Supplementary Notes S1 – Screening criteria

1968, Wilson and Jungner, ten criteria to guide selection of conditions suitable for screening

(1) The condition sought should be an important health problem.
(2) There should be an accepted treatment for patients with recognized disease.
(3) Facilities for diagnosis and treatment should be available.
(4) There should be a recognizable latent or early symptomatic stage.
(5) There should be a suitable test or examination.
(6) The test should be acceptable to the population.
(7) The natural history of the condition, including development from latent to declared disease, should be adequately understood.
(8) There should be an agreed policy on whom to treat as patients.
(9) The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
(10) Case-finding should be a continuing process and not a "once and for all" project.

2008, World Health Organisation, ten criteria additional to the Wilson and Jungner criteria

(1) The screening programme should respond to a recognized need.
(2) The objectives of screening should be defined at the outset.
(3) There should be a defined target population.
(4) There should be scientific evidence of screening programme effectiveness.
(5) The programme should integrate education, testing, clinical services and programme management.
(6) There should be quality assurance, with mechanisms to minimize potential risks of screening.
(7) The programme should ensure informed choice, confidentiality and respect for autonomy.
(8) The programme should promote equity and access to screening for the entire target population.
(9) Programme evaluation should be planned from the outset.
(10) The overall benefits of screening should outweigh the harm.

Supplementary Notes S2 – Definition of grouped outcome parameters

Definition of "Adverse outcome (AO) of the first symptomatic phase" as clinical outcome parameter

No AO: No basal ganglia hyperintensities, or white matter abnormalities or movement disorder
Mild AO: Basal ganglia hyperintensities or white matter abnormalities or movement disorder that can be most likely attributed to the first symptomatic phase, but not wheelchair-bound
Severe AO: Basal ganglia hyperintensities or white matter abnormalities or movement disorder, wheelchair-bound due to movement disorders that most likely arose due to the first symptomatic phase
Death: Death during or due to the first symptomatic phase

Definition of "AMD frequency" as clinical outcome parameter

None: No episode of AMD during first four years of life
Mild: >0.0 $\leq$ 0.5 AMD/PY during first four years of life
Moderate: >0.5 $\leq$ 1.0 AMD/PY during first four years of life
Severe: >1.0 $\leq$ 2.0 AMD/PY during first four years of life
Very severe: >2.0 AMD/PY during first four years of life
Death: Death during or due to AMD, at any moment in life
Not assessed (NA): *if death during or due to first presentation*

Definition of "Cognitive function" as clinical outcome parameter

Group 1: IQ >90 or regular education
Group 2: IQ 60-90 or special education
Group 3: IQ <60 or no education
Not assessed (NA): *Last moment of follow-up <4 years of age*

Definition of "Mitochondrial complications" as clinical outcome parameter

Twenty-one complications considered to be potentially caused by mitochondrial failure based on an extensive literature review [2] were included: hepatomegaly, epilepsy, cardiomyopathy, optic atrophy, pancreatitis, renal failure, sensorineural hearing loss, acute psychosis, stroke-like episodes, prolonged QTc interval, premature ovarian insufficiency, exercise intolerance, autism, feeding disorders, muscular hypotonia, constipation, attention deficit hyperactive disorder, anemia, leukopenia, thrombocytopenia and pancytopenia.

Severity is based on the number of complications recorded at the last moment of follow-up, as follows:

	0-12 year	13-18 year	19-24 year	>25 years
None:	0	0	0	0
Mild:	1-2	1-4	1-6	1-8
Moderate:	3-4	5-6	7-8	9-10
Severe:	5-6	7-8	9-10	11-12
Very severe:	$\geq$7	$\geq$9	$\geq$11	$\geq$13
Death:	Death due to mitochondrial complication			

Not assessed (NA): *If death during or due to first presentation*

Definition of "Treatment-related complications" as clinical outcome parameter

None: No decreased BMD, no growth retardation, no obesity
Mild: 1 of the following: decreased BMD, growth retardation, obesity
Severe: 2 of the following: decreased BMD, growth retardation, obesity
Very severe: Decreased BMD and growth retardation and obesity
Not assessed (NA): *If death during or due to first presentation*

Table S1 – Comparison of baseline characteristics and outcome parameters of patients identified through family screening and their index siblings

	P1.1	**P1.2**	**P2.1**	**P2.2**	**P3.1**	**P3.2**	**P4.1**	**P4.2**	**P5.1**	**P5.2**	**P6.1**	**P6.2**	**P6.3**
Gene	*PCCA*	*PCCA*	*PCCB*	*PCCB*	*PCCB*	*PCCB*	PA, uncl	PA, uncl	PA, uncl	PA, uncl	PA, uncl	PA, uncl	PA, uncl
Sex	M	F	M	M	M	M	F	M	M	F	F	M	M
Age at diagnosis	Day 15	Day 0	0.7 y	Day 2	Day 3	Day 0	Day 18	Day 1	5.0 y	2.0 y	Day 8	Day 4	Day 1
Age at death (years)		8.6	19.1		7.0		12.1				2.9	1.4	
Age last follow-up (years)	18.4	8.6	19.1	19.0	7.0	5.6	12.1	9.7	37.0	33.4	2.9	1.4	11.4
Presentation type	EO	Family	LO	Family	EO	Family	EO	Family	LO	Family	EO	Family	Family
Adverse outc. first sympt. phase	Mild	No	Severe	No	Mild	No	Mild	Mild	No	No	Mild	Mild	Mild
AMD frequency	Severe	Severe	Death	Stable	Death	V. severe	Death	Severe	Stable	Stable	Death	Death	V. severe
Cognitive function	Group 3	Group 3	Group 3	Group 1	NA	Group 3	Group 2	Group 3	Group 1	Group 1	NA	NA	Group 3
Mitochondrial complications	V. severe	Death	Moderate	Mild	V. severe	Severe	Severe	V. severe	None	Mild	V. severe	V. severe	V. severe
Treatment-related complications	V. severe	Mild	Mild	Mild	None	Mild	V. severe	None	None	None	None	None	Mild

Table S1 – Continued

	P7.1	**P7.2**	**P8.1**	**P8.2**	**P9.1**	**P9.2**
Gene	*MUT*	*MUT*	*MUT*	*MUT*	*MMAB*	*MMAB*
Sex	M	M	M	F	F	M
Age at diagnosis	Day 3	Day 0	0.2 y	Day 0	3.9 y	1.6 y
Age at death						
Age last follow-up	13.9	1.7	18.5	13.7	18.3	16.1
Presentation type	EO	Family	LO	Family	LO	Family
Vitamin B12 responsiveness	No	No	No	No	Yes	Yes
Adverse outc. first sympt. phase	Mild	Mild	Mild	Mild	Severe	No
AMD frequency	Severe	NA	Severe	Severe	Stable	Stable
Cognitive function	Group 3	NA	Group 3	Group 2	Group 1	Group 1
Mitochondrial complications	Severe	Moderate	Severe	Moderate	None	Mild
Treatment-related complications	V. severe	Mild	V. severe	Mild	Mild	None

Notes: Early onset: presentation ≤28 days of life; Late onset: presentation >28 days of life. Cognitive function group 1: IQ >90 or regular education, group 2: IQ 60-90 or special education, group 3: IQ <60 or no education. Abbreviations: AMD: acute metabolic decompensation; Adverse outc. first sympt. phase: Adverse outcome of the first symptomatic phase; EO: early onset; Family: family testing; F: female; LO: late onset; M: male; NA: Not assessed; V. severe: very severe; y: years.

Table S2 – All mutations detected in PA and MMA related genes in the Dutch patient cohort

Gene	Mutation (c.)	Mutation (p.)	Mutation type	Reported	Alleles	Origin	Remarks
PCCA	c.625G>C	p.Ala209Pro	Missense/Nonsense	No	1	Dutch	
PCCA	c.923dup	p.Leu308Phefs*35	Duplication	No	1	Dutch	
PCCA	c.1409T>G	p.Leu470Arg	Missense/Nonsense	No	6	Moroccan	2 siblings HO, not reported consanguine; 1 individual HO, consanguine, not knowingly related to the 2 siblings
PCCA	c.2077A>C	p.Met693Leu	Missense/Nonsense	No	1	Dutch	
PCCA	Exon 2 deletion		Gross deletion	No	1	Dutch	
PCCA	Exon 5 and 6 deletion		Gross deletion	No	1	Dutch	
PCCA	Exon 10 deletion		Gross deletion	No	1	Dutch	
PCCA	c.1900-1G>A		Splicing	Yang et al. 2004	2	Afghan	HO, consanguine
PCCA	c.2127delT	p.Val710Cysfs*43	Small deletion	Campeau et al. 1999	1	Dutch	
PCCB	c.644G>C	p.Gly222Arg	Missense/Nonsense	No	2	Turkish	HO, consanguine
PCCB	c.671C>T	p.Ala224Val	Missense/Nonsense	No	1	Dutch	
PCCB	c.703A>C	p.Thr235Pro	Missense/Nonsense	No	4	Moroccan	2 siblings, HO, not reported consanguine
PCCB	c.883_885del	p.Phe295del	Small deletion	No	1	Dutch	
PCCB	c.1127G>A	p.Arg376His	Missense/Nonsense	No	4	Turkish	2 siblings, HO, consanguine
PCCB	c.337C>T	p.Arg113*	Missense/Nonsense	Brosch et al. 2008	2	Turkish	HO, consanguine
MUT	c.623_624del		Small deletion	No	1	Dutch	
MUT	c.730A>C	p.Gln213His	Missense/Nonsense	No	2	Dutch	HO, consanguinity unknown
MUT	c.1022dupA	p.Asn341fs*	Duplication	No	1	Surinamese	
MUT	c.1280G>T	p.Gly427Val	Missense/Nonsense	No	2	Egyptian	HO, consanguine
MUT	c.1311_1312insA	p.Val438Serfs*3	Small insertion	No	4	Turkish/Dutch	1 individual HO, consanguine; 1 individual HO, consanguinity unknown, not knowingly related
MUT	c.1690G>T	p.Glu564*	Missense/Nonsense	No	2	Turkish	HO, consanguine
MUT	c.1962_1963delTC	p.Arg655*	Missense/Nonsense	No	4	Turkish	2 siblings, HO, consanguine
MUT	c.2078delG	p.Gly693Aspfs*12	Small deletion	No	2	Moroccan	HO, consanguine
MUT	c.322C>T	p.Arg108Cys	Missense/Nonsense	Worgan et al. 2006	4	Dutch	HE, 2 siblings, 2 individuals, not knowingly related
MUT	c.454C>T	p.Arg152*	Missense/Nonsense	Martínez et al. 2005	3	Turkish/Dutch	1 individual HO, consanguine; 1 individual HE, not knowingly related
MUT	c.654A>C	p.Gln218His	Missense/Nonsense	Fuchshuber et al. 2000	6	Dutch	2 individuals HO, 2 individuals HE, not reported consanguine, not knowingly related
MUT	c.655A>T	p.Asn219Tyr	Missense/Nonsense	Acquaviva et al. 2005	7	Turkish/Dutch	2 individuals HO (1 consanguine); 3 individuals (2 siblings) HE, not knowingly related
MUT	c.1106G>A	p.Arg369His	Missense/Nonsense	Janata et al. 1997	3	Turkish/Dutch	1 individual HO, consanguine; 1 individual HE, not knowingly related
MUT	c.1160C>T	p.Thr387Ile	Missense/Nonsense	Dündar et al. 2012	2	Syrian	HO, consanguine
MUT	c.1531C>T	p.Arg511*	Missense/Nonsense	Acquaviva et al. 2005	1	Dutch	
MUT	c.1677-1G>C		Splicing	Acquaviva et al. 2005	1	Dutch	
MUT	c.2150G>T	p.Gly717Val	Missense/Nonsense	Crane et al. 1992	1	Surinamese	
MMAA	c.202C>T	p.Gln68*	Missense/Nonsense	No	1	Dutch	
MMAA	c.455del	p.Pro152Leufs*9	Small deletion	No	6	Turkish	2 siblings, 1 cousin, HO, consanguine
MMAA	c.433C>T	p.Arg145*	Missense/Nonsense	Yang et al. 2004	5	Dutch	2 individuals HO (1 consanguine); 1 HE, not knowingly related
MMAA	c.733+1G>A		Splicing	Lerner-Ellis et al. 2004	4	Dutch	1 individual HO (not reported consanguine); 2 individuals HE, not knowingly related
MMAB	c.556C>T	p.Arg186Trp	Missense/Nonsense	Lerner-Ellis et al. 2006	5	Dutch	1 individual HO (not reported consanguine); 3 individuals HE (2 siblings), patients not knowingly related (except the siblings)
MMAB	c.565_577del	p.Cys189Argfs*	Small deletion	No	1	Hindi	
MMAB	c.655T>C	p.Tyr219His	Missense/Nonsense	No	1	Hindi	
MMAB	c.569G>A	p.Arg190His	Missense/Nonsense	Lerner-Ellis et al. 2006	1	Dutch	
MMAB	c.197-1G>A		Splicing	Lerner-Ellis et al. 2006	2	Dutch	HE, 2 siblings
MMAB	c.700C>T	p.Gln234*	Missense/Nonsense	Lerner-Ellis et al. 2006	2	Dutch	HE, 2 individuals, not knowingly related

Mutation (c.) depicts the genetic change in the DNA coding sequence, mutation (p.) depicts the resulting change in the protein coding sequence. HO: homozygous, HE: heterozygous. In one patient with a mutation in *PCCA,* no other mutation at the other allele was identified. References:

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Table S3 – Pregnancy, delivery and birth weight parameters

	Propionic acidemia (*n* = 31)	**Methylmalonic acidemia (*n* = 45)**	**P-value**	**Bonferroni correction**
Maternal health problems during pregnancy	*n* = 2; 6%	*n* = 3; 7%	1.000	NS
Delivery via caesarean section	*n* = 4; 13%	*n* = 2; 4%	0.218	NS
Gemelli	*n* = 1; 3%	*n* = 1; 2%	1.000	NS
Gestational age				
Prematurity (<36 weeks)	*n* = 0; 0%	*n* = 5; 11%	0.075	NS
Serotinity (>42 weeks)	*n* = 0; 0%	*n* = 5; 11%	0.075	NS
Abnormal APGAR scores	*n* = 2; 6%	*n* = 4; 9%	1.000	NS
Birth weight (median ± SD; (n))	0.38 ± 1.61 SDS (23)	-1.48 ± 1.19 SDS (32)	<0.001	**<0.001**
Birth weight < -2 SDS	*n* = 2; 9% (23)	*n* = 10; 31% (32)	0.055	NS

Notes: Student's *t* tests were performed for quantitative data and Fisher's exact tests were performed for qualitative data. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant and are depicted in bold. Abbreviations: NS: not significant; SDS: standard deviation score.

Table S4 – Symptoms and signs, laboratory tests and interventions during the first symptomatic phase

		Early onset (*n* = 29)	**Late onset (*n* = 34)**	**P-value**	**Bonferroni correction**
Symptoms and signs					
Lethargy		*n* = 21; 72%	*n* = 19; 56%	0.199	NS
Anorexia		*n* = 20; 69%	*n* = 18; 53%	0.211	NS
Vomiting		*n* = 6; 21%	*n* = 26; 76%	<0.001	**<0.001**
Hypotonia		*n* = 14; 48%	*n* = 12; 35%	0.318	NS
Dehydration		*n* = 11; 38%	*n* = 8; 24%	0.275	NS
Kussmaul breathing		*n* = 9; 31%	*n* = 10; 29%	1.000	NS
Tachypnea		*n* = 9; 31%	*n* = 2; 6%	0.017	NS
Weight loss		*n* = 5; 17%	*n* = 5; 15%	1.000	NS
Hypothermia		*n* = 7; 24%	*n* = 1; 3%	0.019	NS
Coma		*n* = 1; 3%	*n* = 5; 15%	0.205	NS
Failure to thrive		*n* = 0; 0%	*n* = 6; 18%	0.027	NS
Global developmental delay		*n* = 0; 0%	*n* = 3; 9%	0.243	NS
Laboratory tests		***Median ± SD [Min-Max] (N)***	***Median ± SD [Min-Max] (N)***		
Glucose	mmol/L	5.5 ± 6.2 [1.6 – 25.4] (22)	7.2 ± 7.6 [1.2 – 34.0] (23)	0.465	NS
pH		7.33 ± 0.14 [6.89 – 7.43] (26)	7.28 ± 0.17 [6.81 – 7.42] (27)	0.179	NS
pCO_2	mmHg	28.1 ± 14.0 [12.0 – 62.3] (23)	18.7 ± 12.5 [10.0 – 60.0] (22)	0.023	NS
Bicarbonate	mmol/L	16.1 ± 6.3 [3.2 – 24.9] (25)	7.5 ± 6.7 [2.8 – 23.0] (22)	0.006	NS
Base excess	mmol/L	-9.0 ± 8.5 [-30.0 – 0.1] (23)	-19.1 ± 8.9 [-28.2 – -1.0] (25)	0.052	NS
Lactate	mmol/L	2.2 ± 1.2 [1.0 – 6.0] (19)	2.2 ± 2.0 [0.7 – 6.8] (19)	0.353	NS
Ammonia	µmol/L	934 ± 629 [170 – 2767] (20)	137 ± 104 [49 – 400] (19)	<0.001	**<0.001**
Haemoglobin	mmol/L	9.1 ± 1.7 [5.8 – 13.7] (22)	6.9 ± 1.0 [5.2 – 9.0] (25)	<0.001	**<0.001**
Thrombocytes	$*10^9$/L	292 ± 103 [6 – 358] (22)	321 _ 158 [98 – 892] (22)	0.027	NS
Leukocytes	$*10^9$/L	6.2 ± 4.8 [1.8 – 21.0] (23)	8.0 ± 7.4 [0.8 – 33.6] (25)	0.108	NS
Interventions					
Intensive care unit admission		*n* = 18; 62%	*n* = 15; 44%	0.208	NS
Mechanical ventilation		*n* = 13; 45%	*n* = 10; 29%	0.294	NS
Dialysis (any type) due to hyperammonemia		*n* = 11; 38%	*n* = 0; 0%	<0.001	**<0.001**

Notes: Early onset: presentation ≤28 days of life; Late onset: presentation >28 days of life. Student's *t* tests were performed for quantitative data and Fisher's exact tests were performed for qualitative data. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant and are depicted in bold. Abbreviations: Min.: minimum value; Max.: maximum value; NS: not significant.

Table S5 – Adverse outcome due to the first symptomatic phase

	Propionic acidemia (*n* = 31)			**Methylmalonic acidemia (*n* = 45)**		**P-value**	**Bonferroni correction**
No AO	*n* = 14; 45%			*n* = 26; 58%		0.352	NS
Mild AO	*n* = 15; 48%			*n* = 15; 33%		0.235	NS
Severe AO	*n* = 1; 3%			*n* = 3; 7%		0.641	NS
Death due to AO	*n* = 1; 3%			*n* = 1; 2%		1.000	NS
	Early onset (n = 15)	***Late onset (n = 8)***	**P-value**	***Early onset (n = 14)***	***Late onset (n = 26)***	**P-value**	
No AO	*n* = 4; 27%	*n* = 6; 75%	**0.039**	*n* = 7; 50%	*n* = 17; 65%	0.500	NS; NS
Mild AO	*n* = 10; 67%	*n* = 1; 13%	**0.027**	*n* = 7; 50%	*n* = 5; 19%	0.071	NS; NS
Severe AO	*n* = 0; 0%	*n* = 1; 13%	0.348	*n* = 0; 0%	*n* = 3; 12%	0.539	NS; NS
Death due to AO	*n* = 1; 7%	*n* = 0; 0%	1.000	*n* = 0; 0%	*n* = 1; 4%	1.000	NS; NS
				Vitamin B12 unresponsiveness (n = 24)	***Vitamin B12 responsiveness (n = 21)***	**P-value**	
No AO				*n* = 12; 50%	*n* = 14; 67%	0.366	NS
Mild AO				*n* = 11; 46%	*n* = 4; 19%	0.068	NS
Severe AO				*n* = 1; 4%	*n* = 2; 10%	0.592	NS
Death due to AO				*n* = 0; 0%	*n* = 1; 5%	0.467	NS

Notes: Early onset: presentation ≤28 days of life; Late onset: presentation >28 days of life. Statistical significance was determined by performing Fisher's exact tests. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant. Abbreviations: AO: adverse outcome; NS: not significant.

Table S6 – Acute metabolic decompensations

	Propionic acidemia (*n* = 31) *n* = ; median ± SD /PY [range]	**Methylmalonic acidemia (*n* = 45)** *n* = ; median ± SD /PY [range]	**P-value**	**Bonferroni correction**
Total	394; 0.7 ± 1.6 [0.0 – 7.3]	568; 0.4 ± 1.1 [0.0 – 4.7]	0.257	NS
Early onset	293; 1.2 ± 1.3 [0.0 – 4.2]	254; 0.9 ± 1.4 [0.0 – 4.7]	0.604	NS
Late onset	22; 0.2 ± 0.2 [0.0 – 0.7]	242; 0.2 ± 0.9 [0.0 – 3.5]	0.032	NS
Family testing	79; 0.8 ± 2.7 [0.0 – 7.3]	72; 0.6 ± 1.1 [0.0 – 2.8]	0.327	NS
Vitamin B12 unresponsive		504; 1.0 ± 1.1 [0.0 – 4.7]		
Vitamin B12 responsive		64; 0.1 ± 0.8 [0.0 – 3.5]	0.001	**0.001**
Age				
<1 years	55; 1.0 ± 2.0 [0.0 – 7.0]	38; 0.0 ± 1.4 [0.0 – 5.0]	0.032	NS
1-3 years	136; 1.0 ± 2.0 [0.0 – 8.3]	156; 0.3 ± 1.7 [0.0 – 6.7]	0.485	NS
4-11 years	146; 0.3 ± 0.9 [0.0 – 3.5]	206; 0.1 ± 1.0 [0.0 – 5.0]	0.940	NS
12-17 years	40; 0.0 ± 0.5 [0.0 – 2.0]	85; 0.0 ± 0.8 [0.0 – 4.0]	0.483	NS
≥18 years	17; 0.0 ± 0.4 [0.0 – 1.8]	73; 0.0 ± 1.2 [0.0 – 9.9]	0.145	NS
Admission duration				
1 – 3 days	110; 28%	207; 36%	0.006	**0.026**
4 – 7 days	143; 36%	164; 29%	0.017	NS
8 – 14 days	80; 20%	96; 17%	0.203	NS
≥ 15 days	51; 13%	45; 8%	0.012	**0.047**
NA	10; 3%	56; 10%		
ICU admission	23; 5.8%	18; 3.1%	0.051	NS
Triggers				
Upper RTI	114; 29%	154; 27%	0.559	NS
Unknown	77; 20%	141; 25%	0.060	NS
Gastro-enteritis	75; 19%	111; 20%	0.868	NS
Feeding problems	16; 4%	50; 9%	0.004	**0.033**
Bacterial infection	24; 6%	32; 6%	0.781	NS
Constipation	39; 10%	4; 1%	<0.001	**<0.001**
Chronic instability	13; 3%	9; 2%	0.123	NS
Protein overload	4; 1%	4; 1%	0.723	NS
Other	16; 4%	11; 2%	0.072	NS
NA	16; 4%	52; 9%		
AMD frequency				
None	7; 23%	14; 31%	0.448	NS
Mild	2; 6%	2; 4%	1.000	NS
Moderate	1; 3%	3; 7%	0.641	NS
Severe	6; 19%	6; 13%	0.532	NS
Very severe	8; 26%	10; 22%	0.787	NS
Death	5; 16%	1; 2%	0.038	NS
NA	2; 6%	9; 20%		

Notes: Early onset: presentation ≤28 days of life; Late onset: presentation >28 days of life. Student's *t* tests were performed for quantitative data and Fisher's exact tests were performed for qualitative data. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant and are depicted in bold. Abbreviations: AMD: Acute metabolic decompensation; ICU: intensive care unit; NA: not assessed; NS: not significant; PY: patient year; RTI: respiratory tract infection.

Table S7 – Complications with potential mitochondrial etiology

	Mitochondrial Pathophysiology[a]	Propionic acidemia (*n* = 31)		Methylmalonic acidemia (*n* = 45)		P-value	Bonferroni correction
		This cohort	*Lit.*	*This cohort*	*Lit.*		
Mitochondrial complications during first pres.							
Basal ganglia hyperintensities	Probably	*n* = 10; 32%	22%	*n* = 8; 18%	28%	0.175	NS
White matter lesions		*n* = 6; 19%	39%	*n* = 8; 18%	32%	1.000	NS
Complications related to first presentation							
Cerebral atrophy		*n* = 13; 42%		*n* = 11; 24%		0.135	NS
Movement disorders		*n* = 10; 32%	16%	*n* = 14; 31%	32%	1.000	NS
Psychomotor retardation		*n* = 19; 61%	49%	*n* = 24; 53%	53%	0.638	NS
Cognitive dysfunction		*n* = 20; 65%	69%	*n* = 19; 42%	58%	0.066	NS
Group 1: IQ >90 or regular education		*n* = 7; 23%		*n* = 17; 38%		0.212	NS
Group 2: IQ 60-90 or special education		*n* = 7; 23%		*n* = 13; 29%		0.604	NS
Group 3: IQ <60 or no education		*n* = 13; 42%		*n* = 6; 13%		0.007	**0.021**
NA		*n* = 4; 13%		*n* = 9; 20%			
Mitochondrial complications with acute onset							
Hepatomegaly and/or hyperechogenic liver	Probably	*n* = 11; 35% 3.3 y (0.1 – 57.1)	78%	*n* = 14; 31% 0.4 y (0.0 – 9.9)	33%	0.805	NS
Epilepsy	Probably	*n* = 7; 23% 0.0 y (0.0 – 6.3)	23%	*n* = 2; 4% 2.1 y (0.0 – 4.2)	13%	0.027	NS
Cardiomyopathy	Probably	*n* = 7; 23% 8.5 y (7.5 – 56.3)	14%	*n* = 1; 2% 23.3 y	5%	0.007	NS
Optic atrophy	Probably	*n* = 4; 13% 15.9 y (11.8 – 16.7)	5%	*n* = 4; 9% 20.8 y (12.6 – 26.9)	6%	0.709	NS
Pancreatitis	Probably	*n* = 1; 3% 20.8 y	5%	*n* = 2; 4% 19.1 y (9.5 – 28.7)	4%	1.000	NS
Renal failure	Possibly	*n* = 1; 3% 21.4 y	1%	*n* = 20; 44% 8.9 y (1.2 – 31.3)	29%	<0.001	**<0.001**
Sensorineural hearing loss	Possibly	*n* = 6; 19% 6.6 y (2.0 – 8.5)	4%	*n* = 3; 7% 4.8 y (2.5 – 9.8)	2%	0.434	NS
Acute psychosis	Possibly	*n* = 3; 10% 17.2 y (16.8 – 23.5)		*n* = 0; 0%		0.064	NS
Stroke-like episodes	Possibly	*n* = 2; 7% 13.4 y (9.0 – 17.9)	14%	*n* = 0; 0%	17%	0.163	NS
Prolonged QTc interval	Unknown	*n* = 7; 23% 8.5 y (0.0 – 38.3)	31%	*n* = 4; 9% 14.0 y (3.6 – 28.4)	2%	0.300	NS
Premature ovarian insufficiency	Unknown	*n* = 0; 0%		*n* = 1; 2% 19.1 y		1.000	NS
Mitochondrial complications with chronic onset							
Exercise intolerance	Probably	*n* = 15; 48%		*n* = 10; 22%		0.025	NS
Autism	Probably	*n* = 2; 6%	9%	*n* = 4; 9%		1.000	NS
Feeding problems	Possibly	*n* = 18; 58%		*n* = 22; 49%		0.488	NS
Muscular hypotonia	Possibly	*n* = 13; 42%	45%	*n* = 21; 47%		0.815	NS
Constipation	Unknown	*n* = 14; 45%		*n* = 9; 20%		0.024	NS
Attention deficit hyperactive disorder		*n* = 1; 3%	15%	*n* = 1; 2%		1.000	NS
Mitochondrial complications, intermittent occurr.							
Anemia	Possibly	*n* = 21; 68%	51%	*n* = 28; 62%		0.807	NS
Leukopenia	Possibly	*n* = 19; 61%	31%	*n* = 16; 36%		0.036	NS
Thrombocytopenia	Possibly	*n* = 19; 61%	28%	*n* = 23; 51%		0.483	NS
Pancytopenia	Possibly	*n* = 13; 42%	19%	*n* = 9; 20%	0%	0.044	NS
Mitochondrial complications							
None		*n* = 3; 10%		*n* = 3; 7%		0.683	NS
Mild		*n* = 8; 26%		*n* = 22; 49%		0.057	NS
Moderate		*n* = 3; 9%		*n* = 10; 22%		0.219	NS
Severe		*n* = 5; 16%		*n* = 7; 16%		1.000	NS
Very severe		*n* = 11; 35%		*n* = 3; 7%		0.002	**0.013**
Death		*n* = 1; 3%		*n* = 0; 0%		0.408	NS

Notes: a: Likelihood of mitochondrial pathophysiology [2]; Lit: percentage reported in literature [2]. Statistical significance was determined by performing Fisher's exact tests. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant and are depicted in bold. Abbreviations: NA: not assessed; NS: not significant.

Table S8 – Prevalence of treatment-related and miscellaneous complications

	Propionic acidemia (*n* = 31)	**Methylmalonic acidemia (*n* = 45)**	**P-value**	**Bonferroni correction**
Treatment-related complications				
Reduced bone mineral density	*n* = 9; 29%	*n* = 18; 40%	0.465	NS
Growth retardation/short stature	*n* = 8; 26%	*n* = 13; 29%	0.801	NS
Obesity	*n* = 10; 32%	*n* = 6; 13%	0.084	NS
Treatment-related complications				
None	*n* = 12; 39%	*n* = 21; 47%	0.638	NS
Mild	*n* = 13; 42%	*n* = 15; 33%	0.477	NS
Severe	*n* = 4; 13%	*n* = 5; 11%	1.000	NS
Very severe	*n* = 2; 6%	*n* = 4; 9%	1.000	NS
Miscellaneous complications				
Pes planovalgus	*n* = 9; 29%	*n* = 13; 29%	1.000	NS
Port-a-cath infections	*n* = 3; 10%	*n* = 2; 4%	0.393	NS
Enamel defects	*n* = 1; 3%	*n* = 3; 7%	0.641	NS
Urolithiasis	*n* = 1; 3%	*n* = 1; 2%	1.000	NS
Gout	*n* = 0; 0%	*n* = 2; 4%	0.511	NS

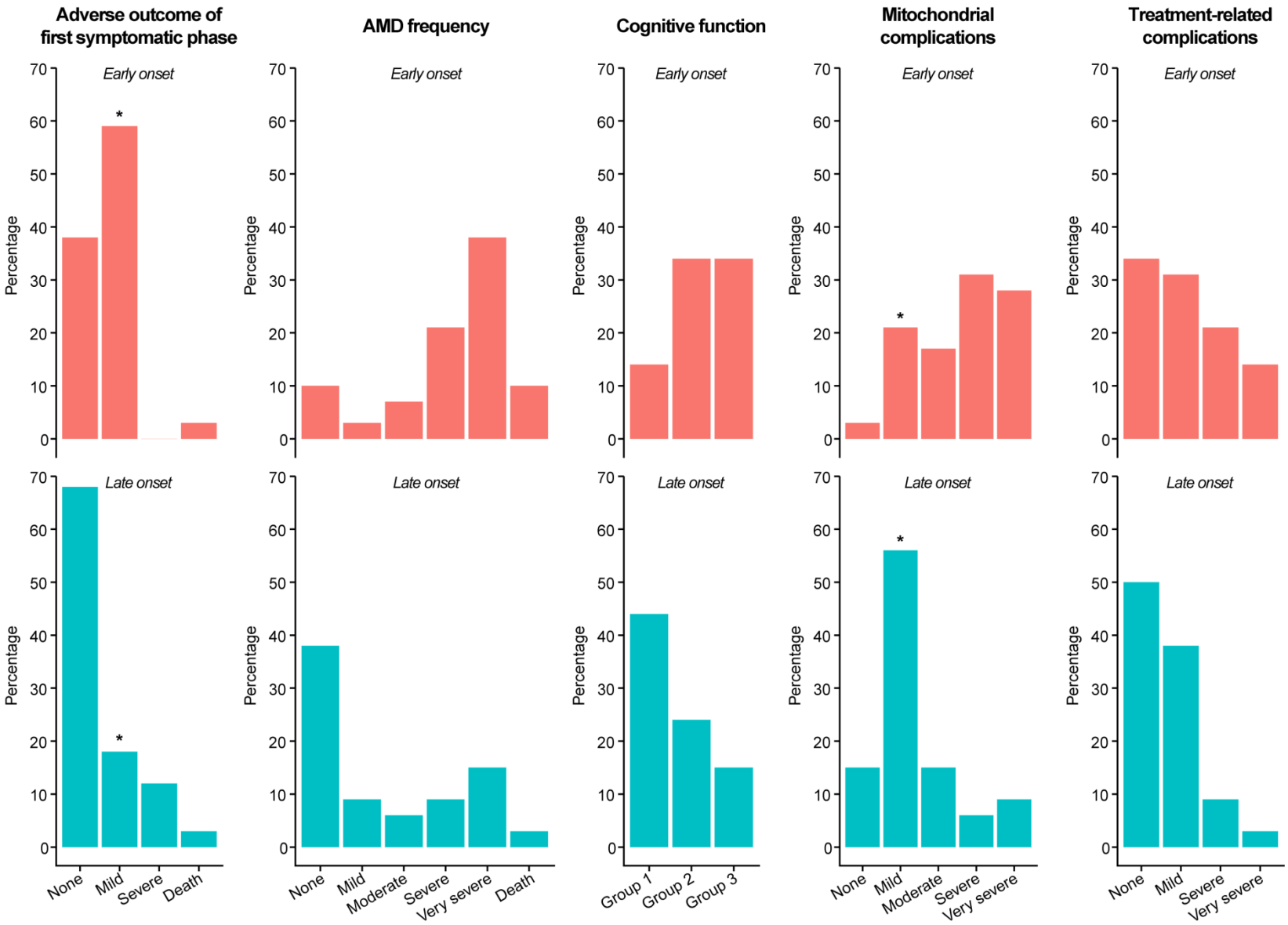
Notes: Statistical significance was determined by performing Fisher's exact tests. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant and are depicted in bold. Abbreviations: NS: not significant.

Supplementary Table 9 – Minimum requirements for follow-up of the post-NBS cohort

	Items to record	**Diagnostics to perform**
Patient characteristics		
Patient	Day of birth	
	Sex	
Family	Ancestry	
	Consanguinity	
Mutation	g. / c. / p. coding sequence	Genetic testing
Vitamin B12 responsiveness	Type enzymatic assay	Enzymatic assay B12 responsiveness
	Results enzymatic assay	
PCC activity	Type enzymatic assay	Enzymatic assay PCC activity
	Results enzymatic assay	
Death	Day of death	
	Cause of death	
Follow-up	Age at last follow-up	
Adverse outcome of first sympt. phase		
First presentation	Symptomatic/asymptomatic	
	Day of first symptoms	
	Day of diagnosis	
Adverse outcome first presentation	Day of brain MRI	Brain MRI at set times
	Brain MRI results	
	Movement disorder	Consult neurologist
AMD frequency		
Number of AMD	Day of admission	
	Day of release	
	Reason of admission	
Cognitive function		
Cognition	Day of neuropsychological tests	Neuropsychological tests at set times
	Neuropsychological test type	
	Neuropsychological test results; IQ	
Education	School career	
	Type of employment	
Mitochondrial complications		
Hepatomegaly	Day of diagnostic study	Liver ultrasound at set times
Epilepsy	Results of diagnostic study	EEG at set times
Cardiomyopathy	Presence complication yes/no	Cardiac ultrasound at set times
Prolonged QTc interval		ECG at set times
Optic atrophy		Consult ophthalmologist at set times
Renal failure		Urine kidney function biochemistry at set times
Pancreatitis		Complete blood count at set times
Sensorineural hearing loss		
Acute psychosis		On indication: consult ENT doctor, gastro-enterologist, neurologist, gynecologist, psychiatrist, physical therapist
Stroke-like episodes		
Premature ovarian insufficiency		
Exercise intolerance		
Muscular hypotonia		
Feeding problems		
Constipation		
Autism		
Attention deficit hyperactive disorder		
Anemia		
Leukopenia		
Thrombocytopenia		
Pancytopenia		
Treatment-related complications		
Bone mineral density	Day of diagnostic study	DEXA-scan at set times
Growth retardation	Results of diagnostic study	
Obesity	Presence complication yes/no	
	Visit date each visit	
	Weight and length each visit	

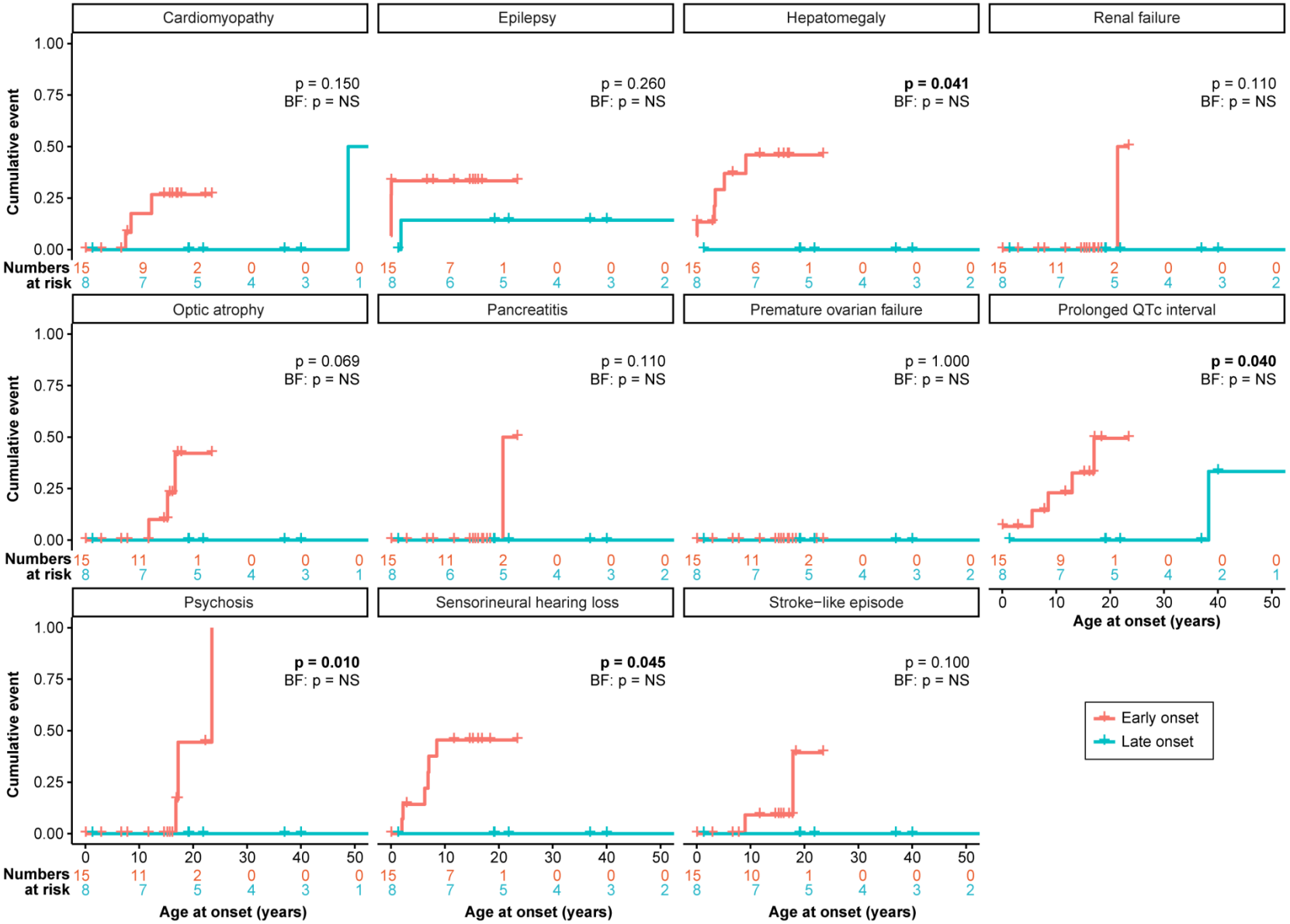
Abbreviations: Adverse outcome of first sympt. phase: Adverse outcome of the first symptomatic phase. DEXA: dual-energy X-ray absorptiometry; ECG: electrocardiogram; EEG: electroencephalogram; ENT: ear-nose-throat; MRI: magnetic resonance imaging; PCC: propionyl-CoA carboxylase; IQ: intelligence quotient. g./c./p. coding sequence: genetic change in the DNA coding sequence and resulting change in the protein coding sequence.

Figure S1 – Grouped outcome parameters according to presentation type



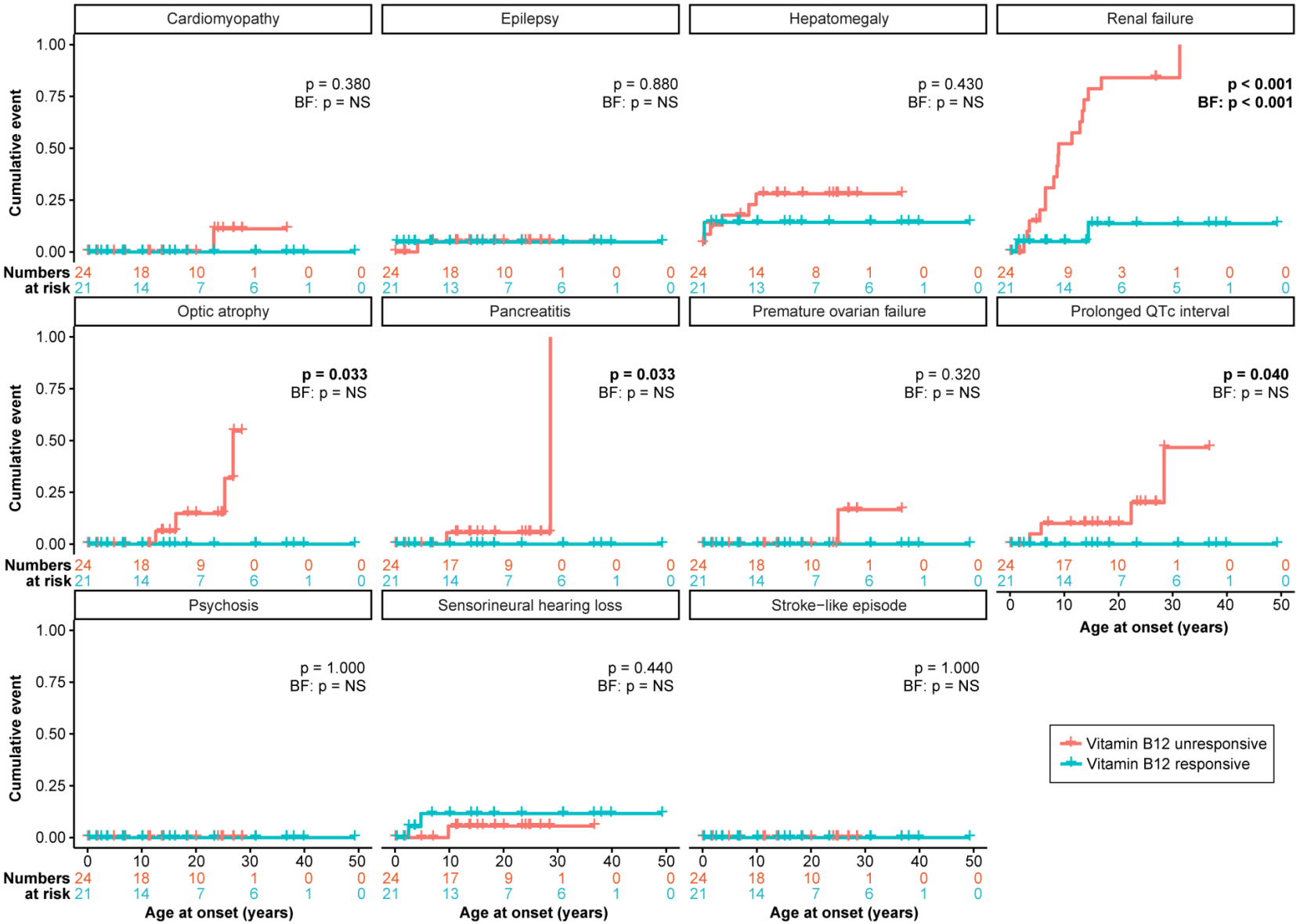
Visual representation of Table 2. Categories of the outcome parameters are depicted on the x-axis, percentages are depicted on the y-axis. Early onset (presentation ≤28 days of life) is depicted in orange, late onset (presentation >28 days of life) is depicted in blue. Statistical significance was determined by Fisher's exact tests. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < .05 were considered statistically significant and are depicted with bold asterisks, as in Table 2.

Supplementary Figure 2 – Early onset presentation in PA tends to be an independent predictor for four mitochondrial complications with acute onset



Kaplan-Meier plots wherein the y-axis depicts the cumulative percentage and the x-axis depicts patient age in years. The panels demonstrate the different mitochondrial complications with acute onset, for patients with late onset presentation in blue versus patients with early onset presentation in orange. Numbers at risk, indicating the number of patients at risk for a certain complication are depicted below the panels, in corresponding colors. Early onset: presentation ≤28 days of life; Late onset: presentation >28 days of life. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < 0.05 were considered statistically significant and are depicted in bold. Abbreviations: BF: Bonferroni; NS: not significant.

Supplementary Figure 3 – Vitamin B12 unresponsiveness in MMA tends to be an independent predictor for three mitochondrial complications with acute onset and is a significant predictor for the occurrence of renal failure



Kaplan-Meier plots wherein the y-axis depicts the cumulative percentage and the x-axis depicts patient age in years. The panels demonstrate the different mitochondrial complications with acute onset, for vitamin B12 responsive patients in blue versus vitamin B12 unresponsive patients in orange. Numbers at risk, indicating the number of patients at risk for a certain complication are depicted below the panels, in corresponding colors. All p-values were adjusted according to the Bonferroni method. Adjusted p-values < 0.05 were considered statistically significant and are depicted in bold. Abbreviations: BF: Bonferroni; NS: not significant.