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REPORT

CAD mutations and uridine-responsive epileptic encephalopathy

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Unexplained global developmental delay and epilepsy in childhood pose a major socioeconomic burden. Progress in defining the molecular bases does not often translate into effective treatment. Notable exceptions include certain inborn errors of metabolism amenable to dietary intervention. *CAD* encodes a multifunctional enzyme involved in *de novo* pyrimidine biosynthesis. Alternatively, pyrimidines can be recycled from uridine. Exome sequencing in three families identified biallelic *CAD* mutations in four children with global developmental delay, epileptic encephalopathy, and anaemia with anisopoikilocytosis. Two died aged 4 and 5 years after a neurodegenerative disease course. Supplementation of the two surviving children with oral uridine led to immediate cessation of seizures in both. A 4-year-old female, previously in a minimally conscious state, began to communicate and walk with assistance after 9 weeks of treatment. A 3-year-old female likewise showed developmental progress. Blood smears normalized and anaemia resolved. We establish *CAD* as a gene confidently implicated in this neurometabolic disorder, characterized by co-occurrence of global developmental delay, dyserythropoietic anaemia and seizures. While the natural disease course can be lethal in early childhood, our findings support the efficacy of uridine supplementation, rendering CAD deficiency a treatable neurometabolic disorder and therefore a potential condition for future (genetic) newborn screening.

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Introduction

Unexplained global developmental delay in childhood, frequently accompanied by epilepsy, poses a major socioeconomic burden (Sillanpaa and Shinnar, 2010). Clinical exome sequencing is revolutionizing diagnosis (Biesecker and Green, 2014; Tarailo-Graovac *et al.*, 2016) and gene discovery in these individually rare Mendelian disorders. However, progress in defining the underlying molecular defects does not often translate into effective treatment beyond genetic counselling and informed decision-making by families and physicians. Notable exceptions include certain genetically defined inborn errors of metabolism amenable to dietary intervention that circumvents the pathomechanism (Tarailo-Graovac *et al.*, 2016).

In this study, we used exome sequencing to analyse four affected children (from three families) with infantile-onset global developmental delay, severe epileptic encephalopathy and anaemia, and identified biallelic mutations in the *CAD* gene. *CAD* encodes a multifunctional enzyme complex (comprising glutamine amidotransferase, carbamoyl phosphate synthase, aspartate transcarbamylase, dihydroorotase) that catalyses the first steps of *de novo* pyrimidine biosynthesis (Fig. 1A); the remaining steps are completed by dihydroorotate dehydrogenase (DHODH) and the bifunctional protein uridine monophosphate synthase (UMPS), to produce the final product uridine monophosphate (Balasubramaniam *et al.*, 2014; Grande-Garcia *et al.*, 2014).

UMPS deficiency (hereditary orotic aciduria, MIM #258900) causes megaloblastic anaemia. Global developmental delay may occur and seizures have been reported in a single case (Grohmann *et al.*, 2015). Treatment with oral uridine, both a product of *de novo* pyrimidine biosynthesis and a substrate of the pyrimidine salvage pathway (Fig. 1A), has been shown to be safe and effective for the haematological and neurological sequelae (Webster, 2001; Grohmann *et al.*, 2015).

CAD was recently reported to be a candidate gene for a congenital disorder of glycosylation (CDG) in a single 17-month-old patient whose clinical features overlapped those of the patients described here; however, no follow-up data were presented (Ng *et al.*, 2015). The patient's fibroblasts showed reduced levels of sugar nucleotides that serve as donors for glycosylation, and this phenotype was rescued by uridine supplementation to the culture medium.

When we treated two of our CAD-deficient patients with oral uridine, both responded with marked clinical improvement, supporting the potential of uridine administration to ameliorate an otherwise severe neurodegenerative disease which can be lethal in childhood.

Materials and methods

Subjects

Clinical features are summarized in Table 1. In all four patients, pregnancy, delivery, and postnatal adaptation were unremarkable and anthropometric parameters were within normal ranges. White blood cell counts and platelets, blood gas analysis, serum transferrin isoelectric focussing, lactate and ammonia, plasma amino acids, urinary organic acids, purines and pyrimidines, and orotic acid were unremarkable in all patients.

Patient F1:II.2

Patient F1:II.2, a male, was the second child born to consanguineous parents of Serbian Roma origin (Fig. 1B). An older sister is healthy. At 20 months, generalized therapy-resistant tonic clonic seizures evolved, which increased in frequency during the third year of life from weekly to several times daily. His development was delayed, with independent sitting and first steps occurring at 3 years. He spoke a few words and was able to eat with his hands. He then developed ataxia and began to lose all acquired skills. After a status epilepticus episode at the age of 3.5 years, he remained in a minimally conscious state and died at 4 years. Parents did not agree to autopsy.

Laboratory testing indicated mild anaemia [haemoglobin 9.7, reference range 10.5–13.5 g/dl; mean corpuscular volume 76–89 (median 82, $n = 111$), reference range 75–87 fl] from the age of 12 months onwards. Abnormal size distribution with anisocytosis and poikilocytosis was first noticed in a peripheral blood smear at the age of 2.75 years.

Brain MRI at 2.7 years was unremarkable; however, follow-up at the age of 3.6 and 3.8 years showed marked global brain atrophy (Fig. 2A–F).

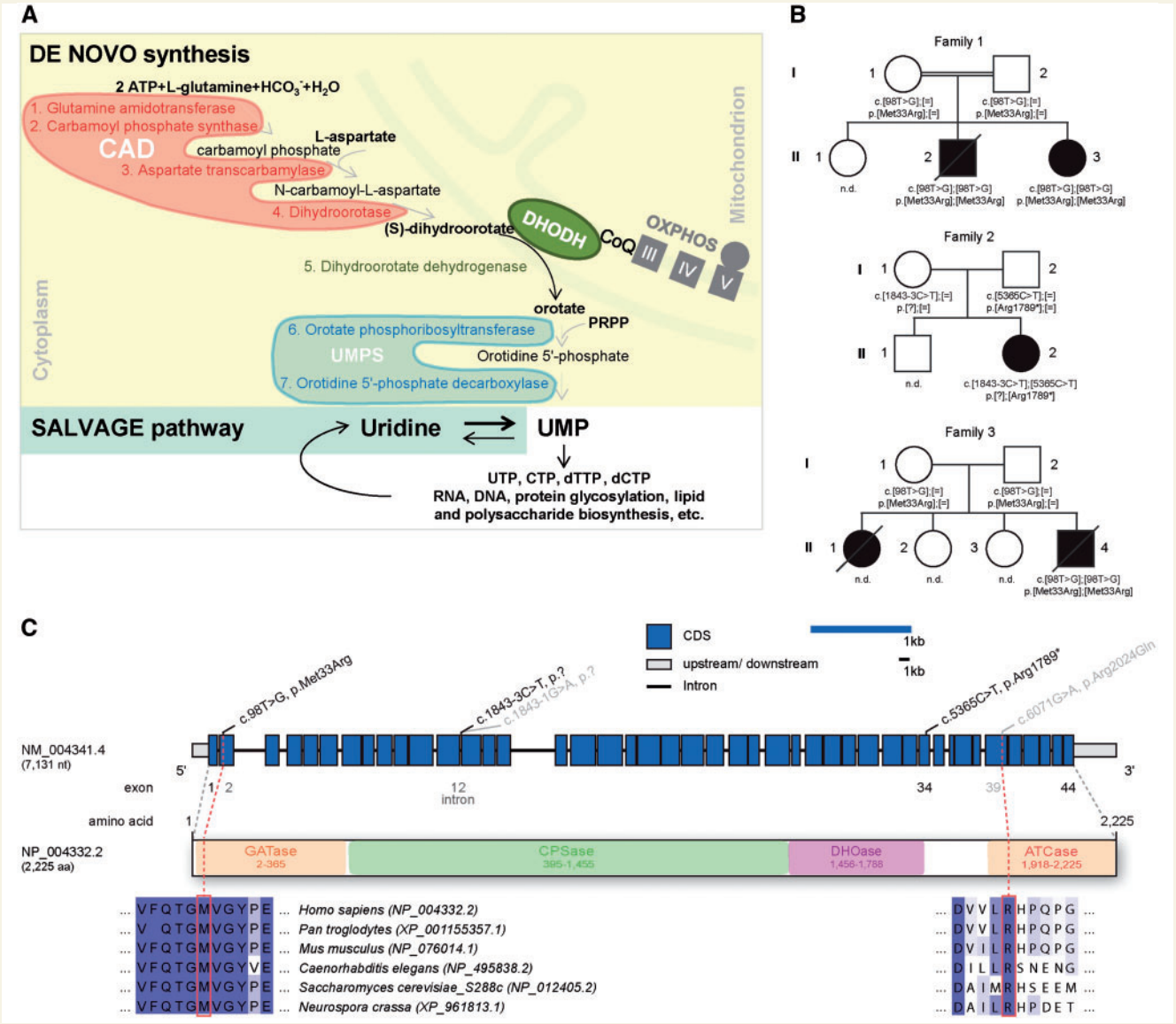

Figure 1 Pyrimidine metabolism and identification of *CAD* mutations. (**A**) Pyrimidine synthesis: *de novo* versus salvage pathway. *De novo* synthesis of uridine monophosphate involving three enzymes localized in two cellular compartments. Cytoplasmic CAD (glutamine aminotransferase, carbamoyl phosphate synthase, aspartate transcarbamylase, dihydroorotase), catalyses the first four steps, Dihydroorotate dehydrogenase (DHODH) the subsequent reduction of dihydroorotate in mitochondria, and UMPS (uridine 5'-monophosphate synthase, orotidine 5'-phosphate decarboxylase) the final two steps in the cytoplasm. UMP can also be formed in a single step from uridine by uridine kinase, as part of the pyrimidine recycling pathway. OXPHOS = oxidative phosphorylation; PRPP = 5-phospho-alpha-D-ribose 1-diphosphate; UMP = uridine monophosphate. (**B**) The pedigrees of the three families with the disease and *CAD* mutation status. Squares denote males, circles females, solid symbols affected persons, and slashes deceased persons. (**C**) Location of the CAD mutations. The open reading frame and architecture of the encoded CAD protein, the position of novel and known *CAD* variants as well as evolutionary conservation of amino acid residues affected by missense changes (Met33 and Arg2024).

Patient F1:II.3

In Patient F1:II.3, the younger sister of Patient F1:II.2, developmental delay was noted at 21 months. At age 2 years she was tested with a standardized developmental testing instrument (ET6-6, https://www.zkpr.uni-bremen.de/forschung/testentwicklung/entwicklungstests/et-6-6-r). Gross motor function was 2 standard deviations (SD) below the age-related mean value; fine motor function was 2–3 SD below the age-related mean value; language, cognition and social-emotional skills were between 1–2 SD below the age-related mean value. From 2 years on, short generalized tonic clonic seizures occurred once a month. Her parents refused antiepileptic medication. Electroencephalography showed multifocal sharp waves and normal background activity. At 2.5 years, seizures occurred weekly or even daily, and her motor, speech and cognitive development stagnated. At 3 years she was barely able to make single independent steps, she used very few words, and her alertness was poor.

Table 1 Clinical characteristics, neuroimaging and laboratory findings in CAD-deficient individuals

	Patient				
Gender	**F1:II.2 Male**	**F1:II.3 Female**	**F2:II.2 Female**	**F3:II.4 Male**	**UDP4003[a] Male**
Clinical findings					
Presenting symptom (age)	DD (< 1 y)	DD (< 1 y)	DD (4 m)	DD (18 m)	FTT (1 m)
Seizure onset	20 m	2 y	6 m	2 y	17 m
Therapy-resistant epilepsy	+	[b]	+	+	n/r
Loss of acquired skills (onset)	+ (3 y)	–/+ (2.5 y)	+ (4 y)	+ (4 y)	n/r
Minimal conscious state	+	–	+	+	n/r
Swallowing problems/gastrostomy	+/+	–/–	+/+	+/+	n/r
Breast feeding	–	–	+	n/a	n/r
Current age / age at death	4 y (deceased)	3.5 y	5.4 y	5 y (deceased)	17 m
Brain MRI findings					
Initial MRI (age, month)	N (2.1 y)	n/a	Mild GA (9 m)	n/a	N (1.4 y)
Follow-up MRI (age)	GA (3.6 and 3.8 y)	n/a	GA (5 y)	GA (5 y)	n/r
Laboratory findings					
Anisopoikilocytosis / anaemia	+/+	+/+	+/+	+/+	+/+
Urinary orotic acid / pyrimidines	N/N	N/N	N/N	n/a	[c]/N
Serum CDG screening (TIEF)	N	n/a	N	n/a	N
Treatment with uridine					
Treatment with uridine (follow-up period)	–	+ (7 m)	+ (5 m)	–	n/r
Seizure-free (time frame)		+ (directly)	+ (directly[d])		
Anisopoikilocytosis resolved (time frame)		+ (3 m)	+/– (2 m)		

[a]Ng *et al.*, 2015.
[b]Not treated with antiepileptic drugs.
[c]Urinary orotic acid 1 × decreased, 1 × normal.
[d]Two clobazam withdrawal-related seizures after 2.5 months.
CDG = congenital disorder of glycosylation; DD = developmental disabilities; FTT = failure to thrive; GA = global cerebral and cerebellar atrophy; m = months; N = normal, unremarkable; n/a = not available; n/r = not reported, TIEF = transferring isoelectric focusing; w = weeks; y = years.

Mild anaemia [haemoglobin 9.1, reference range 10.5–13.5 g/dl; mean corpuscular volume 77–83 (median 79.5, $n = 9$), reference range 75-87 fl] was found at 21 months and thereafter. Abnormal size distribution of the red blood cells with anisocytosis and poikilocytosis in a peripheral blood smear was first noticed at the age of 3 years (Fig. 2G).

Patient F2:II.2

Patient F2:II.2, a female, was the second child born to healthy unrelated German parents (Fig. 1B). An older brother was diagnosed with an autism spectrum disorder. Delayed development was noticed from the age of 4 months and focal and generalized tonic clonic seizures evolved at 6 months. At 8 months, muscular hypotonia, squint, and nystagmus were documented and antiepileptic medication was started. At 2 years, she was barely able to sit independently and was unable to crawl. Her speech was very poor with little vocalizations. Despite multiple anti-epileptic drug treatments, seizure frequency and duration increased. At age 3 years she began to lose her acquired motor and cognitive skills, hypotonia progressed, and she was hypokinetic and unable to sit or swallow. She was fed via gastrostomy. At 5 years old she was in a minimally conscious state.

Her haemoglobin was low (8.8) but normalized under formula feeding by gastrostomy (12.3, reference range 10.8–15.6 g/dl); mean corpuscular volume was normal [85–90 (median 87, $n = 7$), reference range 85–95 fl]. Anisopoikilocytosis was first noticed at 1.75 years and persisted thereafter.

Brain MRI at 9 months showed the first signs of volume loss, which progressed to severe global brain atrophy by the age of 5 years. Electroencephalography showed an encephalopathic pattern with continuous irregular delta activity, multifocal sharp waves and a periodic focal and generalized rhythmic pattern.

Patient F3:II.4

Patient F3:II.4, a male, was the fourth child born to healthy, reportedly unrelated parents of Serbian Roma origin (Fig. 1B). He had three older sisters, one of whom died from severe epilepsy at age 2.5 years, but no additional clinical data or biomaterial were available to establish a diagnosis. Development was reported to be unremarkable until 1.5 years. Beginning at 2 years, epileptic fits, loss of acquired skills, and ataxia were observed. Seizures were mostly generalized, sometimes focal, and often triggered by fever. From 4 years on, his neurological status deteriorated rapidly, accompanied by increasing seizure frequency and intensity. Within a few months he became completely immobile and lost his ability to communicate and swallow. He experienced repeated refractory status epilepticus. After 8 months in a minimally conscious state, he died aged 5 years. Parents did not agree to autopsy. He had mild anaemia [haemoglobin 8.1, reference range 10.8–15.6 g/dl; mean corpuscular volume 91–92 (median 91, $n = 4$) reference range

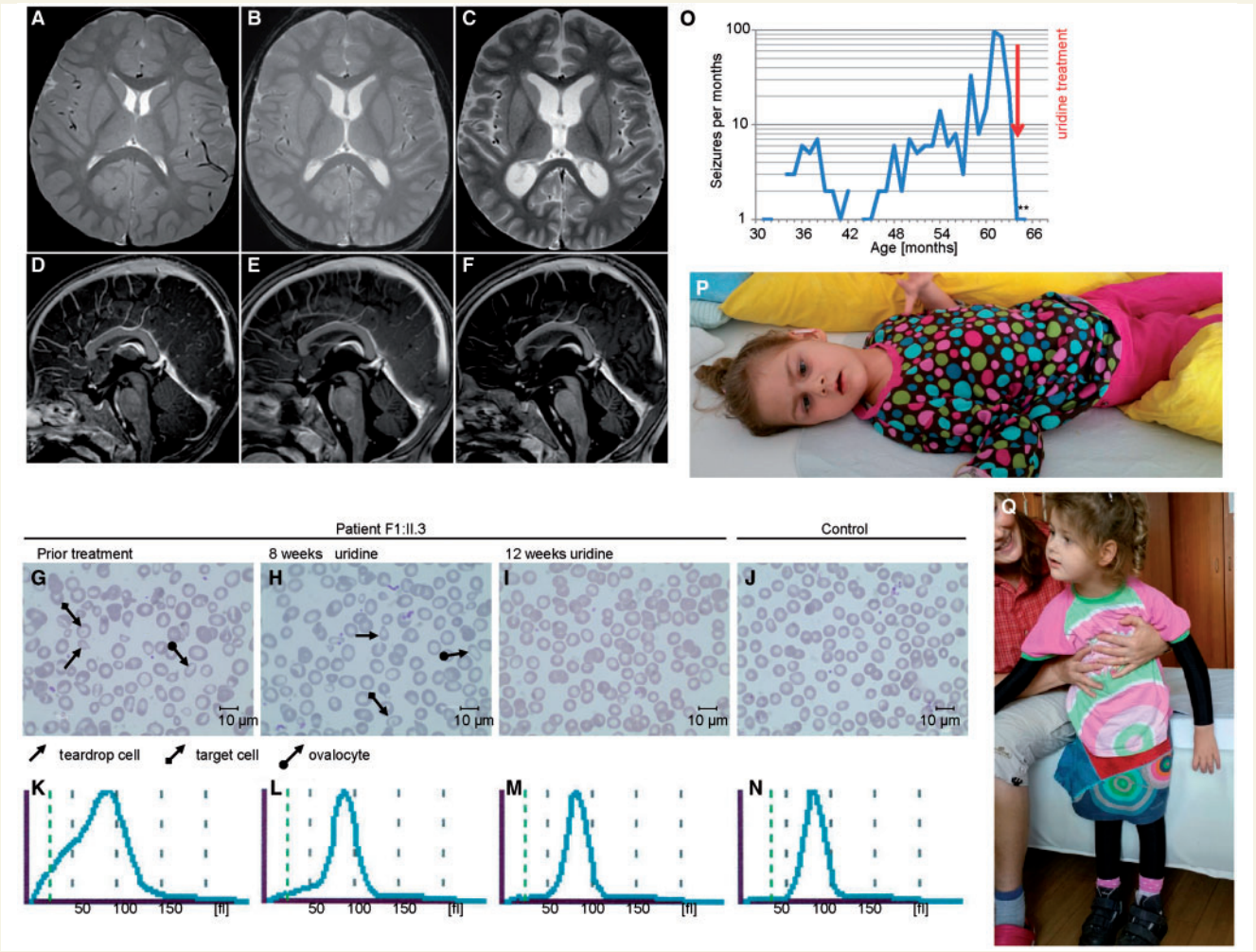

Figure 2 Neuroimaging findings and uridine response. (**A–F**) Neuroimaging findings in *CAD* deficiency. T_2-weighted axial (**A–C**) and T_1-weighted sagittal after contrast (**D–F**) cranial MRI scans in Patient F1:II.2. Brain volume was normal at 2.9 years of age (**A** and **D**) but progressive supra- and infratentorial atrophy was observed at 3.6 years (**B** and **E**) and 3.8 years (**C** and **F**). (**G–J**) Response of anisopoikilocytosis to uridine treatment. May-Grunwald Giemsa staining of peripheral blood smears of Patient F1:II.3. Abnormally shaped erythrocytes (**G**; poikilocytosis with teardrop cells, target cells and ovalocytes) were found reduced after 8 weeks of uridine treatment (**H**) and resolved completely after 12 weeks (**I**) compared to controls (**J**). (**K–N**) Quantification of erythrocyte volume determined by the hydrodynamic focusing detection method revealing an abnormal size distribution of erythrocytes (**K**; anisocytosis: microcytosis and macrocytosis), which normalized on uridine treatment after 8 (**L**) and 12 weeks (**M**), comparable to control levels (**N**). Clinical presentation in CAD deficiency before and after uridine supplementation. (**O–Q**) Seizure frequency (**O**; double asterisks indicates two short, self-limiting seizures presumably triggered by clobazam withdrawal) as well as Patient F2:II.2 at 5 years before (**P**) and after 9 weeks of uridine treatment (**Q**, with her mother).

85–95 fl]. Peripheral blood smears revealed anispoikilocytosis, first seen at 2.75 years.

Brain MRIs in his fifth year of life demonstrated progressive supra- and infratentorial global brain atrophy.

Genetic studies

We performed exome sequencing of peripheral-blood DNA samples from the four affected individuals of Families 1–3 as well as the unaffected parents in Family 1, as described previously (Kremer *et al.*, 2016). In brief, coding regions were enriched using a SureSelect Human All Exon V5 kit (Agilent) followed by sequencing as 100-bp paired-end runs on an Illumina HiSeq2500. Reads were aligned to the human reference genome (UCSC Genome Browser build hg19) using Burrows-Wheeler Aligner (v.0.7.5 a) (Li and Durbin, 2009). Single-nucleotide variants and small insertions and deletions (indels) were detected with SAMtools (version 0.1.19) (Li *et al.*, 2009). Sanger sequencing was used to confirm the identified *CAD* mutations and test the carrier status of unaffected family members. All parents gave written informed consent before undergoing evaluation and testing.

Uridine treatment

The parents of Patients F1:II.3 and F2.II.2 gave written informed consent to treatment with uridine as an unproven intervention according to item 37 of the Declaration of

Helsinki (2013). The FDA-approved drug for treatment of UMPS deficiency [uridine-triacetate, XURIDEN, Wellstat Pharmaceuticals; see: United States Food and Drug Administration (FDA), http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208169s000lbl.pdf] was unavailable in Germany and Austria. Oral uridine (Europepta) was administered in four daily doses for a total of 100 mg/kg/day, according to the lower range of dosage used for treatment of UMPS-deficient patients.

In addition, we tested the effects of uridine treatment on glycosylation patterns *in vitro*. Nucleotide sugars in primary fibroblasts from Patient F1:II.2 were analysed by ion-pair reversed-phased ultra-performance liquid chromatography as described previously (Kevelam *et al.*, 2015) after growth on standard and uridine-enriched culture media (for details see Supplementary material).

Results

Prioritization of *CAD* as a disease gene

Exome sequencing yielded 10.2–14.3 Gb of mappable sequences corresponding to a 124–164-fold coverage, with more than 97% of the target region being covered at least 20-fold (Supplementary Table 1). Based on the recessive pattern of inheritance in Family 1, we prioritized genes carrying rare [minor allele frequency (MAF) < 0.1% in public and in-house databases] biallelic variants common to both affected children in Family 1. This search identified compound heterozygous or homozygous variants in six genes, namely *CAD*, *GALNT14*, *TRIP12*, *TEK*, *C9orf131* and *ZBTB5*. A homozygous missense mutation in *CAD* (GNM_004341.3), c.98T > G (p.Met33Arg) (Fig. 1C), remained the only change predicted to be pathogenic by three algorithms (PolyPhen-2, SIFT, CADD; Supplementary Table 2) (Adzhubei *et al.*, 2010). This mutation is absent from 8000 in-house control exomes and was listed only once in a heterozygous state in 116 914 ExAC control alleles. However, it was also detected in a homozygous state in an in-house database containing a record for Patient F3:II.4, who presented with a similar clinical phenotype. Analysis of ~2000 nucleotides surrounding the mutation in the patients from Families 1 and 3 showed an additional 67 homozygous variants differing from the reference genome, including two variants with a MAF < 1% in public databases. These 67 variants were shared by all subjects carrying the mutation, implicating the disease mutation as a likely single ancestral change in the Serbian Roma population.

Modelling of the p.Met33Arg change using the crystal structure of CPS1 indicated a destabilization of subdomain interactions within the CPS2 moiety of CAD as well as an altered tertiary protein configuration (Supplementary Fig. 1).

In Patient F2:II.2 the same filtering steps prioritized putative compound heterozygous or homozygous variants in seven genes. *CAD* was the only gene carrying biallelic predicted loss-of-function mutations: a maternally inherited splice site mutation, c.1843-3C > T, predicted to affect the splice acceptor site of intron 12, and a paternally inherited nonsense mutation, c.5365C > T (p.Arg1789*), predicted to give rise to a prematurely truncated polypeptide missing the last 438 of 2225 amino acids (Fig. 1C).

The identification of three different biallelic rare variants in three index patients with strikingly similar clinical presentations establishes CAD deficiency to be confidently implicated in this neurometabolic disorder.

Response to uridine treatment

When uridine treatment was started at the age of 3 years in Patient F1:II.3, she was experiencing seizures weekly or even daily. Her development had arrested and she could barely walk and communicate. Retesting with the developmental score tool (ET6-6) directly prior to treatment was not possible due to the patient's very impaired alertness and general condition. Upon uridine administration, no more seizures were observed over a follow-up period of 7 months. When starting uridine, EEG showed irregular slowing, multifocal sharp waves and a periodic sharp wave pattern. During the treatment period this finding improved, the irregular slowing almost disappeared and the periodic pattern was less pronounced (last recording after 6 months of treatment). She became more alert and advanced in her motor, cognitive, and speech development and is now able to walk independently and communicate with words. After 6 months of treatment, retesting with the developmental testing tool (ET6-6) at age 3.5 years showed her gross motor function to be 2 SD below the age-related mean value (stable, no improvement); her fine motor function to be 1–2 SD below the age-related mean value (improvement); her cognition, language and social–emotional skills to be 2–3 SD below the age-related mean value (improvement). One should take into account that developmental testing directly before the start of the treatment was impossible and that all data are compared with the testing at the age of 2 years after which further deterioration occurred. Blood smears normalized within 12 weeks and her anaemia disappeared (Fig. 2G–N).

When uridine supplementation was started at 5.2 years of age in Patient F2:II.2, she was bedridden and in a minimally conscious state (Fig. 2P). She did not communicate and was hypokinetic with only undirected movements. Seizures occurred every second day despite multiple antiepileptic treatments and she was unable to swallow. During the 5 months of follow-up, two very short self-limiting seizures were observed after 2.5 months in the context of clobazam withdrawal (Fig. 2O). In the EEG, the encephalopathic pattern disappeared, background activity improved to the theta range with only occasional sharp waves and without subclinical seizure activity. Her alertness and postural control clearly improved (Fig. 2Q), she began to communicate again, smiled, and her intentional

movements of arms and legs improved. She is now able to take single steps with a walker. Three weeks before treatment with uridine, rating on the Coma Recovery Scale (minimum score 0, maximum score 23) (Kalmar and Giacino, 2005) was 5. After 2 months of treatment, the rating was 16, with improvements in auditory, visual, motor and verbal functions; however, based on the score, she still had not emerged from a minimally conscious state. Within 8 weeks her peripheral blood smear normalized. Her mild anaemia had already normalized during the gastrostomy formula feeding, which she received during the 4 months prior to uridine administration.

In both treated patients we regularly checked total blood counts, ASAT (aspartate amino transferase), ALAT (alanine amino transferase), creatinine, blood gas, glucose and electrolytes in blood; all were within normal limits.

Investigation of fibroblasts from Patient F1:II.2 showed reduced levels of UDP, UDP-glucose (UDP-glc), UDP-*N*-acetylglucosamine (UDP-GlcNac), CTP and UTP compared to a control cell line. Uridine supplementation restored all the reduced metabolites in the patient's fibroblasts to control levels (Supplementary Fig. 2), in line with published data (Ng *et al.*, 2015).

Discussion

The number of epileptic encephalopathies for which there is convincing evidence of beneficial treatment effects is rather limited (Dulac *et al.*, 2014). Based on our observations in two unrelated patients, we suggest that CAD deficiency can now be considered a further example of a treatable neurometabolic disorder that might otherwise lead to death in early childhood. Despite the interventions having been started at an advanced stage of disease, clinical improvement was obvious, immediate, and accompanied by resolving of blood phenotypes.

Uridine monophosphate content is variable in the normal diet (e.g. formula milk contains 3–5-fold more uridine monophosphate than human breast milk) (Janas and Picciano, 1982). Notably, the exclusively breast-fed Patient F2:II.2 had a very early onset of seizures in comparison to the bottle-fed patients (Table 1), and her anaemia improved after she was started on a uridine monophosphate-containing gastrostomy formula. No adverse side effects of uridine administration have been reported in humans, although long-term feeding of high concentrations to a mouse strain resulted in glucose intolerance and hepatic lipid accumulation (Urasaki *et al.*, 2016).

Uridine monophosphate plays a pivotal role in protein glycosylation, lipid metabolism, and polysaccharide biosynthesis (Fig. 1A) (Loeffler and Zameitat, 2004; Wurtman *et al.*, 2006). Dyserythropoiesis might be due to shortage of pyrimidine-dependent nucleotide-lipid cofactors required for erythrocyte membrane synthesis (Bailey, 2009). However, further studies are needed to untangle the cellular mechanisms by which defects in pyrimidine metabolism cause specific clinical signs and symptoms.

We recommend that CAD deficiency should be added to the (short) list of treatable inborn errors of metabolism (van Karnebeek *et al.*, 2014) as well as treatable metabolic epilepsies (Dulac *et al.*, 2014). These disorders should be actively searched for and clinicians must pay attention to co-occurrence of additional phenotypes suggestive of CAD-deficiency in patients with global developmental delay. The following aspects should be taken into account:

First, one should be aware that this anaemia is per definition normocytic, as the mean corpuscular volume is within normal values. Abnormal size distribution and morphology of erythrocytes can easily be missed by automated blood cell count. We recommend a peripheral blood smear and to check the size distribution curve of the counter (Fig. 2K).

Second, despite CAD deficiency being a primary *de novo* pyrimidine biosynthesis disorder with secondarily impaired glycosylation, routine metabolic tests for these disorders (measurement of urinary and serum pyrimidines; transferrin isoelectric focusing) yielded normal results.

Third, although progressive brain atrophy seems to be a consistent feature on neuroimaging, it is rather non-specific and might be only observed at a late stage of the disease; early MRI results in symptomatic Patients F1:II.2 and UDP4003 (Ng *et al.*, 2015) were normal.

Finally, one should also be aware of nutritional factors which might modify the severity and course of CAD deficiency and might mask indicative phenotypes (e.g. erythrocytes).

The three index patients described here were investigated in the context of a clinical exome sequencing study of 700 consecutive patients, including 70 paediatric patients with global developmental delay and epilepsy. To further estimate the prevalence of this phenotype based on Hardy-Weinberg equilibrium, we used carrier frequencies available from the ExAC server (http://exac.broadinstitute.org). We selected variants with a MAF $< 0.5\%$ which either predict a loss of protein function or are rated as functionally relevant *in silico* by PolyPhen-2 (probably damaging), SIFT (score < 0.05), PROVEAN (score > -2.5), and combined annotation-dependent depletion score (CADD PHRED score > 10) (Staufner *et al.*, 2016). Accordingly, 181 variants were considered, predicting a phenotype prevalence of 1/11 273. While this finding suggests that CAD deficiency could be more common, additional experimental data on the functional relevance of the considered variants are needed for more precise estimations. Furthermore, as suggested by our data, founder mutations such as c.98T > G likely contribute to a higher prevalence of CAD deficiency in certain populations.

In summary, CAD deficiency is likely to be an underdiagnosed but clinically recognizable condition due to co-occurrence of anaemia, anisopoikilocytosis, global developmental delay, and seizures. Probatory uridine administration might be considered in severely affected patients with

anisopoikilocytosis and epileptic encephalopathy prior to a confirmed genetic diagnosis. The identification and treatment of additional CAD patients over a longer period is necessary to fully define the phenotypic spectrum and to corroborate the beneficial effects of this therapeutic approach. There is an urgent need to define a reliable biomarker for CAD deficiency (e.g. via an untargeted metabolomics approach) preferably in dried blood spots, as this disorder seems to qualify for newborn screening. However, CAD deficiency might currently represent a potential condition for genetic newborn screening (Andermann *et al.*, 2008).

Web resources

Online Mendelian Inheritance in Man (OMIM, http://www.omim.org).

Exome Aggregation Consortium (ExAC), Cambridge, MA (http://exac.broadinstitute.org).

US Food and Drug Administration, (http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208169s000lbl.pdf).

Zentrum für klinische Forschung und Rehabilitation der Universität Bremen, (https://www.zkpr.uni-bremen.de/forschung/testentwicklung/entwicklungstests/et-6-6-r).

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Supplementary material

Supplementary material is available at *Brain* online.

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