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Maternal vitamin deficiency mimicking multiple acyl-CoA dehydrogenase deficiency on newborn screening

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ABSTRACT

Background: In infancy multiple acyl-CoA dehydrogenase deficiency (MADD) is commonly a severe inherited metabolic disease caused by genetic defects in electron transfer flavoprotein (ETF) or ETF ubiquinone oxidoreductase. Both enzymes require flavin adenine dinucleotide (FAD) as a cofactor. Riboflavin (vitamin B_2) is a precursor in the synthesis of FAD. MADD can be detected by newborn screening (NBS) based on elevation of multiple acylcarnitines.

Methods: We present the results of two children whose NBS results and subsequent confirmatory testing resulted in a suspected diagnosis of MADD. In parallel in both children vitamin B_{12} deficiency was detected.

Results: Biochemical profiles normalized rapidly in both children under supplementation with riboflavin. After extensive work-up of both cases including molecular genetic studies there was no indication of MADD. Vitamin B_{12} deficiency in both children was caused by maternal vitamin B_{12} deficiency and was rapidly corrected by oral supplementation with vitamin B_{12} or (partial) formula feeding. As both vitamin B_{12} and riboflavin have similar food sources we postulate that in these cases positive NBS for MADD was caused by combined maternal vitamin B deficiencies.

Conclusion: The differential diagnosis of maternally caused vitamin B deficiencies should be considered in children with abnormal NBS results for MADD, especially in the presence of normal molecular genetic analysis or in case of associated findings of other maternal vitamin B deficiencies like vitamin B_{12} or folic acid deficiency.

List of abbreviations

tHcy	Total homocysteine
MMA	Methylmalonic acid
MCA	Methylcitric acid
DBS	Dried blood spots
NBS	Newborn screening
MADD	Multiple acyl-CoA dehydrogenase deficiency

1. Introduction

Newborn screening (NBS) is a prerequisite for early diagnosis and treatment for many inborn errors of metabolism. It is an essential part of health care programmes in many countries worldwide [1]. As early treatment after presymptomatic diagnosis allows for normal development in the majority of children identified, NBS has developed into the most successful measure of secondary prevention in medicine [2,3]. Newborn screening panels differ considerably between countries [1]. The study "Newborn Screening 2020" at the Heidelberg Newborn Screening Center started in August 2016 to evaluate a possible extension of the German NBS panel [4], which at this time included 14 target disorders (twelve metabolic disorders and two endocrinopathies; compare supplementary table 1). This study assesses NBS for 26 additional target disorders (25 metabolic disorders and vitamin B_{12}-deficiency) under the application of second-tier strategies [5].

One of the target disorders included in the study is multiple acyl-coA dehydrogenase deficiency (MADD), for which NBS is performed based on elevation of multiple acylcarnitines (C4-C18). MADD (Glutaric aciduria type II, OMIM #231680) is commonly caused by mutations in two genes encoding the subunits of the electron transfer flavoprotein (ETF) (*ETFA* and *ETFB*), or in *ETFDH* which encodes ETF ubiquinone

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oxidoreductase (ETF:QO). Both enzymes, ETF and ETF:QO require FAD as a cofactor. Riboflavin (vitamin B_2) is a precursor in the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The clinical spectrum of MADD is broad including severe neonatal onset with or without congenital anomalies (type I and type II) and late onset forms (type III) [6].

One aim of the study NBS 2020 was to implement and evaluate a systematic screening strategy for vitamin B_{12} deficiency, using a combination of two second-tier strategies. The screening algorithms developed for this study including second-tier strategies for vitamin B_{12} deficiency and cut-offs for all parameters have been recently published in detail [5].

Here we present the results of two children whose initial NBS results and confirmatory testing resulted in a suspected diagnosis of MADD. In addition in both children also vitamin B_{12} deficiency was detected by the NBS pilot study. After extensive work-up of both cases including molecular genetic studies there was no indication of genetic causes of MADD. Results of a third child with functional vitamin B_{12} deficiency are presented, who also showed a slight MADD pattern on first NBS.

We postulate that in these newborns false-positive NBS for MADD was caused by maternal vitamin deficiencies. This observation is of relevance for all NBS programs targeting MADD or vitamin B_{12} deficiency, and the differential diagnosis of maternally caused vitamin deficiencies should be considered in children with abnormal NBS results.

2. Patients and methods

In a prospective single-centre study initiated in August 2016 an extension of the German NBS panel (14 disorders at study initiation, supplementary table 1) by additional 26 conditions (25 metabolic disorders and vitamin B_{12} deficiency, supplementary table 2) is evaluated at the Heidelberg NBS Centre. The aim of the study is to evaluate 1.) technical feasibility of population-based NBS for these disorders 2.) benefit for detected patients, based on their presymptomatic identification.

The Heidelberg NBS Centre performs screening for about 140,000 newborns per year, mainly from South-West Germany, equalling about 17% of children born in Germany. NBS samples in Germany are to be taken between the 36th and 72nd hour of life. NBS was performed including electrospray-ionization tandem-MS (Waters Xevo TQD, Waters, Milford, USA) for determination of amino acids and acylcarnitines [7]. From August 2016 on all hospitals, physicians, and midwives sending samples for regular NBS to the Heidelberg NBS Centre were asked to offer participation in the study free of charge to newborns born at their institution. With parents' written informed consent screening for the additional conditions was performed from the same specimen as regular NBS (Whatman filter paper 903, GE Healthcare, Freiburg, Germany).

For 15 of the studies target conditions abnormal first-tier results are complemented by second-tier testing from the same DBS. One second-tier method analyses MMA, 3-OH-PA, and MCA [8], based on abnormal first-tier results for C3 and C3/C2 (C3 + C3/C2 > cut-off, or C3/C2 > cut-off, or C3 > alarm limit). The second method analyses tHcy [9] after an abnormal first-tier result for Met (< cut-off low) or Met/Phe (< cut-off low or > cut-off high). Patients with vitamin B_{12} deficiency can be detected by elevated tHcy, elevated MMA/MCA, or a combination of both. In cases of suspicion of vitamin B_{12} deficiency, depending on the grade of pathology in the first DBS, it is recommended to either send a repeat DBS for tandem-MS screening and analysis of all second-tier parameters initially out of range, or to also send plasma, serum, and urine samples for additional analyses. In all cases also work-up of the mother's vitamin B_{12} status including functional markers was recommended. Diagnosis of "vitamin B_{12} deficiency" was established in cases with elevation of one or more functional markers of vitamin B_{12} deficiency in confirmatory testing (MMA in plasma and/or urine, homocysteine in plasma), in the presence of vitamin B_{12} serum levels below the norm, and of "functional vitamin B_{12} deficiency" in cases with elevation of one or more functional markers of vitamin B_{12} deficiency and vitamin B_{12} in the (low) normal range [10].

Screening for MADD is performed based on elevation of multiple acylcarnitines (C4-C18). In case of abnormal results in the first NBS sample suggestive of MADD a second DBS sample is requested together with a urine sample for analysis of organic acids in urine. In cases with highly abnormal profiles the child will be immediately referred to a metabolic centre for further diagnostics and treatment. For final confirmation or exclusion of MADD molecular genetic analysis (*ETFA, ETFB, ETFDH*) is recommended [6].

The study "Newborn Screening 2020" has been approved by the ethics committee of the University Hospital Heidelberg (Number S-533/2015). Written informed consent was obtained from parents before participation of children in the study.

3. Results

3.1. Patient 1

Patient 1 is the first child of non-consanguinous German parents. Pregnancy had been complicated by a maternal HELLP-syndrome (Hemolysis, Elevated Liver enzymes, Low Platelet count) leading to delivery via primary cesarian section at 37 + 1 weeks of gestation (birth weight 2800 g, length 48 cm, head circumference 34 cm). Newborn screening (sampled at 62 h) showed an acylcarnitine profile raising suspicion of MADD (for details compare Table 1). At the time the NBS result was reported the child was still in inpatient treatment at a children's hospital. Laboratory investigations, including blood gas analysis, creatine kinase (CK), ammonia, and lactate revealed normal results. Results of confirmatory testing (repeat sample for NBS, organic acid analysis in urine) are shown in Tables 1 and 2. As the results were compatible with MADD the child was referred to a metabolic center. Treatment was started with riboflavin (2 × 100 mg/d orally). Cerebral ultrasound and echocardiography were normal, renal ultrasound was unremarkable except for renomegaly.

In addition, NBS in the context of the study NBS 2020 showed decreased levels of methionine (9 μmol/l, Cut-off low 11) and ratio Met/Phe (0.21, Cut-off low 0.26). According to the second-tier algorithms of the study [5,11] this led to analysis of tHcy in DBS. tHcy was elevated with 21.8 μmol/l (N < 12) in the first NBS specimen, and with 17.3 μmol/l in the repeat specimen (compare Table 1). Therefore, further work-up concerning vitamin B_{12} status in child and mother was initiated and revealed vitamin B_{12} deficiency in mother and child (Table 2).

Treatment was started orally with vitamin B_{12} (0.5 mg/d for 3 days, followed by 0.1 mg/d until normalization of laboratory parameters including functional markers of vitamin B_{12} deficiency) and folic acid (0.4 mg/d for 1 week) according to a supplementation scheme developed for the study NBS 2020 [5,12,13].

Metabolic reinvestigations at age 8 weeks under therapy with riboflavin and vitamin B_{12} and feeding with infant formula (800 ml per day, containing 0.8 μg vitamin B_{12} and 0.8 μg vitamin B_2 per day) showed complete normalization of organic acids in urine and acylcarnitines in DBS. Follow-up measurements concerning vitamin B_{12} status under supplementation showed normalization of homocysteine in plasma age 3 weeks (7.5 μmol/l, N < 15), and of vitamin B_{12} (573 pmol/l, N 160–670), folic acid (41.2 nmol/l, N 4.5–21), and homocysteine in plasma (11 μmol/l, N 2–14) at age 18 weeks. Metabolic profiles stayed normal at reinvestigation of organic acids in urine age 4 months, and acylcarnitines in DBS age 4, 6, and 9 months.

For the mother a balanced diet including meat was reported. The pregnancy had remained unnoticed by the mother for a long time and no vitamin supplementation (neither folic acid nor other B vitamin preparations) had been taken throughout pregnancy. The mother suffered from obesity and was treated with thyroid hormones throughout pregnancy. Pregnancy had been complicated by a HELLP-syndrome leading

Table 1
Newborn screening results of patients' first and second NBS samples.

	Patient 1		Patient 2		Cut-off	Patient 3		Cut-off*
Age at sampling (hours)	62	175	49	176		49	1057 = 44 days	
NBS Markers in DBS								
C0	11	12	25	21	6–65	52	37	6–78.4
C3	1.42	0.64	2.27	1.21	5.5	3.15	1.21	5.5
C5	0.36	0.26	0.61	0.28	0.63	0.19	0.13	0.63
C6	0.23	**0.29**	**0.42**	**0.55**	0.24	0.12	0.07	0.12
C8	**0.34**	**0.39**	**0.38**	**1.0**	0.28	**0.18**	0.06	0.15
C10:1	**0.39**	**0.32**	**0.38**	**0.68**	0.3	**0.14**	0.06	0.07
C10	**0.36**	**0.42**	**0.5**	**1.12**	0.3	0.25	0.09	0.31
Glut	**0.43**	**0.49**	**0.48**	0.32	0.37	**0.53**	0.14	0.46
C12	**0.71**	**0.63**	**1.02**	**1.05**	0.44	0.29	0.08	0.34
C14:1	**1.07**	**1.18**	**0.98**	**1.11**	0.41	0.19	0.04	0.30
C14	**0.8**	**0.78**	**0.75**	**0.87**	0.43	**0.49**	0.14	0.41
C3/C2	0.1	0.06	0.09	0.06	0.22	0.03	0.07	0.22
C8/C2	**0.024**	**0.035**	**0.016**	**0.048**	0.01	0.002	0.004	0.01
C5/C2	**0.03**	**0.03**	**0.03**	0.01	0.02	0	0.009	0.03
C14:1/C4	**2.38**	**3.03**	1.75	**2.47**	2.14	0.34	0.27	1.64
Met	**9**	**9**	14	**10**	11–35	22	21	11–35
Met/Phe	**0.21**	0.26	**0.25**	0.27	0.26–0.56	0.38	**0.87**	0.26–0.56
tHcy	**21.8**	**17.3**	**18.7**	**19.3**	12	8.8	**19.7**	12
MMA	1.75	1.80	0.86	1.21	2.35	0.67	0.84	2.35
MCA	0.06	0.04	0.13	0.16	0.34	0	0.11	0.34

Out of range results are marked in bold.
Cx = respective chain-length of acylcarnitines; tHcy = Total homocysteine; MMA = Methylmalonic acid; MCA = Methylcitric acid; DBS = Dried blood spots; NBS = Newborn screening.
* At the time NBS for patient 3 was performed cut-offs for some parameters in NBS had changed following method adaptation.

Table 2
Results of confirmatory diagnostics in patients and mothers.

Laboratory investigations	**Patient 1**	**Mother 1**	**Patient 2**	**Mother 2**	**Patient 3**	**Mother 3**	**Normal range**
Organic acids in urine							mmol/mol creatinine
Methylmalonic acid	9	2	12	3	**27**	N/A	0–18
Methylcitric acid	6	1	7	2	**23**	N/A	0–9
Ethylmalonic acid	**103**	7	**76**	5	14	N/A	0–19
2-OH-Glutaric acid	**249**	12	**207**	9	**36**	N/A	0–30
3-OH-Glutaric acid	**68**	6	**35**	3	5	N/A	0–8
Glutaric acid	**451**	0	**198**	0	5	N/A	0–8
Adipic acid	**461**	6	**759**	3	12	N/A	0–30
Suberic acid	**298**	1	**217**	2	5	N/A	0–10
Sebacic acid	2	0	**37**	0	2	N/A	0–4
Hexanoylglycine	**10**	1	**17**	1	1	N/A	0–2
Suberylglycine	**3**	0	**7**	0	0	N/A	0–1
2-OH-Sebacic acid	**9**	0	**8**	0	3	N/A	0–3
3-OH-Sebacic acid	**67**	0	**39**	0	6	N/A	0–14
Vitamin B_{12} (S)	**146**	**89**	185	327	164	345	160–670 pmol/l
Holo-Transcobalamin (S)	N/A	N/A	**28**	N/A	N/A	N/A	35–108 pmol/l
Folic acid (S)	N/A	**<3.4**	mv	12	**34**	**2.9** ng/ml ($N > 5.4$)	4,5–21 nmol/l
Hcy (P)	**18**	**21**	**31**	**26**	**27**	**15**	2–14 μmol/l
Methylmalonic acid (P; MMA-I)	N/A	N/A	**0.6**	0.22	**0.74**	0.21	0–0.26 μmol/l
Methylmalonic acid (U; MMA-I)	N/A	1.9	3.4	2.1	**16.1**	N/A	0–10 mmol/mol creatinine

Out of range results are marked in bold.
P = Plasma, U = Urine; mv = missing value; MMA-I = Methylmalonic acid quantification using stable-isotope labelled D3-MMA as internal standard.

to delivery via primary cesarian section at 37 + 1 weeks of gestation. Due to severe pancytopenia (WBC 2.58/nl, Hb 5.7 g/dl; MCV 89.9 fl; MCH 32.9 pg; platelets 87/nl) detected at delivery a bone marrow puncture was performed in the mother after delivery to rule out hematological disorders of erythropoesis. This revealed a picture compatible with severe deficiency of vitamin B_{12} and folic acid, which was confirmed by laboratory work-up (Table 2). Further diagnostics by internal medicine revealed no indication of gastro-intestinal malabsorption for vitamin B_{12}. The mother required two transfusions of red blood cells after delivery, and was treated with vitamin B_{12} parenterally and folic acid orally. This led to rapid normalization of vitamin B_{12} (>1476 pmol/l, N 160–670) and folic acid (9 nmol/l, N 4.5–21) status in the mother.

3.2. Patient 2

Patient 2 is the second child of non-consanguinous German parents born at 39 weeks of gestation after uneventful pregnancy (birth weight 3225 g, length 48 cm, head circumference 34 cm, APGAR 9/10/10, umbilical cord pH 7.40). The patient's brother (2 years and 11 months older) is reported to be healthy. Newborn screening (sampled at 49 h) revealed an acylcarnitine profile raising suspicion of MADD (for details compare Table 1). Consecutively the child was immediately referred to a children's hospital for laboratory investigations (including blood gas analysis, CK, ammonia, lactate) and initiation of confirmatory testing (repeat sample for NBS, organic acid analysis in urine). Treatment was started with riboflavin (2 × 100 mg/d orally).

In addition, NBS in the context of the study NBS 2020 showed a

decreased Met/Phe ratio (0.25, Cut-off low 0.26). According to the second-tier algorithms of the study [5,11] this led to analysis of tHcy in DBS. tHcy was elevated with 18.7 μmol/l ($N < 12$) in the first NBS specimen, and with 19.3 μmol/l in the repeat specimen (compare Table 1). Therefore, further work-up concerning vitamin B_{12} status in child and mother was initiated and revealed decreased holotranscobalamin in the child and elevated homocysteine in plasma in both mother and child compatible with functional vitamin B_{12} deficiency (compare Table 2).

Metabolic reinvestigations under riboflavin therapy and feeding with infant formula containing vitamin B_{12} (350 ml per day, containing 0.7 μg vitamin B_{12} and 0.5 μg vitamin B_2 per day) in addition to breast milk feeding showed complete normalization of organic acids in urine at age 2 weeks and acylcarnitines in DBS at age 7 weeks. Methylmalonic acid in urine stayed normal with 16 mmol/mol creatinine ($N < 18$) at 2 weeks and homocysteine in plasma normalized (13.1 μmol/l, N 2–14), documenting normalization of vitamin B_{12}-status. No additional supplementation with vitamin B_{12} was performed.

Before and during pregnancy, the mother kept a vegetarian diet. Due to hypothyroidism she was treated with thyroid hormones throughout pregnancy.

Riboflavin status was not evaluated in patients or mothers, as this investigation is only available in specialized laboratories.

In one additional case (patient 3), also with functional vitamin B_{12} deficiency detected by NBS, the first NBS sample showed a slight MADD profile. In this child, the acylcarnitine profile normalized on the second DBS and no further confirmatory testing for MADD was indicated also based on unremarkable results of organic acids in urine with regard to MADD. Functional vitamin B_{12} deficiency in this fully breastfed child was corrected with oral vitamin B_{12} supplementation (0.5 mg/d for 3 days, followed by 0.1 mg/d) and folic acid (0.4 mg/d for 1 week) according to a supplementation scheme developed for the study NBS 2020 [5,12]. In the mother folic acid deficiency was diagnosed. The mother of this child was reported to consume meat regularly but had not taken any vitamin supplementation (folic acid or other B-vitamins) during pregnancy.

3.3. Molecular genetic analysis

In both patients 1 and 2 molecular genetic analysis of the most common genetic causes of MADD including analysis of the genes *ETFDH, ETFA, ETFB, FLAD1* (Flavin Adenine Dinucleotide Synthetase 1), and *RFK* (Riboflavin kinase) did not reveal any disease causing variants. Also an additional extended panel analysis including rarer causes of MADD led to normal results. Therefore specific metabolic treatment including riboflavin and recommendations for obligatory frequent feedings was stopped.

3.4. Clinical outcome

Both children showed normal clinical status and normal development for age at last follow-up aged 1 1/2 year and 2 years, respectively.

4. Discussion

MADD is an inborn error of metabolism caused by a defect in ETF or ETF:QO. Both enzymes, ETF and ETF:QO require FAD as a cofactor. Riboflavin is a precursor in the synthesis of FMN and FAD. Therefore riboflavin is an essential part of treatment in MADD [6]. Here we present two children with suspected MADD after abnormal NBS and biochemical confirmatory testing consistent with MADD. Metabolic profiles normalized completely under supplementation with riboflavin, and remained unsuspicious thereafter. After extensive work-up of both cases including extended molecular genetic studies there was no indication of MADD. In addition, in both children NBS had also led to the diagnosis of vitamin B_{12} deficiency, which was of maternal origin in both cases. Vitamin B_{12} status was rapidly corrected by oral supplementation or (partial) formula feeding, respectively. We postulate that in both cases positive NBS for MADD was caused by nutritional maternal B vitamin deficiencies.

Chiong [14], Ho and colleagues [15] previously reported a newborn with clinical and biochemical features of MADD, rapidly corrected by riboflavin supplementation. In this case the mother was found to be persistently riboflavin deficient, which was later explained by a genetic defect in the riboflavin transporter gene *GPR172B* in the mother [15]. Profound riboflavin deficiency in pregnancy has been reported to be associated with adverse pregnancy outcomes, which can be prevented by riboflavin therapy in the mother [16].

In our patients presented here with confirmed (functional) vitamin B_{12} (and folic acid) deficiency in both mothers and their newborns we postulate that false–positive NBS for MADD was caused by nutritional vitamin B deficiencies also affecting the riboflavin status. This is supported by the fact, that biochemical profiles of MADD in both children were rapidly and persistently restored by supplementation of riboflavin and stayed normal after this supplementation had been stopped.

In our patients vitamin B_{12} deficiency was detected due to elevated tHcy in second-tier based NBS. Vitamin B_{12} is an essential cofactor for the enzymes methionine synthase and methylmalonyl-CoA mutase. Metabolic changes due to vitamin B_{12} deficiency result from dysfunction of these enzymes, leading to increased homocysteine and/or methylmalonic acid. The enzyme methionine synthase, which converts homocysteine to methionine, requires 5-methyltetrahydrofolate as a methyl donor, but also vitamin B_{12} as methylcobalamin. It has been reported that the synthesis of methylcobalamin appears to be dependent on flavoproteins, suggesting a link between riboflavin and vitamin B_{12} metabolism [17]. Riboflavin status was reported as being a modulator of plasma homocysteine concentrations [17]. Therefore in addition to vitamin B_{12} deficiency also riboflavin deficiency – explaining transient MADD in our patients 1 and 2 - may have contributed to elevated tHcy in these patients' NBS samples.

Both vitamin B_{12} and riboflavin are mainly included in meat, fish, and dairy foods. Therefore it is plausible that pregnant women with nutritional vitamin B_{12} deficiency are also prone to have other coexisting vitamin B deficiencies including riboflavin. Riboflavin deficiency is endemic in populations consuming little milk or meat products [17]. In women of childbearing age and pregnant women worldwide vitamin B_{12} deficiency has been reported with frequencies of 10%–50% [13].

In mothers 1 and 2 not all aspects of laboratory work-up fit the classical picture of vitamin B_{12} deficiency. This could partly be explained by the time of sampling. Urine sample in mother 1 was obtained after treatment with vitamin B_{12}, possibly explaining normalized MMA. The absence of macrozytosis has been described also in severe vitamin B_{12} deficiency [18] and could theoretically also be explained by combination with e.g. iron deficiency masking hematological changes of vitamin B_{12} deficiency [19]. However, in mother 1 there was no indication of iron deficiency. Findings in mother 2 could be explained by functional vitamin B_{12} deficiency and riboflavin deficiency due to a vegetarian diet. In similar cases in the future, it would be highly desirable to also quantitatively measure riboflavin in both mother and child.

Extensive work-up of affected mothers with nutritional vitamin B_{12} deficiency detected by our NBS pilot study showed that vitamin B_{12} deficiency was frequently caused by feeding difficulties in pregnancy in combination with a lack of vitamin supplementation [5]. Despite a general recommendation in Germany [20] and many other countries to start folic acid supplementation pre-conceptionally - with many preparations containing also vitamin B_{12} or multiple other B vitamins - 59% of affected women in our study had not taken any vitamin supplementation at all [5]. This is consistent with previous national surveys in Germany [21] and a report from Switzerland [22]. In other countries, the adherence to prenatal B-vitamin supplementation is much higher and has been reported with 93% in a Canadian study [23]. Prenatal vitamin supplementation should be encouraged in prenatal care and increased

vigilance for vitamin deficiencies is indicated especially in pregnant women with feeding difficulties. The differential diagnosis of maternally caused vitamin deficiencies should be considered in children with abnormal NBS results. The observations reported here are of relevance for all NBS programs targeting MADD and/or vitamin B_{12}-deficiency.

5. Conclusion

The differential diagnosis of maternally caused vitamin deficiencies should be considered in children with abnormal NBS results for MADD, especially in the presence of normal molecular genetic analysis or in case of associated findings of other maternal B vitamin deficiencies like vitamin B_{12} or folic acid deficiency.

Author statement

All relevant data is already included in the manuscript, therefore no additional raw data is provided.

The research protocol used in this study was approved by the ethics committee of the University Hospital Heidelberg (Number S-533/2015).

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The authors have no financial relationships relevant to this article to disclose.

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Role of the funding source

The funder of the study had no involvement in the study design, the collection, analysis, and interpretation of data, the writing of the report, and the decision to submit the manuscript for publication.

Contributors’ statement

G. Gramer: design of the study “Newborn screening 2020” at the Heidelberg Newborn Screening Center, performance of newborn screening, collection, evaluation and interpretation of data; drafting and writing the manuscript.

G. F. Hoffmann: design of the study “Newborn screening 2020” at the Heidelberg Newborn Screening Center, evaluation and interpretation of data; revision of the manuscript.

J. B. Hennermann: treatment of patients 1 and 2, confirmatory diagnostics, collection, evaluation and interpretation of data; writing and revising the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2021.100738.

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