

*Patient 1* was born after an uneventful pregnancy and delivery. Her birth weight was 3,050 grams (40<sup>th</sup> percentile). She was the second child of healthy non-consanguineous parents. Hypertonia was noticed immediately after birth. At six weeks of age, she was diagnosed with non-febrile generalized seizures. The seizures were frequent and intractable and included multiple seizure types such as infantile spasms, clonic seizures with cyanosis, myoclonic complex partial and absences seizures. Her development was severely delayed. At the age of 11 months her developmental age was 1 month. She had a severe dysregulation of muscle tone. Chromosomal analysis, including genome-wide array analysis, showed no abnormalities. DNA diagnostics for the *POLG* gene (mitochondrial DNA depletion syndrome), blood chemistries and a metabolic screen in urine revealed no abnormalities. At the age of 3 years, DNA sequencing of *FOXG1*, *ARX* and *CDKL5* was performed because of persistent seizures and severe developmental delay. Results were normal. After the age of 3 years, treatment with multiple antiepileptic agents (phenobarbital, phenytoin, levetiracetam, clobazam and zonisamide) led to seizure control.

At the age of 7 years she was wheelchair dependent, and unable to sit independently or speak. She was fed by a percutaneous endoscopic gastrostomic tube. She had to be catheterized intermittently because of a neurogenic bladder due to a tethered cord. Upon physical examination she had a height of 121 cm (>50<sup>th</sup> centile) and a head circumference of 51.5 cm (>50<sup>th</sup> centile). Minor facial dysmorphisms included synophris, broad nasal tip and upslanted palpebral fissures. Hirsutism was probably due to treatment with the anti-epileptic drug phenytoin. A right convex thoracic scoliosis and tapering fingers were observed. MRI demonstrated progressive cerebral atrophy (Supplementary Figure 1).

*Patient 2* was born after uneventful pregnancy and delivery. His birth weight was normal (3,000 grams, 16<sup>th</sup> percentile). At six months, hypertonia and an abnormal head posture were observed. Onset of seizures at 18 months was said to follow an episode of meningitis. He

presented with a severe global developmental delay. At the age of six years, he learned to walk independently and spoke his first words. As an adult, his developmental level was comparable to a child of 4-5 years. Seizures were generally well controlled by treatment with carbamazepine, although he had absence-like episodes and occasional tonic-clonic seizures. After the age of 29 years, he had a gradual decline in motor function and lost the ability to walk independently, to climb stairs and to cycle. He also developed balance problems and ataxia, leading to recurrent falls and swallowing difficulties. In parallel there was a decline in cognitive functioning, albeit less pronounced. Upon the last examination at the age of 31 years he had a head circumference of 55,2 cm (5<sup>th</sup>-10<sup>th</sup> percentile). He showed a gaze-evoked nystagmus, jerky pursuit, and a slight limitation in upward gaze. In addition he had a spastic tetraparesis with a broad based, spastic gait. Lower limb reflexes were brisk with equivocal plantar reflexes. There were no facial dysmorphisms. An MRI brain scan showed cerebellar atrophy (Supplementary Figure 2). Genome-wide chromosomal analysis by 250k SNP array analysis and metabolic tests, including a screen of blood and urine and lysosomal enzyme activity measurements in fibroblasts, revealed no abnormalities.

*Patient 3* was born after an uneventful pregnancy and delivery. He had a normal birth weight of 2,880 grams (25<sup>th</sup> percentile). He was the first child in his family and has two healthy younger brothers. His parents reported an early delay in development, with independent walking after the age of 18 months. Speech development was delayed and he cannot read or write. Eye contact was poor. He had to attend a special school. Formal intelligence tests showed an intelligence quotient of 50. At the age of 15 years he was diagnosed with an autism spectrum disorder (ASD) and at 17 years he was moved to a sheltered home because of problematic behaviours including aggressive outbursts and self-mutilation. He had an anxious and insecure personality and was diagnosed with mild hypothyroidism. He was operated on at six weeks for unilateral inguinal hernia, and at 18 months for an umbilical hernia, but was

otherwise in good medical condition. He had no seizures and a normal electroencephalogram at 4 years of age. A cerebral CT-scan showed atypical anomalies including atrophy of the frontal lobe and paramedian regions. At the age of 31 years his height was 169 cm (2<sup>nd</sup> percentile), with weight of 78 kg (85<sup>th</sup> percentile) and head circumference of 55.8 cm (10<sup>th</sup> percentile). Facial dysmorphisms included a high forehead, full eyelids and ptosis, downturned corners of the mouth and a high and narrow palate. We also observed a sandal gap, flat feet, clinodactyly of both second fingers and a mild pectus excavatum. Genome-wide chromosomal analysis (karyotype and SNP-array), DNA diagnostics for Fragile X syndrome, and metabolic tests in blood and urine revealed no abnormalities. The patient was included in family-based whole-exome sequencing studies.