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ORIGINAL ARTICLE

The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 2: the evolving clinical phenotype

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Abstract
Background The disease course and long-term outcome of patients with organic acidurias (OAD) and urea cycle disorders (UCD) are incompletely understood.
Aims To evaluate the complex clinical phenotype of OAD and UCD patients at different ages.
Results Acquired microcephaly and movement disorders were common in OAD and UCD highlighting that the brain is the major organ involved in these diseases. Cardiomyopathy [methylmalonic (MMA) and propionic aciduria (PA)], prolonged QT_c interval (PA), optic nerve atrophy [MMA, isovaleric aciduria (IVA)], pancytopenia (PA), and macrocephaly [glutaric aciduria type 1 (GA1)] were exclusively found in OAD patients, whereas hepatic involvement was more frequent in UCD patients, in particular in argininosuccinate lyase (ASL) deficiency. Chronic renal failure was often found in MMA, with highest frequency in mut^0 patients. Unexpectedly, chronic renal failure was also observed in adolescent and adult patients with GA1 and ASL deficiency. It had a similar frequency in patients with or without a movement disorder suggesting different pathophysiology. Thirteen patients (classic OAD: 3, UCD: 10) died during the study interval, ten of them during the initial metabolic crisis in the newborn period. Male patients with late-onset ornithine transcarbamylase deficiency were presumably overrepresented in the study population.
Conclusions Neurologic impairment is common in OAD and UCD, whereas the involvement of other organs (heart, liver, kidneys, eyes) follows a disease-specific pattern. The identification of unexpected chronic renal failure in GA1 and ASL deficiency emphasizes the importance of a systematic follow-up in patients with rare diseases.

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Abbreviations

ARG1	Arginase 1
ASL	Argininosuccinate lyase
ASS	Argininosuccinate synthetase

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CPS1	Carbamylphosphate synthetase 1
E-IMD	European registry and network for intoxication type metabolic diseases
GA1	Glutaric aciduria type 1
HHH	Hyperornithinemia-hyperammonemia-homocitrullinuria
IVA	Isovaleric aciduria
MMA	Methylmalonic aciduria (isolated forms)
NAGS	N-acetylglutamate synthase
OAD	Organic aciduria
OTC	Ornithine transcarbamylase
PA	Propionic acidurias
SDS	Standard deviation score
UCD	Urea cycle disorder

Introduction

Patients with organic acidurias (OAD) and urea cycle disorders (UCD) often first present with acute metabolic crises in classic OAD [i.e. methylmalonic aciduria (MMA; OMIM #251000, #251100, #251110, #277400, #277410), propionic acidurias (PA; OMIM #606054), and isovaleric aciduria (IVA; OMIM (#243500)] and UCD, or acute encephalopathic crises in glutaric aciduria type 1 (GA1; OMIM #231670). Such crises may occur as early as the first day of life and as late as adulthood (Kölker et al 2015). Some patients, however, never develop metabolic crises but present with chronic or progressing neurological and/or gastrointestinal signs and symptoms (Kölker et al 2015). The number of untreated patients who remain asymptomatic is usually thought to be low. However, mild clinical variants of IVA, argininosuccinate synthetase (ASS; EC 6.3.4.5; OMIM #215700) and lyase (ASL; EC 4.3.2.1; OMIM #207900) deficiency have been described and are thought to be common in female ornithine transcarbamylase (OTC; EC 2.1.3.3; OMIM #311250) carriers (Ensenauer et al 2004; Mercimek-Mahmutoglu et al 2010; Rüegger et al 2014; Summar et al 2008). Since previous clinical studies have usually been performed in regional or national (Ah Mew et al 2013; Cosson et al 2009; Enns et al 2007; Ensenauer et al 2004; Grünert et al 2012; Grünert et al 2013; Heringer et al 2010; Kölker et al 2007; Nassogne et al 2005; Nizon et al 2013; Seminara et al 2010; Strauss et al 2007; Tuchman et al 2008) rather than international samples (Hörster et al 2007; Hörster et al 2009; Kölker et al 2006a; Rüegger et al 2014), the knowledge about the long-term outcome is still incomplete and there is uncertainty about the risk of developing (multiple) organ dysfunction with increasing age. Evidence is increasing that even patients who have been considered as "metabolically stable" or who have never developed a metabolic crisis might be at risk of developing (fatal) late-onset organ complications (Cavicchi et al 2014; Herskovitz et al 2013; Hörster et al 2007; Komatzusaki et al 2012; Marquard et al 2011; Pena et al 2012; Romano et al 2010; Serrano et al 2010; Williams et al 2009).

The major aim of this study is to elucidate the age-dependent and disease-specific clinical phenotype evolving in patients with OAD and UCD by use of comparative phenotyping.

Patients and methods

The European registry and network for intoxication type metabolic diseases (E-IMD, EAHC no. 2010 12 01) has received funding from the European Union, in the framework of the Health Programme 2008–2013, establishing a network, a patient registry and guidelines. An overview on E-IMD has been published separately (Kölker et al 2015).

Patient registry

The E-IMD registry (URL: https://www.eimd-registry.org) contains comprehensive information on patients with confirmed diagnosis of GA1, IVA, MMA and PA as well as those with inherited deficiency of N-acetylglutamate synthase (NAGS; EC 2.3.1.1; OMIM #237310), carbamylphosphate synthetase 1 (CPS1; EC 6.3.4.16; OMIM #237300), OTC (also including female OTC carriers), ASS, ASL and arginase 1 (ARG1; EC 3.5.3.1; OMIM #207800) and hyperammonemia-hyperornithinemia-homocitrullinuria (HHH; OMIM #238970) syndrome. The study was approved by the ethic committee of the coordinating centre (University Hospital Heidelberg, application no. S-525/2010) and then was approved by all contributing metabolic centres. Written informed consent was obtained from all study patients or carers before enrolment in countries where this was needed by law. A detailed description of the registry has been published separately (Kölker et al 2015).

Statistical analysis

For descriptive statistics and comparison of standard deviation scores (SDS) of anthropometrical data (body length, body weight, head circumference) with t-tests, SPSS (IBM SPSS Statistics 22.0) was used. For multiple testing, we adjusted the α-level according to the number of tests. Standard deviation scores (SDS) of anthropometrical data were calculated with R (R Core Team 2014) according to the LMS method (Cole 1990) and in analogy to a previous study on GA1 (Boy et al 2013). This method provides a way of obtaining normalized growth centiles. Skewed distributions of the measurements are approximated to normal distributions by power transformation. The distribution of each variable is summarized by three

parameters, the Box-Cox power L, the mean M, and the coefficient of variation S. The calculation of SDS for anthropometrical parameters was based on published reference data (Cole et al 1998; Cole et al 2011).

Kaplan Meier analysis and Cox regression analysis were done with R using the statistical package for survival analysis (Therneau 2014). The method used for computation of 95 % confidence intervals for Kaplan Meier analysis is based on the log of the hazard as previously described (Strobl and Dirschedl 2004). The cut-off data for statistical analysis was 22 October 2013.

For some analyses of clinical and biochemical parameters, we dichotomized the group of symptomatic patients by age. According to the international definition of the neonatal period, we used the term "neonatal onset" (or early onset) if first symptoms occurred within 28 days of life and as "late onset" if symptoms started after that period. In addition, GA1 patients were divided into high and low excreters according to the definition of Baric et al (1999) for some analyses.

Results

Brief description of the study population

From 1 February 2011 to 22 October 2013, a total of 795 patients (406 boys/men, 389 girls/women) with confirmed diagnosis of an OAD (n=452) or UCD (n=343) were registered. Information was obtained from 788 baseline and 737 regular (annual) follow-up visits. The median age at baseline visit was 7.4 years (Q1, 3.3; Q3, 13.8 years; range: 0.1–45.8 years) in OAD and 10.3 years (Q1, 4.4; Q3, 18.7 years; range: 0.1–77 years) in UCD patients. A detailed description of the E-IMD study population has been published separately (Kölker et al 2015).

Anthropometrical parameters

Growth is influenced by various endogenous and exogenous factors; anthropometrical parameters reflect the net sum of these factors. In patients with OAD and UCD, body growth and brain development might be negatively affected by secondary mitochondrial dysfunction and brain injury due to accumulation of ammonium and other neurotoxic metabolites, low protein diet, feeding problems and other factors. To identify relevant effects on growth and thrive, we compared SDS of the anthropometrical parameters body weight, body length and head circumference at birth and baseline visit.

In the E-IMD sample, the majority of OAD and UCD patients had normal anthropometrical parameters at birth (Fig. 1) — except for patients with MMA who showed a significantly low birth weight (median SDS, −0.81; p<0.001) and those with GA1 who often presented with macrocephaly (median SDS, 0.88; p<0.001). From birth to baseline visit, body weight decreased in GA1 and ASS deficiency, body length decreased in MMA, PA, GA1 and ASS deficiency and head circumference decreased in MMA, PA, IVA, GA1, ASS deficiency and OTC (male and female) deficiency (Table 1). In NAGS, CPS1, ASL and ARG1 deficiency and HHH syndrome the apparent deceleration in head growth was not confirmed by statistical analysis.

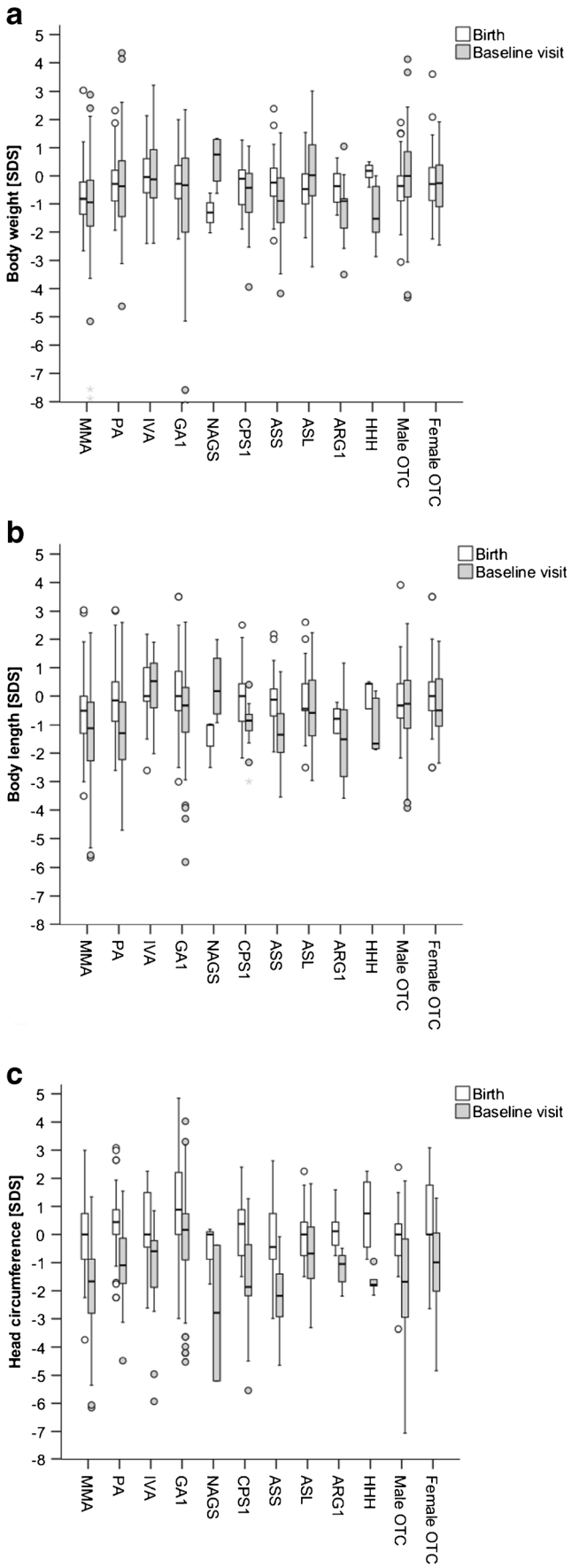
Since anthropometrical parameters were highly variable in individual OAD and UCD, we wondered whether this reflected the variable disease severity. For this purpose we compared growth in early- and late-onset patients and in those with or without a movement disorder. Early- and late-onset groups did not show significant differences in body weight and length; a tendency towards decreased body length was found in early-onset ASS deficiency and for decreased body weight and length in early-onset male OTC deficiency (Suppl. Table 1). MMA patients and — as a tendency — GA1 and male OTC patients with a movement disorder showed a decreased SDS for body length over time compared to those without movement disorders. A significantly decreased SDS for body weight was found for GA1 patients with a movement disorder (Suppl. Table 2).

Poor growth might also be caused by low protein diet. Since the evaluation of dietary treatment on anthropometric parameters requires a longer follow-up of patients, we will study this important question in the future.

Nervous system

The brain is thought to be the most vulnerable organ in OAD and UCD. It can be affected by acute metabolic and encephalopathic crises and/or by chronic neuropathological changes. The vulnerability of the brain is age-dependent and region-specific. Furthermore, endogenously and exogenously disturbed cerebral synthesis of protein, creatine and neurotransmitters has an impact on the neurological phenotype (Braissant et al 2013; Grünert et al 2013; Hörster et al 2007; Kölker et al 2013; Pena et al 2012). In the E-IMD sample, motor dysfunction and impaired motor development were quite common and were usually associated with a movement disorder (Table 2). A movement disorder was most often reported for GA1 (46 %) in the OAD group and for ASL deficiency in the UCD group (27 %). In the 155 patients with a movement disorder, dystonia (n=99) and spasticity (n=76) were most frequent (Suppl. Table 3). Symptomatic patients with HHH syndrome and ARG1 deficiency presented with the previously described pyramidal syndrome.

Kaplan-Meier analysis demonstrated group- and disease-specific differences in the manifestation of a movement disorder (Fig. 2). OAD patients (GA1 > MMA, PA > IVA) developed a movement disorder more often than UCD patients [ASL deficiency > ASS deficiency, OTC deficiency (female,



◀ **Fig. 1** Anthropometrical parameters at birth and baseline visit. **a**, body weight; **b**, body length; **c**, head circumference. *Circles*, outliers; height of box: interquartile range; top end of box: Q3; bottom end of box: Q1; *bold line inside of box*: median; top/bottom whiskers: highest/lowest case within 1.5 times interquartile range; outliers: distance to Q1 or Q3 respectively ≥1.5 and ≤3 times interquartile range; extreme values: distance to Q1 or Q3 respectively >3 times interquartile range. Numbers of patients by disease: MMA (n=127), PA (n=91), IVA (n=45), GA1 (n=131), NAGS deficiency (n=4), CPS1 deficiency (n=14), ASS deficiency (n=51), ASL deficiency (n=37), ARG1 deficiency (n=8), HHH syndrome (n=6), male OTC deficiency (n=66), female OTC deficiency (n=70)

male)]. The low frequency of movement disorders in male OTC patients was unexpected. Since the age at disease manifestation is thought to inversely correlate with the residual enzyme activity, we wondered whether patients with early (neonatal) onset of symptoms more often had movement disorders than those with late onset of symptoms. For OAD, Cox regression analysis between early- and late-onset patients with MMA (p=0.11), PA (p=0.56) and IVA (p=0.68) did not reveal any differences. GA1 was excluded from this analysis since the majority of symptomatic patients had late onset of symptoms. In contrast to OAD, patients with early-onset UCD more often developed movement disorders than patients with late onset of symptoms (Fig. 3).

Seizures and EEG abnormalities were most often reported for PA (OAD group) and for ASL deficiency (UCD group) (Table 2).

Musculoskeletal system

As a consequence of the movement disorder the joint mobility was often decreased in patients with GA1 (n=32) and MMA (n=15) and was associated with contractures and/or hip (sub-)luxations. Immobility and low protein diet may increase the risk for impaired bone mineralization and osteoporosis. Decreased bone age and osteoporosis were seemingly rare in our study cohort. However, these numbers should not be considered as representative, since specific test results for bone age and mineralization have only been reported for a small group of patients.

Behaviour and mental retardation

Behavioral abnormalities and mental retardation are quite common in OAD and UCD patients being a significant burden for patients and their families. These have been studied in detail and will be published separately.

Ocular and auditory system

Ophthalmological problems were reported in 26 patients (19 with OAD, seven with UCD), mostly strabism, hypermetropia

Table 1 Comparison of anthropometric parameters at birth and baseline visit

Disease	Body weight [SDS] Paired *t*-test birth vs. baseline visit	Body length [SDS] Paired *t*-test birth vs. baseline visit	Head circumference [SDS] Paired *t*-test birth vs. baseline visit
MMA	M=0.12, SD=1.63, t(df=110)=0.76, p=0.451	M=0.69, SD=1.71, t(df=92)=3.91, p<0.0001	M=1.63, SD=1.98, t(df=59)=6.40, p<0.0001
PA	M=0.15, SD=1.77, t(df=80)=0.75, p=0.456	M=1.12, SD=1.67, t(df=69)=5.63, p<0.0001	M=1.72, SD=2.23, t(df=46)=5.30, p<0.0001
IVA	M=−0.02, SD=1.45, t(df=39)=−0.07, p=0.947	M=−0.11, SD=1.22, t(df=33)=−0.53, p=0.602	M=1.38, SD=1.40, t(df=20)=4.53, p<0.0001
GA1	M=0.77, SD=2.39, t(df=117)=3.49, p<0.001	M=0.73, SD=1.53, t(df=99)=4.81, p<0.0001	M=1.18, SD=1.73, t(df=70)=5.76, p<0.0001
NAGS-D	M=−2.26, SD=0.32, t(df=2)=−12.37, p=0.006	M=−1.74, SD=2.40, t(df=2)=−1.26, p=0.336	M=1.91, SD=2.17, t(df=1)=1.25, p=0.431
CPS1-D	M=0.65, SD=1.71, t(df=11)=1.32, p=0.213	M=1.23, SD=1.41, t(df=11)=3.02, p=0.012	M=1.87, SD=2.50, t(df=6)=1.97, p=0.096
ASS-D	M=0.77, SD=1.20, t(df=43)=4.25, p<0.0001	M=1.33, SD=1.11, t(df=38)=7.47, p<0.0001	M=1.92, SD=1.76, t(df=26)=5.64, p<0.0001
ASL-D	M=−0.33, SD=1.48, t(df=30)=−1.24, p=0.224	M=0.52, SD=1.82, t(df=25)=1.47, p=0.154	M=0.72, SD=1,38, t(df=21)=2.43, p=0.024
ARG1-D	M=0.48, SD=1.45, t(df=6)=0.87, p=0.416	M=0.32, SD=1.62, t(df=5)=0.48, p=0.652	M=1.25, SD=0.95, t(df=5)=3.22, p=0.024
HHH syndr.	M=1.17, SD=1.40, t(df=3)=1.67, p=0.193	M=0.92, SD=0.59, t(df=3)=3.12, p=0.052	M=2.03, SD=1.03, t(df=2)=3.41, p=0.076
OTC-D (m)	M=−0.24, SD=1.88, t(df=56)=−0.96, p=0.340	M=0.22, SD=2.21, t(df=49)=0.70, p=0.487	M=1.67, SD=2.01, t(df=3)=4.85, p<0.0001
OTC-D (f)	M=0.04, SD=1.20, t(df=48)=0.24, p=0.814	M=0.29, SD=1.24, t(df=41)=1.52, p=0.137	M=1.53, SD=1.35, t(df=24)=5.68, p<0.0001

Sidak-adjusted α=0.00142 (36 tests), mean difference (M) between birth and baseline visit, *df*=N-1. Note that negative values for M indicate an increase in anthropometric parameters from birth to baseline visit, whereas positive values indicate a decrease. *D* Deficiency; *df* Degree of freedom; *f* Female; *m* Male; *SD* Standard deviation; *syndr* Syndrome

and astigmatism which are quite common in the general population. Four OAD patients (three with MMA, one with IVA) presented with optic nerve atrophy. Hypacusis (MMA: one, female OTC carrier: one, male OTC deficiency: one) and deafness (PA: one) were sporadically reported.

Cardiovascular system

During the study interval 153 patients received an electrocardiogram and 149 patients a heart ultrasound study. The majority of these investigations showed normal results or revealed abnormalities that are also common in the general population (e.g. valvular dysfunction in six patients). However, the QT_c interval was often prolonged in PA patients, and dilated cardiomyopathy was identified in PA and MMA (Table 3).

Arterial hypertension was most often found in MMA patients with chronic renal failure (n=11) and was sporadically observed in other diseases (female OTC carriers: five patients; PA and GA1: each three; male OTC deficiency: two; IVA, CPS1, ASL deficiency, HHH syndrome: each one patient; NAGS, ASS and ARG1 deficiency: none). Although ASL deficiency is thought to be a risk factor for systemic hypertension due to impaired systemic nitric oxide production (Erez et al 2011), arterial hypertension was observed in one patient with ASL deficiency only.

Gastrointestinal tract

Vomiting and diarrhoea was found in 25 % and 12 % of patients, respectively. In the majority of OAD and UCD patients these symptoms occurred sporadically or rarely rather than recurrently (Table 4). Hepatomegaly was most often reported for ASL deficiency (37 %) and was usually associated with elevated ALAT and ASAT indicating hepatocellular injury. In OAD patients, albumin, ALAT, ASAT, INR, GGT and bilirubin were usually within the normal range or showed minor deviations (Suppl. Table 4). In UCD patients, however, ASAT, ALAT and — less pronounced — INR values were quite often elevated above the reference range in female OTC carriers and individuals with ASS, ASL and ARG1 deficiency and HHH deficiency during baseline visit and regular follow-up. Plasma albumin was decreased in some of these patients being the consequence of low protein diet or indicating hepatic dysfunction. Fourteen patients received a liver transplantation. All transplanted patients except one with PA had an UCD (CPS1 deficiency: two, female OTC carrier: three, male OTC deficiency: seven, ASS deficiency: one). Although previously described for classic OAD (Marquard et al 2011), pancreatitis was not reported before the cut-off date. Meanwhile, it has been reported for E-IMD patients (MMA, PA) as well.

In total, 13 % of patients received tube feeding via gastrostomy.

Table 2 Neurological manifestation in OAD and UCD

Disease	Decreased muscle strength % (N)	Muscular hypotonia % (N)	Muscular hypertonia % (N)	Abnormal gross motor development % (N)	Abnormal fine motor development % (N)	Disturbed balance % (N)	Movement disorder % (N)	Seizures % (N)	EEG abnormalities % (N)
MMA	17 (126)	23 (129)	5 (129)	31 (129)	36 (115)	27 (117)	26 (131)	3 (128)	38 (24)
PA	25 (80)	38 (82)	2 (82)	30 (83)	45 (73)	39 (74)	22 (82)	13 (87)	63 (16)
IVA	5 (44)	2 (44)	7 (44)	4 (46)	12 (41)	8 (40)	7 (46)	6 (49)	20 (5)
GA1	28 (127)	25 (133)	17 (133)	40 (135)	46 (123)	39 (122)	46 (140)	7 (136)	34 (29)
NAGS-D	0 (4)	0 (4)	25 (4)	50 (4)	50 (4)	25 (4)	25 (4)	0 (4)	100 (1)
CPS1-D	13 (15)	31 (16)	0 (16)	25 (16)	25 (16)	7 (14)	13 (15)	6 (16)	33 (3)
OTC-D (m)	8 (66)	12 (69)	3 (69)	13 (68)	22 (64)	8 (62)	11 (66)	14 (74)	28 (18)
OTC-D (f)	2 (102)	4 (98)	3 (98)	3 (97)	9 (97)	9 (97)	7 (100)	4 (103)	19 (16)
ASS-D	10 (51)	14 (51)	10 (51)	14 (49)	27 (45)	12 (42)	10 (51)	9 (53)	20 (10)
ASL-D	9 (32)	22 (37)	5 (37)	35 (34)	58 (33)	35 (34)	27 (37)	19 (42)	54 (13)
ARG1-D	20 (10)	0 (10)	10 (10)	20 (10)	11 (9)	11 (9)	20 (10)	0 (10)	0 (1)
HHH syndr.	25 (8)	0 (8)	25 (8)	38 (8)	29 (7)	38 (8)	25 (8)	13 (8)	0 (1)

% Percentage of neurologically symptomatic patients; *D* Deficiency; *f* Female; *m* Male; *N* Total number of patients for whom the presence or absence of neurological symptoms have been reported, *syndr.* Syndrome

Urogenital system

In the E-IMD sample, 24 % of children and adolescents with MMA had a creatinine clearance (according to the Schwartz formula) below 60 mL/min [mut^0: 14/23 cases (61 %); mut^-: 1/23 (4 %); cblA deficiency: 2/23 (9 %); cblB deficiency: 3/23 (13 %); unclassified MMA: 2/23 (9 %)] and that 29 % of adult MMA patients suffered from chronic renal failure (glomerular filtration rate below 60 mL/min according to the MDRD formula). Chronic renal failure was sporadically found in patients with PA as well as OTC (male and female) and ASS deficiency. In GA1 and ASL deficiency, Kaplan-Meier analysis unravelled a moderate increase in the frequency of chronic renal failure with age. This was unexpected (Fig. 4). Seven patients received hemodialysis (n=5; three with MMA and each one with PA and male OTC deficiency) or peritoneal

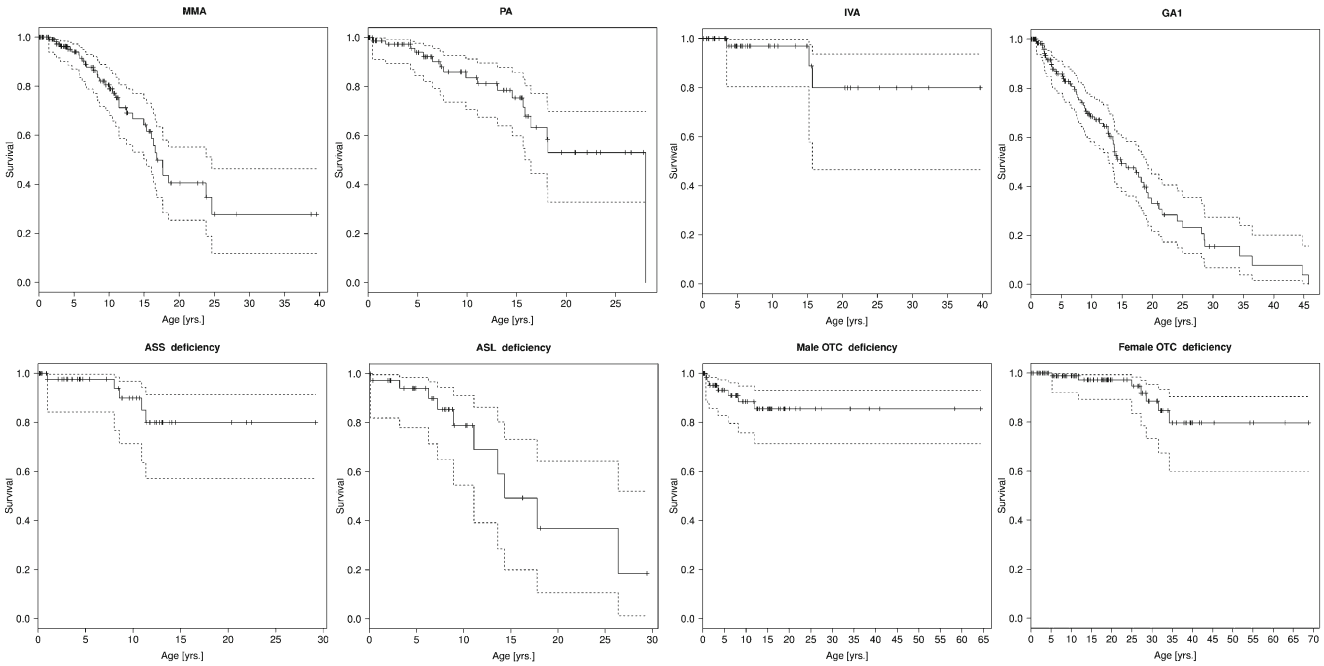


Fig. 2 Movement disorders in OAD and UCD. Results of a Kaplan Meier analysis are depicted. Dashed lines indicate 95 % confidence interval. UCD with a small group size (NAGS, CPS1 and ARG1 deficiency and HHH syndrome) were not included in the Kaplan Meier analysis. Please note that Kaplan Meier analysis is based on the presence or absence of a movement disorder at the time point of the baseline visit. Therefore, Kaplan Meier curves do not reflect the true age-specific progression of symptoms in the OAD and UCD cohorts

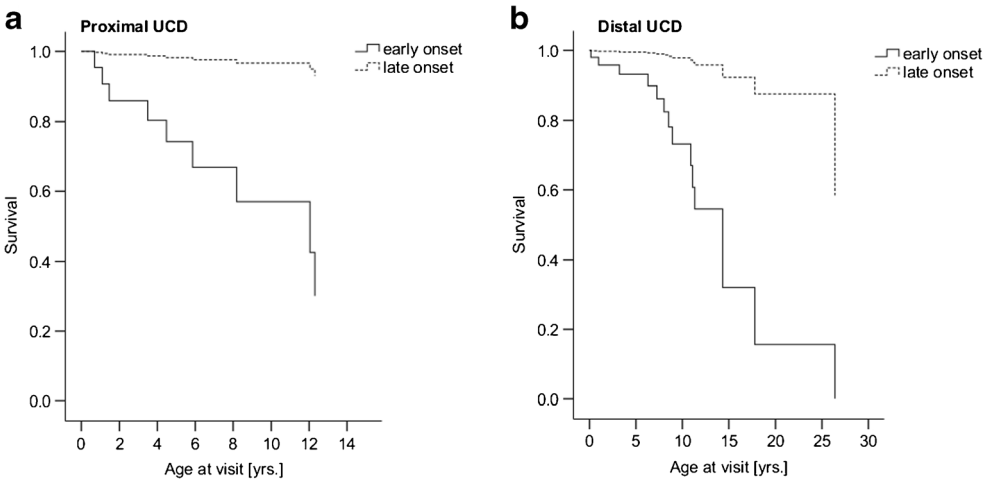


Fig. 3 Effect of onset type on the manifestation of movement disorders in UCD. **a**, proximal (synonym, mitochondrial) UCD (i.e. CPS1 and OTC deficiency). W(*df*=1)=11.27; *p*=0.001 (Cox regression analysis); **b**, distal (synonym, cytosolic) UCD (i.e. ASS and ASL deficiency). W(*df*=1)=5.95; *p*=0.015 (Cox regression analysis)

dialysis (*n*=2; two with MMA). Six MMA patients received a kidney transplantation.

The Cox regression model showed a trend for cobalamin non-responders [HR=3.36, 95 % CI (0.95; 11.92), *p*=0.061] and a significant effect for mut^0-type MMA patients [HR=4.44, 95 % CI (1.24; 15.88), *p*=0.022] having a higher risk for chronic renal failure than cobalamin responders and non-mut^0 patients (mut$^-$, cblA and cblB deficiency), respectively (Fig. 5a and b). The frequency of chronic renal failure did not differ in patients with mut$^-$, cblA and cblB deficiency (not shown). GA1 patients with a movement disorder [HR=1.34, 95 % CI (0.29; 6.13), *p*=0.703; Fig. 5c] or a high excreter phenotype [HR=1.71, 95 % CI (0.33; 8.95), *p*=0.525; Fig. 5d] developed chronic renal failure at a similar frequency as those without a movement disorder or a low excreter phenotype, respectively. Similarly, the manifestation of a movement disorder did not increase the frequency of chronic renal failure in ASL deficiency [HR=1.24, 95 % CI (0.17; 9.28), *p*=0.832] (Fig. 5e). Since serum creatinine rather than cystatin C was commonly used for regular follow-up the frequency of chronic renal failure is likely to be underestimated.

Bone marrow and immune system

Toxic metabolites and insufficient supply with essential nutrients and micronutrients may increase the risk of hematologic abnormalities in individuals with OAD and UCD. In fact, hematologic abnormalities were quite common in OAD, in particular MMA and PA (Suppl. Table 5). Anemia (MMA: 40 %; PA: 22 %) was more common than leukocytopenia (MMA: 7 %; PA: 18 %) or thrombocytopenia (MMA: 6 %; PA: 18 %). Pancytopenia was exclusively found in PA patients

Table 3 Cardiac manifestation

Disease	ECG				Heart ultrasound			
	Patients examined N	Prolonged QT_c time N	Hypertrophy N	Others N	Patients examined N	Hypertrophy N	ASD/VSD N	Others N
MMA	32	0	2	2	36	4	0	1
PA	43	14	0	5	40	3	2	1
IVA	5	0	0	1	4	0	0	0
GA1	22	0	0	1	19	0	0	1
NAGS-D	0	N/a	N/a	N/a	0	N/a	N/a	N/a
CPS1-D	2	0	0	0	3	0	0	0
OTC-D (f)	17	0	0	1	15	0	0	1
OTC-D (m)	16	0	0	2	17	0	2	2
ASS-D	7	0	1	1	6	0	0	2
ASL-D	8	0	0	0	7	0	0	0
ARG1-D	0	N/a	N/a	N/a	1	0	0	0
HHH syndr.	1	0	0	0	1	0	0	0
Total	153	14	3	13	149	7	4	8

ASD Atrial septal defect; *D* Deficiency; *ECG* Electrocardiogram; *f* Female; *m* Male; *N/a* Not applicable; *syndr* Syndrome; *VSD* Ventricular septal defect

Table 4 Gastrointestinal manifestation

Disease	Vomiting				Diarrhoea				Hepatomegaly
	Patients N	Sporadic %	Rare %	Recurrent %	Patients N	Sporadic %	Rare %	Recurrent %	% (N)
MMA	134	20	7	5	133	10	2	2	5 (125)
PA	87	26	12	6	87	12	1	6	1 (84)
IVA	49	16	4	0	49	12	2	2	2 (45)
GA1	142	17	3	2	142	9	1	2	1 (128)
NAGS-D	4	0	25	0	4	0	0	0	0 (4)
CPS1-D	17	6	0	6	17	0	0	0	7 (15)
OTC-D (f)	105	18	5	1	105	7	1	3	3 (97)
OTC-D (m)	75	11	0	4	75	5	1	0	2 (66)
ASS-D	54	19	0	6	53	8	2	0	2 (49)
ASL-D	43	14	2	0	43	9	2	2	37 (41)
ARG1-D	10	0	10	0	10	10	0	10	0 (10)
HHH syndr.	8	0	0	0	8	0	0	0	0 (8)

Definitions: Sporadic, not more than once each month; rare, between once each month and once a week; recurrent, more than once a week

D Deficiency; *f* Female; *m* Male; *N* Number of examined patients; *syndr* Syndrome

(not shown). Hematologic abnormalities in UCD patients were usually associated with iron and/or cobalamin deficiency reflecting inadequate supply with micronutrients (not shown). Alternative causes such as thalassemia ($n=2$), sickle cell disease ($n=1$) or aplastic anemia ($n=1$) were identified sporadically.

Endocrine system

Overall, the frequency of endocrine disorders was low and similar to that found in the general population. Hypothyroidism (OAD: eight patients, UCD: five patients) was most frequent. Sporadically, hyperthyroidism ($n=1$), adrenal gland

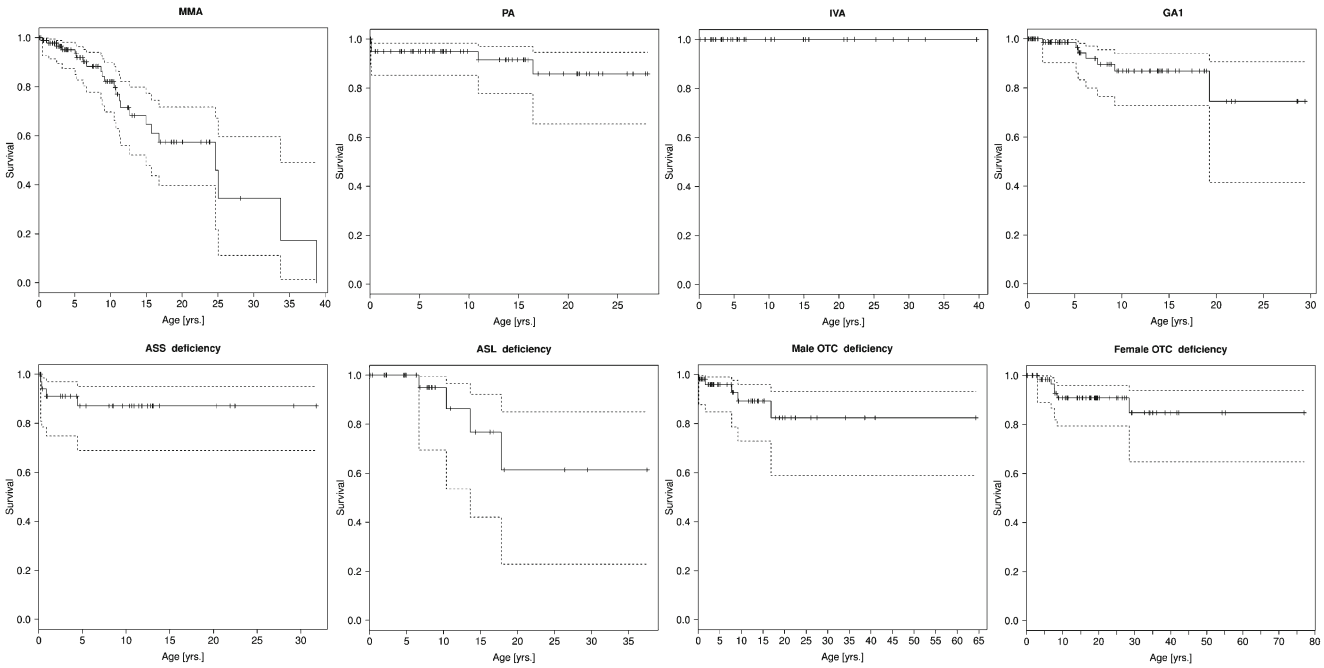


Fig. 4 Chronic renal failure in OAD and UCD. Results of a Kaplan Meier analysis are depicted. UCD with a small group size (NAGS, CPS1 and ARG1 deficiency and HHH syndrome) were not included in the analysis. Chronic renal failure was defined as creatinine clearance (for children and adolescents according to the Schwartz formula) or as glomerular filtration rate (for adults according to the MDRD formula) below 60 mL/min. *Dashed lines* indicate 95 % confidence interval. Please note that Kaplan Meier analysis is based on the presence or absence of chronic renal failure at the time point of the baseline visit. Therefore, Kaplan Meier curves do not reflect the true age-specific progression of symptoms in the OAD and UCD cohorts

dysfunction (n=3), hypopituitarism (n=1), diabetes mellitus (insulin-dependent: 2; insulin-nondependent: 1), and polycystic ovarian syndrome (n=1) were reported. Amenorrhoea, a known adverse effect of prolonged treatment with phenylbutyrate in women with UCD (Batshaw et al 2001), was not reported. Interestingly, only six female UCD patients (age range: 13–25 years) received phenylbutyrate after menarche — likely as a consequence of the known adverse effect of this drug.

Neoplasms

So far, we have not identified an OAD or UCD patient with a malignant tumour. This may reflect the mostly pediatric cohort.

Mortality

During a time period of 34 months, 13 patients died (MMA: one, PA: two, female OTC carrier: one, male OTC deficiency: five, ASS deficiency: four). Ten patients (male OTC deficiency: five, ASS deficiency: three, MMA: one, and PA: one) died during the initial neonatal metabolic decompensation at a median age of 25 days. Two patients died of massive brain edema at 5 years (ASS deficiency, female OTC carrier) during late-onset hyperammonemic crisis. One PA patient with a severe movement disorder died of fatal aspiration at the age of 17 years.

Discussion

The aim of this study was to evalute the evolving clinical phenotypes of OAD and UCD patients. We showed that (1) acquired microcephaly was more often found than poor growth and dystrophy and that poor growth was associated with movement disorders rather than the onset type, (2) neurological symptoms were commonly observed, with a high frequency of movement disorders, (3) the involvement of other organs followed a disease- or group-specific pattern, and (4) chronic renal failure was most frequently found in mut^0 type MMA patients and was unexpectedly observed in patients with GA1 and ASL deficiency.

The E-IMD dataset not only confirms previously described organ-specific manifestations in OAD and UCD, but significantly adds to the understanding of the long-term outcome of these diseases. A detailed clinical synopsis, which is based on a literature review and this study, is provided in Suppl. Table 6. The evolving phenotype of OAD and UCD patients is a combination of a common neurological presentation (e.g. acquired microcephaly, movement disorder) with disease- or disease-group specific manifestations (e.g. chronic renal failure, cardiomyopathy, prolonged QT_c interval, optic nerve atrophy, pancytopenia, liver involvement). This suggests that OAD and UCD share some overlapping pathomechanisms but are separated by disease- or disease group-specific mechanisms.

Toxic metabolites, impaired energy metabolism, and the role of biological membranes

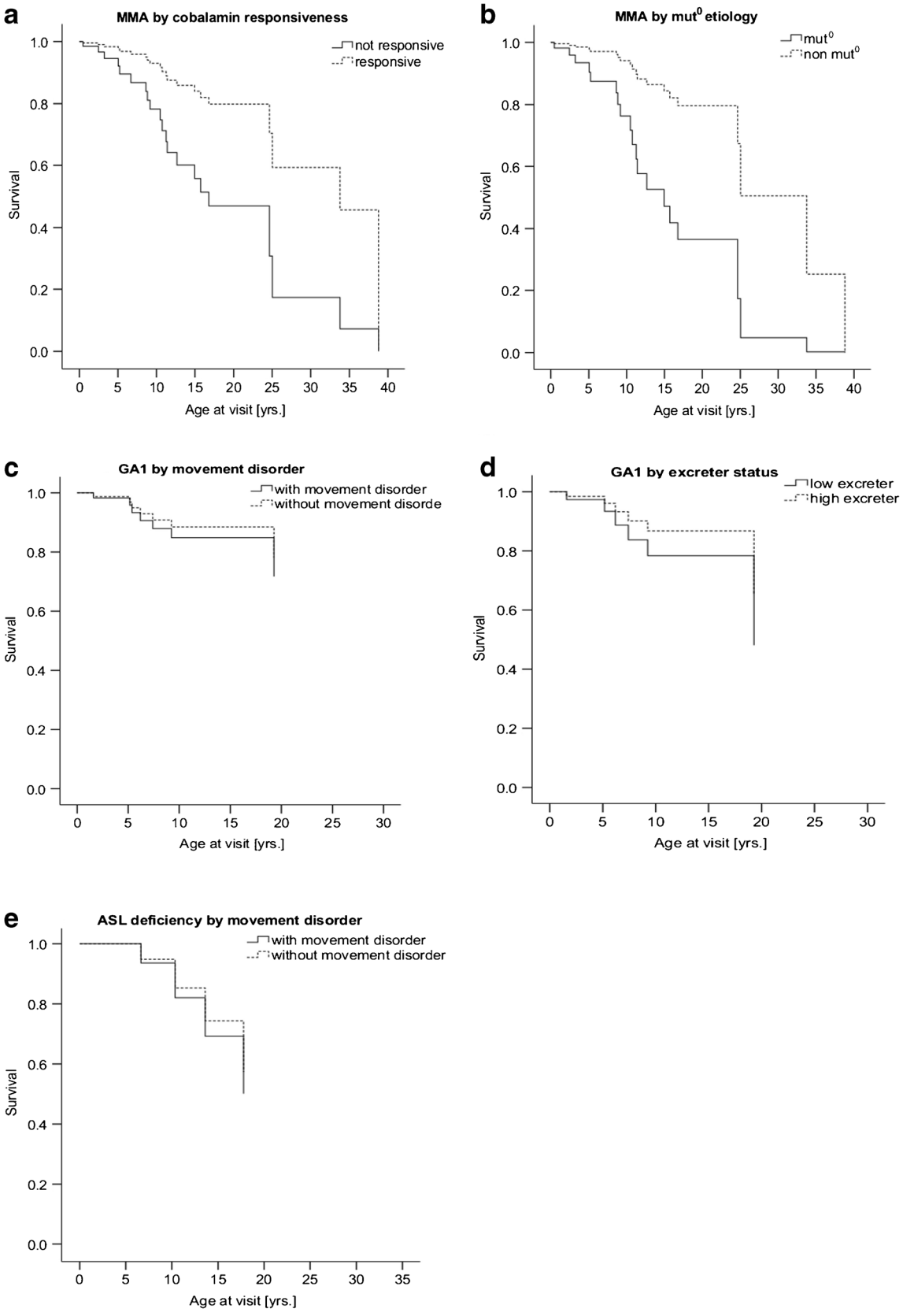
Some accumulating pathologic metabolites in OAD and UCD are thought to act as toxins, many of them inhibit energy metabolism. This inhibitory effect is induced by competition with structurally similar natural substrates at specific enzymes and transporters and sequestration of coenzyme A (Lamp et al 2011; Mirandola et al 2008; Mitchell et al 2008; Sauer et al 2005; Schwab et al 2006). In addition, energy metabolism can be compromised by increased energy consumption and decreased energy supply (Braissant et al 2013; Brunengraber and Roe 2006; Strauss et al 2010).

Primary pathologic metabolites (e.g. acyl-CoA esters) accumulate due to the inherited metabolic block thereby forming a (unique) metabolic signature, whereas secondary toxic metabolites (e.g. lactate) are the consequence of acquired metabolic blocks induced by primary toxic metabolites. For instance, propionyl-CoA induces lactic acidosis and hyperammonemia by inhibition of the pyruvate dehydrogenase complex and NAGS, respectively (Coude et al 1979; Schwab et al 2006).

Although pathologic metabolites have a lower affinity to target enzymes and transporters than their natural substrates, negative effects will become prominent at high metabolite concentrations. The concentrations of pathologic metabolites increase during catabolism and/or increased protein intake which may induce acute metabolic or encephalopathic crises (Kölker et al 2015; Chapman et al 2012; Hörster et al 2009; Kölker et al 2006b; Strauss et al 2007; Zinnanti et al 2007). Since hydrophilic toxic metabolites require a specific transporter to cross biological membranes, accumulation of toxic metabolites in defined compartments is facilitated. Noteworthy, the mitochondrial membrane lacks a transporter for acyl-CoA esters. The blood–brain barrier lacks an effective efflux transport for dicarboxylic acids (Hassel et al 2002; Sauer et al 2010) and has a glutamine efflux transport system that works effectively only if the plasma glutamine concentration is normal (Hawkins et al 2006). These limitations facilitate entrapment of toxic metabolites in mitochondria and the brain (Braissant et al 2013; Hassel et al 2002; Hawkins et al 2006; Kölker et al 2006a; Mitchell et al 2008).

The clinical phenotype of GA1: brain first, but not only

The brain is often the first target in intoxication type metabolic diseases since impairment of brain energy metabolism, disturbance of cerebral autoregulation, activation of excitotoxic pathways and entrapment of key metabolites synergize with



a physiologically high cerebral energy demand (Sokoloff 1960), a lipid-rich environment that is highly susceptible to reactive oxygen species (Dewar et al 2003), and highly vulnerable cell species.

◀ **Fig. 5** Chronic renal failure in subgroups of MMA, GA1 and ASL deficiency. Cox regression model showed a tendency towards significance between cobalamin responders and cobalamin non-responsive MMA patients (**a**), a significant difference between mut^0 and non-mut^0 MMA patients (**b**), but no differences between patients with GA1 (**c**) and ASL deficiency (**e**) with or without a movement disorder and between patients with GA1 (**d**) with low or high excreter status

GA1 is considered a model disease for selective brain involvement in intoxication type metabolic diseases. Our study confirms that the manifestation of a complex movement disorder with predominant dystonia and, as a consequence, musculoskeletal complications such as contractures and (sub-)luxation of joints is the major disease manifestation in infants (Gitiaux et al 2008). Macrocephaly at birth suggests that the disease may already manifest in utero. However, the clinical relevance of this finding is unknown, since macrocephaly as well as temporal hypoplasia and other MRI findings of newborns often improve or even normalize (Harting et al 2009). After age 6 years, most GA1 patients are thought to remain clinically stable and striatal lesions remain unchanged on MRI. In contrast, extrastriatal MRI changes may become more prominent with increasing age, particularly in the periventricular white matter (Garbade et al 2014). Their clinical relevance is still unclear (Harting et al 2009; Garbade et al 2014), but recently reported subependymal mass lesions in adult-onset GA1 necessitate a re-evaluation of these changes (Herskovitz et al 2013).

Fourty years after the description of the index patients (Goodman et al 1975), we report for the first time chronic renal failure in adult GA1 patients. So far, acute renal failure caused by nephrotic syndrome in infancy (Pöge et al 1997) or rhabdomyolysis during status dystonicus during childhood (Jamuar et al 2012), respectively, have been reported in two patients. Interestingly, high-protein diet in a murine model for GA1 caused a renal phenotype (Thies et al 2013), and glutaric and 3-hydroxyglutaric acid interfere with the transport of organic anions and dicarboxylic acids in renal proximal tubule cells (Hagos et al 2008; Stellmer et al 2007) providing a mechanistic link.

The demonstration of an age-specific involvement of brain regions and of chronic renal failure that develops over time in adulthood but is not associated with a dystonic movement disorder highlights that the disease may progress after age 6 years. More work is required to understand the relevance of this finding for long-term management and monitoring (Kölker et al 2011).

The clinical phenotype of classic OAD: multi-organ dysfunction

Accumulation of propionyl-CoA, isovaleryl-CoA, 2-methylcitric acid, methylmalonic acid and other putative toxic metabolites cause synergistic inhibition of pyruvate dehydrogenase complex, tricarboxylic acid cycle, transport of tricarboxylic acid cycle intermediates and ureagenesis as well as coenzyme A sequestration in patients with classic OAD (Kölker et al 2013; Morath et al 2008; Mitchell et al 2008). This explains the manifestation of acute metabolic crises during episodes of catabolism. Evidence is increasing, however, that late-onset organ dysfunction can manifest in MMA and PA patients who have not had a metabolic crisis for years (if at all) (Grünert et al 2013; Hörster et al 2007; Pena et al 2012; Prada et al 2011; Romano et al 2010; Traber et al 2011). This is confirmed by our study. We observed cardiomyopathy (PA, MMA) and a prolonged QT_c interval (PA), optic nerve atrophy (MMA, IVA) and chronic renal failure (MMA, PA). Since we have studied a predominantly pediatric sample and since only a proportion of patients have so far received fundoscopy, echocardiography and ECG, these organ manifestations are presumably underestimated.

Late-onset organ manifestation points to mechanisms that are more sustained than acute (and reversible) inhibition of enzymes and transporters. Noteworthy, decreased activity of bc_1 complex and cytochrome *c*, decreased intracellular content of coenzyme Q_{10} and mtDNA and megamitochondria were observed in PA and MMA patients (Baruteau et al 2014; Chandler et al 2009; De Keyzer et al 2009; Hayasaka et al 1982; Schwab et al 2006). In analogy, in $Mut^{-/-}$ mice (Chandler et al 2009) develop megamitochondria and mitochondrial dysfunction in a tissue-specific and age-dependent fashion (Chandler et al 2009). What drives this process is still unclear. Increased ROS production, glutathione depletion and propionyl-CoA-induced changes in histone acetylation are candidate mechanisms (Chandler et al 2009; Mirandola et al 2008; Nguyen et al 2007; Sauer et al 2010).

The clinical phenotype of UCD: brain, liver (and kidneys)

The biochemical hallmark of NAGS, CPS1, OTC, ASS and ASL deficiency is hyperglutaminemic hyperammonemia and hypoargininemia. Hyperammonemia and subsequently increased astrocytic glutamine production in concert with disturbed autoregulation of cerebral blood perfusion causes impairment of brain energy metabolism, excitotoxicity, oxidative stress, and alters several amino acid and neurotransmitter pathways (Braissant et al 2013; Butterworth 2014). Intracerebral trapping of glutamine is facilitated by polar glutamine transport across the blood–brain barrier (Hawkins et al 2006). The distribution of age-specific MRI lesions is thought to reflect hypoperfusion secondary to hyperammonemia and hyperglutaminemia predominantly affecting brain regions with a high metabolic rate (Takanashi et al 2003; Gropman 2010). The high burden of cerebral involvement in UCD patients is confirmed by this study.

The highest frequency of liver disease was observed in ASL deficiency and female OTC carriers. Whereas acute liver disease was most often found in symptomatic female OTC carriers (Kölker et al 2015), patients with ASL deficiency had the highest frequency of hepatomegaly and biochemical abnormalities suggesting chronic liver disease. This is in line with previous case reports (Mori et al 2002; Mustafa and Clarke 2006; Teufel et al 2011). A recent histopathological study confirmed hepatic involvement in UCD patients showing that neonatal histopathological changes are non-specific, whereas older patients (late-onset OTC, ASL deficiency) developed variable hepatic fibrosis and focal changes resembling glycogen storage disorder and cirrhosis (Yapito-Lee et al 2013). The underlying mechanism remains speculative. Argininosuccinate-induced hepatotoxicity might play a role (Batshaw et al 2001). This might be aggravated by high-dose arginine therapy (Nagamani et al 2012). Furthermore, impairment of the tricarboxylic acid cycle and the malate shuttle secondary to deficient release of fumarate (and arginine) from argininosuccinate might also play a role.

The finding of a moderate frequency of chronic renal failure in patients with ASL deficiency was unexpected and makes this disease unique among UCD. In analogy to chronic liver disease, renal involvement was independent from the neurological phenotype.

In conclusion, the identification of new disease manifestations, such as chronic renal failure in GA1 and ASL deficiency and optic nerve atrophy in IVA, and the broad phenotypic spectrum of OAD and UCD patients emphasizes the importance of deep phenotyping and systematic follow-up. These results necessitate a critical re-evaluation of current strategies for management and care. The disease-specific frequency and severity of specific manifestations (e.g. malignant neoplasms) will be more correctly assessed after a longer follow-up of these patients.

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Compliance with ethics guidelines

Conflict of interest None.

Human and animal rights and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human studies (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients or their legal guardians prior to being included in the study in countries where this was needed by law. This article does not contain animal subjects.

References

Ah Mew N, Krivitzky L, McCarter R, Batshaw M, Tuchman M (2013) Urea Cycle Disorders Consortium of the Rare Diseases Clinical Research Network. Clinical outcomes of neonatal onset proximal versus distal urea cycle disorders do not differ. J Pediatr 162:324–329

Baric I, Wagner L, Feyh P, Liesert M, Buckel W, Hoffmann GF (1999) Sensitivity and specificity of free and total glutaric acid and 3-

hydroxyglutaric acid measurements by stable-isotope dilution assays for the diagnosis of glutaric aciduria type I. J Inherit Metab Dis 22:867–881

Baruteau J, Hargreaves I, Krywawych S et al (2014) Successful reversal of propionic acidemia associated cardiomyopathy: evidence of low myocardial coenzyme Q10 status and secondary mitochondrial dysfunction as an underlying pathophysiological mechanism. Mitochondrion. doi:10.1016/j.mito.2014.07.001

Batshaw ML, MacArthur RB, Tuchman A (2001) Alternative pathway therapy for urea cycle disorders: twenty years later. J Pediatr 138: S46–S54

Boy N, Haege G, Heringer J et al (2013) Low lysine diet in glutaric aciduria type I – effect on anthropometrical and biochemical follow-up parameters. J Inherit Metab Dis 36:525–533

Braissant O, McLin VA, Cudalbu C (2013) Ammonia toxicity to the brain. J Inherit Metab Dis 36:595–612

Brunengraber H, Roe CR (2006) Anaplerotic molecules: current and future. J Inherit Metab Dis 29:327–331

Butterworth RF (2014) Pathophysiology of brain dysfunction in hyperammonemic syndromes: the many faces of glutamine. Mol Genet Metab. doi:10.1016/j.ymgme.2014.06.003

Cavicchi C, Donati M, Parini R et al (2014) Sudden unexpected fatal encephalopathy in adults with OTC gene mutations — clues for early diagnosis and treatment. Orphanet J Rare Dis 9:105

Chandler RJ, Zerfas PM, Shanske S, Sloan J, Hoffmann V, DiMauro S, Venditti CP (2009) Mitochondrial dysfunction in mut methylmalonic acidemia. FASEB J 23:1252–1261

Chapman KA, Gropman A, MacLeod E et al (2012) Acute management of propionic acidemia. Mol Genet Metab 105:16–25

Cole TJ (1990) The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45–60

Cole TJ, Freeman JV, Preece MA (1998) British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Statist Med 17:407–429

Cole TJ, Williams AF, Wright CM (2011) Revised birth centiles for weight, length and head circumference in the UK-WHO growth charts. Ann Hum Biol 38:7–11

R Core Team (2014) R: a language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria, http://CRAN.R-project.org/

Cosson MA, Benoist JF, Touati G et al (2009) Long-term outcome in methylmalonic aciduria: a series of 30 French patients. Mol Genet Metab 97:172–178

Coude FX, Sweetman L, Nyhan WL (1979) Inhibition of propionyl-coenzyme A of N-acetylglutamate synthetase in rat liver mitochondria. A possible explanation for hyperammonemia in propionic and methylmalonic acidemia. J Clin Invest 64:1544–1551

De Keyzer Y, Valayannopoulos V, Benoist JF et al (2009) Multiple OXPHOS deficiency in liver, kidney, heart, and skeletal muscle of patients with methylmalonic aciduria and propionic aciduria. Pediatr Res 66:91–95

Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23:263–274

Enns GM, Berry SA, Berry BT, Rhead WJ, Brusilow SW, Hamosh A (2007) Survival after treatment with phenylacetate and benzoate for urea cycle disorders. N Engl J Med 356:2282–2292

Ensenauer R, Vockley J, Willard JM et al (2004) A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. Am J Hum Genet 75:1136–1142

Erez A, Nagamani SC, Shchelochkov OA et al (2011) Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat Med 17:1619–1626

Garbade SF, Greenberg CR, Demirkol M et al (2014) Unravelling the complex MRI pattern in glutaric aciduria type I using statistical models — a cohort study in 180 patients. J Inherit Metab Dis. doi:10.1007/s10545-014-9676-9

Gitiaux C, Roze E, Kinugawa K et al (2008) Spectrum of movement disorders associated with glutaric aciduria type 1: a study of 16 patients. Mov Disord 23:2392–2397

Goodman SI, Markey SP, Moe PG, Miles BS, Teng CC (1975) Glutaric aciduria: a "new" disorder of amino acid metabolism. Biochem Med 12:12–21

Gropman A (2010) Brain imaging in urea cycle disorders. Mol Genet Metab 100(Suppl 1):S20–S30

Grünert SC, Wendel U, Lindner M et al (2012) Clinical and neurocognitive outcome in symptomatic isovaleric acidemia. Orphanet J Rare Dis 7:9

Grünert SC, Müllerleile S, De Silva L et al (2013) Propionic acidemia: clinical course and outcome in 55 pediatric and adolescent patients. Orphanet J Rare Dis 8:6

Hagos Y, Krick W, Braulke T, Mühlhausen C, Burckhardt G, Burckhardt BC (2008) Organic anion transporters OAT1 and OAT4 mediate the high affinity transport of glutarate derivatives accumulating in patients with glutaric acidurias. Pflugers Arch 457:225–231

Harting I, Neumaier-Probst E, Seitz A et al (2009) Dynamic changes of striatal and extrastriatal abnormalities in glutaric aciduria type I. Brain 132:1764–1782

Hassel B, Brathe A, Petersen D (2002) Cerebral dicarboxylate transport and metabolism studied with isotopically labeled fumarate, malate, and malonate. J Neurochem 82:410–419

Hawkins RA, O'Kane RL, Simpson IA, Viña JR (2006) Structure of the blood–brain barrier and its role in the transport of amino acids. J Nutr 136(Suppl 1):218S–226S

Hayasaka K, Metoki K, Satoh T et al (1982) Comparison of cytosolic and mitochondrial enzyme alterations in the livers of propionic and methylmalonic acidemia: a reduction of cytochrome c oxidase activity. Tohoku J Exp Med 137:329–334

Heringer J, Boy SP, Ensenauer R et al (2010) Use of guidelines improves the neurological outcome in glutaric aciduria type I. Ann Neurol 68: 743–752

Herskovitz M, Goldsher D, Sela BA, Mandel H (2013) Subependymal mass lesions and peripheral polyneuropathy in adult-onset glutaric aciduria type I. Neurology 81:849–850

Hörster F, Baumgartner MR, Viardot C et al (2007) Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (mut^0, mut^-, cblA, cblB). Pediatr Res 62:225–230

Hörster F, Garbade SF, Zwickler T et al (2009) Prediction of outcome in isolated methylmalonic acidurias: combined use of clinical and biochemical parameters. J Inherit Metab Dis 32:630–639

Jamuar SS, Newton SA, Prabhu SP et al (2012) Rhabdomyolysis, acute renal failure, and cardiac arrest secondary to status dystonicus in a child with glutaric aciduria type I. Mol Genet Metab 106:488–490

Kölker S, Dobbelaere D, Häberle J et al (accepted) Networking across borders for individuals with organic acidurias and urea cycle disorders: the E-IMD consortium. J Inherit Metab Dis

Kölker S, Garcia Cazorla A, Valayannopoulos V et al (submitted) The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation. J Inherit Metab Dis

Kölker S, Garbade SF, Greenberg CR et al (2006a) Natural history, outcome, and therapeutic efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. Pediatr Res 59:840–847

Kölker S, Sauer SW, Surtees RA, Leonard JV (2006b) The aetiology of neurological complications of organic acidaemias – a role for the blood–brain barrier. J Inherit Metab Dis 29:701–704

Kölker S, Garbade SF, Boy N et al (2007) Decline of acute encephalopathic crises in children with glutaryl-CoA dehydrogenase deficiency identified by newborn screening in Germany. Pediatr Res 62: 357–362

Kölker S, Christensen E, Leonard JV et al (2011) Diagnosis and management of glutaric aciduria type I — revised recommendations. J Inherit Metab Dis 34:677–694

Kölker S, Burgard P, Sauer SW, Okun JG (2013) Current concepts in organic acidurias: understanding intra- and extracerebral disease manifestation. J Inherit Metab Dis 36:635–644

Komatsuzaki S, Sakamoto O, Fuse N, Uematsu M, Matsubara Y, Ohura T (2012) Clinical reasoning: a young man with progressive subcortical lesions and optic nerve atrophy. Neurology 79:e63–68 doi: 10.1212/WNL.0b013e3182648bb6

Lamp J, Keyser B, Koeller DM, Ullrich K, Braulke T, Mühlhausen C (2011) Glutaric aciduria type 1 metabolites impair succinate transport from astrocytic to neuronal cells. J Biol Chem 286:17777–17784

Marquard J, El Scheich T, Klee D, Schmitt M, Meissner T, Mayatepek E, Oh J (2011) Chronic pancreatitis in branched-chain organic acidurias — a case of methylmalonic aciduria and an overview of the literature. Eur J Pediatr 170:241–245

Mercimek-Mahmutoglu S, Moeslinger D, Häberle J et al (2010) Long-term outcome of patients with argininosuccinate lyase deficiency diagnosed by newborn screening in Austria. Mol Genet Metab 100:24–28

Mirandola SR, Melo DR, Schuck PF, Ferreira GC, Wajner M, Castilho RF (2008) Methylmalonate inhibits succinate-supported oxygen consumption by interfering with mitochondrial succinate uptake. J Inherit Metab Dis 31:44–54

Mitchell GA, Gauthier N, Lesimple A, Wang SP, Mamer O, Qureshi I (2008) Hereditary and acquired diseases of acyl-coenzyme A metabolism. Mol Genet Metab 94:4–15

Morath MA, Okun JG, Müller IB, Sauer SW, Hörster F, Hoffmann GF, Kölker S (2008) Neurodegeneration and chronic renal failure in methylmalonic aciduria - a pathophysiological approach. J Inherit Metab Dis 31:35–43

Mori T, Nagai K, Mori M, Nagao M, Imamura M, Iijima M, Kobayashi K (2002) Progressive liver fibrosis in late-onset argininosuccinate lyase deficiency. Pediatr Dev Pathol 5:597–601

Mustafa A, Clarke JT (2006) Ornithine transcarbamylase deficiency presenting with acute liver failure. J Inherit Metab Dis 29:586

Nagamani SC, Shchelochkov OA, Mullins MA et al (2012) A randomized controlled trial to evaluate the effects of high-dose versus low-dose of arginine therapy on hepatic function tests in argininosuccinate acidurias. Mol Genet Metab 107:315–321

Nassogne MC, Héron B, Touati G, Rabier D, Saudubray JM (2005) Urea cycle defects: management and outcome. J Inherit Metab Dis 28: 407–414

Nguyen NHT, Morland C, Gonzalez SV et al (2007) Propionate increases neuronal histone acetylation, but is metabolized oxidatively via glia. Relevance for propionic acidemia. J Neurochem 101:806–814

Nizon M, Ottolenghi C, Valayannopoulos V et al (2013) Long-term neurological outcome of a cohort of 80 patients with classical organic acidurias. Orphanet J Rare Dis 8:148

Pena L, Franks J, Chapman KA et al (2012) Natural history of propionic acidemia. Mol Genet Metab 105:5–9

Pöge AP, Autschbach F, Korall H, Trefz FK, Mayatepek E (1997) Early manifestation of glutaric aciduria type I and nephrotic syndrome during the first months of life. Acta Paediatr 86:1144–1147

Prada CE, Al Jasmi F, Kirk EP, Hopp M, Jones O, Leslie ND, Burrow TA (2011) Cardiac disease in methylmalonic acidemia. J Pediatr 159: 862–864

Romano S, Valayannopoulos V, Touati G et al (2010) Cardiomyopathies in propionic aciduria are reversible after liver transplantation. J Pediatr 156:128–134

Rüegger CM, Lindner M, Ballhausen D et al (2014) Cross-sectional observational study of 208 patients with non-classical urea cycle disorders. J Inherit Metab Dis 37:31–30

Sauer SW, Okun JG, Schwab MA et al (2005) Bioenergetics in glutaryl-coenzyme A dehydrogenase deficiency: a role for glutaryl-coenzyme A. J Biol Chem 280:21830–21836

Sauer SW, Opp S, Mahringer A et al (2010) Glutaric aciduria type I and methylmalonic aciduria: simulation of cerebral import and export of accumulating neurotoxic dicarboxylic acids in in vitro models of the blood–brain barrier and the choroid plexus. Biochim Biophys Acta 1802:552–560

Schwab MA, Sauer SW, Okun JG et al (2006) Secondary mitochondrial dysfunction in propionic aciduria: a pathogenic role for endogenous mitochondrial toxins. Biochem J 398:107–112

Seminara J, Tuchman M, Krivitzky L et al (2010) Establishing a consortium for the study of rare diseases: The Urea Cycle Disorders Consortium. Mol Genet Metab 100(Suppl 1):S97–105

Serrano M, Martins C, Pérez-Dueñas B et al (2010) Neuropsychiatric manifestation in late-onset urea cycle disorder patients. J Child Neurol 25:352–358

Sokoloff L (1960) The metabolism of the central nervous system in vivo. In: Field J, Magoun HW, Hall VE (eds) Handbook of physiology, Sect 1, Vol II. Raven, New York, 161–168

Stellmer F, Keyser B, Burckhardt BC et al (2007) 3-Hydroxyglutaric acid is transported via the sodium-dependent dicarboxylate transporter NaDC3. J Mol Med (Berl) 85:763–770

Strauss KA, Lazovic J, Wintermark M, Morton DH (2007) Multimodal imaging of striatal degeneration in Amish patients with glutaryl-CoA dehydrogenase deficiency. Brain 130:1905–1920

Strauss KA, Donnelly P, Wintermark M (2010) Cerebral hemodynamics in patients with glutaryl-coenzyme A dehydrogenase deficiency. Brain 133:76–92

Strobl R, Dirschedl P (2004) Comparison of simultaneous and pointwise confidence intervals for survival functions. Biomet J 46(Suppl 1):90

Summar ML, Dobbelaere D, Brusilow S, Lee B (2008) Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicenter study of acute hyperammaemic episodes. Acta Paediatr 97:1420–1425

Takanashi J, Barkovich AJ, Cheng SF et al (2003) Brain MR imaging in neonatal hyperammonemic encephalopathy resulting from proximal urea cycle disorders. Am J Neuroradiol 24:1184–1187

Teufel U, Weitz J, Flechtenmacher C et al (2011) High urgency liver transplantation in ornithine transcarbamylase deficiency presenting with acute liver failure. Pediatr Transpl 15:E110–E115

Therneau T (2014) A package for survival analysis in S. R package version 2.37-7, http://CRAN.R-project.org/package=survival

Thies B, Meyer-Schwesinger C, Lamp J et al (2013) Acute renal proximal tubule alterations during induced metabolic crises in a mouse model of glutaric aciduria type 1. Biochim Biophys Acta 1832:1463–1472

Traber G, Baumgartner MR, Schwarz U, Pangalu A, Donath MY, Landau K (2011) Subacute bilateral visual loss in methylmalonic acidurias. J Neuroophthalmol 31:344–346

Tuchman M, Lee B, Lichter-Konecki U et al (2008) Cross-sectional multicentre study of patients with urea cycle disorders in the United States. Mol Genet Metab 94:397–402

Williams ZR, Hurley PE, Altiparmak UE, Feldon SE, Arnold GL, Eggenberger E, Mejico LJ (2009) Late onset optic neuropathy in methylmalonic and propionic acidemia. Am J Ophthalmol 147:929–933

Yapito-Lee J, Chow CW, Boneh A (2013) Histopathological findings in livers of patients with urea cycle disorders. Mol Genet Metab 108: 161–165

Zinnanti WJ, Lazovic J, Housman C et al (2007) Mechanism of age-dependent susceptibility and novel treatment strategy in glutaric acidemia type I. J Clin Invest 117:3258–3270

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