated axons.

Case 3 (Age, 6 Months): The cerebral cortex and hippocampus were unremarkable. The cerebral white matter, including fimbria, internal capsule, and frontal periventricular regions, was evenly myelinated, although the intensity of myelin staining was not strong. Other regions were not available for examination. Abundant PAS-positive granular cells were spread diffusely in the white matter. Small perivascular calciospheres and granular cells were found in the putamen. The external granular layer of the cerebellum was negligible. The cerebellar white matter was atrophic and rare PAS-positive granular cells were seen. Myelin staining in the brainstem and spinal cord tracts was appropriate for the age. The central canal of the lumbar spinal cord was bifid, but no other anomalies were seen. The eye exhibited mild pigmentary retinopathy and retinal ganglion cell degeneration, and scattered Morgagnian globules in the lens suggestive of early cataract formation.

Cases 4 to 7 (Age, 4 Years 2 Months to 5 Years 8 Months): These 4 cases of roughly the same age exhibited similar abnormalities, albeit to different extents. Hippocampal architecture was normal. Cortical lamination was intact, but there was a diffuse loss of neurons and the cortical thickness was clearly less than in the control brains (Table 2). Many small foci of mineralization were present alongside capillaries, particularly at the depths of sulci. The cerebral white matter was generally atrophic. Myelin staining was diminished or absent in irregular patchy areas (Fig. 5), or in one case almost the entire cerebrum. In the demyelinated areas there were fewer glial cells overall and scattered reactive astrocytes. In all cases, and in particular the most severely affected case, there were multinucleate astroglia. Silver staining and immunohistochemical demonstration of neurofilament showed that axons were at least partially spared in the areas of myelin loss. Neither oil red O staining material nor autofluorescent material were identified in cortex or white matter. Minimal myelin debris was observed in Marchi-stained sections of the periventricular white matter (Cases 6 and 7). Microglial activation was negligible, with no HLA-DR-immunoreactive cells and only rare cells with CD68 immunoreactive processes. Uniformly distributed pericapillary mineralization was observed in the globus pallidus of all cases. Mild perivascular mineralization was found in the thalamus and pons of 2 cases. In all cases, many medium-sized arteries exhibited thickened walls with foci of mineralization (Fig. 6). This material stained positively for iron and calcium. Reactive gliosis was severe in the globus pallidus bilaterally, but minimal in the cerebral cortex, even at sites with extensive mineralization. Ependymal loss was almost complete throughout the lateral ventricles, and to a lesser extent in the third and fourth ventricles. Pyramidal tracts in the midbrain and pons were small. Focal myelin loss was identified in rare, apparently random foci of brainstem white matter tracts. Rare PAS-positive, ubiquitinated
granular cells were seen in the middle cerebellar peduncle, pontine tegmentum, and inferior olive (Case 5 only) and the cerebellar white matter (Case 6 only). The cerebellum was atrophic, with wide sulci, a very sparse internal granular layer, and thin giotic white matter (Table 2). The Purkinje cell population was mildly to moderately depleted and very rare binucleate Purkinje cells were found (Fig. 7). Extensive branching mineralization of Purkinje cell dendrites was seen in the molecular layer (Fig. 8). This was accompanied by reactive changes among Bergmann glia. Silver-staining, neurofilament-immunoreactive swollen axons (torpedoes) were common in the granular layer and there were occasional dendritic "asteroids" (Fig. 9). The dentate nucleus was normal. The spinal cord of Case 7 exhibited rare neurofilament-immunoreactive axonal swellings in the posterior horns and corticospinal tracts. The walls of major blood arteries at the base of the brain were unremarkable. Electron microscopic examination of moderately well-preserved specimens of gray and white matter revealed no cellular inclusions or storage material. The lamellar pattern of myelin appeared normal. The pituitary was histologically normal.

**Case 9 (Age, 3 Years 2 Months):** In the cerebrum, there was general preservation of the large neurons, with loss of smaller neurons, reactive astrocytosis, parenchymal calcification, and severe vascular sclerosis and calcification. The lenticular nuclei were more severely affected with regard to the capillary and vascular mineralization. The corpus callosum was thin and the cerebral white matter was diffusely sclerotic, with multifocal mineral deposition, severe reactive astroglial changes, and loss of oligodendrocytes and myelinated axons. Some multinucleate cells and granular bodies were identified. Brainstem structures were variably affected. In the cerebellum, Purkinje and granular neurons were decreased in number, with microcalcification of parenchyma and capillaries in the molecular layer, and activation of both astrocytes and microglia in all layers including the white matter. The loss of internal granular neurons was not as severe as that seen in cases 4 to 7. Neuronal loss was identified in the spinal cord anterior horns and there was degeneration of the descending corticospinal tracts with prominent reactive astrocytosis, including large gemistocytic forms. Examination of the eyes revealed bilateral blunting of the
ciliary processes, chorioretinal scarring, preretinal glial membrane, and ganglion cell and nerve fiber layer atrophy. There were mild lens changes in the right eye. Both optic nerves were myelinated but small.

**DISCUSSION**

In summary, the neuropathological findings in COFS syndrome include (a) severe microcephaly and mild ventriculomegaly evident from birth, (b) possible delayed myelination in cerebral structures, (c) swollen, PAS-positive ubiquinated granular cells in the white matter that appear shortly after the onset of myelination, (d) subsequent patchy or diffuse loss of myelin with atrophy and gliosis in the white matter, (e) progressive cortical atrophy, (f) parenchymal, pericapillary, and vascular mineralization in the globus pallidus and putamen, cerebral cortex at the depths of sulci, and white matter, (g) rare binucleate Purkinje cells, (h) severe degenerative changes in the internal granular and Purkinje cell layers of the cerebellum, and (i) retinal degeneration with optic nerve atrophy. At birth and in infancy the brains have a normal gyral pattern, cortical architecture, and cortical thickness, suggesting that basic developmental processes are intact.

Myelin structure is apparently normal. However, the small brain size with ventriculomegaly at birth suggests that the degenerative process begins in utero. The presence of PAS-positive granular cells in 5/8 brains further suggests that the degenerative process in white matter begins shortly after the onset of myelination. Because we could not unequivocally demonstrate markers of monocyte lineage, these cells could represent degenerating glial cells with autophagic organelles. The subsequent severe neuronal loss indicates that the degenerative process accelerates in infancy.

COFS syndrome is currently diagnosed on the basis of phenotype (1). Among the children described herein with that clinical diagnosis, there was remarkable similarity in the neuropathological findings. Seven were North American aboriginal children from a well-defined inbred community, and one child was Caucasian from a distant geographic location. Other authors have only briefly documented the neuropathological or neuroradiological features in children with COFS syndrome (2). A 5-month-old Japanese male infant had a 230 g brain with partial agenesis of the posterior corpus callosum, dilated
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Fig. 9. Photomicrograph showing an argyrophilic axonal swelling ("torpedo") (large arrow) in the internal granular layer and a dendritic asteroid (small arrow) in the molecular layer of the cerebellum from Case # 5 (modified Bielschowsky method). Bar = 50 μm.

lateral ventricles, scattered white matter heterotopia, generalized poor myelination but no astrogliosis, "irregular neuronal arrangement" in the cerebellum, and microphthalmia. These changes were considered to be indicative of disordered migration, although there was also focal polymicrogyria believed to be due to a hypoxic-ischemic insult sustained in utero (6). A 3 year 9 month old Finnish child had a 600 g brain with firm atrophic white matter, cortex of normal thickness with paradoxically "few or no neurons" and microglossis, and cerebellar "inclusion bodies." There were no illustrations in that report (7). A CT scan of the younger brother at age 2 years 6 months demonstrated symmetric calcifications in the lenticular nuclei, patchy calcification in the frontal and occipital white matter, enlarged ventricles, and cerebellar atrophy (7). The CT scan of a 1-week-old infant of Muslim Arab parents showed dilatation of the cerebral ventricles and mild hypodensity of white matter "denoting dysmyelination." It is not clear, however, that this should represent a problem with myelin, considering that no cerebral myelin deposition would be expected at the age of 1 week (5). A muscle biopsy of the same child showed mild fibrosis and disorganized myofilaments. The child died at 38 days, but no autopsy was permitted (8). The CT scan of an 8-day-old Mexican child with COPS syndrome and infantile spasms showed "prominent ventricles and cisterns" (9). These 5 cases could probably be considered as within the spectrum of neuropathological changes that we have described. In two other published reports, the brains of children with a clinical diagnosis of COPS syndrome have been described, but they did not display any of the features we describe here. An African-American child who died at 33 hours had a small brain with partial agenesia of the corpus callosum; however, no details of the microscopy were presented (10). A 3-month-old child of Turkish Jewish parents with some phenotypic features of COPS syndrome and nonspecific myopathic changes had no abnormalities of the brain (11). The widespread severe demyelination observed in our Cases #6 and #9 bears some similarity to the changes in a previously described familial infantile encephalopathy with cerebral calcifications and diffuse leukodystrophy (12). However, in those cases the subcortical white matter was spared, the cortical calcification was more severe, there was no cerebellar degeneration, and the phenotype was entirely different. Another sporadic childhood leukodystrophy with basal ganglia calcifications but no cerebellar degeneration has been described. The damage was speculatively attributed to a primary microangiopathy (13).

The neuropathological changes in these children with COPS syndrome resemble those in persons with Cockayne syndrome (CS), specifically the early-onset type described by Lowry and coworkers in which the neurological deficit is evident at or shortly after birth (14, 15). The abnormalities have been described in 11 children ranging in age from 2 years 9 months to 8 years (16–24). As in older patients with CS, these children exhibited microencephaly, enlarged ventricles, discontinuous or patchy demyelination ("tigroid" pattern), gliosis, multifocal calcifications in cerebral capillaries and blood vessel walls, degenerative changes in the cerebellum including swollen axons ("torpedoes"), dendritic asteroid formations, and mineralized dendritic trees, and pigmentary retinopathy (25, 26). On all counts the findings in COPS syndrome were similar. Multinucleate astrocytes, which we observed, have also been reported in patients with CS (15, 20). We reviewed the pathological findings in the brain of a 14-year-old male with CS and found the typical patchy white matter loss and cerebellar degeneration, as well as the large cells with PAS-positive granules similar to those seen in the COPS syndrome cases.

The similarity between COPS and CS has been previously recognized on the basis of other phenotypic features; for example, facial appearance, cataracts, deafness, and dwarfism (27). Lowry suggested that the syndromes might be variants of the same entity (28). Baraitser and

Winter stated that "there is good evidence that some infants diagnosed initially as COFS subsequently develop Cockayne syndrome" (29). However, the evidence alluded to was not presented. The neuropathological similarities do not prove that they are variants of the same entity. COFS syndrome patients do not exhibit the sensitivity of skin to ultraviolet light observed in CS (26). In CS, cultured cells have impaired ability to repair damaged DNA following exposure to ultraviolet radiation. Results of similar tests have not yet been reported in COFS syndrome, although fibroblasts from one child with COFS syndrome did exhibit deficient growth in culture (3). The molecular defects in CS have been shown to be due to heterogeneous mutations in genes, termed CS group A and group B, that play a role in transcription-dependent DNA repair processes (30–34). These defects in DNA repair are closely related to the molecular defects in xeroderma pigmentosum (XP), in which at least 7 different involved genes, designated complementation groups XP-A to XP-G, have been identified (35–37). Mutations in 3 of these, groups B, D, and G, have occasionally been associated with a phenotypic complex of CS and XP. The children have diffuse cerebral demyelination and peripheral neuropathy, but no intracerebral calcifications (38, 39). Two infants with typical early onset CS had DNA repair defects similar to XP-A, although the mutations were not proven to be identical (40). Patients with XP-A frequently suffer severe neurological degeneration, referred to as De Sanctis-Cacchione syndrome, with death in childhood. However, this disorder is associated with diffuse neuronal loss from the cerebral cortex, pons, and Purkinje layer, but no demyelination or brain calcification such as are seen in CS and COFS (41–44). Another disorder with gene defects related to XP, trichothiodystrophy, is associated with neurological degeneration and diffuse cerebral demyelination but no calcifications. These features have been demonstrated by radiologic studies (45, 46), but no pathological studies are reported.

To conclude, the neuropathological features of COFS syndrome appear to be due to degeneration of white matter, cerebellum, and cerebrum, which likely begins in utero and accelerates in infancy as the brain matures. Gliosis and extensive mineralization are reactive or secondary features. The changes should be viewed in terms of a cellular defect involving multiple cell types throughout the central nervous system. The similarity of the pathological features to those of early-onset Cockayne syndrome suggests that a similar cellular mechanism may be defective; however, as indicated above, similarity in the biochemical or molecular defect does not necessarily dictate a similar neuropathological outcome. As has been suggested for xeroderma pigmentosum (47), we hypothesize that neurons, glia, and endothelial cells in COFS syndrome might have impaired ability to repair DNA, which results in their gradual dysfunction. The observation that transcription-coupled DNA repair is also involved in the repair of oxidative DNA damage, and not just that following ultraviolet exposure (48), makes it easier to understand why the brain would be particularly vulnerable with such a mutation. Direct testing of this hypothesis in COFS syndrome will require fortuitous sampling of fresh brain specimens and definition of the basic genetic defect in this disorder.

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