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Successful Treatment of Molybdenum Cofactor Deficiency Type A With cPMP

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KEY WORDS

newborn, molybdenum, molybdenum cofactor deficiency, treatment, sulfite oxidase, cyclic pyranopterin monophosphate

ABBREVIATIONS

MoCo—molybdenum cofactor
MoCD—molybdenum cofactor deficiency
cPMP—cyclic pyranopterin monophosphate
HPLC—high-performance liquid chromatography
MRS—magnetic resonance spectroscopy

Drs Veldman and Santamaria-Araujo contributed equally to this article.

Dr Veldman developed the treatment plan, performed the treatment and monitoring, and co-wrote the manuscript; Dr Santamaria-Araujo developed cPMP production and purification, performed cPMP stability analysis, and determined compound Z content; Dr Sollazzo purified cPMP; Dr Pitt performed metabolite analysis; Dr Gianello prepared cPMP for infusion and supervised quality control of cPMP on intravenous medication standards; Dr Yaplito-Lee was responsible for the diagnosis of MoCD; Dr Wong was responsible for follow-up and neurodevelopmental assessment; Dr Ramsden contributed to the treatment plan and supervised treatment; Dr Reiss performed genetic analysis; Drs Cook and Fairweather were responsible for the analysis of cPMP identity and purity, endotoxin assays, and certificate of analysis; and Dr Schwarz developed cPMP purification, production, and characterization, proposed treatment strategy, was principal investigator of the study, and co-wrote the manuscript.

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abstract

Molybdenum cofactor deficiency (MoCD) is a rare metabolic disorder characterized by severe and rapidly progressive neurologic damage caused by the functional loss of sulfite oxidase, 1 of 4 molybdenum-dependent enzymes. To date, no effective therapy is available for MoCD, and death in early infancy has been the usual outcome. We report here the case of a patient who was diagnosed with MoCD at the age of 6 days. Substitution therapy with purified cyclic pyranopterin monophosphate (cPMP) was started on day 36 by daily intravenous administration of 80 to 160 μg of cPMP/kg of body weight. Within 1 to 2 weeks, all urinary markers of sulfite oxidase (sulfite, S-sulfocysteine, thiosulfate) and xanthine oxidase deficiency (xanthine, uric acid) returned to almost normal readings and stayed constant (>450 days of treatment). Clinically, the infant became more alert, convulsions and twitching disappeared within the first 2 weeks, and an electroencephalogram showed the return of rhythmic elements and markedly reduced epileptiform discharges. Substitution of cPMP represents the first causative therapy available for patients with MoCD. We demonstrate efficient uptake of cPMP and restoration of molybdenum cofactor–dependent enzyme activities. Further neurodegeneration by toxic metabolites was stopped in the reported patient. We also demonstrated the feasibility to detect MoCD in newborn-screening cards to enable early diagnosis. *Pediatrics* 2010;125:e1249–e1254

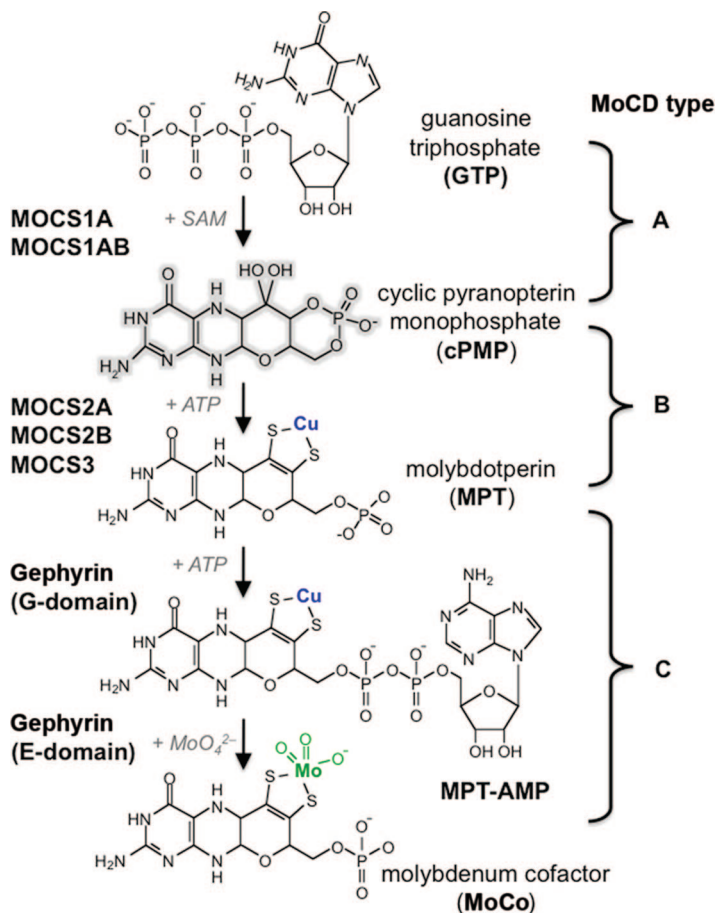


FIGURE 1

MoCo biosynthesis, involved gene products, and MoCD types. Shown is a simplified schematic presentation of MoCo biosynthesis in man with the 3 known intermediates (cPMP, molybdopterin, and molybdopterin-adenosine monophosphate [MPT-AMP]) and the proteins catalyzing the individual steps within the biosynthetic pathway. On the basis of genetic and biochemical analyses, MoCD can be grouped into 3 types (A, B, and C). Note that step 1 (guanosine triphosphate to cPMP) is catalyzed by proteins expressed from the *MOCS1* locus that are either terminated early (MOCS1A) or fully translated as a fusion protein (MOCS1AB). Steps 3 and 4 are catalyzed by the multifunctional and multidomain protein gephyrin.

Molybdenum cofactor (MoCo) deficiency (MoCD) is a rare metabolic disorder that is characterized by severe progressive neurologic damage, disordered autonomic function, exaggerated startle reactions, dysmorphic facial features, alterations in muscle tone, progressive cerebral palsy, microcephaly, seizures, and early death,¹ and caused by the functional loss of sulfite oxidase, 1 of 4 molybdenum-dependent enzymes in humans.

MoCo is synthesized by a complex biosynthetic pathway that involves 4 steps (Fig 1).² Approximately two-thirds of the patients with MoCD lack cyclic pyranopterin monophosphate (cPMP) and

are classified as having MoCD type A.^{1,3} More than 100 patients have been described with MoCD, but no effective treatment has been available.³

We previously constructed an animal model for human MoCD type A⁴; these *MOCS1*-knockout mice display a severe phenotype that reflects the biochemical characteristics of human MoCD. They fail to thrive and die early, with an average life span of 7.5 days. We showed that this lethal phenotype can be neutralized efficiently by injection of cPMP, the first isolatable intermediate in MoCo biosynthesis, which was puri-

fied from *Escherichia coli*,⁵ as its structure is conserved in all kingdoms of life.⁶ We describe here the first (to our knowledge) replacement therapy with the MoCo precursor cPMP in a human patient with type A MoCD.

METHODS

Metabolic Analysis

Urine metabolites were measured by direct-injection electrospray tandem mass spectrometry.⁷ For the analysis of newborn-screening dried-blood-spot samples, metabolites were extracted and analyzed by liquid chromatography/tandem mass spectrometry. In addition, sulfite was semi-quantified in fresh urine by using a colorimetric dipstick test (Merckoquant test sulfite, Merck Chemicals, Darmstadt, Germany). Urine compound Z (cPMP oxidation product) levels were determined by high-performance liquid chromatography (HPLC) analysis, as described previously.⁸

MoCD Mutation Analysis

DNA was extracted from EDTA peripheral blood according to standard procedures. Polymerase-chain-reaction amplification of individual exons was performed as previously described.⁹

Production of cPMP

cPMP was produced in 12 L-expression cultures of *Escherichia coli* and purified by 2-step HPLC chromatography as we described elsewhere⁵ with the exception that 1 mM EDTA was added to the first purification buffer. Purified cPMP from different purifications was pooled, aliquoted, and stored at -80°C in concentrations that ranged from 35 to 180 $\mu\text{g}/\text{mL}$.

Analysis of cPMP Solutions and Preparation for Injection

cPMP content of frozen stocks was determined to be >95% in respect to other organic components according

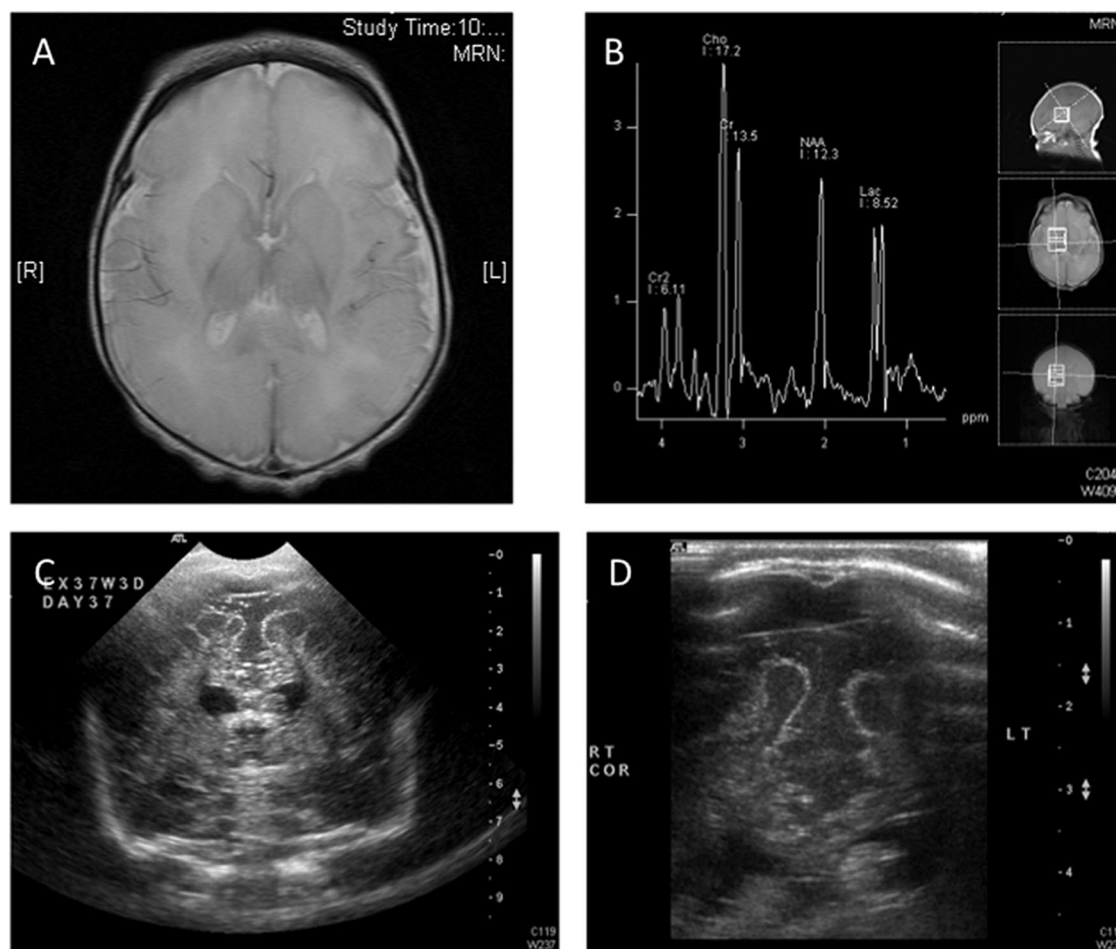


FIGURE 2

Cranial MRI of the patient on day 6 and cranial ultrasound at 1 month of age. Shown are MRI with diffuse cerebral edema (A) and MRS showing an elevated lactate peak (B). A cranial ultrasound at 1 month of age showed cerebral atrophy with enlargement and ventricles and loss of gray/white-matter differentiation (C) and enlargement of the subarachnoid space (D).

to HPLC and nuclear magnetic resonance. An endotoxin assay was performed. For intravenous administration, aliquots of frozen cPMP solution were thawed rapidly, and predetermined volumes of 10-times phosphate-buffered saline and 0.5 M NaOH were added such that the final solution had a pH of 7.3 and was of isotonic strength. Before injection, the cPMP solution was sterile-filtered.

Patient Presentation

An infant girl was born to a healthy, primigravida mother after an uneventful pregnancy and delivery at 37 weeks of gestation. She was not dysmorphic and had no eye lens abnormalities. She became symptomatic within 24 hours

after birth with jerkiness, twitching, and poor sucking. Frank seizures were observed at 60 hours of age, for which she was transferred to a NICU. After increasing doses of anticonvulsant medications, she was mechanically ventilated. At one week of life, the infant was maintained on phenobarbitone and valproate and weaned from the ventilator after 5 days to room air without the need for further respiratory support.

The infant had markedly abnormal electroencephalography results, which showed generalized suppression of electrical activity and multi-form epileptiform discharges throughout the recording, at 7 days of age.

General hypertonia, frequent startle reactions, and myoclonic twitching were observed. MRI at day 6 showed diffuse cerebral edema with an elevated lactate peak in the magnetic resonance spectroscopy (MRS) and diffuse hyperintensity in the basal ganglia, cortex, and the subjacent white matter (Fig 2A and 2B). She required nasogastric tube feeding of standard newborn infant formula. Although weight gain was normal, her head circumference did not increase in the first month of life. Typical of patients with MoCD,¹ cranial ultrasound examinations at 1 month showed cerebral atrophy with enlargement of subarachnoid space and ventricles

and loss of gray/white-matter differentiation throughout the brain (Fig 2C and 2D).

The infant's urine revealed markedly elevated levels of *S*-sulfocysteine, thiosulfate, and xanthine, low plasma and urine uric acid levels, an elevated urine sulfite level, and undetectable cPMP oxidation product compound Z (data not shown). The diagnosis of MoCD type A was confirmed by gene analysis, which showed a homozygous G175R (GGG-to-AGG) change in exon 10 of the *MOCS1* gene, which has been described.¹⁰ Retrospective analysis of the infant's newborn-screening dried-blood-spot sample revealed increased xanthine (144 $\mu\text{mol/L}$ [controls: $<4 \mu\text{mol/L}$]) and decreased uric acid (39 $\mu\text{mol/L}$ [controls: 156–432 $\mu\text{mol/L}$]) levels.

After approval by the local bioethics committee, the patient began intravenous replacement therapy with cPMP on day 36 of life. The cPMP starting dose of 80 $\mu\text{g/kg}$ per day was calculated by extrapolating the dose that most efficiently abolished symptoms and lethality in the mouse model.⁵ Before use, cPMP stock solutions were neutralized by NaOH and phosphate-buffered saline and were given as a continuous intravenous infusion, initially through peripheral intravenous access and later through a Broviac catheter. On day 1, 3 test doses, each consisting of 10% of the total daily dose, were administered over 30 minutes followed by a 30-minute observation time. Afterward, the remaining 70% of the first dose was infused over 3 hours. In the following days, the 80 $\mu\text{g/kg}$ per day dose was infused over 1 hour. After 12 days, the dose was increased to 120 $\mu\text{g/kg}$ per day, and after 35 and 75 days of treatment, changes in the metabolites led to another dose increase to 160 $\mu\text{g/kg}$ once or twice per day respectively.

Organ function (liver-function tests, renal parameters, blood counts), urine

markers for sulfite oxidase activity (*S*-sulfocysteine and thiosulfate), and xanthine oxidase activity (xanthine and uric acid) were monitored daily to estimate treatment response and allow for dose adjustments.

Within a few days of injecting cPMP, a rapid, dramatic, and sustained decline toward normal levels of urine *S*-sulfocysteine (Fig 3A) and thiosulfate (Fig 3B) was recorded. After reaching a nadir around days 8 to 9, a slow but continuous increase of *S*-sulfocysteine was observed (Fig 3A), and the dose of cPMP was increased in 2 steps to 160 $\mu\text{g/kg}$ per day. A second plateau was observed around day 60, which led to an increase of the daily dose by administering 160 μg of cPMP/kg twice per day. From the second day of treatment onward, sulfite dipstick test results became and have remained negative. Purine metabolites responded more slowly than sulfite metabolites: after 15 to 20 days, xanthine had normalized, whereas uric acid was variably slightly below or within the control range (Fig 3C).

Clinically, treatment resulted in the infant becoming more alert within 2 days; within 1 week the infant took full bottle feeds. Her head growth improved and, although still plotting well below the percentiles, reached 40.5 cm at 18 months of age. The infant was discharged from the hospital on daily cPMP infusion 28 days after the start of therapy. For a period of 1 week (days 28–35), cPMP solution was prepared in the hospital and delivered to the infant's home (45-minute transfer time), which probably resulted in increased cPMP oxidation and, consequently, a temporary rise in *S*-sulfocysteine and thiosulfate levels caused by oxidation-triggered loss of active cPMP (Fig 3A). Subsequently, cPMP was prepared at home and given immediately, which resulted in less cPMP oxidation and, therefore, further normalization of

metabolite levels (Fig 3A). After treatment was started, oxidized cPMP (compound Z) was detected in the patient's urine; the levels varied depending on the time of urine collection (data not shown).

An electroencephalogram on day 12 of treatment showed significant improvement (return of rhythmic elements and markedly reduced epileptiform discharges). Twitching and startling decreased from a frequency of ~ 10 events per hour before treatment to fewer than 5 events per day. The infant was weaned from the phenobarbitone and kept on monotherapy with sodium valproate. MRS and MRI, recorded 24 days after starting cPMP infusion, showed that the previously abnormal lactate peaks were markedly reduced. As in the head ultrasound investigations before treatment, this MRI revealed the presence of marked loss of brain volume with extensive destruction/gliosis of white matter within the hemispheres.

The cPMP infusions were well tolerated, and no adverse clinical events or routine laboratory alterations were observed. When last seen by a developmental pediatrician at 18 months of age, the infant was thriving and interactive with squealing and vocalizations. She is clinically free of seizures and has spontaneous, voluntary movements in all limbs. She has signs of quadriplegic cerebral palsy, with generalized increase in truncal and peripheral tone, brisk reflexes, and tendency for an extensor posture. At an age of 18 months, she has achieved level 3 on the Gross Motor Functions Classification System. Complete neurodevelopmental examination using the Bayley Scales of Infant Development will be conducted at 2 years of age. The finding is consistent with the cerebral injury documented on the last MRI at 6 weeks of age. No evidence of progressive neurologic deficit or func-

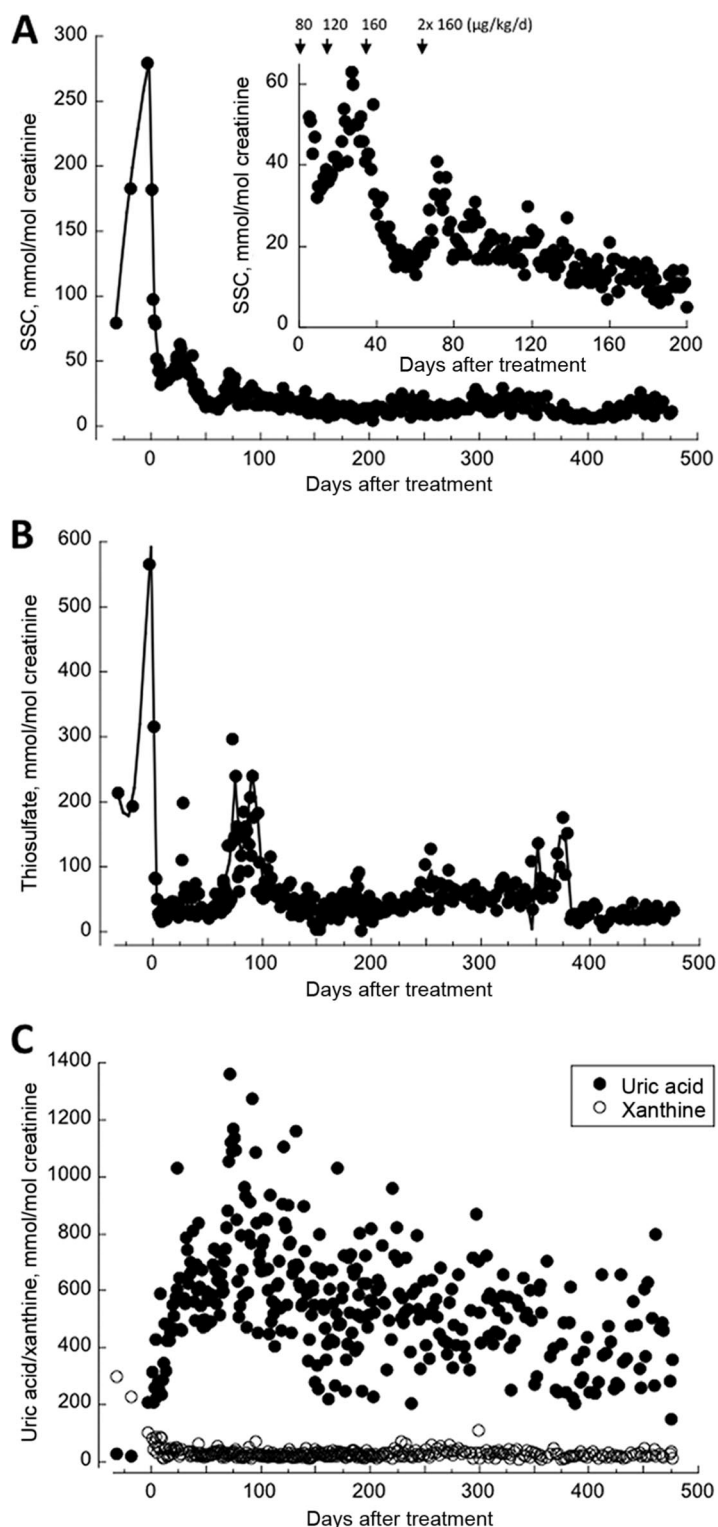


FIGURE 3

Urinary metabolite levels ($\mu\text{mol}/\text{mmol}$ creatinine) in the patient before and after therapy. A, *S*-sulfocysteine (controls: <9 $\mu\text{mol}/\text{mmol}$ creatinine) (enlarged view shown in the inset), including changes in dosing. B, Thiosulfate (controls: <54 $\mu\text{mol}/\text{mmol}$ creatinine). C, Xanthine (controls: <38 $\mu\text{mol}/\text{mmol}$ creatinine) and uric acid (controls: 820–2813 $\mu\text{mol}/\text{mmol}$ creatinine). Metabolite levels were determined by tandem mass spectroscopy as described in “Methods.”

tional deterioration after the patient was commenced on treatment reaffirms that there is no ongoing neurotoxicity. She passed formal audiology examination; she has myopic astigmatism but is fixing and following well.

DISCUSSION

MoCD type A is a lethal condition; individuals who become symptomatic during the neonatal period rarely live beyond late infancy.^{11,12} In the past, treatment offered little more than symptomatic relief (eg, anticonvulsant medications) and supportive care, because no effective therapy for MoCD was available.¹ Here, we demonstrate that cPMP substitution resolves the metabolic abnormalities and results in dramatic clinical improvement.

Administration of cPMP was remarkably successful in restoring *S*-sulfocysteine, thiosulfate, xanthine, and uric acid to control or near-control levels. cPMP was well tolerated in the doses administered, which ranged from 80 to 320 $\mu\text{g}/\text{kg}$ per day. There is no evidence of adverse events, at least for more than 20 months. Dosing was guided by clinical improvement and by the goal of returning urine metabolite levels to the normal range. It is unclear what urinary levels of *S*-sulfocysteine and thiosulfate are optimal for brain development. Although cPMP is presently given as a daily infusion through a central venous catheter, alternative ways of administration might become available in the future. Initial pilot data on subcutaneous administration in rodents has shown a plasma profile comparable to that of intravenous administration (data not shown) and will be explored further. The case described here was a single case study; hence, well-designed clinical trials will be necessary to investigate this therapeutic option for an up-to-now fatal disease. An international, single-arm, phase

I/II clinical trial for cPMP substitution in patients with MoCD type A is currently in the final stage of development, but an initial program of animal toxicology and dose-finding work has been initiated.

Given the marked neurotoxicity of sulfite and its related metabolites, it seems likely that early diagnosis and initiation of treatment will be crucial to achieve a favorable long-term outcome. In the patient described here, we documented significant cerebral atrophy within the first month of life before commencing cPMP substitution. Thus, diagnosis and treatment should optimally occur well before the

end of the first month of life. Despite a late start of therapy, our patient has made remarkable clinical improvement. To achieve an earlier diagnosis it would be necessary to perform urine screening with mass spectrometry in newborns with suspected inborn errors of metabolism (eg, in a clinical scenario of seizures of unknown origin).

We have demonstrated a characteristic metabolic profile, namely increased xanthine and decreased uric acid levels on day 3 of life in the sample of dried blood obtained for newborn screening by evaluation with tandem mass spectroscopy. If the ef-

ficacy of cPMP can be established, MoCD may join the small number of metabolic disorders for which effective therapy is available and for which extended newborn screening may be life-saving.

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FINANCIAL DISCLOSURE: Dr Veldman was the principal investigator of an upcoming phase I study on cPMP and the chief medical officer of Orphatec Pharmaceuticals GmbH; Dr Santamaria-Araujo has filed patents for the clinical use of cPMP and is a shareholder of Orphatec Pharmaceuticals GmbH; Dr Gianello is a shareholder of Orphatec Pharmaceuticals GmbH; and Dr Schwarz has filed patents for the clinical use of cPMP and is a shareholder of Orphatec Pharmaceuticals GmbH. The other authors have indicated they have no financial relationships relevant to this article to disclose.

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