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ORIGINAL ARTICLE

A scoring system predicting the clinical course of CLPB defect based on the foetal and neonatal presentation of 31 patients

Ewa Pronicka[1,2] · Mariola Ropacka-Lesiak[3] · Joanna Trubicka[1] · Magdalena Pajdowska[4] · Markus Linke[5] · Elsebet Ostergaard[6] · Carol Saunders[7,8] · Sandra Horsch[9] · Clara van Karnebeek[10] · Joy Yaplito-Lee[11] · Felix Distelmaier[12] · Katrin Õunap[13,14] · Shamima Rahman[15] · Martin Castelle[16] · John Kelleher[17] · Safa Baris[18] · Katarzyna Iwanicka-Pronicka[19] · Colin G. Steward[20,21] · Elżbieta Ciara[1] · Saskia B. Wortmann[22,23,24] · Additional individual contributors[1,2,25,26,27,28,29]

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Abstract Recently, CLPB deficiency has been shown to cause a genetic syndrome with cataracts, neutropenia, and 3-methylglutaconic aciduria. Surprisingly, the neurological presentation ranges from completely unaffected to patients with virtual absence of development. Muscular hypo- and hypertonia, movement disorder and progressive brain atrophy are frequently reported. We present the foetal, peri- and neonatal features of 31 patients, of which five are previously unreported, using a newly developed clinical severity scoring system rating the clinical, metabolic, imaging and other findings weighted by the age of onset. Our data are illustrated by foetal and neonatal videos. The patients were classified as having a

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✉ Saskia B. Wortmann
s.wortmann-hagemann@salk.at

[1] Department of Medical Genetics, Children's Memorial Health Institute, Warsaw, Poland

[2] Department of Pediatrics, Nutrition and Metabolic Diseases, Children's Memorial Health Institute, Warsaw, Poland

[3] Department of Perinatology and Gynaecology, University of Medical Sciences, Poznań, Poland

[4] Department of Biochemistry and Experimental Medicine, Children's Memorial Health Institute, Warsaw, Poland

[5] Department of Neonatology, DRK Children's Hospital Siegen, Siegen, Germany

[6] Department of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark

[7] Center for Pediatric Genomic Medicine, Children's Mercy Hospital, Kansas City, MO 64108, USA

[8] Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, Kansas City, MO 64108, USA

[9] Department of Neonatology, Helios Klinikum, Berlin-Buch, Germany

[10] Division of Biochemical Diseases, Department of Pediatrics, B.C. Children's Hospital, Treatable Intellectual Disability Endeavour, Vancouver, BC V6H 3N4, Canada

[11] Department of Metabolic Medicine, Murdoch Childrens Research Institute, The Royal Children's Hospital Melbourne, Parkville, VIC 3052, Australia

[12] Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, Heinrich-Heine University, Moorenstr. 5, 40225 Duesseldorf, Germany

[13] Department of Genetics, United Laboratories, Tartu University Hospital, 51014 Tartu, Estonia

[14] Department of Pediatrics, Institute of Clinical Medicine, University of Tartu, 51014 Tartu, Estonia

[15] UCL Institute of Child Health, London WC1N 1EH, UK

[16] Department of Hemato-Immunology, Hospital Necker-Enfants malades, Paris, France

[17] Department of Neonatology, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland

[18] Division of Pediatric Allergy/Immunology, Marmara University, Istanbul, Turkey

[19] Department of Audiology and Phoniatrics, Children's Memorial Health Institute, Warsaw, Poland

mild (n = 4), moderate (n = 13) or severe (n = 14) disease phenotype. The most striking feature of the severe subtype was the neonatal absence of voluntary movements in combination with ventilator dependency and hyperexcitability. The foetal and neonatal presentation mirrored the course of disease with respect to survival (current median age 17.5 years in the mild group, median age of death 35 days in the severe group), severity and age of onset of all findings evaluated. CLPB deficiency should be considered in neonates with absence of voluntary movements, respiratory insufficiency and swallowing problems, especially if associated with 3-methylglutaconic aciduria, neutropenia and cataracts. Being an important differential diagnosis of hyperekplexia (exaggerated startle responses), we advise performing urinary organic acid analysis, blood cell counts and ophthalmological examination in these patients. The neonatal presentation of CLPB deficiency predicts the course of disease in later life, which is extremely important for counselling.

Keywords Hyperekplexia · Prenatal seizures · Prenatal movement disorder · 3-methylglutaconic aciduria · Cataracts · Neutropenia

Introduction

Recently autosomal recessive mutations in *CLPB* have been shown to cause a genetic syndrome with a broad phenotypic spectrum (MIM #616254) (Wortmann et al 2015; Saunders et al 2015; Kanabus et al 2015; Capo-Chichi et al 2015). The neurological presentation ranges from normal development without intellectual deficits to a severe and progressive encephalopathy associated with muscular hypertonia, progressive brain atrophy and movement disorder. Additionally, cataracts, neutropenia, infections and leukaemia are reported. All patients share an elevated urinary excretion of 3-methylglutaconic acid (3-MGA) as a characteristic biomarker.

CLPB encodes caseinolytic peptidase B homologue ClpB, a member of the AAA+ protein family with mitochondrial localization (Wortmann et al 2015). The exact function of human ClpB remains elusive, although the bacterial homologue acts as a chaperone involved in disaggregation of misfolded proteins (Rosenzweig et al 2013)[5]. In two independent zebrafish models of CLPB defects, neurological features (cerebellar hypoplasia, abnormal touch-evoked response with increased swim velocity and tail beat frequency) reflect the human phenotype (Wortmann et al 2015; Capo-Chichi et al 2015).

In this paper we present the foetal and neonatal data of 31 CLPB deficient patients and a clinical scoring system that can predict the further disease course based on these data.

Subjects and methods

Patients and data collection

Both the data of the previously unreported (P16, P17, P24, P25 and P31) as well as of the previously reported individuals (P1, 2, 5, 6, 7, 9, 10, 11, 18–23 are (Wortmann et al 2015) individuals 1–14; P3, 4 are (Kanabus et al 2015) patients 1, 2; P12–15, 26 are (Saunders et al 2015) subjects 1–5; P8 is (Kiykim et al 2016)) were collected via an anonymized online questionnaire completed by the respective physicians of the patients. The data of the patients P 27–30 (individuals II-1 – II-IV, Capo-Chichi et al 2015) were extracted from the literature. During the work on the manuscript one additional patient was diagnosed who is mentioned briefly in the discussion. Informed consent was obtained from the parents or guardians of the children serving as subjects of the investigation. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Clinical severity scoring

For the following signs and symptoms previously reported in CLPB deficient patients, 2 points were assigned for neonatal onset and 1 point for onset in later life: cataracts, neutropenia, 3-MGA-uria, altered muscle tone (hyper- or hypotonia), movement disorder (dystonia, tremor, ataxia etc.), seizures and brain atrophy on MRI or autopsy. For any foetal problem, or any APGAR score ≤ 5, 1 point was given. Additionally, the age at

[20] School of Cellular & Molecular Medicine, Medical Sciences Building, University of Bristol, Bristol, UK

[21] Department of Haematology, Oncology and BMT, Royal Hospital for Children, Bristol, UK

[22] Department of Pediatrics, Salzburger Landeskliniken and Paracelsus Medical University, Müllner-Hauptstraße 48, 5020 Salzburg, Austria

[23] Institute of Human Genetics, Technical University Munich, Munich, Germany

[24] Institute of Human Genetics, Helmholtz Zentrum Munich, Neuherberg, Germany

[25] Department of Neonatology Polish Mother's Memorial Hospital Research Institute, Łódź, Poland

[26] Department of Neonatology, University of Medical Sciences, Poznań, Poland

[27] Department of Neonatology, Medical University of Silesia, Zabrze, Poland

[28] Department of Clinical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland

[29] School of Medicine and Medical Science, University College Dublin, Dublin, Ireland

death was rated (10 points = neonatal death, 5 points = death later in life). Intellectual disability/developmental delay was rated as follows: 0 = no, 1 = mild, 2 = moderate, 3 = severe. The maximal score achievable was 28 points. Patients were designated as having a mild (clinical score < 5), moderate (5–15) or a severe (> 15) phenotype. The diagnostic criteria for bedside use can be found as Suppl. Table 1.

Foetal video ultrasound

The ultrasound videos of P23 were performed with (Voluson E8, General Electric Healthcare, Europe). Foetal activity and muscle tone were evaluated using the respective parameters of the biophysical profile during 20 min' observation (Baskett et al 1987). Normal foetal activity/gross body movements includes at least two movements of the torso or limbs. A normal foetal muscle tone is defined by at least one episode of active bending and straightening of the limbs or trunk. Slow extension with return to partial flexion, limb movement in full extension, absent foetal movement or partially open foetal hand indicate decreased foetal activity and muscle tone. The presence of an abnormal biophysical variable implies significant central nervous system hypoxemia at the time of testing.

Genetic investigations

The alterations in *CLPB* (RefSeq NM_030813.3*)* in the previously unreported individuals were identified by exome sequencing followed by Sanger sequencing (P17) or directly by Sanger sequencing (P16, 24, 25, 31) using standard procedures as described previously (Wortmann et al 2015; Saunders et al 2015; Kanabus et al 2015). Alamut® Visual Software and the ExAC Browser (http://exac.broadinstitute.org/*)* were used to interpret the variants found.

Results

Clinical details of all patients

The detailed case reports of the previously unreported (P16, 17, 24, 25, 31) patients can be found in the Supplemental data. Supplementary Video 1 shows P16 shortly after birth and supplementary Videos 2 and 3 show P23 as a foetus. Further details are given below.

Clinical severity scoring (Table 1)

Four patients were classified as mild (mean clinical severity score 3.5, range 3–4), 13 as moderate (mean score 10 range 5–14) and 14 as having a severe phenotype (mean score 21, range 17–26). The current median age (or age at death if deceased) was 17.5 years (range 9–25 years, all alive) in the mild group, 3.0 years (0.9–20 years, seven alive, six deceased) years in the moderate group and 35 days (1 day − 3.8 years, all deceased) in the severe phenotype (Fig. 1).

General pre- and perinatal findings (Table 2)

No prenatal abnormalities were reported in association with the mild phenotype. Conception difficulties were reported in 17% of the moderate and 40% of the severe subgroup. Polyhydramnios and foetal contractures were only reported in relation with the severe phenotype (50% and 30% respectively). IUGR was noted in 45% of all cases (23% of moderate, 79% of severe cases). The mothers of 14% of all cases (8% of the moderately affected and 18% of the severely affected patients) reported increased foetal movements. Decreased movements were reported for 14% of all cases (moderate 8%, severe 27%).

A low birth weight (< p3) was noted in 20% of all and 29% of the severely affected patients. Microcephaly (< p3) was seen only in the severely affected subgroup (43%). Low APGAR scores (≤ 5) were also seen predominantly in the severe subgroup.

Neonatal features (Table 2)

The following features were most frequently reported in the neonatal period: 3-MGA-uria, neutropenia and cataracts (in 74%, 52% and 28% of all patients and in 100%, 92% and 44% of the severly affected patients, respectively).

The most frequent neonatal neurological findings in all patients were swallowing problems (59%), burst suppression on EEG (56%), abnormalities on neonatal brain MRI (56%) and respiratory insufficiency (45%). The clinical presentation shows overlap with hyperekplexia, especially in the severe subgroup: excessive startle reflex followed by a period of stiffness where voluntary movements are impossible were seen in 44% of individuals. Most striking in the severe subgroup is that all patients show an absence of voluntary movements. This is mostly (86%) combined with generalized muscular hypertonia, some patients show contractures at birth, and in 79% of patients involuntary movements, ranging from limb tremor via jitteriness to dystonia often upon tactile or acoustic stimuli are reported. Additionally, unresponsiveness to pain was reported in 56% of the severely affected patients.

Foetal video ultrasound

During the biophysical profile assessment of P23 at 36 weeks gestation, no spontaneous foetal movements were seen during 20 min' observation. The muscle tone was abnormal with constantly bent joints of the upper and lower limbs, clenched

Table 1 Clinical severity scoring in CLPB deficient patients

	Mild				Moderate												
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
Foetal problem	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1
APGAR <5	n/a	n/a	n/a	n/a	0	0	0	n/a	n/a	0	0	n/a	n/a	n/a	0	0	0
Cataracts	2	2	1	1	0	1	0	n/a	1	1	0	1	1	1	n/a	1	0
Neutropenia	1	1	0	0	1	0	0	1	1	0	0	1	1	1	1	2	2
3-MGA-uria	1	1	1	1	n/a	2	2	1	1	1	1	1	1	1	n/a	2	2
Altererd muscle tone*	0	0	0	0	2	2	2	1	1	2	1	1	1	1	1	2	2
Movement disorder	0	0	0	0	1	0	0	0	1	1	1	1	1	0	0	0	2
Seizures	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	2	2
Brain atrophy	n/a	0	0	n/a	n/a	n/a	n/a	1	1	1	1	n/a	1	0	0	n/a	0
DD/ID	0	0	0	0	1	1	1	1	1	3	3	3	3	3	3	3	2
Age at death	0	0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	0
Total clinical score (max 28)	4	4	3	3	5	6	6	6	7	10	8	13	14	13	11	13	13
Current age in days**	3960	3240	9000	8640	2520	1080	1080	1080	7200	6480	3600	330*	1080*	900*	1440*	4680	450
Median clinical score (range)	3.5 (3–4)				10 (5–14)												
Median age (range)	17.5 (9–25) years				3 (0.9–20) years												
	Severe																
	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	P31			
Foetal problem	1	1	0	1	1	1	0	1	1	1	1	1	1	1			
APGAR <5	0	1	0	1	1	1	1	0	1	1	0	1	1	1			
Cataracts	1	n/a	n/a	0	0	2	1	1	2	n/a	n/a	0	n/a	0			
Neutropenia	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
3-MGA-uria	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Altererd muscle tone*	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Movement disorder	1	0	2	2	2	2	2	1	2	2	2	2	2	2			
Seizures	0	0	0	2	1	0	2	2	2	2	2	2	2	2			
Brain atrophy	1	1	1	1	n/a	n/a	1	1	2	0	0	0	0	2			
DD/ID	3	3	3	3	3	3	3	3	n/a	n/a	n/a	n/a	n/a	n/a			
Age at death	5	5	5	5	5	10	5	5	10	10	10	10	10	10			
Total clinical score (max 28)	18	17	17	21	19	25	21	20	26	22	21	22	22	24			
Current age in days** (at death)	1380*	90*	105*	150*	57*	24*	98*	45*	8*	5*	9*	4*	1*	15*			
Median clinical score (range)	21 (17–26)																
Median age (range)	36 days (1 day − 3.8 years)																

For any of the signs and symptoms listed 2 points were given for neonatal onset, 1 point for onset in later life; with exception of foetal problems and APGAR score (0 = no, 1 = yes), DD/ID (0 = no, 1 = mild, 2 = moderate, 3 = severe) and age at death (10 points = neonatal death, 5 points = later life). *either decreased or increased, **at death, B = behavioural problems, DD/ID = developmental delay/intellectual disability, n/a = not available.

hands and closed mouth. The foetus was surrounded by excessive amniotic fluid. Video 2 shows the motionless foetus with bent elbows and clenched hands. Upon external stimulation the foetus reacts with persistent high muscle tone and excessive trembling of the upper limbs. The 3D–Video 3 shows the tense muscles of the face, including the masseter muscle (lockjaw) as well as the clenched hands.

Genetic results of newly described patients (Table 3)

In P16 one previously reported (c.1222A > G, p.(Arg408Gly)) (Wortmann et al 2015) and one unreported variant (c.1383dupA, p.(Asp462Arg*11)) were detected. This duplication creates a frame shift starting at codon Asp462. The new reading frame ends in a stop codon 10 amino acids downstream, and the mRNA produced may be targeted for nonsense mediated decay. Evaluation of P17 revealed two previously reported mutations (c.1249C > T, p.(Arg417*), c.1222A > G, p.(Arg408Gly)), and in P24 the previously reported mutation (c.1249C > T, p.(Arg417*) was detected in homozygous state (Wortmann et al 2015; Saunders et al 2015). Both mutations are predicted to have deleterious impact on protein function.

A previously unreported homozygous mutation, c.1949G > C, p.(Arg650Pro), was detected in P25. This

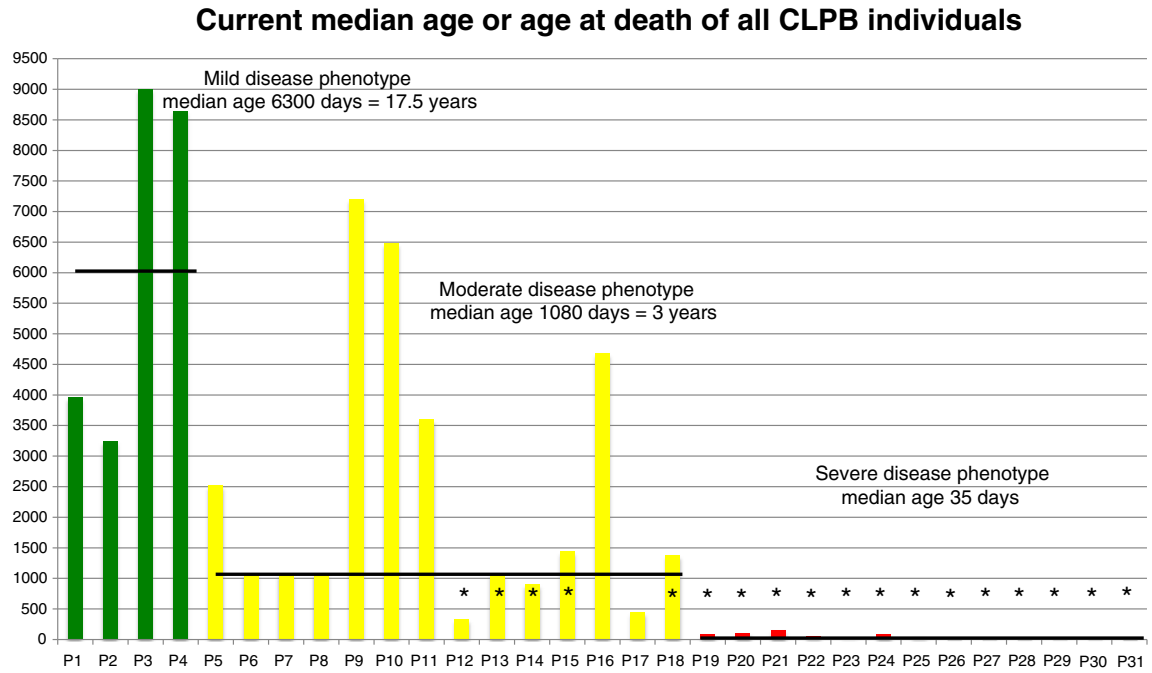

Fig. 1 Current age or age at death (*) of every patient

variant is located in the P-loop containing nucleoside triphosphate hydrolase of the Clp ATPase domain. The amino acid is highly conserved, down to zebrafish (considering 12 species). It is evaluated as deleterious by SIFT (score 0.04, median 3.38) (Adzhubei et al 2010). P31 presented a previously unreported homozygous missense variant, c.1424G > A, p.Arg(475Gln). The amino acid is highly conserved, down to crustacean (considering nine species). None of the newly reported variants was reported in the ExAC browser.

All parents were found to be heterozygous for one variant each.

Genetic spectrum in all patients

A total of 22 different mutations (Table 3) were found in 31 patients from 18 families. These were 16 missense, three nonsense and three frameshift mutations. The four nonsense mutations are predicted to lead to an mRNA product prone to nonsense mediated decay. Absence of CLPB protein in fibroblasts (p.(Thr268Met), p.(Ile562Thrfs*23) and in liver (p.(Arg417*), p.(Lys321*) has been shown for the respective mutations (Saunders et al 2015; Capo-Chichi et al 2015).

Discussion

CLPB deficiency can present with a mild, moderate or severe phenotype

This study confirms the initial suspicion that the clinical phenotype of CLPB deficiency can range from mild to severe (Wortmann et al 2015). We developed a clinical scoring system, and based on that we describe four patients with a mild, 13 with a moderate and 14 with a severe phenotype (Table 1). At present it is difficult to estimate which phenotype is most frequent since ascertainment bias may favour the detection of more severe cases. Due to the novelty of this syndrome it will be underdiagnosed in general and especially in mildly affected patients. Given the high frequency of conception difficulties and miscarriages reported in parents of CLPB deficient patients, especially within the subgroup of the most severely affected, one could speculate whether mutations in CLPB may lead to a lethal in utero phenotype. In addition, higher expression of CLPB transcripts observed in testicular cell lines of both Leydig and Sertoli origin may suggest a paternal contribution to conception difficulties (Wortmann et al 2015). One could also speculate whether maternal haploinsufficiency of CLPB has an influence on the foetal environment.

Genetic spectrum, genotype-phenotype correlation

CLPB deficiency is found in different ethnic backgrounds with patients of European, Australasian and North American descent reported. Two of the variants were found in more than one family: p.Arg417* was reported in five families (Australia, Poland, Turkey, Asia/Northern Europe), whereas p.Arg408Gly was found in three families (Poland, Australia, France). In the ExaC database this variant has only been reported in Europeans. Nonsense and frameshift mutations are predominantly found in the severe subgroup, whereas in patients with the mild phenotype only missense mutations are reported to date. This might indicate a genotype-phenotype relation, where missense mutations are

Table 2 Pre-, peri- and neonatal findings in CLPB deficient patients

	Mild (n = 4)	Moderate (n = 13)	Severe (n = 14)	All (n = 31)
Prenatal findings				
Conception difficulties	0/4 (0%)	2/12 (17%)	4/10 (40%)	6/26 (23%)
Placental calcifications		1/9 (11%)	2/8 (25%)	3/21 (14%)
Altererd placental blood flow		1/9 (11%)	3/7 (43%)	4/20 (20%)
Polyhydramnios		0/13 (0%)	7/14 (50%)	7/31 (23%)
Foetal edema		1/13 (8%)	1/10 (10%)	2/27 (7%)
Foetal contractures		0/13 (0%)	3/10 (30%)	3/27 (11%)
Foetal brain abnormalities (US)		1/5 (20%)	2/9 (25%)	3/18 (17%)
Intra-uterine growth retardation		3/13 (23%)	11/14 (79%)	14/31 (45%)
Increased foetal movements		1/13 (8%)	2/11 (18%)	3/28 (11%)
Decreased foetal movements		1/13 (8%)	3/11 (27%)	4/28 (14%)
Perinatal findings				
Prenatal birth	0/4 (0%)	1/13 (8%)	5/14 (36%)	6/31 (19%)
Normal vaginal delivery	4/4 (100%)	11/13 (85%)	11/14 (79%)	26/31 (84%)
Assisted delivery	0/4 (0%)	1/13 (8%)	1/14 (7%)	1/31 (3%)
Caesarean section	0/4 (0%)	1/13 (8%)	1/14 (7%)	4/31 (13%)
Birth weight < p3	2/4 (50%)	0/12 (0%)	4/14 (29%)	6/30 (20%)
Birth length < p3	0/2 (0%)	0/10 (0%)	1/9 (11%)	1/21 (48%)
Birth head circumference < p3	0/2 (0%)	0/10 (0%)	6/14 (43%)	6/26 (23%)
Apgar score 1 min <5	n/a	2/8 (25%)	10/13 (77%)	12/21 (57%)
Apgar score 5 min <5	n/a	0/8 (0%)	7/13 (54%)	7/21 (33%)
Apgar score 10 min <5	n/a	0/6 (0%)	6/9 (67%)	6/17 (35%)
Neonatal findings				
3-Methylglutaconic aciduria	0/0 (0%)	4/9 (44%)	10/10 (100%)	14/19 (74%)
Neutropenia	0/2 (0%)	2/12 (17%)	12/13 (92%)	14/27 (52%)
Infection(s)	0/4 (0%)	0/13 (0%)	7/14 (50%)	7/31 (23%)
Cataracts	2/4 (50%)	1/12 (8%)	4/9 (44%)	7/25 (28%)
Jaundice	1/4 (25%)	5/13 (38%)	1/14 (7%)	7/31 (23%)
Hypoglycemia	0/4 (0%)	4/13 (31%)	2/14 (14%)	6/31 (19%)
Abnormalities on brain US	0/2 (0%)	0/4 (0%)	8/14 (57%)	8/20 (40%)
Abnormalities on brain MRI	0/2 (0%)	1/1 (100%)	8/13 (62%)	9/16 (56%)
Burst suppression on EEG	0/2 (0%)	0/1 (0%)	9/13 (69%)	9/16 (56%)
Seizures	0/4 (0%)	1/13 (8%)	4/10 (40%)	5/27 (19%)
Generalized hypertonia/stiffness	0/4 (0%)	1/13 (8%)	12/14 (86%)	13/31 (42%)
Generalized hypotonia	0/4 (0%)	4/13 (31%)	5/14 (36%)	9/31 (29%)
Excessive startle reflex, period of stiffness in which voluntary movements are impossible	0/4 (0%)	0/12 (0%)	4/9 (44%)	4/25 (16%)
Artificial ventilation	0/4 (0%)	2/13 (15%)	12/14 (86%)	14/31 (45%)
Swallowing problems/feeding difficulties	0/4 (0%)	6/13 (46%)	10/10 (100%)	16/27 (59%)
Absence of voluntary movements	0/4 (0%)	1/13 (8%)	14/14 (100%)	15/31 (48%)
Limb tremor/ jitteriness/ involuntary movements	0/4 (0%)	2/13 (15%)	11/14 (79%)	13/31 (42%)
Unresponsiveness to pain	0/4 (0%)	1/13 (8%)	5/9 (56%)	6/25 (24%)

n/a = not available, US = ultrasound

associated with a milder phenotype, which could be explained by partially functional CLPB protein, although in western blot analysis of fibroblast protein from patients with the missense variant Thr268Met, no detectable CLPB protein was seen. Also, the finding that patients with the same genotype have similar clinical severity both within families (Tables 1 and 3, P5–7, P10–11, P19–20, P22–23 or P27–30), and between families (P5–7 and P17, P12–15) indicates a genotype-phenotype correlation. This report broadens the genetic spectrum of CLPB defect by reporting three novel mutations, one frameshift mutation

Table 3 Mutational spectrum in CLPB deficient patients

Patient	Clinical severity	Allele 1	Mutation type	Allele 2	Mutation type
P1, 2	mild	Met411Ile	missense	Tyr617Cys	missense
P3, 4	mild	Arg628Cys	missense	Glu639Lys	missense
P5–7	moderate	Arg417*	nonsense	Arg408Gly	missense
P8		Ala269Thr	missense	Ala269Thr	missense
P9	moderate	Glu435_436delins AspPro	missense	Gly646Val	missense
P10, 11	moderate	Cys486Arg	missense	Cys486Arg	missense
P12–15	moderate	Thr268Met	missense	Thr268Met	missense
P16	moderate	Arg408Gly	missense	**Asp462Arg*11**	frameshift
P17	moderate	Arg417*	nonsense	Arg408Gly	missense
P18	severe	Ala591Val	missense	Ala591Val	missense
P19,20	severe	Tyr272Cys	missense	Tyr272Cys	missense
P21	severe	Cys647Leufs*26	frame shift	Ile682Asn	missense
P22, 23	severe	Arg417*	nonsense	Arg250*; Glu501Lys	nonsense; missense
P24	severe	Arg417*	nonsense	Arg417*	nonsense
P25	severe	**Arg650Pro**	missense	**Arg650Pro**	missense
P26	severe	Arg417*	nonsense	Lys321*	nonsense
P27–30	severe	Ile562Thrfs*23	frame shift	Ile562Thrfs*23	frame shift
P31	severe	**Arg475Gln**	missense	**Arg475Gln**	missense

Previously unreported variants in bold.

(leading to p.Asp462Arg*11) and two missense mutation predicted to be pathogenic (p.Arg650Pro, p.Arg475Gln).

Pre- and perinatal findings in patients with CLPB deficiency

Our data (Tables 1 and 2) indicate that CLPB deficiency can lead to prenatal onset of signs and symptoms. This foetal presentation is not reported in the mildly affected patients, but it is frequently seen in the severely affected subgroup where IUGR, abnormal foetal movements (both increased and decreased), as well as polyhydramnios and contractures were observed (Table 2, Video 2). One could speculate that the periodically increased foetal movements represent foetal seizures or the prenatal onset of a movement disorder. Decreased foetal movements, contractures and arthrogryposis are reported in neuromuscular and mitochondrial disorders, but the occurrence of increased movements could be indicative of CLPB deficiency (Video 1). The finding of alterations in the placental blood circulation are difficult to interpret in this context as they represent maternal findings, but it could be speculated that CLPB haploinsufficiency plays a role.

The neonatal presentation of CLPB deficiency predicts the clinical course in later life

In all patients, irrespective of the severity of the disease, the most frequent neonatal findings were 3-MGA-uria, neutropenia and cataracts (Table 2). Therefore, we advise urinary organic acid screening and ophthalmological screening for any neonate with unexplained neutropenia, especially in the presence of neurological findings.

The findings in CLPB patients in the foetal and neonatal period appear to predict the course of disease in later life. This is true mainly for the neurological findings, since cataracts do not lead to clinically important acute problems and neutropenia in CLPB deficiency often does not lead to corresponding clinical problems (e.g. P1, 2, 5, 9). None of the 31 patients reported here showed an uncomplicated neonatal period and a severe course of disease, or vice versa. This is a very important finding for the counselling of parents of patients diagnosed in the future, especially as it may influence decisions concerning continuation of intensive care treatments in the neonatal period.

The most striking feature of the severe subtype is absence of voluntary movements in combination with ventilator dependency and a hyperexcitability to tactile stimuli. Interestingly, most patients also show unresponsiveness to pain.

CLPB mutations should be added to the differential diagnosis of "stiff baby" and hyperekplexia

Several of the patients reported at the very severe end of the spectrum show substantial clinical overlap with patients with hyperekplexia (Thomas et al 2013). One difference is that hyperekplexia patients are hypertonic, whereas patients with CLPB deficiency may be hypotonic, and some may have alternating periods of hypo- and hypertonia. In hyperekplexia, glycinergic signalling is disturbed. Further investigations are

necessary to investigate the role of glycinergic signalling in CLPB deficiency. Since CLPB deficiency should be considered a differential diagnosis in hyperekplexia, we suggest that urinary organic acid analysis, blood cell counts and ophthalmological examination are performed in all patients with a neonatal hyperekplexia phenotype. Identifying the underlying basis of hyperekplexia is important for prognostication, since patients with isolated hyperekplexia have a much more favourable outcome than those with CLPB deficiency (Thomas et al 2013).

In conclusion, biallelic *CLPB* mutations show a broad phenotypic spectrum, the hallmarks of which are 3-methylglutaconic aciduria, neutropenia and cataracts in combination with neurological findings of varying severity. CLPB deficiency can lead to a foetal phenotype and a severe neonatal phenotype which is quite characteristic and should be recognized by neonatologists, paediatric neurologists, metabolic physicians and geneticists. A clinical scoring system is available for predicting the clinical course based on foetal and neonatal findings (for bedside use, see Suppl. Table 1).

During the work on this manuscript another patient has been diagnosed with CLPB deficiency (*CLPB* (NM_030813.5) c.216del (p.Arg73Alafs*168) and c.1222A > G (p.Arg408Trp), both previously unreported). His clinical score was 5 (moderate phenotype) which mirrors the clinical course well. Problems started with delayed development and he learned to walk aged 5 years. He was diagnosed with 3-MGA-uria and severe chronic neutropenia when he required ventilation for croup but has not had recurrent infections. He is currently aged 22 years, has mild learning difficulties and an autistic spectrum disorder with hospital phobia which has precluded performance of brain MRI. Due to the combination of fidgety movements, joint laxity and an evolving thoracic scoliosis/kyphosis he has an abnormal gait which compromises his walking. It is notable that he had an older brother presenting with low birth weight, feeding problems and 3-MGA-uria neonatally. This sib died in his first months of sudden infant death syndrome (SIDS). Unfortunately no data on neutrophil counts nor stored tissues are available to confirm if he also suffered CLPB defect. This case could potentially illustrate a variability in disease severity or progression within different siblings in the same family.

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Additional individual contributors Dorota Piekutowska-Abramczuk[1], Dariusz Rokicki[2], Olga Fałek[25], Anna Nowak[26], Krystyna Brązert[27], Andrew Green[28], Johannes A. Mayr[29]

[1]Department of Medical Genetics, Children's Memorial Health Institute, Warsaw, Poland, [2]Department of Pediatrics, Nutrition and Metabolic Diseases, Children's Memorial Health Institute, Warsaw, Poland, [25]Department of Neonatology Polish Mother's Memorial Hospital Research Institute, Łódź, Poland, [26]Department of Neonatology, University of Medical Sciences, Poznań, Poland, [27]Department of Neonatology, Medical University of Silesia, Zabrze, Poland, [28]Department of Clinical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland, [29]School of Medicine and Medical Science, University College Dublin, Dublin, Ireland.

Compliance with ethical standards

Conflict of interest E. Pronicka, M. Ropacka-Lesiak, J. Trubicka, M. Pajdowska, M. Linke, E. Ostergaard, C. Saunders, S. Horsch, C. van Karnebeek, J. Yaplito-Lee, F. Distelmaier, K. Õunap, S. Rahman, M. Castelle, J. Kelleher, S. Baris, K. Iwanicka-Pronicka, C. G. Steward, E. Ciara, and S. B. Wortmann declare that they have no conflict of interest.

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