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ORIGINAL ARTICLE

Age at disease onset and peak ammonium level rather than interventional variables predict the neurological outcome in urea cycle disorders

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Abstract

Background Patients with urea cycle disorders (UCDs) have an increased risk of neurological disease manifestation.

Aims Determining the effect of diagnostic and therapeutic interventions on the neurological outcome.

Methods Evaluation of baseline, regular follow-up and emergency visits of 456 UCD patients prospectively followed between 2011 and 2015 by the E-IMD patient registry.

Results About two-thirds of UCD patients remained asymptomatic until age 12 days [i.e. the median age at diagnosis of patients identified by newborn screening (NBS)] suggesting a potential benefit of NBS. In fact, NBS lowered the age at diagnosis in patients with late onset of symptoms (>28 days), and a trend towards improved long-term neurological outcome was found for patients with argininosuccinate synthetase and lyase deficiency as well as argininemia identified by

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NBS. Three to 17 different drug combinations were used for maintenance therapy, but superiority of any single drug or specific drug combination above other combinations was not demonstrated. Importantly, non-interventional variables of disease severity, such as age at disease onset and peak ammonium level of the initial hyperammonemic crisis (cut-off level: 500 μmol/L) best predicted the neurological outcome.

Conclusions Promising results of NBS for late onset UCD patients are reported and should be re-evaluated in a larger and more advanced age group. However, non-interventional variables affect the neurological outcome of UCD patients. Available evidence-based guideline recommendations are currently heterogeneously implemented into practice, leading to a high variability of drug combinations that hamper our understanding of optimised long-term and emergency treatment.

Abbreviations

ARG1	Arginase 1
ASL	Argininosuccinate lyase
ASS	Argininosuccinate synthetase
CPS1	Carbamylphosphate synthetase 1
E-IMD	European registry and network for intoxication type metabolic diseases
EO	Early onset
HHH syndrome	Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome
IR	Interquartile range
LO	Late onset
NAGS	N-acetylglutamate synthase
NBS	Newborn screening
OR(s)	Odds ratio(s)
OTC	Ornithine transcarbamylase
Q1	First quartile
Q3	Third quartile
UCD(s)	Urea cycle disorder(s)

Introduction

UCDs are caused by inherited deficiencies of six enzymes and two transporters that are involved in irreversible detoxification of ammonium to urea: N-acetylglutamate synthase (NAGS; OMIM # 237310), carbamylphosphate synthetase 1 (CPS1; OMIM # 237300), ornithine transcarbamylase (OTC; OMIM # 311250), argininosuccinate synthetase (ASS; OMIM # 215700), argininosuccinate lyase (ASL; OMIM # 207900), arginase 1 (ARG1; OMIM # 207800), citrin or aspartate/glutamate carrier (OMIM # 603471 and # 605814) and the mitochondrial ornithine transporter 1 causing

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hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHH syndrome; OMIM # 238970). The clinical phenotype of UCD patients is highly variable with first symptoms starting as early as the first days of life [early onset (EO): ≤ 28 days] or as late as adulthood [late onset (LO): > 28 days]. In addition, patients with atypical disease course or who have remained asymptomatic without treatment have also been reported (Summar et al 2008; Kido et al 2012; Rüegger et al 2014; Kölker et al 2015a, b).

Diagnosis is made by prenatal testing, high-risk family screening in families with a previously identified index patient, selective metabolic investigation in patients with symptoms, or newborn screening (NBS). However, UCDs are still rarely implemented in national NBS disease panels (Loeber et al 2012), and where they are, very few include screening for NAGS-, CPS1- and OTC-deficiency, since the sensitivity for these disorders is certainly low. Guidelines for the diagnosis and management of UCDs have been proposed for use of single therapeutic means as well as their combinations (Häberle et al 2012). However, unlike in diseases such as glutaric aciduria type 1 (Kölker et al 2011) where the positive effect of adherence to guidelines on the neurological outcome was clearly shown (Heringer et al 2010), guideline evaluation is still lacking for UCDs.

The aim of this study is to describe and evaluate currently used diagnostic and therapeutic strategies for UCDs and their impact on the neurological outcome.

Patients and methods

Patient registry and inclusion/exclusion criteria

The European registry and network for intoxication type metabolic diseases (E-IMD) project (URL: https://www.eimd-registry.com) collects comprehensive information of patients with inherited deficiency of NAGS, CPS1, OTC, ASS, ASL, ARG1 and HHH syndrome. Asymptomatic female OTC carriers were also included in the study sample. Citrin deficiency is not included. A detailed description of the registry has been published previously (Kölker et al 2015a, b, c). Patients with other metabolic diseases, unconfirmed suspicion of an UCD, unrelated serious comorbidities (Down syndrome, intraventricular hemorrhage grade III-IV in the newborn period, birth weight below 1500 grams, kernicterus, and embryofetal disease due to maternal alcohol or drug abuse) or patients who died before 1st January 2011 (starting date of E-IMD) were excluded from the analysis. The phenotype in patients with UCD was studied by use of standardised electronic forms comprising common data elements for all patients as well as disease-specific follow-up parameters using a predefined algorithm (Kölker et al 2015a, b, c). The cut-off data for the statistical analysis was 16th March 2015.

The study was approved by the ethics committee of the coordinating centre (University Hospital Heidelberg, application no. S-525/2010), and consecutively by all contributing metabolic centres. Written informed consent was obtained from all study patients before enrolment and baseline visit in countries where this was needed by law.

Statistical analysis

Descriptive statistics [mean, median, interquartile range (IR), range] and *phi* coefficients were calculated with SPSS (IBM SPSS Statistics 22.0). For the following R (R Core Team 2015) was used. For recursive partitioning the method "Conditional inference trees" (Hothorn et al 2006, 2015) was applied according to previous studies (Kölker et al 2006; Garbade et al 2014). Age-adjusted and -unadjusted odds ratios (ORs) were computed using Firth's bias reduced logistic regression approach with penalized profile likelihood based confidence intervals for parameter estimates to take into account the small numbers in unfavourable levels of almost all outcome variables for some groups (Heinze and Schemper 2002; Heinze et al 2013). The adjusted ORs were used when there was a significant influence of age on the outcome variable as evaluated by the penalized likelihood ratio, otherwise the unadjusted ORs were chosen (Heinze et al 2013). It was also tested if there was a significant interaction effect between mode of diagnosis and age on the outcome variable. In all other cases a model using diagnosis and age as selective predictors was used. Parameters were compared by randomised median difference tests programmed in R (Richter and McCann 2007); 100,000 permutations were chosen for these tests to ensure the stability of the probability estimates (Smucker et al 2007); p-values below 0.05 were considered statistically significant, values between 0.05 and 0.1 are reported as trends.

Results

Brief description of the study population

From 1st February 2011 to 16th March 2015, a total of 456 UCD patients (229 males, 227 females) were enrolled in 36 metabolic centres in 18 countries (Suppl. Table S1). The most frequent diagnosis was OTC deficiency (56%) followed by ASS (19%) and ASL deficiency (13%). Median chronological age at the last regular visit was 11 years (IR 4.9-19.2 years, range 0.1-77 years) (Table 1, Suppl. Table S2). A detailed description of the study population has been published previously (Kölker et al 2015a, b, c).

Table 1 Age and mode of diagnosis

Disease	Patients	Gender	Chronological age at last visit	Age at diagnosis (all)	Age at diagnosis (NBS)	Age at diagnosis (selective)		Randomised median test for age at diagnosis†	
	n	m/f	Median (n) in years	Median (n) in days	Median (n) in days	Median (n) in days			
						EO	LO	NBS vs. Selective, EO group	NBS vs. Selective, LO group
NAGS-D	9	3/6	8.7 (9)	8 (7)	n.r.	5 (4)	1930 (2)	n/a	n/a
CPS1-D	21	14/7	9.8 (21)	90 (21)	14(1)	4(12)	1260 (7)	n/a	n/a
OTC-D (m)	109	109/0	10.3 (109)	435 (106)	7 (1)	4 (22)	815 (48)	n/a	n/a
OTC-D (f)	146	0/146	18.3 (145)	1095 (140)	1 (1)	11 (4)	840 (72)	n/a	n/a
ASS-D	87	49/38	6.3 (87)	5 (83)	11(4)	5 (45)	270 (18)	p=0.114	p=0.153
ASL-D	61	39/22	9 (61)	12 (56)	12(9)	4 (21)	876(17)	p=0.021	p=0.036
ARG1-D	12	5/7	7.9 (12)	156 (12)	17(4)	270 (1)	1545 (6)	n/a	p=0.076
HHH syndr.	11	10/1	12 (11)	570 (10)	6 (1)	n.r.	690 (7)	n/a	n/a
Total	456	229/227	11 (455)	360 (435)	12 (21)	4(109)	730 (177)	p<0.001	p=0.019

Data are shown as median (n); † Based on 100,000 permutations; *D*, deficiency; *EO*, early onset; *f*, female; *LO*, late onset; *m*, male; *n/a*, not applicable; *n.r.,* not reported; *NBS*, newborn screening; selective, selective diagnostic work-up started after the onset of first symptoms; *syndr.*, syndrome. For descriptive statistical information including median, mean, interquartile range and range see Suppl. Table S2. The supplementary table also includes data of high-risk family screening, i.e. families with a previously identified index patient

Effect of diagnostic mode on age at diagnosis and neurological outcome

Since UCDs are rarely implemented to NBS programmes (ASL in six countries; ASS in five countries, ARG1 in four countries; citrin deficiency in two countries; Loeber et al 2012), most patients were diagnosed by selective metabolic investigation after the onset of symptoms (71%), whereas identification by high risk family screening (20%), NBS (5%) or prenatal testing (1%) was less frequent. In a small group of UCD patients (3%), diagnostic mode was not reported. In the NBS group, ASS (n=6), ASL (n=9) and ARG1 deficiency (n=4) were the most common diagnoses, whereas all other UCDs were not or sporadically (n=1) reported (Table 1, Suppl. Table S2).

Age at diagnosis was influenced by the proportion of patients with EO or LO of first symptoms and by the diagnostic mode. As expected, the overall median age at diagnosis was lower in the NBS group (median: 12 days; IR: 6-14 days) than in the LO group of patients identified after manifestation of first symptoms (median: 730 days; IR: 365-2160 days). However, this effect was only significant for ASL deficiency (p=0.036) and showed a trend for ARG1 deficiency (p=0.076). In contrast, age at diagnosis was similar or even lower in the EO group (median: 4 days; IR: 3-9 days) than in the NBS group (median: 12 days; IR: 6-14 days) highlighting that for the majority of these UCD patients a positive screening result would not have been available before the manifestation of first symptoms (Table 1, Suppl. Table S2).

Next, we calculated how many UCD patients in the E-IMD sample might have been successfully diagnosed by NBS while being asymptomatic. For this purpose, we examined the numbers of patients in the NBS and selective metabolic investigation groups who have remained asymptomatic until day 6 (i.e. Q1, age at diagnosis in the NBS group; IR: 6-14 days) and day 12 (i.e. median, age at diagnosis in the NBS group; IR: 6-14 days). Overall, 70% (day 6) and 65% (day 12) of UCD patients identified by NBS and selective metabolic investigation, respectively, have remained asymptomatic and, theoretically, the manifestation of first symptoms could have been prevented by NBS in this group (Table 2, Suppl. Table S3).

To increase the statistical power, data were aggregated across newborns identified by NBS, asymptomatic newborns diagnosed by high-risk family screening or newborns diagnosed by prenatal testing to an "early diagnosis" group. Altogether, 52% of patients identified by NBS and 50% of patients from the "early diagnosis" group have remained asymptomatic until the last reported visit, whereas all patients in the selective metabolic investigation group became symptomatic. Since the E-IMD sample includes a high proportion of LO patients, in particular male patients with OTC deficiency (Kölker et al 2015a), these numbers do not necessarily match with the case mix of single countries (Summar et al 2008, 2013; Kido et al 2012; Batshaw et al 2014; Kölker et al 2015a).

We have previously demonstrated that the manifestation of neurological symptoms such as movement disorders and intellectual disability is a common finding of UCD

Table 2 Frequency of asymptomatic patients and age at first symptoms

Disease	Patients	Age at first symptoms (NBS)	Age at first symptoms (selective)		Asymptomatic patients at day 6		Asymptomatic patients at day 12	
	n	Median (n) in days	Median (n) in days		n (%)		n (%)	
			EO	LO	NBS	Selective	NBS	Selective
NAGS-D	9	n.r.	2 (4)	3650 (1)	n/a	2 (33)	n/a	2 (33)
CPS1-D	21	3 (1)	3 (12)	510 (7)	0 (0)	8 (42)	0 (0)	7 (37)
OTC-D (m)	109	n.r.	2 (22)	690 (45)	1 (100)	55 (79)	1 (100)	50 (71)
OTC-D (f)	146	n.r.	3 (4)	720 (68)	1 (100)	74 (96)	1 (100)	73 (95)
ASS-D	87	15 (1)	2 (44)	300 (15)	5 (100)	25 (40)	5 (100)	22 (35)
ASL-D	61	4 (3)	3 (21)	210 (15)	4 (67)	25 (63)	4 (67)	21 (53)
ARG1-D	12	n.r.	n.r.	1440 (5)	3 (100)	6 (100)	3 (100)	6 (100)
HHH syndr.	11	6 (1)	n.r.	480 (6)	0 (0)	7 (100)	0 (0)	7 (100)
Total	456	5(6)	3 (107)	630 (162)	15 (79)	202 (70)	15 (79)	188 (65)

Data are shown as median (n) and as n (%); *D*, deficiency; *EO*, early onset; *f*, female; *LO*, late onset; *m*, male; *n/a*, not applicable; *n.r.*, not reported; *NBS*, newborn screening; selective, selective diagnostic work-up started after the onset of first symptoms; *syndr.*, syndrome. For descriptive statistical information including median, mean, interquartile range and range see Suppl. Table S3

patients in the E-IMD sample (Jamiolkowski et al 2015; Kölker et al 2015b). We therefore wondered whether NBS could improve the neurological outcome. Only patients with ASS, ASL and ARG1 deficiency were included in this analysis due to small numbers of early diagnosed patients in other UCDs (Table 1). Overall, ORs showed a trend towards lower odds for movement disorder in the NBS and "early diagnosis" groups compared to the selective metabolic investigation group (Table 3; Suppl. Tables S4a-c). A similar result was found for delayed milestones, however, with ARG1 deficiency reaching statistical significance (Table 4; Suppl. Tables S4d-f).

Since IQ data so far have been reported only for a small number of patients in the NBS group, it remains unclear whether NBS has a beneficial effect on intellectual abilities.

Metabolic treatment: variability of current strategies

Early diagnosis is the precondition of early start of treatment aiming to reduce acute and chronic intoxication and organ damage. A combination of low protein diet with or without essential amino acid supplements, citrulline and/or arginine, oral antibiotics (metronidazole, colistin) and nitrogen scavengers (sodium benzoate, sodium and glycerol phenylbutyrate) has been recommended for the maintenance treatment. In addition, carglumic acid is licensed for NAGS deficiency. Consistent with these recommendations, the majority of UCD patients in the E-IMD sample received a calculated diet (286/456; 63%) or a protein-controlled diet (i.e. solely reduction of the intake of protein-rich food without performing calculation; 50/456; 11%), whereas some patients with a mild clinical phenotype did receive no

Table 3 Movement disorder (NBS versus selective metabolic investigation group)

Disease	Mode of diagnosis	Total n	No MD n (%)	MD n (%)	OR from logistic regression† with covariate age*
ASS-D	NBS	6	6 (100)	0 (0)	MoD: OR_u=3.55, 95%CI [0.38;473.21], p=0.321
	Selective	62	49 (79)	13 (21)	LR (df=1)=0.69, p=0.407
ASL-D	NBS	8	7 (87)	1 (12)	MoD: OR_u=2.45, 95%CI [0.46;25.08], p=0.317
	Selective	37	25 (68)	12 (32)	LR (df=1)=0.01, p=0.905
ARG1-D	NBS	4	4 (100)	0 (0)	MoD: OR_u=11.57, 95%CI [0.78;1741.93], p=0.078
	Selective	7	3 (43)	4 (57)	LR (df=1)=0.31, p=0.580

Only cases with a value for the covariate age were included; † according to Firth's bias reduced logistic regression approach with penalized profile likelihood based confidence intervals for parameter estimates (Heinze and Schemper 2002; Heinze et al 2013); *age at first time of MD for those with MD and age at last regular visit for those without MD; *CI*, confidence interval; *D*, deficiency; *LR*, penalized likelihood ratio test for comparison of nested models (Heinze et al 2013); *MD*, movement disorder; *MoD*, mode of diagnosis; *NBS*, newborn screening; *OR*, odds ratio (reference group: NBS, reference category: no MD); OR_u, unadjusted OR (if LR was not significant); selective, selective metabolic investigation. Age distribution is specified in Suppl. Table S4b

Table 4 Delayed milestones (NBS versus selective metabolic investigation group)

Disease	Mode of diagnosis	Total n	No DM n (%)	DM n (%)	OR from logistic regression† with covariate age*
ASS-D	NBS	6	3 (50)	3 (50)	MoD: OR_u=0.21, 95%CI [0.04;1.13], p=0.068
	Selective	59	49 (83)	10 (17)	LR (df=1)=0.28, p=0.596
ASL-D	NBS	8	8 (100)	0 (0)	MoD: OR_u=2.78, 95%CI [0.25;382.78], p=0.457
	Selective	31	27 (87)	4 (13)	LR (df=1)<0.01, p=0.982
ARG1-D	NBS	4	4 (100)	0 (0)	MoD: OR_a=0.43, 95%CI [<0.01;126.28], p=0.736
	Selective	7	3 (43)	4 (57)	LR (df=1)=5.36, p=0.021

Only cases with a value for the covariate age were included; †according to Firth's bias reduced logistic regression approach with penalized profile likelihood based confidence intervals for parameter estimates (Heinze and Schemper 2002; Heinze et al 2013); *age at last regular visit; *CI*, confidence interval; *D*, deficiency; *DM*, delayed milestones; *LR*, penalized likelihood ratio test for comparison of nested models (Heinze et al 2013); *MoD*, mode of diagnosis; *NBS*, newborn screening; *OR*, odds ratio (reference group: NBS, reference category: no delayed milestones); OR_a, adjusted OR (if LR was significant); OR_u, unadjusted OR (if LR was not significant); selective, selective metabolic investigation. Age distribution is specified in Suppl. Table S4e

dietary treatment at all (47/456; 10%). Detailed information on dietary treatment was not reported for 73 patients (16%). Essential amino acid supplements were most often administered to patients receiving a calculated diet (111/286; 39%). Tube feeding was used in 36 patients; the majority of them (72%) were EO patients. The underlying disease severity influenced the intensity of dietary management. Natural protein intake of EO and LO patients was often below international recommendations (World Health Organization et al 2007), however, EO patients with a calculated diet had an apparently lower natural protein and significantly higher caloric intake than LO patients (Table 5; Suppl. Table S5). Essential amino acid supplements were more frequently used in EO (62%) than in LO patients (29%). Median intakes of total protein of EO and LO patients were similar but often remained below the safe values of WHO recommendations (2007). Also LO patients often received fewer calories than recommended. Noteworthy, a different calculated diet pattern was found for symptomatic female OTC carriers. Median intakes of natural and total protein were unexpectedly higher for EO than for LO patients and were above WHO recommendations (2007) in EO patients. However, caloric intake in symptomatic female OTC carriers did not differ from the whole sample (Table 5; Suppl. Table S5).

Maintenance metabolic pharmacotherapy was specified for 296 patients and overall, up to seven different drugs have been used with numbers of different drug combinations ranging from three (NAGS deficiency) to 17 (female OTC deficiency) (Table 6). This may reflect adaptation of pharmacotherapy to individual requirements but alternatively may be interpreted as uncertainty of treating physicians. Sodium benzoate was more often used than (sodium or glycerol) phenylbutyrate; and arginine more often than citrulline.

Table 5 Dietary management of UCD patients receiving calculated diet (maintenance treatment)

Disease	Patients	Natural protein				Total protein				Calories			
	n	Median (n) in %WHO*				Median (n) in %WHO*				Median (n) in %FAO**			
		Total	EO	LO	EO vs. LO†	Total	EO	LO	EO vs. LO†	Total	EO	LO	EO vs. LO†
NAGS-D	9	98 (3)	80 (2)	98 (1)	n/a	137 (3)	157 (2)	98 (1)	n/a	87 (3)	98 (2)	87 (1)	n/a
CPS1-D	21	61 (13)	58 (8)	63 (4)	p=0.465	88 (13)	100 (8)	71 (4)	p=0.316	96 (11)	102 (8)	96 (3)	p=0.873
OTC-D (m)	109	102 (61)	70 (8)	103 (41)	p=0.065	116 (61)	103 (8)	113 (41)	p=0.591	95 (45)	107 (6)	86 (32)	p=0.153
OTC-D (f)	146	73 (71)	106 (4)	72 (60)	p=0.164	89 (73)	138 (5)	84 (61)	p=0.029	92 (56)	101 (4)	90 (50)	p=0.361
ASS-D	87	83 (60)	78 (37)	85 (13)	p=0.555	97 (60)	95 (37)	90 (13)	p=0.678	96 (50)	101 (28)	84 (13)	p=0.133
ASL-D	61	86 (44)	76 (19)	104 (20)	p=0.073	93 (45)	90 (20)	113 (20)	p=0.033	95 (29)	95 (11)	86 (14)	p=0.591
ARG1-D	12	65 (11)	63 (1)	64 (6)	n/a	103 (11)	79 (1)	79 (6)	n/a	112 (7)	102 (1)	154 (2)	n/a
HHH syndr.	11	100 (11)	77 (2)	110 (7)	p=0.248	100 (11)	77 (2)	110 (7)	p=0.251	103 (10)	85 (2)	93 (6)	p=0.748
Total	456	82 (274)	73 (81)	86 (152)	p=0.088	100 (277)	94 (83)	98 (153)	p=0.616	95 (211)	102 (62)	89 (121)	p=0.008

Data are shown as median (n); *according to WHO recommendations (2007); **according to FAO (2001); for adults a physical activity level (PAL) of 1.76 according to Table 5.1 in FAO (2001) was taken, which is equivalent to the mean PAL of an active or moderately active life style; this life style is in between the extreme lifestyles "sedentary or light activity lifestyle" and "vigorous and vigorously active lifestyle"; † randomised median test, based on 100,000 permutations; *D*, deficiency; *EO*, early onset; *f*, female; *LO*, late onset; *m*, male; *n/a*, not applicable; *syndr.*, syndrome. For descriptive statistical information including median, mean, interquartile range and range see Suppl. Table S5

Table 6 Maintenance metabolic pharmacotherapy

Disease	All patients n	Patients with medication n	BZA n	PBA n	CBG n	ARG n	CIT n	MTN and/or COL n
NAGS-D	9	6	1	1	6	1	1	0
CPS1-D	21	11	7	9	2	8	7	0
OTC-D (m)	109	63	30	21	n/a	37	37	1
OTC-D (f)	146	79	52	46	n/a	43	46	2
ASS-D	87	69	53	24	n/a	68	n/a	0
ASL-D	61	47	20	14	n/a	47	n/a	0
ARG1-D	12	10	6	6	n/a	n/a	n/a	0
HHH syndr.	11	11	3	3	n/a	4	5	0
Total	456	296	172	124	8	208	96	3

ARG, arginine; *BZA*, sodium benzoate; *CBG*, carbamylglutamate; *CIT*, citrulline; *COL*, colistin; *D*, deficiency; *f*, female; *m*, male, *MTN*, metronidazole; *n/a*, not applicable; *PBA*, (sodium or glycerol) phenylbutyrate; *syndr.*, syndrome. Liver transplanted UCD patients were omitted from the analysis

Combinations of sodium benzoate and phenylbutyrate were applied to 67 patients and those of arginine and citrulline to 28 patients. Intestinal bacterial decontamination with metronidazole or colistin was rarely used for maintenance treatment. As expected, carglumic acid was solely applied to patients with NAGS deficiency and patients with partially responsive CPS1 deficiency (off-license use). Liver transplantation was reported for 18 patients (five CPS1 deficiency, eight male OTC deficiency, three female OTC deficiency, and two ASS deficiency). They did not receive metabolic drug therapy after transplantation.

Emergency treatment is used to prevent or reverse metabolic decompensation. At present, the E-IMD registry includes detailed information on 87 emergency visits of 47 patients, the majority of them (38 emergency visits, 14 patients) are female with OTC deficiency (Suppl. Table S6). Therefore, the disease distribution is skewed and data should be regarded as preliminary. The overall pattern of emergency interventions was transient reduction or stop of protein intake and increased carbohydrate intake. Noteworthy, caloric intake was not increased within the first 24 hours. In fact, initial emergency caloric intake (Suppl. Table S6) was lower than caloric intake during maintenance treatment (Table 5) and below age-adapted recommendations indicating that carbohydrates were used as the primary if not sole energy source during the first 24 hours of emergency treatment, whereas fat was often omitted from initial emergency treatment. The median dosages of sodium benzoate, phenylbutyrate and/or arginine hydrochloride were in the range of the standard starting doses of these drugs (Häberle et al 2012). Intravenous sodium benzoate/sodium phenylacetate was sporadically used, the increased dosage indicating severe hyperammonemic crises (Suppl. Table S6). Extracorporeal detoxification during emergency treatment was used in two male patients with OTC deficiency (data not shown).

Impact of onset type, mode of diagnosis and therapy on the disease course

Since the onset type reflects the natural disease course, it was included in all further analyses as an independent variable. To investigate whether a specific intervention was more effective to improve the neurological outcome than others, we used recursive partitioning analysis. This procedure allows identifying predictors for clinical outcomes and can handle numerical, ordinal and categorical data which may even have highly skewed or multimodal distributions. Diseases with small numbers of patients and different initial and evolving phenotypic presentation—NAGS, and ARG1 deficiency as well as HHH syndrome—were excluded from this analysis. Independent variables showing high correlations were selectively excluded from the analysis to reduce co-linearity (Suppl. Table S7).

For the analysis, we used diagnostic mode (NBS, selective metabolic investigation, asymptomatic newborns diagnosed by high risk family screening, prenatal testing), diet (no diet, calculated diet), essential amino acid supplements (no, yes), UCD drugs (level 1: no metabolic pharmacotherapy; level 2: CPS1 and OTC deficiency: arginine or citrulline; ASS and ASL deficiency: arginine; level 3: CPS1 and OTC deficiency: one scavenger with or without arginine or citrulline; ASS and ASL deficiency: arginine and one scavenger; level 4: CPS1 and OTC deficiency: two scavengers with or without arginine or citrulline; ASS and ASL deficiency: arginine and two scavengers), onset type (asymptomatic, EO, LO), literature-based initial peak ammonium level (≤200 μmol/L, 201-500 μmol/L, 501-1000 μmol/L, >1000 μmol/L) and impaired consciousness at initial presentation (no, yes) as independent variables. The independent variable "sex" (male, female) was not included in the analysis because it did not correlate with any of the other variables. Furthermore, initial plasma glutamine concentrations and emergency management of the initial crisis

were not included due to lack of data. Movement disorder (no, yes), abnormal fine motor development (no, yes), abnormal gross motor development (no, yes), muscular hypotonia (no, yes) and delayed milestones (no, yes) were used as dependent outcome variables. Motor abnormality (no, yes) was used as superordinate dependent variable subsuming four single motor variables because they were highly correlated (Suppl. Tables S7 and S8).

No particular diagnostic and therapeutic intervention could be identified as a significant predictor for any of the outcome variables. This does not necessarily mean that these interventions are not effective. However, an in-depth analysis of these interventions was hampered by the strong effect of the initial peak ammonium level on the outcome and by the heterogeneity of drug combinations used for the treatment of UCD patients in our sample. Recursive partitioning using single UCDs did not provide more detailed results (data not shown).

For CPS1-, OTC-, ASS- and ASL-deficient patients, initial peak ammonium level and onset type had the strongest influence on motor outcome. Initial peak ammonium levels above 500 μmol/L were associated with a higher frequency of movement disorders (dystonia, spasticity, chorea and ataxia) (Fig. 1) as well as impaired fine and gross motor development (data not shown). Early onset of symptoms was associated with the highest frequency of motor abnormalities (Fig. 2). As shown before, peak ammonium level is associated with the onset type (Kölker et al 2015a), supporting that age at and severity of the initial crisis are of utmost importance for the neurological outcome of above mentioned UCD patients.

Discussion

Analysis of comprehensive follow-up data of 456 UCD patients revealed five main results: (1) Peak plasma ammonium concentration during the initial hyperammonemic crisis (cut-off: 500 μmol/L) and age at disease onset are important predictors of the neurological outcome, both of them reflecting the disease severity. (2) NBS reduces the age at diagnosis in LO but not in EO patients. Hypothetically, two thirds of all UCD patients in the E-IMD sample could have been diagnosed by NBS until day 12 while being asymptomatic. (3) ASS-, ASL- and ARG1-deficient patients identified by NBS have an apparently reduced risk for developing movement disorders than those identified by selective metabolic investigation. However, a larger number of patients and additional outcome parameters (e.g. intellectual disability, mortality, liver disease) are required to evaluate the effect of NBS on the outcome more thoroughly. (4) The intensity of metabolic treatment correlates with the age at disease onset. (5) At present, high and unsystematic variability of drug combinations hamper the understanding of optimal metabolic treatment.

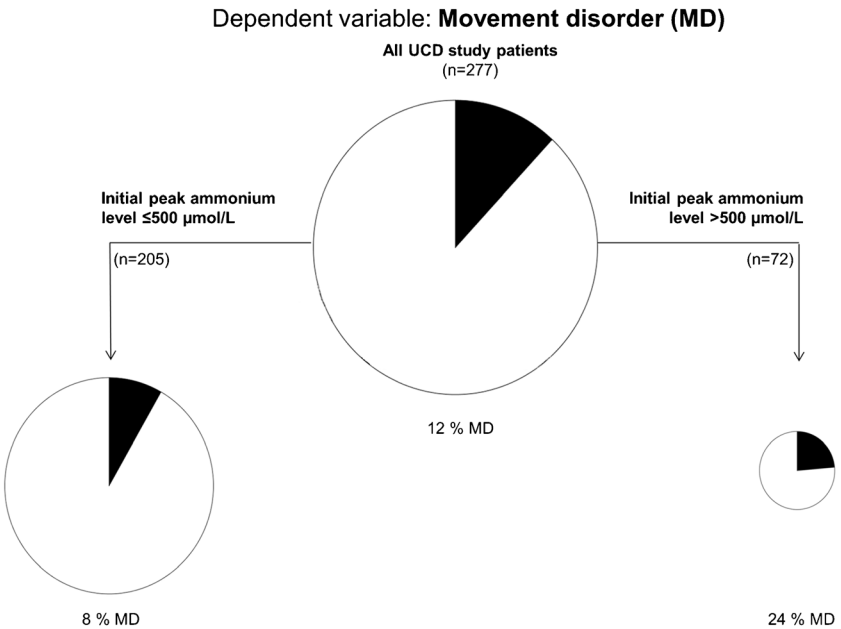

Fig. 1 Results of recursive partitioning (movement disorder). Recursive partitioning demonstrates that the initial peak ammonium level is an important predictor of the neurological outcome in surviving UCD patients. Patients with initial peak ammonium level above 500 μmol/L more often develop movement disorders. In this analysis patients with CPS1, OTC, ASS and ASL deficiency were included. Patients with NAGS, ARG1 deficiency as well as HHH syndrome were excluded due to small group size and/or discrepant initial and evolving phenotypic presentation. *MD*, movement disorder; *UCD*, urea cycle disorder

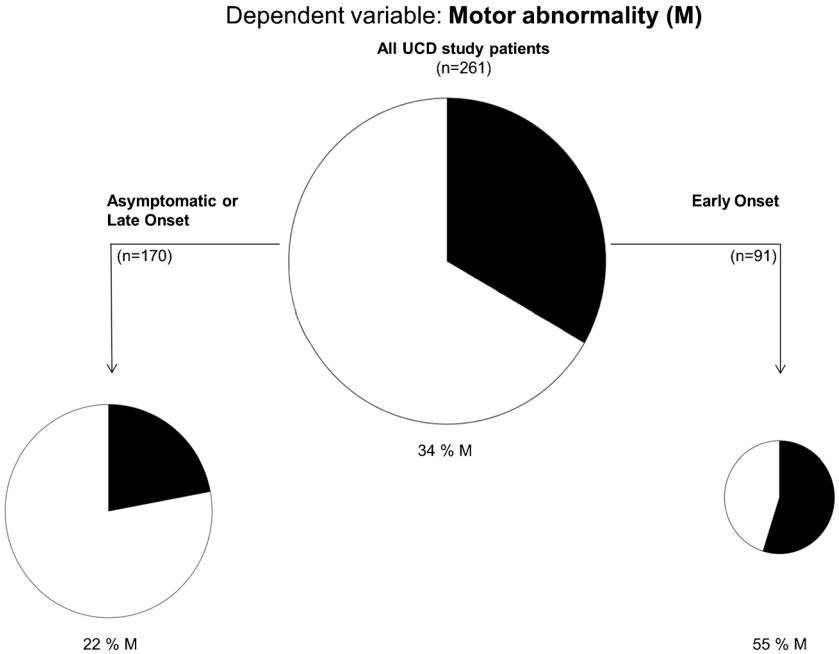

Fig. 2 Results of recursive partitioning (motor abnormality). Recursive partitioning demonstrates that the onset type is an important predictor of the neurological outcome in surviving UCD patients. Patients with early onset of symptoms more often develop motor abnormalities. In this analysis patients with CPS1, OTC, ASS and ASL deficiency were included. Patients with NAGS, ARG1-deficiency as well as HHH syndrome were excluded due to small group size and/or discrepant initial and evolving phenotypic presentation. *M*, motor abnormality; *UCD*, urea cycle disorder

Peak plasma ammonium concentration and age at disease onset rather than interventional variables best predict the neurological outcome

Among the independent variables tested, peak plasma ammonium concentration during the initial hyperammonemic crisis and the age at disease onset best predicted the neurological disease outcome for CPS1-, OTC-, ASS- and ASL-deficient patients. Both variables should be regarded as inter-related aspects of the intrinsic disease severity with peak ammonium levels being higher in EO than LO patients with UCDs (Kölker et al 2015a). Initial peak ammonium concentrations above 500 μmol/L and disease manifestation during the newborn period were clearly associated with a poor neurological outcome. The relevance of the initial crisis for the outcome is in line with previous studies. Enns and coworkers demonstrated that UCD patients with neonatal hyperammonemic crisis, peak ammonium concentrations above 1000 μmol/L and coma on admission had the lowest survival rate, whereas highest survival rates were found in patients with symptoms starting after the newborn period and with peak ammonium levels less than 200 μmol/L (Enns et al 2007). Similar results were obtained in a longitudinal study from Japan and a questionnaire-based outcome study from Europe showing that peak plasma ammonium exceeding 350 μmol/L (Uchino et al 1998) or 480 μmol/L (Bachmann 2003a), respectively, increased the risk of severe neurological deficits and intellectual disability. Others showed that onset of first symptoms during the newborn period is associated with severe brain damage and poor neurological prognosis (Nagata et al 1991). In contrast, a recent study of male and female OTC patients was not able to demonstrate a correlation between peak ammonium levels and onset type with neurological outcome parameters (Brassier et al 2015). Although the severity of hyperammonemia-induced cerebral damage might be influenced by the developmental age of affected individuals, the interaction between plasma ammonium level and maturation needs to be further elucidated. Another study highlighted that duration of hyperammonemic coma but not peak ammonium levels predicted neuroradiological abnormalities and concurrent IQ (Msall et al 1984). In analogy, it was recently reported that duration of the initial hyperammonemic event and coma duration before start of hemodialysis predict the outcome (Picca et al 2015). The analysis of peak ammonium concentrations is a pragmatic approach, whereas the integration of plasma ammonium concentrations and duration of hyperammonemia more precisely reflect the severity of a hyperammonemic episode. However, since the start of a hyperammonemic episode usually remains vague and thus, the duration of the episode can only be estimated, this parameter is not included in the E-IMD registry.

These studies highlight that the disease severity, in particular the severity of the initial presentation, should be included as important independent variables in all studies that aim to

elucidate the proposed benefit of diagnostic and therapeutic interventions for UCD patients. However, for ARG1 deficiency and HHH syndrome rarely presenting with acute hyperammonemia but with chronic neurological and developmental deterioration, early diagnosis and adherence to metabolic treatment might be more important for a favourable long-term outcome (Crombez and Cederbaum 2005; Martinelli et al 2015).

The effect of early diagnosis and therapy on the neurological outcome

A relevant but so far unanswered question is whether and to what extent the natural disease course of UCD patients can be modulated by diagnostic and therapeutic interventions. NBS programmes aiming to identify patients with a high *a priori* risk of an unfavourable disease course may be promising, but substantial variation in national NBS panels exists (Burgard et al 2012) and UCDs are rarely included in those panels (McHugh et al 2011; Loeber et al 2012). In a previous questionnaire-based cross-sectional study, patients with ASS and ASL deficiency identified by NBS had less often clinical symptoms, episodes of hyperammonemic encephalopathy and epilepsy than those identified by selective metabolic investigation (Rüegger et al 2014). The results of our study are less convincing. In 23 patients identified by NBS, the majority of them having ASS (n=6), ASL (n=9) or ARG1 deficiency (n=4), the age at diagnosis was significantly lower in the NBS group compared to LO patients of the selective metabolic investigation group, whereas EO patients mostly presented with first symptoms before NBS results could have been available. Although not all UCD patients would thus benefit from NBS, we showed that 65% of UCD patients identified by selective metabolic investigation and 79% of those identified by NBS remained asymptomatic until day 12. The positive effect of early diagnosis, however, was not sustained in all patients over time. At latest report, 50% of patients in the early diagnosis group and 52% of patients in the NBS group have remained asymptomatic. This may reflect inappropriate long-term efficacy of current treatment strategies. Furthermore, EO patients with a fulminant disease course might die before being diagnosed correctly and thus, could be underrepresented in the E-IMD registry. Despite these shortcomings the neurological outcome of early identified and treated ASS-, ASL- and ARG1-deficient patients is better than for those identified after the manifestation of first symptoms.

To understand the impact of therapeutic interventions on the outcome, we included various therapeutic and phenotypic independent variables. Liver transplantation was not included since the number of liver transplanted patients is still small in the E-IMD sample. The analysis did not elucidate a clear-cut superiority of any of the various treatment combinations used in the study sample. This does not mean that metabolic treatment is not at all effective. The implementation of low protein diet with or without essential amino acid supplements (Adam et al 2013), alternative pathway therapy (Brusilow et al 1979; Enns et al 2007; Berry et al 2014), arginine and citrulline supplementation (Brusilow et al 1979; Brusilow and Batshaw 1979), hemodialysis and filtration (Schaefer et al 1999; Picca et al 2015), nonabsorbable antibiotics (Foster et al 2010), carglumic acid for NAGS deficiency (Schubiger et al 1991), early liver transplantation (Morioka et al 2005; Kimura et al 2013), and timely emergency and/or maintenance treatment have improved the therapeutic options and are thought to improve the survival and neurological outcome in UCD patients (Batshaw et al 2014).

However, some studies did not unequivocally confirm improved outcome in UCD patients (Bachmann 2003b; Nassogne et al 2005). In fact, a recent meta-analysis did not show improved survival of EO patients over more than 35 years including studies from the USA, Europe and Japan (Burgard et al 2015). Study cohorts and results of clinical studies must be compared with great caution, since the proportions of patients with EO and LO often differ. To avoid misinterpretation, the therapeutic benefit must be adjusted to the assumed natural disease course, particularly if the sample contains a large number of LO patients (Uchino et al 1998; Enns et al 2007; Summar et al 2008; Kido et al 2012).

The impact of the natural disease courses and the large numbers of currently used treatment combinations are a challenge for the study design and remain a bottle neck for any attempt of treatment optimisation in UCD patients. It is hoped that more evidence will provide evidence-based guidelines, harmonising diagnostic and therapeutic interventions, to reduce the current interventional variability and to improve our understanding of optimal emergency and long-term management in the future (Häberle et al 2012).

Conclusions

The major predictors of the neurological outcome in UCD patients are non-interventional parameters reflecting the underlying disease severity such as age at disease onset and initial peak ammonium level. Other variables not included in our analysis such as plasma glutamine/glutamate concentration, mode and efficacy of ammonium detoxification as well as duration of the first hyperammonemic episode may be similarly useful. NBS helps to prepone the age at diagnosis in LO patients but unlikely in EO patients. Although early diagnosis and intervention seems beneficial for many UCD patients, the long-term benefit of NBS is still unclear and requires re-

evaluation in a larger sample and at a more advanced age, using additional outcome parameters. The large number of drug combinations and the correlation of disease severity with treatment intensity hamper our understanding of optimised long-term management.

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Conflict of Interest None.

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Animal rights This article does not contain animal subjects.

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