**SUPPLEMENTARY DATA**

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

Ewa Pronicka1,2, Mariola Ropacka-Lesiak3, Joanna Trubicka1, Magdalena Pajdowska4, Markus Linke5, Elsebet Ostergaard6, Carol Saunders7,8, Sandra Horsch9, Clara van Karnebeek10, Joy Yaplito-Lee11, Felix Distelmaier12, Katrin Õunap13,14, Shamima Rahman15, Martin Castelle16, John Kelleher17, Safa Baris18, Katarzyna Iwanicka-Pronicka19, Colin G. Steward20,21, Elżbieta Ciara1, Saskia B. Wortmann22,23,24

Additional individual contributors: Dorota Piekutowska-Abramczuk1, Dariusz Rokicki2, Olga Fałek25, Anna Nowak26, Krystyna Brązert27, Andrew Green28,29, Johannes A. Mayr22

1Department of Medical Genetics, Children’s Memorial Health Institute, Warsaw, Poland; 2Department 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; 4Department of Biochemistry and Experimental Medicine, Children’s Memorial Health Institute, Warsaw, Poland; 5Department of Neonatology,DRK Children’s Hospital Siegen, Siegen, Germany; 6Department of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark; 7Center for Pediatric Genomic Medicine, Children’s Mercy Hospital, Kansas City, MO 64108, USA; 8Department of Pathology and Laboratory Medicine, Children’s Mercy Hospital, Kansas City, MO 64108, USA; 9Department of Neonatology, Helios Klinikum, Berlin-Buch, Germany; 10Division of Biochemical Diseases, Department of Pediatrics, B.C. Children’s Hospital, Treatable Intellectual Disability Endeavour, Vancouver, BC V6H 3N4, Canada; 11Department of Metabolic Medicine, Murdoch Childrens Research Institute, The Royal Children’s Hospital Melbourne, Parkville, VIC 3052, Australia; 12Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children’s Hospital, Heinrich-Heine University, Moorenstr. 5, 40225 Duesseldorf, Germany; 13Department of Genetics, United Laboratories, Tartu University Hospital, Tartu 51014, Estonia; 14Department of Pediatrics, Institute of Clinical Medicine, University of Tartu, Tartu 51014, Estonia; 15UCL Institute of Child Health, London WC1N 1EH, United Kingdom; 16Department of Hemato-Immunology, Hospital Necker-Enfants malades, Paris, France; 17Dept. of Neonatology, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland; 18Marmara University, Division of Pediatric Allergy/Immunology, Istanbul,Turkey; 19Department of Audiology and Phoniatrics, Children’s Memorial Health Institute, Warsaw, Poland; 20School of Cellular & Molecular Medicine, Medical Sciences Building, University of Bristol, Bristol, United Kingdom; 21Dept of Haematology, Oncology and BMT, Royal Hospital for Children, Bristol, United Kingdom; 22Department of Pediatrics, Salzburger Landeskliniken and Paracelsus Medical University, Salzburg, Austria; 23Institute of Human Genetics, Technical University Munich, Munich, Germany; 24Institute of Human Genetics, Helmholtz Zentrum Munich, Neuherberg, Germany;

25Department of Neonatology Polish Mother’s Memorial Hospital Research Institute, Łódź, Poland; 26Department of Neonatology, Medical University of Silesia, Zabrze, Poland; 27Department of Neonatology, University of Medical Sciences, Poznań, Poland; 28Dept. of Clinical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland; 29School of Medicine and Medical Science, University College Dublin, Dublin, Ireland

Correspondence: Saskia B. Wortmann, MD, PhD, Department of Pediatrics, Salzburger Landeskliniken and Paracelsus Medical University, Müllner-Hauptstraße 48, 5020 Salzburg, Austria, T +43-(0)662-4482-2601, F +43-(0)662-4482-2604, [s.wortmann-hagemann@salk.at](mailto:s.wortmann-hagemann@salk.at)

**Case reports of previously unreported patients**

**P 16 (**male) was born as the first child to healthy unrelated French parents after conception difficulties. An earlier pregnancy had ended in a miscarriage. The pregnancy was complicated by intrauterine growth retardation (IUGR). No altered placental blood flow was detected on prenatal ultrasounds, and postnatal histological evaluation of the placenta showed minimal vascular changes. By 34 weeks of gestation (wg) a Cesarean section (CS) was performed because of fetal rhythm abnormalities. The Apgar scores were 5, 9, 9 at 1, 5, and 10 minutes. At birth a neonate with a distinctive “seagull cry”, axial hypotonia and peripheral hypertonia was observed. He did not show any spontaneous movements, the response to pain and startle reflex were not reported. Visual contact was described as poor, but ophthalmological investigation was unremarkable. A severe vagal hyperreflexia (e.g. upon naso-gastric tube introduction, or oculo-cardiac reflex) with bradycardia and hypoxia necessitated artificial ventilation. The EEG showed maturation delay, and periods of hypoactivity with concomitant apnea. Neonatal brain ultrasound was unremarkable, and MRI was not performed. 3-MGA-uria and neutropenia were noted in the neonatal period. The first infection occurred in the second month of life and cataracts were noted after several months. The patient is currently aged 13 years, shows severe intellectual disability and autistic/aggressive features. He first walked at 12 years and has limited verbal communication. He developed severe kypho-scoliosis and hip dislocation requiring orthopedic surgery. He has persistent severe neutropenia, requiring granulocyte colony stimulating factor (G-CSF) (clinical scoring 13 points, moderate phenotype).

**P17** (female) was born after two miscarriages and one healthy female to unrelated Polish parents. The mother underwent prolonged hormonal treatment for conception difficulties. From 35 wg onwards the mother felt repetitively abrupt "trembling” movements of the fetus. In wg 39 ultrasound revealed fetal edema and impaired placental flow, CS was performed because of previous CS. Apgar scores were 7, 5, 8, 8 at 1, 3, 5 and 10 minutes. She was resuscitated and subsequently required nasal oxygenation. Anthropometric data were unremarkable, but a heart murmur and edema of the legs were observed. The dominant finding was a generalized muscular hypertonia with tremor mainly in the lower extremities, and generalized marked hyperreflexia (video 1). Furthermore, the patient was hyperreactive upon tactile stimulation. Seven hours postnatally generalized convulsions with tonic flexion of the upper limbs, clamping hands and extension of lower limbs occurred, and lasted for hours despite treatment. In the next days feeding difficulties, transient hypoglycemias, bradycardia and impaired temperature regulation as well as neutropenia occurred. The patient was referred to a tertiary center at day 7 where the clinical state improved spontaneously and remained stable. Persistent 3-MGA-uria was observed, cerebral ultrasound was inconclusive and an MRI suggested delayed myelination. The further course has been complicated by several infections (septicemia, urinary tract infection, otitis media). Currently, she is aged 15 months, has prominent generalized muscular hypotonia and her development is significantly delayed (unable to sit independently, no sounds or words, very limited interaction with parents). She currently has no cataracts, no seizures or recurrent infections (without antibiotics or G-CSF) (clinical scoring 15 points, moderate phenotype).

**P24 (**female) was born as the first child to healthy unrelated German parents after conception difficulties. The pregnancy was uncomplicated, but she was delivered prematurely by CS because of premature rupture of membranes. Apgar scores were 1, 3, 3 at 1, 5, and 10 minutes. She was primarily intubated and ventilated because of apnea. Poor swallow leading to drooling, necessitated tube feeding. Muscle tone varied between severe spasticity with clonus myoclonic salves upon tactile stimuli, to hypotonia without any movements against gravity. She showed an excessive startle reflex with eye blinking and flexor spasm of the trunk followed by a period of stiffness in which voluntary movements were impossible. In addition, 3-MGA-uria, hypertrophic cardiomyopathy, cholestasis, nephrocalcinosis, hypothyroidism, neutropenia, and severe infections were noted. Cataracts were not detected. Brain ultrasound and MRI were normal at birth, but showed progressive cerebellar and cerebral atrophy during the disease course. EEG showed a mildly immature pattern at birth, but progressed to a burst suppression pattern at the age of five weeks. She died at the age of 14 weeks (clinical scoring 21 points, severe phenotype).

**P25 (**male) was born as the third child to healthy related Turkish parents after conception difficulties. An earlier pregnancy ended in a stillbirth, one older sibling is healthy. An altered placental blood flow was detected. He was born prematurely (wg 36+0) by normal vaginal delivery after induction of labour due to IUGR and polyhydramnios. Apgar scores were 6, 7, 9 at 1, 5, and 10 minutes. At birth hypertonic muscle tone was observed. He was hyperexcitable, but showed no excessive startle response. Physical examination detected multiple hematomata on his back and arms, petechiae all over the trunk, lowered mobility of all large joints, low-set ears, retrognathia, a micropenis and retractile testes. He had swallowing problems and excessive drooling as well as periods of hypo- and hyperthermia. The first generalized tonic clonic convulsion occurred five hours after birth. He further suffered recurrent seizures, mainly myoclonic, sometimes nearly continuously. EEG showed a continuous burst suppression pattern. Hypoglycemia, neutropenia, recurrent infections, 3-MGA-uria and bilateral cataracts were also noted. He died at the age of 5.5 months due to ileus followed by multiorgan failure. Cerebral ultrasound showed pachygyria and progressive widening of the internal and external liquor spaces (clinical scoring 20 points, severe phenotype).

**P31** (male) was born to consanguineous parents (first cousins) from the Irish Traveller. There was no known neurometabolic pathology in the family history however an older sibling had learning difficulties and a “small head” that had never been formally investigated. At 35 weeks gestation polyhydramnios and clenched fists were seen on antenatal ultrasound. He was a vaginal delivery at 38+4 weeks and weighed 1.9 kg (<0.4th centile). Microcephaly was noted with a head circumference of 31.5cm (0.4th – 2nd centile) consistent with severe intra-uterine growth restriction. His APGARs were 5, 5 and 7 at 1, 5 and 10 minutes respectively. Marked hypertonia and diffuse, evolving petechiae were noted at delivery and he was intubated for poor respiratory effort. Initial blood results showed a profound neutropenia, coagulopathy and a mixed acidosis. He was transferred to the NICU for further management.

On further examination immature male genitalia with impalpable testes and an anterior positioned anus were noted. Initial abdominal ultrasound was normal with no clinical/radiological organomegaly. His hypertonia progressed to spasticity and contractures (“stiff baby”). Full septic and serologic work-up were negative. Bilateral microspherophakia was seen on ophthalmology assessment. An EEG demonstrated severe, global cerebral dysfunction with increased epileptogenicity although no clinical or electrographic seizures were ever noted. MRI Brain revealed immature pattern of gyri with loss of white matter volume and high CSF lactate.

**Supplementary video legends**

Video 1) This video of P16 was taken shortly after birth. Note the muscular hypertonia, the absence of voluntary movements and the hyperreactivity upon tactile stimuli.

Video 2) This ultrasound video, taken at week of gestation 36 of P23, initially shows the upper limbs of a motionless fetus suspended by excessive amniotic fluid (polyhydramnios). The placenta is at the upper margin of the video. Note the bent elbows and clenched hands. Upon external stimulation, note the persisting high muscle tone and excessive trembling movements of the upper limbs.

Video 3) This 3D ultrasound video, taken at week of gestation 36, shows the face and hands of fetus P23. Note the tense muscles of the face, including the masseter muscle (lockjaw). It is possible that the polyhydramnios could be a consequence of the lockjaw.

**Supplementary Table 1**

Clinical scoring card for patients with CLPB defect

|  |  |  |
| --- | --- | --- |
| Any fetal problem | 1 = yes, 0 = no,n/a = not available |  |
| Any APGAR <5 | 1 = yes, 0 = no, n/a = not available |  |
| Cataracts | 2 = neonatal onset, 1 = onset later in life, 0 = no cataracts, n/a = not available |  |
| Neutropenia | 2 = neonatal onset, 1 = onset later in life, 0 = no neutropenia, n/a = not available |  |
| 3-Methylglutaconic aciduria | 2 = neonatal onset, 1 = onset later in life, 0 = no 3-methylglutaconic aciduria, n/a = not available |  |
| Altered muscle tone (hypo- or hypertonia) | 2 = neonatal onset, 1 = onset later in life, 0 = normal muscle tone, n/a = not available |  |
| Movement disorder (dystonia, tremor, ataxia etc.), | 2 = neonatal onset, 1 = onset later in life, 0 = no movement disorder, n/a = not available |  |
| Seizures | 2 = neonatal onset, 1 = onset later in life, 0 = no seizures, n/a = not available |  |
| Brain atrophy | 2 = neonatal onset, 1 = onset later in life, 0 = no brain atrophy, n/a = not available |  |
| Developmental delay/ intellectual disability | 2 = severe, 1 = moderate, 0 = mild, n/a = not available |  |
| Age at death | 10 = neonatal death, 5 = death later in life, 0 = alive, n/a = not available |  |
|  | Total clinical score (max 28) |  |
| **Clinical phenotype** | **Mild (clinical score <5)**  **Moderate (clinical score 5-15)**  **Severe (clinical score >15)** |  |