

***De novo* stoploss variants in *CLDN11* cause hypomyelinating leukodystrophy**

Korbinian M. Riedhammer,^{1,2} Sylvia Stockler,³ Rafal Ploski,⁴ Maren Wenzel,⁵ Burkhard Adis-Dutschmann,⁶ Uwe Ahting,¹ Bader Alhaddad,¹ Astrid Blaschek,⁷ Tobias B. Haack,^{1,8} Robert Kopajtich,^{1,9} Jessica Y. Lee,¹⁰ Victor M. Pienkowski,⁴ Agnieszka Pollak,⁴ Krystyna Szymanska,¹¹ Robin van der Lee,¹⁰ Clara D. van Karnebeek,^{10,12} Thomas Meitinger,^{1,9} Ingeborg Krägeloh-Mann,¹³ Katharina Vill,⁷

¹Institute of Human Genetics, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, 81675, Germany

²Department of Nephrology, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, 81675, Germany

³Division of Biochemical Diseases, Department of Pediatrics, B.C. Children's Hospital, The University of British Columbia, Vancouver, BC V6H 0B3, Canada

⁴Department of Medical Genetics, Medical University of Warsaw, Warsaw, 02-106, Poland

⁵Genetikum, Genetic Counseling and Diagnostics, Neu-Ulm, 89231, Germany

⁶Center for pediatrics, Ulm, 89073, Germany

⁷Dr. v. Hauner Children's Hospital, Department of Pediatric Neurology and Developmental Medicine, LMU – University of Munich, 80337, Germany

⁸Institute of Medical Genetics and Applied Genomics, University of Tübingen, 72076 Tübingen, Germany

⁹Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, 85764, Germany

¹⁰Centre for Molecular Medicine and Therapeutics, BC Children's Hospital Research Institute, Department of Medical Genetics, The University of British Columbia, Vancouver, BC V6H 0B3, Canada

¹¹Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw, 02-106, Poland

¹²Department of Pediatric Metabolic Diseases, Amalia Children's Hospital, Institute for Life Sciences, Radboud University Medical centre, Nijmegen, 6525 GA, The Netherlands

¹³Department of Pediatric Neurology and Developmental Medicine, University Children's Hospital, Tübingen, 72076, Germany

Supplementary case reports

Individual 1 is a girl of Non-Finnish European descent and the oldest child of healthy, non-consanguineous parents. Two younger sisters are not affected. The family history is inconspicuous for movement disorders. Premature contractions occurred during pregnancy from the 30th week, the child was born in the 37th week of pregnancy. Postnatal adaptation was unremarkable. Growth was within normal range and there was no microcephaly [age 13 years: length 153cm (15P), head circumference 57 cm (97P; father and paternal grandfather have large head circumference)]. The BMI is slightly obese with 22.5 (weight 53kg). Age at last examination was 13 years.

In the first year of life, a delayed motor development and leg dominated spasticity with truncal hypotonia became apparent. Motor milestones were reached with great delay - sitting without support was possible at 4.5 years, walking without help became possible only over a distance of a few meters. At the age of 13 years, she showed knee and ankle contractures, flat feet and no scoliosis.

Speech development was delayed, first words were spoken at the age of 26 months, at the age of 13 years she spoke sentences with correct grammar and had a good vocabulary, difficult to understand because of oral dyspraxia. ID is maximally mild, IQ testing was not performed.

Repeated ophthalmological examination showed nystagmus, a hyperopic astigmatism of +2 dpt and strabismus, the latter was surgically treated at the age of 3 years .

Hearing was normal. At the age of 3 years a seizure occurred during a small operation with topic anaesthesia - anticonvulsant medication was taken over a period of several years. At 13 years she was free of seizures without medication. In the past, the EEG showed ETP, the latest EEG was normal without medication. Diagnostic work-up included amino acids in plasma and

neurotransmitters in cerebrospinal fluid, which were inconspicuous. Previous genetic tests included chromosomes and micro-array, which stayed without any indicative findings. Electrophysiology at age 3 years showed disturbed SEP, AEP und VEP, nerve conduction studies stayed normal.

Individual 2 is a boy of Non-Finnish European descent and the first child of healthy, non-consanguineous parents. He has no siblings. Family history was inconspicuous for movement disorders. Pregnancy was unremarkable until the 36th week. Birth was triggered after 37 weeks due to suspicion of preeclampsia. Postnatal adaptation was normal.

Growth was within normal range and there was no microcephaly [age 9 years: length 130cm (14P), weight 26kg (14P), head circumference 52cm (19P)]. Last examination was at age 9 years.

At the age of 4 months, movement seemed not age-appropriate and „stiff” and first symptoms of strabismus appeared. On follow-up, a clear spastic movement disorder with increased tone of all extremities and truncal hypotonia became apparent. In the course of the disease, he developed fluctuating muscular rigidity in the upper extremities, no dystonia or ataxia. The trunk shows rigidity, especially in the shoulder belt. Motoric milestones were reached with great delay, sitting without support was possible at 3.5 years. Walking without help over a distance of few meters was achieved age 5 years and was lost again 6 months later, mainly due to increasing weight and growth. At the age of 8 years he showed abnormal shoulder belt, short neck, concave chest, contractures but no scoliosis.

Speech was severely delayed. At the age of 4 years, first syllables and words were spoken. At the age of 8 years he spoke in 5-6-word sentences difficult to understand because of oral dyspraxia with pronounced restriction of arbitrary tongue and lip mobility. He shows severe drooling. The intelligence is in a low-normal range, IQ testing was not performed.

The repeated eye examination showed a nystagmus, a strabismus, which was surgically treated three times, and a hypermetropia of +4.5/+4.25 dpt (age 8 years).

Hearing was normal. EEG age 8 years showed unspecific findings with low spatial differentiation and abnormally low basal activity, paroxysmal changes localized in the middle

region and the fronto-central area of the right hemisphere, worsening during falling asleep and while asleep without any clinical fits on video. Seizures never occurred. Electrophysiology age 8 years showed normal nerve conduction velocity studies and EMG.

Diagnostic work-up included broad previous genetic (including *PLP1* sanger sequencing and deletion/duplication analysis) and metabolic tests which remained without any indicative findings.

Individual 3 is a boy of Non-Finnish European descent and the second child of healthy, non-consanguineous parents. The older brother is unaffected. Family history was unremarkable for movement disorders. Pregnancy was complicated by pre-eclampsia, with a spontaneous vaginal delivery at term. The postnatal adaptation was insufficient with APGAR values 4/5/6, requiring intubation and ventilation for less than 12h and discharge from NICU at day 3. He had an umbilical hernia, and a right thigh congenital naevus. In infancy, recurrent obstructive bronchitis occurred.

Growth was within normal range and there was no microcephaly [age 4 years: length 101cm (24P), head circumference 50cm (18P)]. Age at last examination was 6.5 years.

In the first year of life a delayed motor development was noticeable; at age 11 month he showed a spastic movement disorder with increased tone of the extremities (pronounced on the lower extremities), arching of neck and plagiocephalus, and was diagnosed with CP. The trunk muscles were normal. Motor milestones were reached with great delay, sitting without support was possible at 4 years, standing without help at 6 years and walking without help was never possible. He has contractures with a hamstrings- and gastrocnemius-shortening, no scoliosis.

Speech was severely delayed. At the age of 4 years, he babbled and communicated mainly using a combination of gestures, pointing and crying, but at 6.5 years he spoke sentences up to 6 words (dysarthric) and had good receptive speech. IQ was not tested, but ID seems to be mild. There is constant drooling and he shows oral hypotonia.

Eye examination, latest at the age of 5.5 years showed strabism with exotropic eye alignment, a fine nystagmus, decreased visual acuity near and far (Teller acuity cards 20/70; Lea symbol recognition 20/130) and a hyperopic astigmatism+ 3.25 OD and + 2.75 OS.

Hearing is normal. No seizures occurred so far. EEG at age 1 year was normal. Electrophysiology age at 18 month revealed normal BAER, and normal nerve conduction velocity studies.

Broad diagnostic work-up was performed according to the TIDE diagnostic recommendations for treatable inborn errors of metabolism causing intellectual disability superimposed to App-guidelines (van Karnebeek *et al.*, 2014). This included plasma aminoacids, blood acylcarnitines, copper, ceruloplasmin, very long chain fatty acids, transferrin isoelectric focusing; urine HVA, VMA; urine oligosaccharides, glycosaminoglycans, which remained inconspicuous. Blood lactate was high on repeat occasions (mmol/L): 6.1, 4.7, 3.0 (elevated lactate-pyruvate ratio 54, ref 10-20), latest 2.3. Enzyme activity measurements included NCL1, NCL2, beta galactosidase, arylsulfatase, hexosaminidase. Galactocerebrosidase 1.6 noml/hr/mg protein (normal 2.1-10.4, likely hypomorphic allele).

Previous genetic tests included *PLP1* sanger sequencing and deletion/duplication analysis as well as microarray which was found to be without any indicative findings.

References

van Karnebeek CD, Shevell M, Zschocke J, Moeschler JB, Stockler S. The metabolic evaluation of the child with an intellectual developmental disorder: diagnostic algorithm for identification of treatable causes and new digital resource. *Mol Genet Metab* 2014; 111(4): 428-38.