Sulfite Intoxication Disorders

Mahmoud Reza Ashrafi
Professor of Pediatric Neurology
Children’s Medical Center
Pediatrics Center of Excellence
Tehran University of Medical Sciences
• Sulfite oxidase is one of three enzymes (xanthine dehydrogenase and aldehyde oxidase) in man that require the trace metal molybdenum.

• In this enzyme, the metal is complexed to a unique pterin, molybdopterin, forming the molybdenenum cofactor. (Rajagopalan and Johnson, 1992)
Metabolic pathway of cysteine and xanthine metabolism

Sulfite oxidase is localized in the mitochondrial intermembrane space and catalyzes the oxidation of sulfite to sulfate, the terminal reaction in the pathway of degradation of sulfur amino acids. (Methionine, cysteine, homocysteine, taurine)
Metabolic pathway of cysteine and xanthine metabolism

- Molybdenum cofactor (MoCo) is also required for xanthine dehydrogenase and aldehyde oxidase.
- Xanthine dehydrogenase catalyzes the hydroxylation of hypoxanthine and xanthine producing uric acid, and aldehyde oxidase oxidizes aldehydes to carboxylic acids in the cytoplasm and functions in detoxification.
Isolated sulfite oxidase deficiency (ISOD) and Molybdenum cofactor deficiency (MoCoD), are autosomal recessive inherited diseases.

Of the two variants, isolated sulfite oxidase deficiency (1967) appears to be much less common.

The prevalence of molybdenum co-factor deficiency is estimated as being between 1 in 100,000 and 1 in 200,000.

Loss of activity of SOX is sufficient to cause the neurodegenerative phenotype of both ISOD and MoCoD.
Isolated sulfite oxidase deficiency (ISOD) Molybdenum cofactor deficiency (MoCoD)

- ISOD affects the metabolism of sulfated amino acids due to SOX deficiency, while MoCoD affects SOX and xanthine dehydrogenase.
- Deficiency of either xanthine oxidase or aldehyde oxidase is not known to be associated with neurologic disorders.
The spectrum of sulfite intoxication disorders ranges from classic early-onset (severe) disease to late-onset (mild) disease.

Individuals affected with sulfite intoxication disorders most commonly present in the neonatal period with a broad range of signs including encephalopathy, intractable seizures, feeding difficulties, high-pitched cry, metabolic acidosis, exaggerated startle response, axial hypotonia, and characteristic dysmorphic facial features.
Patients with GPHN deletions and splice site mutations show hyperekplexia.
Key Findings on Examination

• Abnormalities in facial features also become increasingly pronounced with lack of normal head growth.
• These facial differences include a relatively long and narrow face; deep-set, widely-spaced eyes; elongated openings of the eyes (palpebral fissures); puffy cheeks; a small nose; a large space between the nose and upper lip (a long philtrum); and thick lips.
Ectopia lentis

- A significant diagnostic clue, ectopia lentis, is seen in some patients if they progress beyond the neonatal period.
- In those infants that survive, subsequent lens dislocation may occur, with the youngest report of dislocation occurring at 8 weeks of age.
- The association of ectopia lentis with a movement disorder, even without psychomotor regression or epilepsy, should therefore prompt us to look for this diagnosis.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marfan syndrome</td>
<td>AD</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>AR</td>
</tr>
<tr>
<td>Weill-Marchesani</td>
<td>AD/AR</td>
</tr>
<tr>
<td>Dominant spherophakia</td>
<td>AD</td>
</tr>
<tr>
<td>Simple ectopia lentis et pupillae</td>
<td>AR</td>
</tr>
<tr>
<td>Hyperlysinemia</td>
<td>AR</td>
</tr>
<tr>
<td>Sulfite oxidase deficiency</td>
<td>AR</td>
</tr>
<tr>
<td>Aniridia*</td>
<td>AD</td>
</tr>
<tr>
<td>Conradi syndrome*</td>
<td>XL/AD</td>
</tr>
<tr>
<td>Crouzon syndrome*</td>
<td>AD</td>
</tr>
<tr>
<td>Ehler’s–Danlos syndrome*</td>
<td>AD/AR</td>
</tr>
<tr>
<td>Kniest syndrome*</td>
<td>AR</td>
</tr>
<tr>
<td>Mandibulofacial dysostosis*</td>
<td>AR</td>
</tr>
<tr>
<td>Megalophthalmos*</td>
<td>AR</td>
</tr>
<tr>
<td>Pierre Robin syndrome*</td>
<td>AR</td>
</tr>
<tr>
<td>Oxycephaly*</td>
<td>AD/AR/XL</td>
</tr>
<tr>
<td>Refsum disease*</td>
<td>AR</td>
</tr>
<tr>
<td>Retinitis pigmentosa*</td>
<td>AD/AR/XL</td>
</tr>
<tr>
<td>Wildervanck syndrome*</td>
<td>AR</td>
</tr>
</tbody>
</table>

AD = autosomal dominant; AR = autosomal recessive; XL = X-linked.

* Less frequent.
Key Findings on Examination

- Other uncommon features include renal stones, intermittent microscopic hematuria, skeletal abnormalities, laryngomalacia, autonomic dysfunction, and metabolic and lactic acidosis.
- Macrocephaly may occur exceptionally due to hydrocephalus.

<table>
<thead>
<tr>
<th>Eye findings</th>
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</thead>
<tbody>
<tr>
<td>• Dislocated lenses (may develop after the neonatal period)</td>
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<tr>
<td>• Lack of response to light</td>
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</table>

<table>
<thead>
<tr>
<th>Cardiovascular</th>
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<tbody>
<tr>
<td>• Hypotension</td>
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<table>
<thead>
<tr>
<th>Gastrointestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Feeding difficulties</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Nephrolithiasis</td>
</tr>
</tbody>
</table>
Late-onset ISOD

- Rarely, milder cases have been reported.
- Late-onset ISOD usually begins between the ages of 6 and 18 months, often after a febrile illness.
- Individuals with this form of the disorder may not have the seizures and ectopia lentis that usually occur in the classic form.
- They have developmental delay and may lose skills that they had already developed (developmental regression).
- Movement problems occur in this form of the disorder, including dystonia, choreoathetosis, and ataxia.
- The signs and symptoms of late-onset ISOD can gradually get worse (progress), or they can be episodic, which means that they come and go.
- Some individuals with this form of ISOD survive into childhood or adolescence; because of the rarity of this disorder, their life expectancy is unknown.
Late-onset MoCoD

- The phenotypic spectrum of MoCoD was recently further delineated by Misko, et al. into 2 phenotypes based on clinical and radiographic distinctions.
- In the first group are those who have “classic MoCoD” defined as clinical presentation from DOL 1-50 with acute onset of neurologic symptoms.
- In this case scenario, MoCoD may mimic HIE in the newborn and needs to be considered.
- The second group of patients with MoCoD, accounting for 13% of patients, present between 50 days and 23 years.
- The prominent neurologic condition involves a movement disorder, and MRI shows selective injury of the basal ganglia and cerebellum.
- This overlaps clinically with many other IEMs and needs to be included in the differential.

Molybdenum cofactor deficiency – phenotypic variability in a family with a late-onset variant

Diagnosis and Testing

• When sulfite intoxication is in the differential diagnosis, specific laboratory testing may offer clues.
• One should consider screen for the presence of urine sulfite and low or decreasing plasma urate.
• Next, one should screen for elevations of purine metabolites (xanthine and hypoxanthine) and elevations of S-sulfocysteine (SSC), which are due to the deficiency of MoCodependent enzymes.
Urine Sulfite dipstick

- A positive sulfite dipstick finding of very fresh urine is highly suggestive of sulfite intoxication disorders; however, a negative dipstick finding should not eliminate suspicion, as urinary sulfite is an unstable compound and prone to false-negative results related to drugs and bacterial degradation.
S-Sulfocysteine Panel, Urine

- This test provides a quantitative report of s-sulfocysteine, xanthine, hypoxanthine and uric acid identified via liquid chromatography-mass spectrometry.
- Useful for diagnosis of molybdenum cofactor deficiency, isolated sulfite oxidase deficiency and hereditary xanthinuria.

Test ID: SSCTU
S-Sulfocysteine Panel, Urine
Hereditary Xanthinuria

- Xanthinuria is a descriptive term for excess urinary excretion of the purine base xanthine.
- Two inherited forms of xanthinuria principally result from a deficiency of the enzyme xanthine dehydrogenase, which is the enzyme responsible for degrading hypoxanthine and xanthine to uric acid.
- Related signs and symptoms can include abdominal pain, recurrent urinary tract infections, and hematuria.
- Less commonly, xanthine crystals build up in the muscles, causing pain and cramping.
- In some people with hereditary xanthinuria, the condition does not cause any health problems.
Biochemical Findings of ISOD

• Biochemical features of the isolated form include increased urinary excretion of sulfite, thiosulfate, taurine and S-sulfocysteine and low plasma cystine and homocysteine.
• Urinary excretion of xanthine and hypoxanthine is normal, and so is uric acid.
• Biochemical abnormalities that characterize MoCoD are a reflection of deficient functioning of molybdenum cofactor–dependent enzymes xanthine oxidase and sulfite oxidase.

• The most consistent and classical biochemical abnormality is markedly reduced or undetectable uric acid in serum secondary to deficiency of xanthine dehydrogenase.

• Urinary purine metabolites, namely xanthine and hypoxanthine, are markedly elevated.

• A word of caution is that milder phenotypes may have plasma uric acid at just the lower end of the laboratory reference range.
# Diagnosis and Testing Requirements for Diagnosis of MoCoD

<table>
<thead>
<tr>
<th></th>
<th>Quantity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfite dipstick</td>
<td>N/A</td>
<td>Fresh urine</td>
</tr>
<tr>
<td>S-sulfocysteine</td>
<td>1 mL</td>
<td>Frozen</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1 mL</td>
<td>Frozen</td>
</tr>
<tr>
<td>Xanthine</td>
<td>1 mL</td>
<td>From purine/pyrimidinium panel</td>
</tr>
<tr>
<td><strong>Plasma studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-sulfocysteine</td>
<td>1 mL</td>
<td>EDTA tube</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1 mL</td>
<td>EDTA tube</td>
</tr>
<tr>
<td>Xanthine</td>
<td>1 mL</td>
<td>From purine/pyrimidinium panel</td>
</tr>
</tbody>
</table>

Abbreviation: EDTA, ethylenediabminetetraacetic acid.
### Metabolic Differences Between Subtypes of MoCoD

<table>
<thead>
<tr>
<th>Molybdenum cofactor deficiency type</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine laboratory</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Special laboratory</td>
<td>N</td>
<td>↑↑</td>
<td>n-↑</td>
</tr>
<tr>
<td>Cystine (P)</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Homocysteine (P)</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↑</td>
</tr>
<tr>
<td>PLP (P)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Sulfocysteine (P, U)</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Sulfite (U)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Taurine (P, U)</td>
<td>↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Urothione (U)</td>
<td>↓↓↓</td>
<td>↓</td>
<td>n-↑</td>
</tr>
<tr>
<td>Xanthine (P, U)</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
</tbody>
</table>

*Abbreviations: P, plasma; U, urine*
In the absence of neonatal asphyxia, MR findings showing the rapid development of severe white matter cavitary changes may suggest ISOD or MoCoD, and they should prompt appropriate biochemical evaluation.
FIG 1. Axial MR image obtained when the patient was aged 5 days shows extensive areas of abnormal signal intensity, which suggest edema involving the bilateral cerebral white matter and bilateral parietal and occipital gray matter. The bilateral frontal and temporal cortex, as well as parts of the basal ganglia, are probably spared. Ventricular size is normal. FIG 2. Axial MR image obtained when the patient was aged 12 days. FIG 3. Axial MR images obtained when the patient was aged 31 days demonstrate dramatic changes compared with findings on initial images.

Fig. 1. — MR imaging performed at age 3 days. A, axial T2-weighted image; B, axial IR image; C, coronal T2-weighted image; D, sagittal T1-weighted image. There is abnormal high signal in the central and peripheral white matter in the T2-weighted images (A, C) and low signal in the inversion recovery T1-weighted image (B), with a cystic appearance of the white matter and a corresponding ex-vacuo enlargement of the ventricular system and subarachnoid spaces. Notice severe thinning of the corpus callosum (D, arrows) and flattening of the pontine protuberance (D, arrowhead).
Brain Imaging

• ISOD and MoCoD should be considered when HIE is in the differential diagnosis.
• Typical brain MRI findings include cerebral edema, cystic encephalomalacia, and involvement of the globus pallidus and subthalamic nuclei, with cortical and white matter atrophy.
• HIE involves deep gray matter: posterolateral putamina and ventrolateral thalami or more diffuse basal ganglia injury.
Sagittal magnetic resonance imaging of proband showing diffuse white matter abnormality.
The brainstem and cerebellum are hypoplastic with extensive surrounding cisternal fluid.
Prenatal enlarged cisterna magna has been observed in a patient who was ultimately diagnosed with an MOCS1 gene mutation.
The prominent neurologic condition involves a movement disorder, and MRI shows selective injury of the basal ganglia and cerebellum. (a) Axial PD-weighted image shows bilateral hyperintense signal of globus pallidi. (b) Sagittal T1-weighted image, showing a thin corpus callosum.
The pathogenesis of neurotoxicity is not completely understood but could comprise the accumulation of glutamate, an excitotoxic neurotransmitter, due to the combined inhibition of glutamate dehydrogenase (GDH) and possibly alfa-ketoglutarate dehydrogenase by sulfites.

It is believed that SSC is responsible for progressive excitotoxic neurodegeneration.

S-sulfocysteine (SSC) is a structural analog of glutamate that accumulates in the plasma and urine of patients.

Studies show it can function as an N-methyl-d-aspartate receptor (NMDA-R) agonist, thereby promoting calcium influx and downstream events leading to neurotoxicity.

2-Kayal Vijayakumar et al. Pediatric Neurology 45 (2011) 246e252
Cystic brain destruction might already be present prenatally, and subsequent development of gyration and differentiation of the cortical layers in the developing brain can be affected by sulfite accumulation early during the third trimester.

Prenatal appearance of molybdenum cofactor deficiency in the fetus at 35 weeks' gestation.

(A) Transvaginal ultrasound; paramedian plane shows the multilocular pattern of the multiple cysts.

(B) Transvaginal ultrasound. Coronal plane at the level of the third ventricle shows lateral and third ventricle dilatation; the subcortical white matter has been replaced by multiple encephaloclastic lesions.

Genetic Testing

- Patients with ISOD carry biallelic mutation in SUOX.
- Patients with MoCoD harbor mutation in one of the molybdenum cofactor synthesis genes MOCS1, MOCS2, GPHN, or MOCS3.

<table>
<thead>
<tr>
<th>Complementation Group</th>
<th>Gene Symbol</th>
<th>Chromosomal Locus</th>
<th>Protein Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated sulfite oxidase</td>
<td>SUOX</td>
<td>12q13.2</td>
<td>Sulfite oxidase</td>
</tr>
<tr>
<td>Complementation group A</td>
<td>MOCS1</td>
<td>6p21.3</td>
<td>MoCo synthesis protein 1 A and AB</td>
</tr>
<tr>
<td>Complementation group B</td>
<td>MOCS2</td>
<td>5q11</td>
<td>MoCo synthesis protein 2</td>
</tr>
<tr>
<td>Complementation group B</td>
<td>MOCS3</td>
<td>20q13.13</td>
<td>MoCo synthesis protein 3</td>
</tr>
<tr>
<td>Complementation group C</td>
<td>GPHN</td>
<td>14q23.3</td>
<td>Gephyrin</td>
</tr>
</tbody>
</table>
Case Presentation

• An 18-month old girl presented with abnormal movement and motor regression from 8 month-old after an URI.
• The only child of a consanguineous marriage, Term, no perinatal insult.
• Hypotonic, poor Fix and follow, Neck holding + Rolling –, Sitting –, HC=46
• The diagnosis provided to the family was **mitochondrial disorders** and treatment with Madopar 125, ¼ BD, Biotin 5 mg daily, Vitamin B1, B6, B2 and Q10 provided.
Second Case Presentation - MRI

• Bilateral GP, dentate nucleus
Case Presentation - Biochemical Findings

- Ammonia 33
- Lactate 29
- Pyruvate 1.3
- CSF Gluc 58, pro 9, lactate 9
- Uric acid 2.2 mg/dL (normal range, 2-7)
- Homocysteine 5 (5-15)
- Normal acylcarnitine profile
- Increased urine s-sulfocysteine
Case Presentation – Genetic Analysis

RESULT SUMMARY

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant Coordinates</th>
<th>Zygosity</th>
<th>In Silico Parameters</th>
<th>Allele Frequencies*</th>
<th>Type and Classification**</th>
</tr>
</thead>
</table>

*Genome Aggregation Database (gnomAD) Genomes version.3.0, Exome Aggregation Consortium (ExAC) version.1.0 and Innomie **Variant classification is based on ACMG recommendations: Class 1: Pathogenic; Class 2: Likely pathogenic, Class 3:Variant of uncertain significance (VUS); Class 4: Likely benign, Class 5: Benign

VARIANT INTERPRETATION

SUOX . c.429C>A p.(His143Gln)

CLINICAL INFORMATION

The proband was healthy until 8 months old, after which she regressed due to recurrent viral infections and diarrhea. Metabolic profile was normal.

Genes related to the following HPO terms were applied in the analysis: Hypotonia, Developmental regression, Brain imaging abnormality.

Consanguinity: Yes

POSITIVE RESULT

Likely pathogenic variant identified

INTERPRETATION

A homozygous likely pathogenic variant in the SUOX gene was identified. The genetic diagnosis of autosomal recessive Isolated Sulfite Oxidase Deficiency is possible.

As an incidental finding, we detected a heterozygous pathogenic variant in the KCNJ5 gene. The genetic diagnosis of autosomal dominant Familial Hyperaldosteronism type III, and autosomal dominant Long QT syndrome-13 is possible.

No copy number variants (CNVs) were detected in the analyzed genes.
• As it is believed that sulfite and related compounds account, in part, for disease related neurotoxicity, early diagnosis is likely to be essential to maximize outcomes.

• In support of this theory, retrospective data on patients treated shortly after birth suggested that early treatment appeared to be associated with better seizure control and required fewer anticonvulsants, and with improved clinical and neurodevelopmental outcomes.

• Therefore, rapid recognition and diagnosis is critical to allow for early treatment and to maximize clinical treatment outcomes.
Treatment

- In the absence of treatment, patients with MoCoD and ISOD typically die within the first few years of life without achieving developmental milestones.
- A dietary approach with low sulfur containing amino acids (low-cysteine and methionine-containing foods) has been tried but has not resulted in any significant improvements in symptoms.
- Theoretically, uric acid supplementation may reduce or limit neuronal injury occurring in MoCoD due to hypouricemia and increased oxidative stress. (Its practical utility remains to be established).
- NMDA receptor inhibition with dextromethorphan and thiamine have been tried without any benefit.
- **Pyridoxine** has been shown to improve seizure frequency without affecting the underlying metabolic defect.
In 2009, Monash Children's Hospital at Southern Health in Melbourne, Australia reported that a patient known as Baby Z became the first person to be successfully treated for molybdenum cofactor deficiency type A. The patient was treated with cPMP, a precursor of molybdopterin.
The first mechanistic therapy for MoCoD type A patients was recently approved in the US and involves IV treatment with **synthetic cyclic pyranopterin monophosphate (cPMP)**, the first intermediate in the MoCo synthesis pathway.

This compound is ideally initiated at birth at a dose of 80 µg/kg/day and increased to 240 µg/kg/day.

Neurocognitive outcome is markedly improved and lifelong therapy is recommended.

**Molybdate therapy** may benefit those with mutations in the GEPH gene.

**NULIBRY Rx**

**Generic Name & Formulations:**
Fosdenopterin 9.5mg (equivalent to 12.5mg fosdenopterin hydrobromide as a dihydrate); per vial; lyophilized pwd or cake for IV infusion after reconstitution; preservative-free; contains mannitol.

**Company:**
Origin Biosciences, Inc.
Prenatal diagnosis

• Prenatal diagnosis has been accomplished by assay of sulfite oxidase activity in cultured amniocytes and in uncultured CVS tissue.

• Gene-based prenatal diagnosis of molybdenum cofactor deficiency and isolated sulfite oxidase deficiency is more informative than enzyme or metabolite assays but generally should be attempted only in those families in which the affected gene and specific defect have been characterized.

• Prenatal diagnosis has the advantage of permitting decision regarding continuation of pregnancy and initiation of early treatment in the newborn child to arrest the illness.

Take Home Message

• The detection of inborn errors of metabolism (IEM) depends upon a high index of suspicion.
• Sulfite Intoxication Disorders should be considered in any infant with neonatal onset seizures.
• Uric acid is a useful screening tool and should form a part of routine evaluation of a patient with neonatal seizures.
• Rapid recognition and diagnosis is critical to allow for early treatment and to maximize clinical treatment outcomes.
• cPMP is a novel treatment for MoCD type A
Thank you For your Attention