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ORIGINAL ARTICLE

Impact of age at onset and newborn screening on outcome in organic acidurias

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Abstract

Background and aim To describe current diagnostic and therapeutic strategies in organic acidurias (OADs) and to evaluate their impact on the disease course allowing harmonisation.

Methods Datasets of 567 OAD patients from the E-IMD registry were analysed. The sample includes patients with methylmalonic (MMA, $n=164$), propionic (PA, $n=144$) and isovaleric aciduria (IVA, $n=83$), and glutaric aciduria type 1 (GA1, $n=176$). Statistical analysis included description and recursive partitioning of diagnostic and therapeutic strategies, and odds ratios (OR) for health outcome parameters. For some analyses, symptomatic patients were divided into those presenting with first symptoms during (i.e. early onset, EO) or after the newborn period (i.e. late onset, LO).

Results Patients identified by newborn screening (NBS) had a significantly lower median age of diagnosis (8 days) compared to the LO group (363 days, $p<0.001$], but not compared to the EO group. Of all OAD patients 71 % remained asymptomatic until day 8. Patients with cobalamin-nonresponsive MMA (MMA-Cbl$^-$) and GA1 identified by NBS were less likely to have movement disorders than those diagnosed by selective screening (MMA-Cbl$^-$: 10 % versus 39 %, $p=0.002$; GA1: 26 % versus 73 %, $p<0.001$). For other OADs, the clinical benefit of NBS was less clear. Reported age-adjusted intake of natural protein and calories was significantly higher in LO patients than in EO patients reflecting different disease severities. Variable drug combinations, ranging from 12 in MMA-Cbl$^-$ to two in isovaleric aciduria, were used for main-

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tenance treatment. The effects of specific metabolic treatment strategies on the health outcomes remain unclear because of the strong influences of age at onset (EO versus LO), diagnostic mode (NBS versus selective screening), and the various treatment combinations used.

Conclusions NBS is an effective intervention to reduce time until diagnosis especially for LO patients and to prevent irreversible cerebral damage in GA1 and MMA-Cbl$^-$. Huge diversity of therapeutic interventions hampers our understanding of optimal treatment.

Abbreviations

AAM(s)	Amino acid mixture(s)
E-IMD	European registry and network for intoxication type metabolic diseases
EO	Early onset (i.e. onset of first symptoms during the newborn period)
GA1	Glutaric aciduria type 1
HRF	High-risk family screening
IVA	Isovaleric aciduria
IVD	Isovaleryl-CoA dehydrogenase
LO	Late onset (i.e. onset of first symptoms after the newborn period)
MMA	Methylmalonic aciduria (isolated forms)
MMA-Cbl$^+$	Cobalamin-responsive methylmalonic aciduria
MMA-Cbl$^-$	Cobalamin-nonresponsive methylmalonic aciduria
NBS	Newborn screening
OAD(s)	Organic aciduria(s)

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OR	Odds ratio
PA	Propionic aciduria
Q1	First quartile
Q3	Third quartile
WHO	World Health Organisation

Introduction

Most organic acid disorders (OADs) are caused by inherited enzyme deficiencies in the catabolic pathways of specific amino acids such as branched-chain amino acids and lysine. First symptoms start during (early onset, EO) or after (late onset, LO) the newborn period (Kölker et al 2015a). Although most OAD patients develop symptoms without treatment, individuals have been identified who remained well without treatment such as those with p.Ala282Val mutations in the *IVD* gene (Ensenauer et al 2004). "Classic" OADs including isolated forms of methylmalonic aciduria (MMA; OMIM #251000, #251100, #251110, #277400, #277410), propionic aciduria (PA; OMIM #606054) and isovaleric aciduria (IVA; OMIM #243500) are characterized by acute and/or chronic neurological impairment in combination with variable visceral organ manifestations such as chronic renal failure and cardiomyopathy (Grünert et al 2012a, 2013; Hörster et al 2007, 2009; Kölker et al 2015b; Mardach et al 2004; Nizon et al 2013). Glutaric aciduria type 1 (GA1; OMIM #231670), a "cerebral" OAD, is clinically characterized by the acute or insidious onset of secondary dystonia due to striatal damage in infancy. Preliminary evidence points to progressive disease in patients diagnosed late with the onset of dementia, cerebral neoplasms, peripheral neuropathy and chronic renal failure (Herskovitz et al 2013; Kölker et al 2015b, 2006).

Although some of the OADs are already included in national newborn screening (NBS) programmes of some countries, many patients, in particular those with PA and MMA, are still diagnosed after the manifestation of initial symptoms (Loeber et al 2012). One argument for excluding patients with "classic" OADs from NBS is that the first symptoms often manifest during the first days of life so that not all patients can be identified early enough to prevent acute metabolic crises (Kölker et al 2015a). In addition, with the exception of GA1 patients, it is not clear whether patients with OADs benefit from NBS (Dionisi-Vici et al 2006; Grünert et al 2012a, b; Kölker et al 2006, 2007; Strauss et al 2007).

Guidelines for MMA, PA and GA1 for diagnostic work-up, maintenance and emergency treatment as well as long-term follow-up and care have recently been published (Baumgartner et al 2014; Kölker et al 2011; Sutton et al 2012). General recommendations for maintenance treatment include (1) L-carnitine supplementation and (2) a low protein diet supplemented with precursor-free synthetic amino acid mixtures (AAMs), minerals and micronutrients. In addition, treatment protocols variably include oral antibiotics (MMA and PA), glycine (IVA) and hydroxycobalamin (cobalamin-responsive MMA, MMA-Cbl^{+}) (Baumgartner et al 2014; Grünert et al 2012a; Kölker et al 2011; Sutton et al 20124). Feeding problems are common in OAD patients, particularly in MMA and PA patients, and tube feeding is often necessary to supplement the diet and to ensure adequate intake of medication (Evans et al 2012). Emergency treatment aims to prevent or reverse catabolism, to restore acid base balance, to reduce the production of toxic metabolites and facilitate their detoxification and urinary excretion and thus to protect OAD patients from irreversible organ damage and death (Baumgartner et al 2014; Kölker et al 2011; Sutton et al 2012). Diagnostic and therapeutic recommendations are often based on clinical studies with a low level of evidence.

The major aim of this study is to describe current practices of diagnostic work-up and treatment for OAD patients and to evaluate the impact of different strategies on the disease outcome.

Patients and methods

Patient registry and inclusion/exclusion criteria

The European registry and network for intoxication type metabolic diseases (E-IMD, EAHC no. 2010 12 01) received funding from the European Union in the framework of the Health Programme 2008–2013. The patient registry includes pseudonymised data of patients with MMA ($n = 164$), PA ($n = 144$), IVA ($n = 83$) and GA1 ($n = 176$) who were followed by 35 centres in 20 countries (Suppl. Table 1). A detailed description of the registry (URL: https://www.eimd-registry.org) has been published previously (Kölker et al 2015a, b, c). The study was approved by the ethic committee of the coordinating centre (University Hospital Heidelberg, application no. S-525/2010) and consecutively by those of all contributing metabolic centres. Written informed consent was obtained for all study patients before enrolment and baseline visit in countries where this was needed by law. Patients with unconfirmed suspicion of an OAD unrelated serious comorbidities and patients who died before 1st January 2011 (starting date of E-IMD) were excluded.

Statistical analysis

SPSS (IBM SPSS Statistics 22.0) was used for descriptive statistics (mean, median, interquartile range, range) and computation of *phi* coefficient. For recursive partitioning the method "Conditional inference trees" (Hothorn et al 2006, 2015) was used according to previous studies (Garbade et al 2014; Kölker et al 2006). Age-adjusted and -unadjusted odds ratios (OR) were computed using 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) to take into account the small numbers in unfavourable levels of almost all outcome variables for some groups. The adjusted OR was used when there was a significant influence of age on the outcome variable as evaluated by the penalized likelihood ratio test (Heinze et al 2013), otherwise the unadjusted OR was chosen. It was also tested if there was a significant effect of the interaction between mode of diagnosis and age on the outcome variable. When it was not significant the model with only the predictors mode of diagnosis and age was included in the paper. Parameters were compared by randomised median difference tests using the programming language R (Richter and McCann 2007). In order to ensure the stability of the probability estimates 100,000 permutations were chosen for these tests (Smucker et al 2007). p-values ≤ 0.05 were considered statistically significant, values between 0.05 and 0.1 are reported as trends. The cut-off date for the statistical analysis was 16th March 2015. For some analyses, we divided the group of symptomatic patients into those presenting with first symptoms during (EO group) or after the newborn period (LO group).

Results

Study sample

From 1st February 2011 to 16th March 2015, a total of 567 OAD patients (303 males, 264 females) were registered by 35 metabolic centres in 20 countries. For further details see previous publication (Kölker et al 2015a). The cohort consists of patients with MMA ($n=164$) — among them 47 MMA-Cbl$^+$ and 117 cobalamin-nonresponsive MMA patients (MMA-Cbl$^-$) — PA ($n=144$), IVA ($n=83$) including 12 patients with a putatively mild form (Ensenauer et al 2004) and GA1 ($n=176$). The median age at the last reported visit was 8.3 years (interquartile range: 3.9-14.1 years, range: 0.1-48.9 years). Six patients died during the study period ($n=4$ PA, $n=1$ GA1, $n=1$ MMA-Cbl$^-$), two of them ($n=1$ PA, $n=1$ MMA-Cbl$^-$) during the initial metabolic crisis.

Age at and mode of diagnosis

Most OAD patients ($n=320$) were diagnosed after the manifestation of symptoms (selective screening), whereas 180 patients were identified by NBS, 49 patients by high-risk family (HRF) screening (i.e. families with a previously diagnosed index patient) and eight patients by prenatal testing. In ten patients, the mode of diagnosis was not reported. All patients diagnosed by selective screening were born in countries without existing NBS programmes for these diseases.

The overall median age at diagnosis was 21 days (interquartile range: 6–300 days, range: 1–13,140 days). Disease specific medians varied from 9 (IVA) to 105 days (MMA-Cbl$^+$) (Table 1; Suppl. Table 2). The age at diagnosis was mostly related to two variables: (1) the mode of diagnosis and (2) the proportion of EO and LO patients by disease (Table 1). Noteworthy, across all OADs the age at diagnosis was consistently lower in the NBS group than in LO patients diagnosed by selective screening, whereas in the EO group an analogous effect was observed for IVA and GA1 but not for MMA and PA patients (Table 1; Suppl. Table 2). This indicates that some patients had already presented clinically before results of NBS were available. In fact, 77 of 180 patients identified by NBS exhibited their first symptoms during the newborn period [median age at first symptoms (interquartile range, range): 6 (3–21, 1–2370) days] in the E-IMD sample (Table 2; Suppl. Table 3). To estimate the number of patients who might have remained asymptomatic before NBS results were available, we calculated the proportion of asymptomatic patients at day 5 [i.e. quartile 1 (Q1), age at diagnosis, NBS group] and 8 (i.e. median, age at diagnosis, NBS group) in the NBS and selective screening group. The results were strongly influenced by the proportion of EO and LO patients in specific OADs. As expected, the highest frequency of asymptomatic patients at days 5 and 8 was found for GA1 (Table 2; Suppl. Table 3), followed by IVA and MMA-Cbl$^+$. Proportions of asymptomatic patients at days 5 and 8 were similar in the NBS and selective screening groups suggesting a similar case mix of EO and LO in both groups. However, we cannot exclude that both groups are discrepant since patients who have remained asymptomatic would not be included in the selective screening group but could have been identified by NBS, whereas an unknown number of patients might have died undiagnosed in both groups. Based on this we calculated that

Table 1 Age and mode of diagnosis

Disease	Patients *n*	Gender *m/f*	Chronological age at last visit, median (n) in years	Age at diagnosis (all), median (n) in days	Age at diagnosis (NBS), median (n) in days	Age at diagnosis (selective), median (n) in days		Randomized median test† for age at diagnosis	
						EO	LO	NBS vs. selective, EO group	NBS vs. selective, LO group
MMA-Cbl$^-$	117	54/63	7.4 (117)	17 (115)	8 (32)	6 (31)	224 (35)	$p=0.430$	$p<0.001$
MMA-Cbl$^+$	47	25/22	8.9 (47)	105 (47)	7 (7)	7 (11)	348 (18)	$p=1.000$	$p=0.032$
PA	144	80/64	9.5 (142)	11 (137)	8 (43)	8 (37)	210 (35)	$p=1.000$	$p<0.001$
IVA	83	41/42	7.5 (83)	9 (82)	7 (37)	18 (15)	1410 (17)	$p=0.003$	$p<0.001$
GA1	176	94/82	8.1 (175)	270 (173)	11 (59)	60 (4)	450 (85)	$p<0.001$	$p<0.001$
Total	567	303/264	8.3 (564)	21 (554)	8 (178)	9 (98)	363 (190)	$p=0.810$	$p<0.001$

Data are shown as median (n); † Based on 100,000 permutations; data are shown as median (n); EO, early onset, f, female; LO, late onset; m, male; NBS, newborn screening; Selective, selective diagnostic work-up started after the onset of first symptoms. For descriptive statistical information including median, mean, minimum, maximum, interquartile range see Suppl. Table 2. The supplementary table also includes data of high-risk family screening, i.e. families with a previously identified index patient

at least 73 % of symptomatic OAD patients identified by selective screening in the E-IMD sample could have been identified by NBS if screening results had been available before 8 days of age. This proportion varied for the different OADs (Table 2; Suppl. Table 3).

Newborn screening: impact on health outcomes

To investigate the effect of NBS on health outcomes of neonatally identified OAD patients we compared the frequencies of developmental delay, movement disorders (including dystonia, chorea, ataxia, spasticity), intellectual impairment and renal and cardiac manifestations of patients identified by NBS with those diagnosed after the onset of first symptoms (selective screening group), taking into account the different age distributions between the two groups (if any). The major beneficial effect of NBS was found for neurological outcome parameters. For MMA-Cbl$^-$ and GA1 patients identified by NBS achievement of motor milestones was less often delayed than in the selective screening group. For IVA patients, we found a statistical trend ($p=0.075$) for normal development in the NBS group compared with the selective screening group. However, this disappeared ($p=0.141$), when patients with an assumingly mild clinical phenotype were omitted from the analysis (Table 3, Suppl. Table 4). In addition, the manifestation of a movement disorder (MD) was significantly reduced in the NBS group for MMA-Cbl$^-$ and GA1. For all other OADs, odds ratios (ORs) were in the same direction of improved outcome, however, the logistic regression did not confirm significance (Table 4, Suppl. Table 5, Suppl. Fig. 1).

Intellectual impairment is an additional or alternative indicator of cerebral disease manifestation. Since IQ tests have so far only been reported for a small number of OAD patients

Table 2 Frequencies of symptomatic patients and age at first symptoms

Disease	Patients *n*	Age at first symptoms (NBS), median (n) in days	Age at first symptoms (selective), median (n) in days		Asymptomatic patients at day 5 n (%)		Asymptomatic patients at day 8 n (%)	
			EO	LO	NBS	Selective	NBS	Selective
MMA-Cbl$^-$	117	3 (19)	3 (30)	210 (34)	17 (59)	41 (62)	15 (52)	39 (59)
MMA-Cbl$^+$	47	4 (3)	3 (11)	300 (17)	4 (67)	21 (72)	4 (67)	21 (72)
PA	144	6 (37)	4 (36)	195 (32)	23 (56)	51 (72)	20 (49)	45 (63)
IVA	83	6 (9)	4 (15)	570 (17)	30 (88)	24 (73)	26 (77)	20 (61)
GA1	176	240 (9)	5.5 (4)	360 (72)	32 (100)	87 (98)	32 (100)	86 (97)
Total	567	6 (77)	3.5 (96)	300 (172)	106 (75)	224 (78)	97 (68)	211 (73)

Data are shown as median (n) and as n (%);EO, early onset; LO, late onset; NBS, newborn screening; Selective, selective diagnostic work-up started after the onset of first symptoms. For descriptive statistical information including median, mean, minimum, maximum, interquartile range see Suppl. Table 3

Table 3 Achievement of motor milestones

Disease	MoD	Total *n*	Normal development *n (%)*	Delayed development *n (%)*	OR from logistic regression† with covariate age at last regular visit
MMA-Cbl$^-$	NBS	30	28 (93)	2 (7)	MoD: $OR_a = 5.63$, 95 % CI [1.52;30.95], $p = 0.008$ LR (df = 1) = 8.18, $p = 0.004$
	Selective	63	47 (75)	16 (25)	
MMA-Cbl$^+$	NBS	7	6 (86)	1 (14)	MoD: $OR_u = 1.71$, 95 % CI [0.29;18.24], $p = 0.574$ LR (df = 1) = 0.55, $p = 0.459$
	Selective	29	21 (72)	8 (28)	
PA	NBS	39	35 (90)	4 (10)	MoD: $OR_u = 0.73$, 95 % CI [0.19;2.88], $p = 0.639$ LR (df = 1) = 0.01, $p = 0.903$
	Selective	64	59 (92)	5 (8)	
IVA (with IVA mild)	NBS	35	35 (100)	0 (0)	MoD: $OR_u = 9.04$, 95 % CI [0.83;1234.58], $p = 0.075$ LR (df = 1) = 1.24, $p = 0.266$
	Selective	30	27 (90)	3 (10)	
IVA (w/o IVA mild)	NBS	25	25 (100)	0 (0)	MoD: $OR_u = 6.49$, 95 % CI [0.59;889.22], $p = 0.141$ LR (df = 1) = 1.23, $p = 0.267$
	Selective	30	27 (90)	3 (10)	
GA1	NBS	51	36 (71)	15 (29)	MoD: $OR_a = 3.38$, 95 % CI [1.47;8.09], $p = 0.004$ LR (df = 1) = 4.23, $p = 0.040$
	Selective	78	40 (51)	38 (49)	

† 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); CI, confidence interval; MoD, mode of diagnosis; LR, penalized likelihood ratio test for comparison of nested models (Heinze et al 2013); NBS, newborn screening; OR, odds ratio (reference group: NBS, reference category: normal development); OR_a, age-adjusted OR (if LR was significant); OR_u, unadjusted OR (if LR was not significant); Selective, selective screening. Age distribution is specified in Suppl. Table 4

($n = 98$) including those identified by NBS ($n = 38$), results should be regarded as preliminary. ORs showed shifts towards improved cognitive outcome in the NBS group of IVA and GA1 patients compared with the selective screening group (IVA: 92 % for NBS versus 64 % for selective screening group; GA1: 86 % versus 73 % for selective screening group). This shift, however, did not reach significance for IVA ($n = 23$ test results; OR = 0.22, 95 % CI [0.02;1.49], $p = 0.123$) or GA1

Table 4 Movement disorder

Disease	MoD	Total *n*	No MD *n (%)*	MD *n (%)*	OR from logistic regression† with covariate age*
MMA-Cbl$^-$	NBS	31	28 (90)	3 (10)	MoD: $OR_u = 5.20$, 95 % CI [1.72;20.76], $p = 0.002$ LR (df = 1) = 0.03, $p = 0.862$
	Selective	67	41 (61)	26 (39)	
MMA-Cbl$^+$	NBS	7	7 (100)	0 (0)	MoD: $OR_u = 4.33$, 95 % CI [0.42;590.22], $p = 0.256$ LR (df = 1) = 0.05, $p = 0.820$
	Selective	28	22 (79)	6 (21)	
PA	NBS	40	31 (78)	9 (23)	MoD: $OR_u = 2.02$, 95 % CI [0.86;5.01], $p = 0.106$ LR (df = 1) = 0.36, $p = 0.547$
	Selective	69	43 (62)	26 (38)	
IVA	NBS	33	31 (94)	2 (6)	MoD: $OR_u = 2.72$, 95 % CI [0.60;16.16], $p = 0.199$ LR (df = 1) = 1.18, $p = 0.278$
	Selective	30	25 (83)	5 (17)	
GA1	NBS	57	42 (74)	15 (26)	MoD: $OR_u = 7.22$, 95 % CI [3.50;15.59], $p < 0.001$ LR (df = 1) = 0.73, $p = 0.394$
	Selective	88	24 (27)	64 (73)	

† 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; LR, penalized likelihood ratio test for comparison of models (Heinze et al 2013); MoD, mode of diagnosis; MD, movement disorder; NBS, newborn screening; OR, Odds ratio (reference group: NBS, reference category: no MD); OR_u, unadjusted OR (if LR was not significant); Selective, selective screening. Age distribution is specified in Suppl. Table 5

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

Disease	Patients *n*	Natural protein, median in %WHO*(n)				Total protein, median in %WHO* (n)				Calories, median in %FAO** (n)			
		Total	EO	LO	EO vs. LO†	Total	EO	LO	EO vs. LO†	Total	EO	LO	EO vs. LO†
MMA-Cbl -.	117	92 (96)	83 (46)	93 (37)	$p = 0.281$	140 (97)	135 (47)	154 (37)	$p = 0.121$	107 (86)	104 (41)	114 (34)	$p = 0.067$
MMA-Cbl+	47	114 (31)	95 (7)	114 (16)	$p = 0.262$	132 (31)	163 (7)	124 (16)	$p = 0.316$	90 (24)	80 (5)	94 (13)	$p = 0.178$
PA	144	87 (108)	77 (66)	100 (34)	$p = 0.003$	131 (108)	119 (66)	143 (34)	$p = 0.016$	98 (102)	93 (62)	110 (32)	$p = 0.027$
IVA	83	98 (35)	95 (14)	103 (15)	$p = 0.480$	144 (36)	144 (14)	135 (15)	$p = 0.489$	95 (22)	85 (11)	92 (8)	$p = 0.750$
GA1	176	104 (111)	97 (3)	106 (83)	$p = 0.673$	171 (114)	97 (3)	187 (86)	$p = 0.033$	108 (74)	122 (2)	110 (54)	$p = 0.652$
Total	567	98 (381)	82 (136)	105 (185)	$p < 0.001$	143 (386)	128 (137)	154 (188)	$p < 0.001$	103 (308)	96 (121)	108 (141)	$p = 0.011$

*According to WHO safe values (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"; † randomized median test for age at diagnosis, based on 100,000 permutations; EO, early onset; LO, late onset. Patients who solely avoided excess protein intake were excluded from the analysis since the exact protein and caloric intake was unknown. For descriptive statistical information including median, mean, minimum, maximum, interquartile range see Suppl. Table 9

patients ($n = 38$ test results; OR = 0.46, 95 % CI [0.09;2.22], $p = 0.330$).

As with IQ, the evaluation of renal and cardiac manifestations did not convincingly show a positive effect of NBS (Suppl. Tables 6 and 7). For MMA-Cbl$^-$ patients we observed a statistical trend ($p = 0.091$) for patients identified by NBS to show a lower probability of chronic renal failure than those in the selective screening group. For PA patients there was a significant interaction between mode of diagnosis and age on the probability of cardiac manifestation, with only patients in the selective screening group showing a higher risk for cardiac manifestation with increasing age (Suppl. Table 7, Suppl. Fig. 2). These results need to be re-evaluated in a larger sample.

Metabolic treatment of OADs

Age at onset of first symptoms and age at diagnosis are two factors influencing the above described health outcomes of OAD patients. Treatment is the third important modulator of disease course. In the statistical analysis we took into consideration the potential confounder that patients with a more severe disease course are likely to receive more intensive treatment than those with a less severe phenotype.

The basis of metabolic therapy of OAD patients is dietary restriction of precursor amino acids of toxic metabolites. The majority of OAD patients either received a prescribed low protein diet (404/567; 71 %) or simply avoided protein-rich foods (57/567; 10 %) for maintenance treatment. Almost half of the patients ($n = 266$; 47 %) received synthetic AAMs, and 131 patients (23 %) were tube fed. EO patients were more likely to receive a prescribed diet (MMA-Cbl$^-$ and PA), AAMs (MMA Cbl$^+$, PA and IVA) and tube feeding (MMA Cbl$^-$, MMA Cbl$^+$, PA and GA1) than LO patients, reflecting a higher disease severity in the EO group resulting in low protein tolerance and feeding difficulties (Suppl. Table 8). In line with this, EO and LO groups also differed in the composition of their diet. Overall, LO patients received higher amounts of natural protein, total protein and calories than EO patients. This effect was most pronounced for PA patients. Interestingly, median natural protein intake of LO patients was almost identical

Table 6 Pharmacotherapy (maintenance treatment)

Disease	All patients	Patients with medication	Carnitine	Glycine	Vitamin B12	Vitamin B2	Sodium benzoate	Arginine	Metronidazole and/or colistin
	n	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*
MMA-Cbl$^-$	117	95	92	n/a	19	n/a	4	2	34
MMA-Cbl$^+$	47	34	33	n/a	24	n/a	n/a	n/a	7
PA	144	118	117	n/a	2	n/a	8	11	56
IVA	83	65	65	37	0	n/a	n/a	n/a	0
GA1	176	142	142	n/a	1	35	n/a	n/a	n/a
Total	567	454	449	37	48	35	12	13	97

Vitamin B12, hydroxocobalamin and/or cyanocobalamin; n/a, not applicable

to the minimum safe values of the World Health Organization (WHO) 2007 recommendation, whereas reported natural protein intake for EO patients was below this level. Addition of AAMs increased the total protein intake beyond safe values for the majority of OAD patients (Table 5; Suppl. Table 9).

In total, 454 (80 %) of 567 OAD patients received specific drug treatment (Table 6). The most frequently used medication was carnitine (449/567 patients, 80 %) which was used alone (all OADs) or in combination with glycine (IVA only). Cofactor treatment with hydroxy- or cyanocobalamin (MMA) and riboflavin (GA1) was given if cofactor responsiveness was reported to be confirmed (MMA-Cbl^{+}) or suggested (GA1). Notably, only half of MMA-Cbl^{+} patients, but 20 of 95 patients with MMA-Cbl^{-} received cobalamin supplementation. Oral metronidazole was used in PA ($n = 56/144$) and MMA patients ($n = 41/164$ patients) with the aim to reduce the intestinal preload of propionate and ammonia. The nitrogen scavenger sodium benzoate (MMA and PA, $n = 12$ patients) and arginine supplementation ($n = 13$, mostly PA, with the aim of stimulating carbamylphosphate synthetase 1) were only sporadically used. Carbamylglutamate, a licensed drug for the treatment of hyperammonemia in MMA, PA and IVA, was used for maintenance treatment in a single MMA patient only. Allopurinol was used to treat hyperuricemia in MMA patients with chronic renal failure. So far six MMA patients who underwent kidney transplantation and one PA patient with liver transplantation have been reported (data not shown).

Emergency treatment was documented for 271 emergency visits of 92 OAD patients, with highest frequencies for PA, GA1 and MMA-Cbl^{-} patients (Table 7; Suppl. Table 10). Since emergency treatment is highly individualised on a day-to-day basis, we only evaluated the first day of emergency treatment, assuming that the initial emergency treatment best reflects centre-specific standard protocols. As expected, emergency treatment followed a similar pattern for all OADs: natural protein was strictly reduced or transiently withheld, AAMs were continued (if used for maintenance treatment) in GA1 as recommended in the guideline, whereas carbohydrate intake and carnitine supplementation was increased. Continuation of AAMs during emergency treatment in MMA-Cbl^{-} and PA patients was less consistent among patients and centres: the current guideline for MMA and PA does not recommend continuation of AAMs in hyperammonemic patients (Baumgartner et al 2014). Despite increased carbohydrates, caloric intake was often below age-adjusted energy recommendations (Food and Agricultural Organization 2001). Pharmacological treatment of hyperammonemia using sodium benzoate, arginine and—sporadically—carbamylglutamate was used

Table 7 Emergency treatment (first day)

Disease	Patients *n*	Patients with emergency treatment *n*	Emergency episodes *n*	Dietary management					Pharmacotherapy			
				Carbohydrates Median in % Recommendation* (n)	Natural protein Median in %WHO** (n)	Total protein Median in %WHO** (n)	AAM Median in g/kg/d (n)	Calories Median in %FAO*** (n)	BZA (incl. bolus) Median in mg/kg/d (n)	CNT (incl. bolus) Median in mg/kg/d (n)	CBG Median in mg/kg/d (n)	ARG-HCl (incl. bolus) Median in mg/kg/d (n)
MMA-Cbl^{-}	117	26	54	151 (49)	0 (51)	52 (51)	0.3 (49)	65 (49)	265 (2)	161 (50)	8 (1)	n.r.
MMA-Cbl^{+}	47	4	6	94 (4)	0 (3)	0 (3)	0 (3)	38 (1)	n.r.	100 (3)	n.r.	n.r.
PA	144	25	111	166 (108)	35 (104)	91 (105)	0.4 (102)	98 (109)	153 (21)	101 (108)	118 (3)	126 (60)
IVA	52	8	11	156 (9)	0 (8)	0 (8)	0 (8)	73 (9)	n.r.	143 (10)	n.r.	n.r.
GA1	176	29	89	133 (61)	0 (79)	107 (79)	0.9 (75)	93 (69)	n.r.	163 (75)	n.r.	149 (1)
Total	452	92	271	149 (231)	28 (245)	92 (246)	0.5 (237)	88 (237)	222 (23)	113 (246)	116 (4)	127 (61)

Data are shown as median (n); *According to recommended carbohydrate management for emergency treatment of GA1 patients (Kölker et al 2011); **according to WHO safe values (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"; AAM, amino acid mixture; ARG-HCl, arginine hydrochloride; BZA, sodium benzoate; CBG, carbamylglutamate; CNT, carnitine; n.r., not reported. For descriptive statistical information including median, mean, minimum, maximum, interquartile range see Suppl. Table 10

for PA patients ($n=3$) and in one MMA-Cbl$^-$ patient. Extracorporeal detoxification was reported for a single emergency episode (after the initial presentation) in a patient with MMA-Cbl$^+$.

Impact of therapy on the disease course

To investigate whether a specific metabolic treatment regimen was more effective than others to improve the health outcomes of OAD patients, we used recursive partitioning. This statistical procedure was chosen to identify predictors that are useful to make clinical decisions in OADs because this method can handle numerical data that are highly skewed or multimodal, as well as categorical predictors with either ordinal or categorical structure. Correlating independent variables were selectively excluded from the analysis to reduce co-linearity (Suppl. Table 11). Patients detected by NBS, selective screening, prenatal screening or high-risk family screening, the latter diagnosed during the neonatal period and being asymptomatic at last visit, were included in this analysis. "Motor abnormality" was used as the superordinate dependent variable, subsuming four single motor variables because they were highly correlated (Suppl. Table 12). Recursive partitioning did not discriminate a special beneficial treatment form. This result does not mean that metabolic treatment is not effective at all, but that we found no evidence that any of the treatment regimens used was superior to others. However, mode of diagnosis and onset type had much stronger effects than any of the single and combined therapeutic interventions (Fig. 1). Analysis for classic OADs (i.e. excluding GA1 patients) and for single OADs revelead similar patterns (data not shown).

Discussion

Newborn screening reduces the time to diagnosis

Patients with OADs carry a life-long risk of cerebral and extracerebral disease manifestations (Grünert et al 2013; Hörster et al 2007; Kölker et al 2006; Nicolaides et al 1998; Pena et al 2012; Prada et al 2011; Schreiber et al 2012). The aim of NBS programmes is to prevent irreversible organ damage and death by allowing treatment to be started, ideally, before the onset of symptoms. Screening panels of existing NBS programmes vary from one to over 20 inherited metabolic diseases and currently there is no international harmonisation. Benefit and cost effectiveness of NBS have been studied for some OADs (Dionisi-Vici et al 2006; Heringer et al 2010; Kölker et al 2007; Pfeil et al 2013; Wilcken 2010). This present study

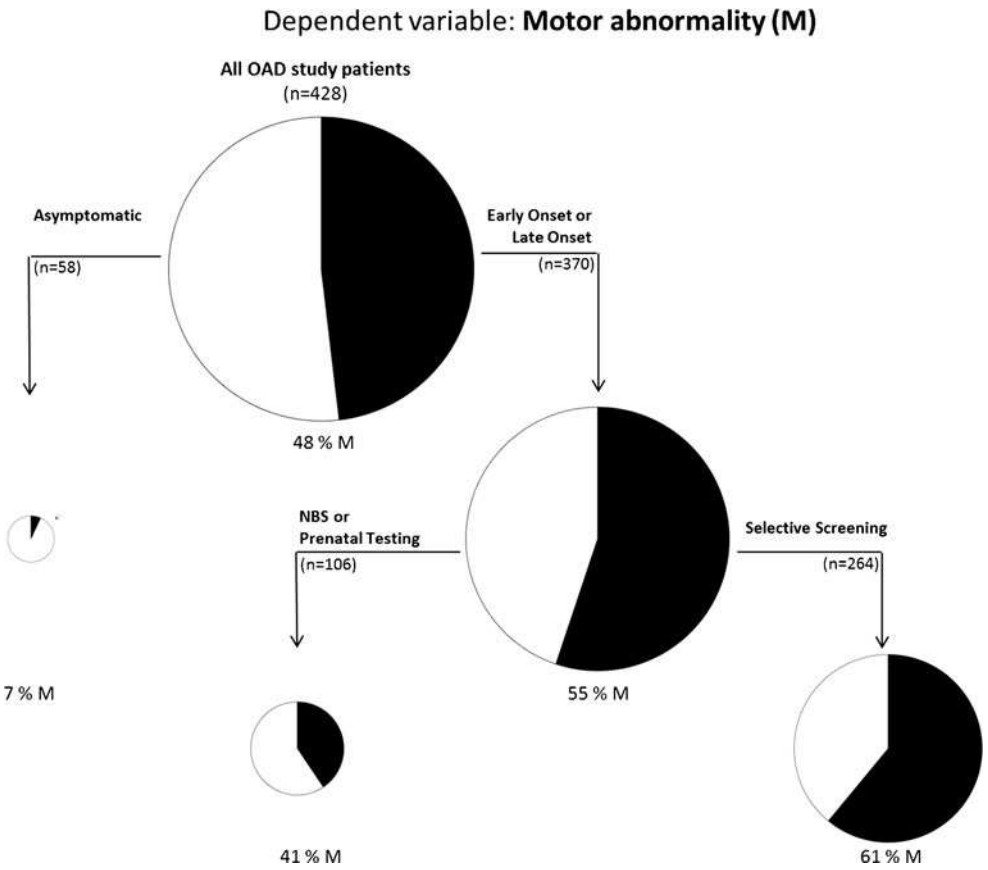

Fig. 1 Effect of type of onset and diagnostic mode on the neurological outcome (all organic acidurias) after recursive partitioning. Motor abnormality was used as superordinate dependent variable subsuming four single motor variables (muscular hypotonia, abnormal gross and fine motor development, movement disorders). Percentages for "yes" refers to the proportion of patients with motor abnormality. Note that patients with transient developmental delay of motor functions were assigned to the asymptomatic group ($n=4$; 7 %) since they were asymptomatic at the last reported visit. Similar results of recursive partitioning were obtained for classic organic acidurias (i.e. excluding glutaric aciduria type 1) and single organic acidurias (not shown). EO, early onset (i.e. during the newborn period); LO, late onset (i.e. after the newborn period); NBS, newborn screening

includes 180 patients identified by NBS, the largest NBS sample of OADs studied so far. We clearly demonstrate that NBS reduces the age at diagnosis for all OADs, in particular for LO patients. Statistical modelling of the selective screening group showed that 77 % of OAD patients remained asymptomatic at day 5 and 73 % at day 8. Major arguments against the inclusion of classic OADs to NBS programmes are low specificity of propionylcarnitine as screening parameter for MMA and PA and the early onset of symptoms. However, our results indicate that a significant proportion of OAD patients could have been identified by NBS whilst still asymptomatic. Interestingly, the percentage of patients who could have been identified by NBS before the onset of symptoms is even higher than in two previously published studies from smaller patient cohorts with PA (Grünert et al 2012a) and classic OADs (Dionisi-Vici et al 2006). However, we cannot exclude a sampling bias due to an unknown number of OAD patients who might have died undiagnosed. Our findings provide valuable information for ongoing discussions about the inclusion of OADs in existing NBS programmes.

The effect of early diagnosis on health outcomes

The evaluation of the effects of diagnostic and therapeutic interventions on disease course relies on reliable clinical endpoints. Whilst GA1 and IVA patients mostly or exclusively present with neurological symptoms during infancy, patients with MMA or PA characteristically show a combination of symptoms due to multi-organ involvement which develops over time (Kölker et al 2015b; Mardach et al 2004; Marquard et al 2011; Martinez Alvarez et al 2015; Pena et al 2012). This is a challenge for the study design since effects of interventions can be organ- and age-specific and thus may result in a broad spectrum of therapeutic benefits being superimposed on a variable natural disease course. Since neurological symptoms such as developmental delay and movement disorders are most commonly found in OAD patients and often manifest early in the disease course (Kölker et al 2015b), we mostly focused on the neurological outcome. In line with previous studies (Bijarnia et al 2008; Boneh et al 2008; Heringer et al 2010, 2015; Kölker et al 2007; Strauss et al 2007; Viau et al 2012), we showed that NBS improves the neurological outcome in GA1 patients. Similarly, NBS reduces the occurrence of movement disorders, and increases normal motor development in MMA-Cbl$^-$ patients ($n=31$). This supports the preliminary findings of a previous report providing evidence for improved neurological outcome and mortality in OAD patients including four MMA patients identified by NBS (Dionisi-Vici et al 2006). For IVA patients, a tendency towards improved achievement of motor milestones was found in the NBS group. However, this disappeared when patients with predicted mild phenotype were excluded from the analysis ($p=0.141$). Overall, the neurological outcome of IVA patients was relatively good in the selective screening group and even better than previously reported for symptomatic IVA patients (Grünert et al 2012a). For PA and MMA-Cbl$^+$ patients, neurological outcome was not improved by NBS compared to the selective screening group. The negative result for PA patients is in line with a previous report (Grünert et al 2012b).

The effect of NBS on cognitive development could not be evaluated with appropriate strength since psychological test results have so far been reported for only small groups of patients. This is a real shortcoming since impaired neurocognitive development is often found in patients with classic OADs, particularly in PA and MMA-Cbl$^-$ (De Baulny et al 2005; Dionisi-Vici et al 2006; Grünert et al 2012a, 2013; Hörster et al 2007, 2009; Nicolaides et al 1998; Nizon et al 2013; Schreiber et al 2012).

It was less clear whether extracerebral disease manifestation, in particular chronic renal failure and cardiac manifestation (prolonged QT_c interval, cardiomyopathy), were influenced by early diagnosis in patients with classic OADs. In line with previous studies, cardiac manifestations were most often found in PA patients and renal manifestation in MMA-Cbl$^-$ patients (Grünert et al 2013; Hörster et al 2007; Kölker et al 2015b; Romano et al 2010). However, we found no difference between patients with MMA-Cbl$^-$ identified by NBS versus selective screening, whereas PA patients identified by NBS showed significantly lower risk of progressive cardiac disease than those identified by selective screening. Since OAD patients in the E-IMD sample have a median age of 8.3 years (interquartile range: 3.9-14.1 years) and extracerebral disease manifestation may not appear before adolescence or adulthood, it remains to be seen whether NBS has a protective effect on these and other late organ manifestations in older cohorts of OAD patients.

Diagnostic mode and onset type are more important than the effects of treatment on health outcomes

Early diagnosis is a prerequisite for early therapeutic intervention. A combination of low protein diet, adequate caloric intake and cofactor (in responsive patients) and drug treatment is usually applied in OAD patients with the aim of reducing or preventing the accumulation of toxic metabolites, metabolic decompensations and long-term complications. During intercurrent illness, and other events that might trigger catabolism, emergency treatment with transient intensification of the above mentioned therapies is recommended (Baumgartner

et al 2014; Kölker et al 2011; Sutton et al 2012). Although adherence to evidence-based recommendations was shown to improve the neurological outcome in GA1 patients (Heringer et al 2010; Kölker et al 2012; Strauss et al 2011), this has not yet been studied for classic OADs. Furthermore, late onset of symptoms could still appear in patients with classic OADs thought to have been "metabolically stable" for years (Kölker et al 2015b, Mardach et al 2011, Martinez Alvarez et al 2015; Pena et al 2012).

Dietary management in the E-IMD sample was mostly in line with current recommendations for OADs (Baumgartner et al 2014; Kölker et al 2011; Sutton et al 2012). As expected, differences in the disease severity between EO and LO patients were reflected in a higher intake of natural protein, total protein and calories in the LO group. This effect was most pronounced for PA patients. Notably, for EO patients a total protein intake above WHO minimum safe values was often achieved only when AAMs were administered. For emergency treatment, EO and LO patients were treated similarly. Transiently reducing or stopping natural protein, continuing AAMs (in MMA Cbl^{-}, PA and GA1) and increasing carbohydrate intake was the most common dietary management—except for MMA-Cbl^{+} patients who followed a more relaxed emergency regime due to cofactor responsiveness. Of note, continuation of AAMs is in line with guideline recommendations for GA1 but in disagreement with recommendations for MMA and PA (Baumgartner et al 2014; Kölker et al 2011). Continuation of AAMs in MMA and PA is thought to increase the risk of hyperammonemia. Despite an overall increase of carbohydrates to 149 % of those recommended in the guidelines, this did not result in an increase in the total amount of calories during the first day of emergency treatment. In fact, caloric intake was below (88 %) the FAO recommendations (FAO 2001) indicating that carbohydrates were used as the major or even the sole energy source. This is in disagreement with the recently published guideline for MMA and PA which recommends early implementation of intravenous lipids (Baumgartner et al 2014). This discrepancy might reflect the uncertainty of metabolic specialists about the use of intravenous lipids in ketoacidotic patients with PA and MMA. Future investigations of the E-IMD sample will evaluate whether the recently published guideline recommendations for MMA and PA help to harmonise emergency treatment.

To improve removal of toxic acyl-CoA, carnitine (all OADs) and glycine (IVA) were administered as expected. Furthermore, oral antibiotics (metronidazole, colistin), sodium benzoate and arginine (given less often) and carbamylglutamate (given only in a single MMA patient) were used to prevent hyperammonemia. Interestingly, cofactor treatment such as hydroxy- or cyanocobalamin in MMA-Cbl^{+} patients was not only used for patients with explicit cofactor responsiveness, but was sometimes administered to patients with unproven cofactor responsiveness (e.g. hydroxy- or cyanocobalamin in MMA-Cbl^{-} or riboflavin in GA1). The rationale for this treatment remains unclear. On the other hand, only 25 of 47 MMA-Cbl^{+} patients received cofactor treatment, which might reflect limited availability of this drug, patient refusal or discontinuation of intramuscular or subcutaneous administration. Among the MMA-Cbl^{+} patients who did not receive cobalamin seven patients were classified as mut^{-}, nine as cblA, three as cblB and two remained unclassified. During emergency treatment, carnitine supplementation was often increased to facilitate the detoxification of toxic acyl-CoA and to prevent carnitine depletion, and sodium benzoate and arginine (mostly PA) were used to treat hyperammonemia. In contrast, carbamylglutamate, which is licensed for the treatment of hyperammonemia in classic OADs, was only sporadically used in PA ($n=3$) and MMA patients ($n=1$).

A further in-depth analysis on the impact and superiority of specific therapeutic protocols on the outcome of OAD patients was not possible for at least two reasons: firstly, the mode of diagnosis and type of onset had strong effects on outcome so that any small to moderate effect resulting from different treatment protocols was likely to have been overshadowed. Secondly, various combinations and doses of drugs and cofactor treatment were used for metabolic maintenance treatment in MMA-Cbl^{-} ($n=12$ combinations), MMA-Cbl^{+} ($n=8$ combinations), PA ($n=13$), IVA ($n=2$) and GA1 ($n=4$) patients resulting in small group sizes and consequently low statistical power. In addition, national health systems differ between countries and are changing over time. Although these infrastructure differences are likely to influence outcomes, their true impact on individual patients with rare diseases like OADs is difficult to assess. It is important to note that these results do not question the evidence of existing guidelines and recommendations at all, nor was it an aim of this study to evaluate these recommendations.

Observational studies do not reduce existing variability; they document the status of current practice. However, since guidelines for MMA, PA and GA1 have been published during the course of the E-IMD project, the use of these evidence-based recommendations may reduce therapeutic variability in the future. Prospective follow-up of the E-IMD patient cohort and evaluating the long-term outcome of patients whose treatment is in agreement with current recommendations will lead to a better understanding of the effect of therapeutic interventions. However, the impact of non-interventional variables needs to be carefully considered.

Conclusions

NBS shortens the diagnostic process, in particular for LO patients, and improves the neurologic outcome and motor development in particular for GA1 and MMA-Cbl$^-$ patients. The impact of NBS on visceral disease manifestation needs further attention. Since the mode of diagnosis and the type of onset have strong effects on the disease course, the identification of the most effective treatment strategy (if any) is an important goal for future research.

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Conflict of interest None.

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 inclusion in the study in countries where this was needed by law.

Animal rights This article does not contain animal subjects.

References

Baumgartner MR, Hörster F, Dionisi-Vici C et al (2014) Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. Orphanet J Rare Dis 9:130

Bijarnia S, Wiley V, Carpenter K, Christodoulou J, Ellaway CJ, Wilcken B (2008) Glutaric aciduria type I: outcome following detection by newborn screening. J Inherit Metab Dis 31:503–507

Boneh A, Beauchamp M, Humphrey M, Watkins J, Peters H, Yaplito-Lee J (2008) Newborn screening for glutaric aciduria type I in Victoria: treatment and outcome. Mol Genet Metab 94:287–291

De Baulny HO, Benoist JF, Rigal O, Touati G, Rabier D, Saudubray JM (2005) Methylmalonic and propionic acidaemias: management and outcome. J Inherit Metab Dis 28:415–423

Dionisi-Vici et al (2006) 'Classical' organic acidurias, propionic aciduria, methylmalonic aciduria and isovaleric aciduria: long-term outcome and effects of expanded newborn screening using tandem mass spectrometry. J Inherit Metab Dis 29:383–389

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

Evans S, Alroqaiba N, Daly A, Neville C, Davies P, Macdonald A (2012) Feeding difficulties in children with inherited metabolic disorders: a pilot study. J Hum Nutr Diet 25:209–216

FAO (2001) Human energy requirements: Report of a Joint FAO/WHO/UNU Expert Consultation. FAO Report Series, No. 1. FAO, Rome

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 37: 763–773

Grünert SC, Müllerleile S, de Silva L et al (2012a) Propionic acidemia: neonatal versus selective metabolic screening. J Inherit Metab Dis 35:41–49

Grünert SC, Wendel U, Lindner M et al (2012b) 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

Heinze G, Schemper M (2002) A solution to the problem of separation in logistic regression. Stat Med 21(16):2409–2419

Heinze G, Ploner M, Dunkler D, Southworth H (2013) Logistf: Firth's bias reduced logistic regression. R package version 1.21. http://CRAN.R-project.org/package=logistf

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

Heringer J, Boy N, Burgard P, Okun JG, Kölker S (2015) Newborn screening for glutaric aciduria type I: benefits and limitations. Int J Neonatal Screen 1:57–68

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

Hothorn T, Hornik K, Zeileis A (2006) Unbiased recursive partitioning: a conditional inference framework. J Comput Graph Stat 15(3):651–674

Hothorn T, Hornik K, Strobl C, Zeileis A (2015) Party: a laboratory for recursive partitioning. R package version 1.0-23. http://CRAN.R-project.org/package=party

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

Kölker S, Garbade SF, Boy N, Maier EM 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, Boy SP, Heringer J et al (2012) Complemetary dietary treatment using lysine-free, arginine-fortified amino acid supplements in glutaric aciduria type I—a decade of experience. Mol Genet Metab 107:72–80

Kölker S, Garcia Cazorla A, Valayannopoulos V et al (2015a) The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation. J Inherit Metab Dis 38:1041–1057

Kölker S, Valayannopoulos V, Burlina AB et al (2015b) The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 2: the evolving clinical phenotype. J Inherit Metab Dis 38:1059–1074

Kölker S, Dobbelaere D, Häberle J et al (2015c) Networking across borders for individuals with organic acidurias and urea cycle disorders: the E-IMD consortium. JIMD Rep 22:29–38

Loeber JG, Burgard P, Cornel MC et al (2012) Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 1. From blood spot to screening result. J Inherit Metab Dis 35:603–611

Mardach R, Verity MA, Cederbaum SD (2004) Clinical, pathological, and biochemical studies in a patient with propionic acidemia and fatal cardiomyopathy. Mol Genet Metab 85:286–290

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

Martinez Alvarez L, Jameson E, Parry NR, Lloyd C, Ashworth JL (2015) Optic neuropathy in methylmalonic and propionic acidemia. Br J Ophthalmol. doi:10.1136/bjophthalmol-2015-306798

Nicolaides P, Leonard J, Surtees R (1998) Neurological outcome of methylmalonic acidaemia. Arch Dis Child 78:508–512

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

Pfeil J, Listl HGF, Kölker S, Lindner M, Burgard P (2013) Newborn screening by tandem mass spectrometry for glutaric aciduria type 1: a cost-effectiveness analysis. Orphanet J Rare Dis 8:167

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

Richter SJ, McCann MH (2007) Multiple comparison of medians using permutation tests. J Mod Appl Stat Methods 6:399–412

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

Schreiber J, Chapman KA, Summar ML et al (2012) Neurologic considerations in propionic acidemia. Mol Genet Metab 105:10–15

Smucker MD, Allan J, Carterette B (2007) A comparison of statistical significance tests for information retrieval evaluation. CIKM'07 Proceedings of the sixteenth ACM conference on information and knowledge management. 623–632

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, Brumbaugh J, Duffy A et al (2011) Safety, efficacy and physiological actions of a lysine-free, arginine-rich formula to treat glutaryl-CoA dehydrogenase deficiency: focus on cerebral amino acid influx. Mol Genet Metab 10:93–106

Sutton VR, Chapman KA, Gropman AL et al (2012) (2012) Chronic management and health supervision of individuals with propionic acidemias. Mol Genet Metab 105:26–33

Viau K, Ernst SL, Vanzo RJ, Botto LD, Pasquali M, Longo N (2012) Glutaric acidemia type 1: outcomes before and after expanded newborn screening. Mol Genet Metab 106:430–438

Wilcken B (2010) Expanded newborn screening: reducing harm, assessing benefit. J Inherit Metab Dis 33(Suppl 2):S205–S210

World Health Organization, Food and Agriculture Organization of the United Nations, United Nations University (2007) Protein and amino acid requirements in human nutrition. Report of a joint WHO/FAO/UNU expert consultation. WHO Technical Report Series, No. 935, WHO Press