Clinical Neuroimaging Features and Outcome in Molybdenum Cofactor Deficiency

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Molybdenum cofactor deficiency (MOCOD; OMIM 252150) is a rare autosomal-recessive neurodegenerative disorder characterized by a combined deficiency of molybdenum-dependent enzymes xanthine oxidase, sulfite oxidase, nitrogenases, and nitrate reductase [1].

Over 100 cases have been reported, from approximately 50 unrelated families of diverse ethnic groups. More than 30 distinct disease-causing mutations in the cofactor biosynthesis genes MOCO1, MOCO2, and GEPH have been identified [2]. The global incidence of molybdenum cofactor deficiency is likely to be higher as a result of failure to diagnose and underreporting [3].

The original case reports include descriptions of intractable neonatal seizures progressing to severe global developmental delay, feeding difficulties, and death by infancy [4,5]. Milder phenotypes with late presentation and survival into the third decade of life are now recognized [6-9]. Neurologic features include epileptic encephalopathy, motor disorder, microcephaly, and visual impairment in addition to general features such as dysmorphism, marfanoid habitus, and lens dislocation [5-9]. The neurologic sequelae typically resemble those of hypoxic-ischemic brain injury, the most common form of neonatal encephalopathy [10,11].

Early precise identification is important in view of the emerging potential for therapeutic substitution as a treatment before the onset of neuronal damage. With wide use of brain magnetic resonance imaging (MRI), recognition of a specific imaging pattern could aid early mutational confirmation, resulting in appropriate therapeutic interventions and prenatal diagnosis [12,13].

Methods

Children with a confirmed biochemical (sulfite oxidase deficiency and xanthine oxidase deficiency) or genetic diagnosis of molybdenum cofactor deficiency referred...
to our institution over the past decade were identified by clinical records. They were systematically reviewed for demographic details, clinical features, neurodevelopmental outcome, and results of investigations. Clinical features of patients are summarized in Table 1. The study was approved by the ethics committee at Great Ormond Street Hospital, London, UK.

The criteria for diagnosis are shown in Figure 1. Biochemical analysis was performed in our laboratory. Two patients had enzyme assay performed at the metabolic laboratory of Pédriatique Hôpital Debrousse, Lyon, France. Four patients had mutational confirmation at the Purine Research Laboratory, Guy’s and St Thomas' Hospital, London, UK.

Five patients underwent electroencephalogram (EEG) in the referral hospital soon after the onset of seizures. These were repeated in 2 cases at Great Ormond Street Hospital.

All children had conventional brain MRI scans, which were reassessed independently by 2 pediatric neuroradiologists (R.G. and K.C.). Four patients (patients 1, 4, 6, and 8) had MRI performed at Great Ormond Street Hospital on a 1.5 T Symphony scanner (Siemens, Germany). This included T1-weighted and fast spin echo T2-weighted sequences, FLAIR (a 1.5 T Symphony scanner (Siemens, Germany). This included T1-weighted and fast spin echo T2-weighted sequences, FLAIR (patient 2), dysmorphism (patients 3 and 4), and typical MRI features (patients 1, 3, and 4).

### Results

Four of the 8 patients studied were boys. All were born in good condition, except patient 5, who needed 3 days' ventilatory support for presumed severe hypoxic-ischemic injury. The age range at presentation was 1 day to 24 months. Two groups were distinguished on the basis of their predominant presenting features: early presentation with encephalopathy and atypical late presentation with global delay.

#### Early presentation with encephalopathy

Patients 1 through 6 manifested classical symptoms with encephalopathy. Patients 1 through 5 had neonatal seizures. The diagnosis of molybdenum cofactor deficiency was considered because of seizure intractability (patients 1 and 2), family history (patient 2), dysmorphism (patients 3 and 4), and typical MRI features (patients 1, 3, and 4).

### Table 1. Clinical and investigation profile in 8 children diagnosed with molybdenum cofactor deficiency

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Early Onset (Classical) Encephalopathy</th>
<th>Late onset (atypical) GDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset/presenting feature</td>
<td>Patient 1</td>
<td>Patient 2</td>
</tr>
<tr>
<td>Ethnicity/consanguinity</td>
<td>Afghanistan/Y</td>
<td>India/Y</td>
</tr>
<tr>
<td>Gestation/delivery/BW</td>
<td>36 wk/ES/NA</td>
<td>Term/ES/3.2</td>
</tr>
<tr>
<td>OFC percentile at birth/follow-up</td>
<td>98th/2nd</td>
<td>98th/2nd</td>
</tr>
<tr>
<td>Reason for consideration of MOCOD</td>
<td>Dysm3, SS, MRI features</td>
<td>Dysm3, SS, MRI features</td>
</tr>
<tr>
<td>Establishment of abnormal biochemistry</td>
<td>Day 9</td>
<td>Day 9</td>
</tr>
<tr>
<td>Plasma urate (range 90-370 μmol/L)</td>
<td>&lt;30/positive</td>
<td>&lt;12/positive</td>
</tr>
<tr>
<td>Enzyme assay/genetic analysis</td>
<td>ND/MOCS1</td>
<td>ND/MOCS1</td>
</tr>
<tr>
<td>Age at MRI Outcome (age at follow-up)</td>
<td>Day 7/3 mo</td>
<td>Day 7/3 mo</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- **BLO** = Bilateral lens dislocation
- **BS** = Burst suppression
- **BW** = Birth weight (kg)
- **Dysm** = Dysmorphism
- **ECS** = Emergency caesarean section
- **FD** = Feeding difficulties
- **GDD** = Global developmental delay
- **HIE** = Hypoxic-ischemic encephalopathy
- **MOCOD** = Molybdenum cofactor deficiency
- **NA** = Not available
- **ND** = Not done
- **NVD** = Normal vaginal delivery
- **SOD** = Sulfite oxidase deficiency
- **Sz** = Seizures
- **VI** = Visual impairment

1. Older sibling died at the age of 9 years from MOCOD
2. Two maternal uncles had unexplained death in infancy
3. Older sibling has BLD and mild motor/speech delay
4. Frontal bossing, adducted thumb, dysmorphism
5. Frontal bossing, high arched palate, small chin, downsizing palpebral fissures, deep-set ears, dysmorphism
6. Frontal bossing, depressed nasal bridge, retrognathia, low posterior hairline, prominent calcaneum, widespread nipples, long fifth finger, dysmorphism
7. Large ears, anteverted nose, and high arched palate (features evident at age 4)
Patient 5 was assumed to have a diagnosis of severe hypoxic-ischemic injury until 4 years of age, when dysmorphic features and evolving neurologic signs prompted a search for an alternative diagnosis. Patient 6 developed an encephalopathy in the form of extreme irritability with feeding difficulties from day 7. Similar to patient 5, progressive pyramidal and extrapyramidal neurologic (mixed spastic and dystonic) signs led to the diagnosis of molybdenum cofactor deficiency at 26 months.

**Atypical late presentation with global delay**

Patients 7 and 8 manifested global developmental delay at age 8 months and 24 months, respectively. Patient 7 developed seizures at 26 months after an intercurrent illness, with subsequent loss of mobility and postural skills. Patient 8 developed visual impairment due to lens subluxation at age 5 years, 6 months. He had a sibling with similar clinical features, which prompted the diagnosis.

**Neurologic features in both groups**

In the 7 children with microcephaly, serial occipitofrontal measurements crossed more than 2 standard deviations below the mean (severe progressive microcephaly). One patient with early manifestation of symptoms (patient 1) experienced ventricular dilatation resulting from global cerebral atrophy and at 4 months of age required ventriculoperitoneal shunt placement for symptomatic hydrocephalus.

All 8 children had moderate to severe motor disorder comprising one or more of the following: axial hypotonia, peripheral hypertonia, orobulbar dysfunction, dystonia, and verbal dyspraxia. In addition, all had varying degrees of language and visual impairment. The latter was due to cortical damage in all except patient 8, who had myopia/lens dislocation. Except patient 8, all showed progressive pyramidal and extrapyramidal neurologic impairment that evolved at a variable rate, and they developed epilepsy and orobulbar dysfunction requiring alternative methods of feeding (gastrostomy, nasogastric tube) or modified puree. Two children became free of seizures, and 5 experienced seizure control with one antiepileptic drug. Of the 3 children with kyphoscoliosis, 1 required spinal fusion at 14 months (patient 3).

**Outcome in both groups**

The duration of follow-up ranged from 12 months to 8 years (median 4 years). There were no deaths in the study period. The outcome was classified as severe in 7 patients (6 with early and 1 with late presentation), including nonambulation, spastic quadriplegia, progressive microcephaly, global delay, orobulbar dysfunction, blindness, and epilepsy. Outcome was moderate in a patient with late presentation (patient 8), with visual impairment and severe verbal dyspraxia but no feeding difficulties or seizures. He is ambulant, independent with daily activities, and attends mainstream school with support.

**Investigation**

The latency between symptom onset and establishment of biochemical diagnosis ranged from 4 days to 4 years (median 3 months) (Table 1, Fig 1). Initial EEG in patients 1, 3, and 4 revealed a burst suppression pattern that resolved at 12 days, 3 months, and 4
weeks, respectively. Electrical seizure activity was observed on EEG in patient 5. Patient 7 underwent a muscle biopsy at 4 years, which revealed type 2 fiber atrophy and secondary complex 1 deficiency.

Age at which brain MRI was performed ranged from 1 day to 5 years 6 months (median 10 months). Six patients had imaging available for review (5 with early presentation [patients 1, 2, 4, 5, and 6] and 1 with late presentation [patient 8]). For patients 3 and 7, the imaging features were extracted from the radiology reports from our institution. In 3 patients (patients 1, 4, and 7), sequential MRI data were available.

In all 6 patients with early presentation with encephalopathy (patients 1-6), brain MRI demonstrated diffuse cerebral hemisphere infarction of the cortex and white matter. Imaging was performed in patients 1, 3, and 4 at days 7, 1, and 9 respectively. These scans showed acute changes consisting of global cerebral swelling and edema (Fig 2). Patients 2, 5, and 6 had imaging at 2, 10, and 26 months, respectively, which revealed cystic encephalomalacia and/or cortical and white matter atrophy similar to the changes observed at follow-up imaging of patients 1 and 4.

The 2 children with early brain MRI scans available for review (patients 1 and 4) had edema within the cerebral hemispheres, basal ganglia, and thalami with more acute, focal, and symmetrical changes within the globus pallidi, cerebral peduncles of the midbrain, and subthalamic regions. Focal changes included swelling with hyperintense signal observed on dual-echo short time inversion recovery and on diffusion-weighted imaging, with hypointense signal on calculated apparent diffusion coefficients maps in keeping with restricted diffusion (Fig 2). In patient 3, the radiology report documented chronic changes of cystic encephalomalacia, including basal ganglia and thalamic injury on the scan acquired on the first day of postnatal life indicating that a prenatal insult had occurred.

Patients 2 and 6 had brain MRI in the chronic phase of their illness (2 and 26 months, respectively) that revealed mature injury of the globus pallidi with atrophy and T1 shortening. Patient 6 had additional subtle features of injury within the cerebral peduncles and subthalamic regions. Patient 5, imaged 10 months after his acute illness, had mature anterior, posterior parasagittal, and deep watershed infarction in a pattern indistinguishable from the pattern observed in hypoxic-ischemic injury at term, without involvement of the deep gray matter, cerebral peduncles, or subthalamic regions.

Global cerebral infarction with greater involvement of the parieto-occipital white matter was observed in patients 1, 4, and 5, including the 2 patients with early brain MRI (Fig 2f). A distinctive band of intermediate signal intensity at the junction between cortex and white matter was noted on dual-echo short time inversion recovery images (Fig 2C). Of the 3 patients who were imaged late, 2 (patients 2 and 6) did not manifest any posterior-anterior gradient of severity, and 2 (patients 5 and 6)
did not manifest presence of a distinctive band at the interface between subcortical white matter and cortex.

Four of 6 patients (patients 1, 2, 4, and 6) with early clinical presentation had pontocerebellar hypoplasia and posterior fossa retrocerebellar cyst. In 2 children who underwent sequential imaging, we observed maturation of extensive cerebral hemisphere and deep gray matter infarction. Progressive pontocerebellar atrophy (Fig 3) and enlargement of retrocerebellar cyst was observed. There were no other features of progression.

One of the 2 children (patient 8) with atypical late presentation with global developmental delay had imaging available for review. In this child, who had no acute neurologic illness, there was symmetrical signal abnormality within the cerebellar deep nuclei (Fig 4) and mild lack of white matter bulk with a thin corpus callosum. Bilateral lens subluxations were also present.

Figure 3. Patient 4. Brain MRI performed at day 9 after onset of encephalopathy (a, b) reveals a small pons, a slightly small cerebellar vermis, and small posterior fossa retrocerebellar cyst. Brain MRI at age 11 months (c, d) and ventricular puncture for hydrocephalus reveal progressive pontine atrophy and enlargement of the cyst.

Figure 4. Patient 7 with atypical clinical presentation of global developmental delay. Axial dual-echo image (a) reveals bilateral signal abnormality within the cerebellar deep nuclei and hiliar white matter. Sagittal T1-weighted image (b) reveals lens subluxation.
Radiology reports for patient 7, with brain MRI scans acquired at age 10, 26, 33, and 51 months, indicated progressive cerebral atrophy with involvement of the globus pallidi observed from the initial scan. A scan performed during a noninfective encephalopathic illness in this patient demonstrated more focal changes within the globus pallidi, subthalamic regions, and cerebral peduncles.

Discussion

To our knowledge, this is the largest reported series of MOCOD with an outcome spanning over 10 years. Previous phenotypic variability [4-9] has now been extended to include severe verbal dyspraxia.

We have identified 2 groups on the basis of distinctive neurologic features at presentation: neonatal encephalopathy and late-onset developmental delay. Except in patient 8, progression to long-term neurologic phenotype in both groups is consistent. However, variations are observed in the severity of the motor, visual, and language impairment. MOCOD is a debilitating disease, particularly in cases of early manifestation [5,14-16]. We had no deaths in our series, but 7 children had a severe outcome, including 1 with late presentation.

MOCOD is screened for the presence of positive urine sulfite and low plasma urate. This is followed by evidence for high purine metabolites (xanthines and hypoxanthines) and high S-sulfoysteine, which are due to the deficiency of xanthine oxidase and sulfite oxidase, respectively (Fig 1). Plasma urate can be normal in the mild phenotype, and urine sulfites can be falsely negative if a fresh sample is not analyzed [3,7]. In isolated sulfite oxidase deficiency (OMIM 2722300), a related condition, there is no xanthine oxidase deficiency, and hence urate and purine metabolites are normal. These 2 conditions are often not clearly distinguished in the literature, resulting in heterogenous data [11].

One of the major difficulties in the management of MOCOD patients is the ability to control seizures at their onset, the earliest and most common manifesting feature in our cohort. The burst suppression pattern—a marker of severe neuronal insult—was observed in the acute EEG in 3 of the children with early presentation; 2 of these also had imaging features of diffuse irreversible cytotoxic edema during this acute phase. This pattern, however, resolved on sequential EEGs. These infants appeared in relatively good condition at birth compared to neonates with a similar extent of brain injury due to severe hypoxic-ischemic injury, who may have similar patterns of electrical activity and frequently do not survive. In vitro studies suggest that endogenous toxic sulfite induces glutamate-mediated excitotoxicity (via NMDA [N-methyl-D-aspartic acid] receptors) and inhibits tricarboxylic-cycle substrates of mitochondrial energy metabolism [17,18]. This hypothesis is supported by observation of complex 1 deficiency (NADH) in patient 7, the symmetrical involvement with restricted diffusion of the basal ganglia in previous series [10,14,19,20], changes in globus pallidi, subthalamic and dentate nuclei in our patients, and magnetic resonance spectroscopy data [20,21]. These data suggest that mitochondrial dysfunction and oxidative stress may contribute to cerebral infarction.

Pyramidal signs emerge during disease progression, as observed in our study as well as previous ones [10,11]. The clinical and imaging features of progressive white matter loss may be explained by the accumulation of sulfite impairing biosynthesis of sphingolipids necessary for myelination [17,18]. Previous histopathology studies have revealed widespread neuronal loss, gliosis, and severe cystic necrosis in the white matter and basal ganglia in MOCOD patients [16-18]. In most children with MOCOD, growth parameters seem to be proportionate until birth. This has been attributed to protective effects in utero from the maternal clearance of cytotoxic sulfites, rather than placental transfer of molybdenum cofactor, precursor Z (now termed cyclic pyranopterin monophosphate [cPMP]) or sulfates [22]. All children in our series exhibited either slowing of head growth or cortical atrophy at imaging. However, progressive microcephaly becomes apparent postnatally in most of our cases. The sequential MRI scans from 3 of these infants showed evidence of cerebral atrophy being the likely cause of poor head growth, with the earliest finding of cortical atrophy being on day 1 of postnatal life. To our knowledge, to date, there are only a few descriptions of MRI features, and little information exists about its natural history on sequential or diffusion-weighted imaging [8-10,20,21]. Previous studies have described the difficulty of distinguishing MRI features of global cerebral infarction in MOCOD from that due to global hypoxic-ischemic injury [10,14].

In children with encephalopathy, the imaging appearances depended mostly on the time after onset of encephalopathy. On the basis of our observations and those of others, the initial acute manifestations are diffuse brain swelling and edema, in keeping with acute global cerebral infarction. The exception was patient 3, who already had chronic changes of diffuse cerebral infarction by the time of the first clinical episode of encephalopathy on day 1 of postnatal life. Diffusion-weighted MRI supports the concept of diffuse and irreversible cytotoxic edema occurring in the acute stages of encephalopathy. Restricted diffusion depicted as dark regions on apparent diffusion coefficient maps matched by hyperintensity on diffusion-weighted images is observed as early as a few minutes to hours after the onset of the ischemic insult: the dark regions gradually increase over a period of days to become similar to the adjacent brain (that is, they “pseudonormalize”) by 7-10 days. Interestingly, the other 2 neonates with early MRI had both acute (swelling and restricted diffusion within the deep gray matter and midbrain) and subacute (cortical T2 shortening) changes, suggesting 2 different time points of brain injury rather than a single insult.

Although the onset of the primary pathogenetic mechanisms is different in severe forms of hypoxic-ischemic injury [23] and MOCOD, the net mechanisms leading to neuronal cell death due to energy failure, glutamate release, intracellular calcium, and free radical accumulation are remarkably similar [24,25]. This explains the striking clinical and imaging appearances of hypoxic-ischemic injury. However, the more selective symmetrical pallidal and subthalamic changes compared to the rest of the basal ganglia observed in our cases are not a feature of severe hypoxic-ischemic injury in which deep gray matter injury ranges from selective symmetrical postero lateral putamina and ventrolateral thalami changes to more diffuse basal ganglia involvement [26-29]. These pallidal and subthalamic changes with restricted diffusion were observed during the acute neurologic illness, whereas later scans showed less of these regional abnormalities and were less specific. This underlines the importance of performing early brain MRI in neonatal encephalopathy, so that it is sufficiently early (<1 week) to detect restricted diffusion within the pallidi, cerebral peduncles, and subthalamic regions.

Another sign that could suggest the diagnosis of MOCOD in the context of global cerebral infarction is the presence of a distinctive cortical/subcortical band of intermediate signal intensity on T2-weighted images, outlined by edema on either side. It is not clear precisely what causes this, although it could represent relatively spared brain parenchyma. Pontocerebellar hypoplasia with or without progressive atrophy also appears to distinguish MOCOD from hypoxic-ischemic injury. In the latter, the acute brain stem injury has a different pattern comprising acute swelling or edema and/restricted diffusion, and is observed only in the most severe cases, usually in neonates who do not survive. Previous reports
describe Dandy-Walker malformation or Dandy-Walker variant in MOCOD [14,15,19,30], but they do not indicate the presence of vermian rotation or cystic fourth ventricle enlargements, which define these conditions. Instead, they appear to indicate the presence of a small cerebellum/pons with a retrocerebellar arachnoid cyst. In 2 cases, we found progressive pontocerebellar atrophy with enlargement of the retrocerebellar cyst.

With advances in neuroimaging and molecular genetics, it has been increasingly recognized that the phenotypic expression in MOCOD is broad. An important challenge is to raise awareness to aid in early diagnosis. Furthermore, the clinical and brain MRI phenotypes typically resemble those of hypoxic-ischemic injury and may lead to misdiagnosis, with implications for family and health care providers. In this context, performing early and possibly sequential MRI could help to identify specific features of MOCOD.

The early diagnosis of MOCOD is crucial in the context of recently emerging therapeutic option: substitution of cyclic pyranopterin monophosphate (cPMP; comparable to tetrahydrobiopterin) produced in bacteria and currently evaluated in preclinical studies. To reduce the potential harm to the central nervous system, supplementation should be commenced early.[12,13] The findings in our cohort support the need for early recognition of the condition because severe damage to the brain tissue may happen in early, possibly fetal, life.[31]

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References