# Pyridoxine-dependent Epilepsy With Elevated Urinary α-Amino Adipic Semialdehyde in Molybdenum Cofactor Deficiency


## abstract

α-Amino adipic semialdehyde (α-AASA) accumulates in body fluids from patients with pyridoxine-dependent epilepsy because of mutations in *antiquitin* (*ALDH7A1*) and serves as the biomarker for this condition. We have recently found that the urinary excretion of α-AASA was also increased in molybdenum cofactor and sulfite oxidase deficiencies. The seizures in pyridoxine-dependent epilepsy are caused by lowered cerebral levels of pyridoxal-5-phosphate (PLP), the bioactive form of pyridoxine (vitamin $B_6$), which can be corrected by the supplementation of pyridoxine. The nonenzymatic trapping of PLP by the cyclic form of α-AASA is causative for the lowered cerebral PLP levels. We describe 2 siblings with clinically evident pyridoxine-responsive seizures associated with increased urinary excretion of α-AASA. Subsequent metabolic investigations revealed several metabolic abnormities, all indicative for molybdenum cofactor deficiency. Molecular investigations indeed revealed a known homozygous mutation in the *MOCS2* gene. Based upon the clinically evident pyridoxine-responsive seizures in these 2 siblings, we recommend considering pyridoxine supplementation to patients affected with molybdenum cofactor or sulfite oxidase deficiencies. *Pediatrics* 2012;130:e1716–e1719



**AUTHORS:** Eduard Alexander Struys, PhD,[a] Benjamin Nota, PhD,[a] Abdellatif Bakkali, BSc,[a] Saad Al Shahwan, MD,[b] Gajja Sophi Salomons, PhD,[a] and Brahim Tabarki, MD[b]

*[a]Metabolic Unit, Department of Clinical Chemistry, VU Medical Center, Amsterdam, Netherlands; and [b]Division of Pediatric Neurology, Department of Pediatrics, Riyadh Military Hospital, Riyadh City, Saudi Arabia*



**KEY WORDS**

seizures, molybdenum cofactor deficiency, α-amino adipic semialdehyde, pyridoxine-dependent epilepsy


**ABBREVIATIONS**

α-AASA—α-amino adipic semialdehyde
cPMP—cyclic pyranopterin monophosphate
P6C—piperideine-6-carboxylate
PLP—pyridoxal-5-phosphate

Dr Struys performed the analysis of urinary α-AASA and drafted the initial manuscript, and approved the final manuscript as submitted; Dr Nota performed the molecular studies, revised the manuscript, and approved the final manuscript as submitted; A. Bakkali performed the biochemical studies, and approved the final manuscript as submitted; Dr Al Shahwan was the clinical caretaker of the patients and approved the final manuscript as submitted; Dr Salomons performed and supervised the molecular studies, revised the manuscript, and approved the final manuscript as submitted; and Dr Tabarki was the clinical caretaker of the patients, revised the manuscript, and approved the final manuscript as submitted.


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Address correspondence to Eduard Alexander Struys, PhD, Metabolic Unit, Clinical Chemistry, VUMC Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, Netherlands. E-mail: e.struys@vumc.nl



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The assessment of urinary $\alpha$-amino adipic semialdehyde ($\alpha$-AASA) has become the gold standard to evaluate the biochemical diagnosis of $\alpha$-AASA dehydrogenase deficiency (MIM 107323), a defect leading to pyridoxine-dependent epilepsy.[1] Since the genetic entity of this disease was unraveled in 2006, the urinary assessment of $\alpha$-AASA has proved itself in both sensitivity and selectivity.[2] In almost all cases in which we measured an increased excretion of $\alpha$-AASA, subsequent genetic testing of the corresponding gene, that is, *ALDH7A1*, revealed pathogenic mutations. $\alpha$-AASA is in equilibrium with its cyclic form piperideine-6-carboxylate (P6C) and the latter nonenzymatically reacts with pyridoxal-5-phosphate (PLP), the active form of vitamin $B_6$. This trapping mechanism leads to a PLP deficit, which can be corrected by the supplementation of pyridoxine. Recently, we noticed that $\alpha$-AASA is not exclusively increased in $\alpha$-AASA dehydrogenase deficiency, but also in 2 other inborn errors of metabolism, that is, molybdenum cofactor deficiency (MIM 252150) and sulfite oxidase deficiency (MIM 272300).[3] We have demonstrated that sulfite in vitro strongly inhibits $\alpha$-AASA dehydrogenase activity, resulting in an accumulation of $\alpha$-AASA, with subsequent decreased formation of the enzyme's product $\alpha$-amino adipic acid. The clinical features and the disease severity of both molybdenum cofactor deficiency and sulfite oxidase deficiency resemble one another, pointing to a devastating role of sulfite, which accumulates in both diseases. The mechanisms by which sulfite accumulation leads to neurotoxicity are poorly understood, although it has been shown that sulfite inhibits glutamate dehydrogenase.[4] Recently, it has been described that PLP, the active vitamer of pyridoxine (vitamin $B_6$), is decreased in cerebrospinal fluid from patients with molybdenum cofactor deficiency, and it was postulated that this decrease was the result of the nonenzymatic reaction between sulfite and PLP.[5] Our recent findings that, next to sulfite, $\alpha$-AASA also accumulates in molybdenum cofactor deficiency and sulfite oxidase deficiency indicate that PLP is exposed to a double hit in these diseases, that is, inactivation by sulfite and inactivation by P6C. This novel comprehension of pathophysiology leads us to speculate that patients with either molybdenum cofactor deficiency or sulfite oxidase deficiency might benefit from pyridoxine supplementation.[3] We here report 2 siblings with a strong clinical suspicion of $\alpha$-AASA dehydrogenase deficiency based upon proven pyridoxine-responsive epilepsy. Subsequent biochemical and genetic testing revealed that these children had molybdenum cofactor deficiency caused by a known homozygous mutation in the *MOCS2* gene.

## PATIENT PRESENTATION

Sibling 1 is a girl born at term in 2010 after an unremarkable pregnancy from first-degree consanguineous parents. At the first day of life, she presented with jerkiness, twitching, myoclonic reactions, and poor sucking. Seizures were refractory to phenobarbitone, phenytoin, and levetiracetam, and the girl was hypotonic. EEG recording at 3 days of life showed continuous electrographic seizures on both temporal areas. MRI diffusion-weight imaging studies revealed extensive diffusion restriction in both hemispheres involving gray and white matter, which was most pronounced in the temporal, occipital, and posterior parietal regions. Axial fluid-attenuated inversion recovery (FLAIR) studies revealed a mild degree of cortical edema with normal ventricles and basal cisterns. Supplementation of pyridoxine (30 mg/kg) and folinic acid (3 mg/kg) was initiated at day 6 of life, resulting in a clear decrease in the frequency of seizures, that is, from 20 to 30 attacks per day to ~1 to 2 attacks per day, accompanied with improved sucking. A subsequent EEG recording (while on pyridoxine and folinic acid supplementation) showed well-organized and well-developed activities at 5 to 6 Hz, bilaterally synchronous and symmetrical. Intermixed are the $\delta$ brushes, sharp $\alpha$ and $\theta$ on the temporal area with suppression of activity up to 26 seconds. Sharp waves were noted on the temporal and central vertex areas. Pyridoxine and folinic acid supplementation was discontinued at 13 days of life, resulting in an increased frequency of seizures, which subsequently responded to the reintroduction of pyridoxine and folinic acid supplementation. Since then, the girl is seizure free, with the exception of breakthrough seizures during febrile illness. The results of neurologic examination at 6 months of life are abnormal with poor sucking, microcephaly, and hypotonia.

Sibling 2 is a male infant born in 2008 who was referred at 2 years of age with severe developmental delay and frequent seizures. EEG showed multifocal discharges, and brain MRI studies showed extensive brain damage with significant loss of bilaterally cerebral white matter with ex vacuo dilatation of the ventricles and major cerebrospinal fluid spaces. The ventricular margin reached the cortex, which showed stunted/retarded mushroom-shaped gyri representing ulegyria. There was abnormal hyperintensity seen more or less symmetrically in the posterior limbs of the internal capsule extending down to the brainstem along the corticospinal tracts. This study also demonstrated significantly destroyed/thinned out corpus callosum. Diffuse atrophy of the cerebellum was also noted.

After the successful suppression of the seizures for his younger sister,

pyridoxine and folinic acid supplementation was initiated, which led to a significant decrease in seizure frequency. On EEG, no epileptiform discharges were noticed, with poor background activity. The results of neurologic evaluation of this boy, however, were severely abnormal. Only from the male sibling, a urine sample became available (see Table 1). Urinary $\alpha$-AASA excretion was found to be increased: 4.5 mmol/mol creatinine (normal, $<1$) as well as its cyclic form P6C: 0.86 mmol/mol creatinine (normal, $<0.05$). Amino acids analysis revealed that *S*-sulfocysteine was strongly increased 193 mmol/mol creatinine (normal, nondetectable), with an accompanying increase of taurine 245 mmol/mol creatinine (normal, $<137$). Purine and pyrimidine analysis revealed an increased excretion of xanthine of 315 mmol/mol creatinine (normal, $<31$) and of hypoxanthineof 267 mmol/mol creatinine (normal, $<33$). Uric acid was nondetectable (normal, 0.21–1.36 mol/mol creatinine). Interestingly, the sulfite dipstick test results were negative, probably because the urine was transported at room temperature for $>72$ hours.

Sequencing analysis of the coding region (including splice sites) of *ALDH7A1* from genomic DNA from the affected siblings revealed no mutations. Subsequent sequencing of the *MOCS1* and *MOCS2* genes revealed a known homozygous mutation (c.564+1G>A) in *MOCS2*, which is predicted to cause skipping of exon 5 of the long transcript (NM_004531.3) encoding MOCS2B.[6] The parents were found to be carriers of this mutation, confirming homozygosity in the affected siblings.

**TABLE 1** Biochemical Data of Sibling 2

| Metabolite | Urinary Concentration, mmol/mol Creatinine | Reference Values for Age, mmol/mol Creatinine |
|---|---|---|
| $\alpha$-AASA | 4.5 | $<1$ |
| P6C | 0.86 | $<0.05$ |
| *S*-Sulfocysteine | 193 | ND |
| Taurine | 245 | $<137$ |
| Xanthine | 315 | $<31$ |
| Hypoxanthine | 267 | $<33$ |
| Uric acid | 0 | 210–1360 |

ND, nondetectable.

## DISCUSSION

Here, we report on 2 siblings affected with molybdenum cofactor deficiency because of a pathogenic homozygous mutation in the *MOCS2* gene. Sibling 1 presented with evident pyridoxine/folinic acid–dependent epilepsy, with reoccurrence of seizures when pyridoxine/folinic acid was terminated. This clinical presentation strongly suggested $\alpha$-AASA dehydrogenase deficiency, and, indeed, increased excretion of $\alpha$-AASA was noticed. However, subsequent molecular investigations of the *ALDH7A1* gene did not reveal mutations in this child. Our latest finding that $\alpha$-AASA is also increased in molybdenum cofactor deficiency and sulfite oxidase deficiency prompted us to biochemically evaluate these conditions for this child. The constellation of the observed metabolic abnormalities was highly indicative for molybdenum cofactor deficiency. Genetic studies revealed a homozygous c.564+1G>A mutation in the *MOCS2* gene.

Recently, an innovative treatment was reported for those patients in whom molybdenum cofactor deficiency was caused by mutations in the *MOCS1* gene.[7] As a consequence, these patients are unable to synthesize cyclic pyranopterin monophosphate (cPMP), the initial intermediate in molybdenum cofactor biosynthesis, from guanosine triphosphate molybdenum. As a therapeutic strategy, cPMP was produced by *Escherichia coli* expression cultures, and the obtained cPMP was subsequently purified and given intravenously. This resulted in a positive clinical response and almost normalization of the biochemical abnormalities related to molybdenum cofactor deficiency. This treatment option, however, is only applicable when the impairment of the molybdenum cofactor biosynthesis is at the level of the conversion of cPMP into guanosine triphosphate molybdenum. No treatment options are currently available for individuals with mutations in the *MOSC2* or *GPHN* genes, which are involved in the subsequent steps in molybdenum cofactor biosynthesis. For this group of patients, supplementation of pyridoxine will likely normalize the deficiency of the active form of vitamin $B_6$, PLP. Recently, it was reported that in 2 patients with genetically proven molybdenum cofactor deficiency, the PLP concentrations in cerebrospinal fluid were decreased.[5]

The secondary increase of $\alpha$-AASA observed in patients with molybdenum cofactor deficiency and sulfite oxidase deficiency might accelerate the severity of these conditions because the cyclic form of $\alpha$-AASA (ie, P6C) chemically traps PLP, thereby lowering its bioavailability. Because inorganic sulfite is also capable of inactivating PLP, patients with molybdenum cofactor deficiency and sulfite oxidase deficiency are exposed to a double hit, that is, direct intoxication by the accumulation of sulfite with a subsequent secondary increase of $\alpha$-AASA.

This case report argues in favor of pyridoxine supplementation to patients with molybdenum cofactor and sulfite oxidase deficiencies. It is important to note that the treatment modification by pyridoxine supplementation, in contrast to the cPMP treatment, does not correct for the biochemical defect, but it will alleviate the patient's clinical condition.

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