

Patient 1

The female patient was the first child of non-related parents. The family history was unremarkable except for a labial pit in her mother and a unilateral cleft lip and palate in her maternal grandfather. Antenatal karyotype was performed due to high serum markers, which showed a normal result. She was born at 34 weeks of gestation, with normal birth weight (30th centile) and length (60th centile). Examination revealed bilateral cleft lip and palate, hypo-pigmented areas on the back along Blaschko's lines, and a bilateral hip dislocation. Because of the cleft lip and palate in the context of the family history, Van Der Woude syndrome was suspected; *IRF6* testing by Sanger sequencing was negative. Early milestones were reported as normal until 7 months, when she developed generalized epilepsy and psychomotor regression. When she was referred to a clinical geneticist, at 14 years, she had severe intellectual disability. She could not walk independently, had no speech. She had growth retardation, with length at -4.5 S.D. and obesity (body mass index (BMI) +3 S.D.). OFC was average. Orthopedic examination showed slightly asymmetric and short lower limbs, flat feet and hyperlordosis. Facial dysmorphism was striking with bifid nose, hypertelorism, hypertrichosis and coarse features. Echocardiography demonstrated asymptomatic aortic insufficiency.

Patient 2

She was the first child of unrelated parents, born at 39 weeks of gestation following an uneventful pregnancy. Birth weight was on 5th centile, length on 10th centile, OFC on 10th centile. Neonatal examination showed bilateral clubfeet. Her first year of life was marked by recurrent infections and by rapid weight gain. Psychomotor development was normal until 9 months; she then made very slow progress, walked at 40 months, and then went in a school for children with special needs. She developed generalized epilepsy from 10 years with tonic-clonic seizures. Behavioural issues were noted from teenage, treated by antipsychotic drug. On the last examination at 20 years old, she could speak a few words. Her weight was on +4 S.D., length -4 S.D, BMI 30. She had coarse facial features, hypertelorism, flat nasal bridge, anteverted nares, full mouth, thick gums, protruding tongue, facial hypertrichosis and convergent strabismus. Orthopedic examination showed hyperlordosis, limitation in

elbow extension, genu valgum, flat feet, small hands and feet. Bone age on the X-ray, as well as the spine, were normal. Blood glucose level was normal, and there was no hepatomegaly on the abdominal ultrasound.

Patient 3

She was the first child of nonrelated healthy parents. Pregnancy was complicated by oligohydramnios and third trimester intra-uterine growth retardation. She was born at 41 weeks of gestation with a low birthweight on the 2nd percentile, and was admitted for hypoglycemia in the first two weeks of life. At 6 months she was noted to have delayed milestones and a poor eye contact. She developed tonic seizures from 18 months. Brain MRI brain was normal, EEG showed an abnormal pattern with epileptic encephalopathy and multifocal spikes. She had chronic upper airway infections in the winter, periods during which the parents reported she did not make any developmental progress or even regressed. At examination at 3 years old, she could roll over but could not walk or grasp objects; she made sounds but no words. She had linear and whorled hyperpigmentation all over the body except on the face, palms and soles. Growth parameters were in the normal ranges. She was noted to have umbilical hernia, strabismus and a single palmar crease. The facial features were remarkable by synophris, hypertrichosis, full cheeks and lips, flat nasal bridge, hypertelorism and thick eyebrows. In the context of the pigmentary changes, chromosomal mosaicism was considered, and a skin biopsy was performed, showing a mosaic translocation t(X;11) with a microdeletion of Xp22.3 in 1 out of 2 cell-cultures; the deletion contained no genes of which haploinsufficiency was known to cause developmental delay and seizures and was therefore not considered to be causative for the phenotype.

Patient 4

She was the first child of her healthy and not related parents from European decent. She was born after uncomplicated pregnancy by spontaneous vaginal delivery at 41+2 weeks. The birth weight was 4370 g (+1.8 SD, the birth length 58 cm (+2.6 SD) and the OFC at birth 36 cm (+0.6 SD). APGAR 10 and 10 at minute five and ten, respectively. The neonatal period was described as uncomplicated despite long-lasting feeding. At 6 weeks of life physiotherapy was initiated due to scull asymmetry and pigeon

toes. At the age of two months poor fixation with eyes and interaction were obvious. At 9 months global development delay, muscular hypotonia and hearing deficits were diagnosed. An extended work up at age of 12 months revealed abnormal profile in aminoacids in plasma (elevated alanine-lysine ratio), elevated lactate in blood, inconclusive hearing testing and hyperopia. Karyotyping gave normal result; CGH-array discovered a 0.1 Mb duplication in 19p13.3 of uncertain significance. The further development was slow. At last examination at age of 18 months we saw a restless girl with severe global developmental delay, stereotypic movements, poor social interaction, severe truncal muscular hypotonia and hypertonia in legs. The head control was insufficient and she was not able to sit or to crawl but she could roll over from belly to back. She did not grasp but transferred objects from one hand to the other. She expressed emotions by sounds but she did not spoke a word. Facial dysmorphism consisted in coarseness, flat profile, high forehead, hypertelorism and small eyes, depressed nasal bridge and short nose, small mouth with small teeth, full and pink cheeks. The measurements were above the age related average with a weight of 14.7 kg (+2.4 SD), length 86 cm (+1.4 SD) and OFC 48 cm (+0.7 SD). MRI of brain was normal.

Patient 5

She was born from unrelated healthy parents. She was born full term (36 WG) after uncomplicated pregnancy. Birth weight was +1.5 SD, length was +0.5 SD., OFC +0.5 SD. She had transient neonatal hepatomegaly and jaundice. She developed severe ID, with central hypotonia and peripheral spastic paraplegia. She had bilateral congenital hearing loss. On examination she had hypopigmentation following Blaschko's lines, and facial dysmorphism with progressive coarsening and hirsutism. She developed epilepsy at 6 years old. At 10 years old she had growth retardation on -4 SD. Bone X-rays demonstrated moderate metaphyseal enlargement. Metabolic workup, including lactate, glycosaminoglycans, very long chain fatty acid, transferrin, plasmatic aminoacids, urinary organic acids, phytanic and pristanic acids, lysosomal enzymes and neurotransmitters in the cerebrospinal fluid, was normal. Muscle biopsy showed an irregular size of the fibers with increased lipid accumulation, no COX-negative fibers, no Ragged Red fibers, no structural anomalies. Mitochondrial

DNA sequencing on DNA extracted from muscle tissue was normal. Brain MRI showed a short corpus callosum.

Patient 6

She was the first child of unrelated parents from Portugal. Family history was unremarkable. She was born at term after a normal pregnancy. Birth weight was 2690g (0 SD), length 46.5 cm (-1 SD), and OFC 33 cm (-1 SD). Apgar was 10/10. She had feeding difficulties and laryngomalacia in the first days of life. At seven months she had surgery for triventricular hydrocephalus due to aqueductal stenosis. Profound congenital hearing loss was diagnosed. She was referred to the genetics clinic at 8 months for developmental delay and pigmentation anomalies. She could smile but had no head control. She had hypopigmentation on Blaschko's lines, hypotonia and facial dysmorphism with and synophrys, thick eyebrows, and hypertelorism. Array CGH was normal on blood and fibroblasts. She had chronic bronchic infections. At last examination at 29 months, head control was unstable, she made sounds, could roll over. Growth parameters were normal (weight on 0SD, length on -1SD, OFC on 0SD). She had normal heart and abdominal ultrasound. Ophthalmological exam showed an iridian depigmented macule. Metabolic workup (including very long chain fatty acids and lysosomal enzymes) was normal.

Patient 7

She was the first child born from unrelated parents. The mother has deafness as well as her two brothers. Pregnancy was marked by the discovery of ureteral dilatation at 5 months of gestation in a context of maternal gestational diabetes. She was born at 36 weeks of gestation, with fetal macrosomia (weight +3 SD, length +2 SD occipitofrontal circumference +2.5 SD). She presented with neonatal hypotonia and feeding difficulties. She sat at 12 months of age. At last examination at 4 years and 3 months of age she was unable to stand and walk alone, had no speech. She presented sleep disturbance and hand stereotypies. Her weight was +1.5 SD, length was +2.5 SD with a parental target size at +1.2 SD and her OFC was +1 SD. BMI was 15.4. She had surgery for an histiocytofibroma. Brain MRI, skeletal X-rays, eye and auditive exams were normal.

Patient 8

She was born following the first uncomplicated pregnancy of healthy non consanguineous parents. Younger's sister was healthy. She was born at term 39 weeks of gestation and 3 days, with eutocic delivery. Relevant hypotonia was detected and she received support since 10 months of age. Motor milestones were delayed. A metabolic disorder was suspected but without any anomaly on the metabolic workup. Mucopolysaccharidosis were ruled out, lysosomal enzymatic activities in leucocytes were normal. Genetic tests were performed: karyotype, CGH array in dark and light skin and *MECP2* sequencing gave normal results.

Patient 9

Patient 13 is a 10-year-old female born at 39 weeks of gestation after an uneventful pregnancy with normal growth parameters (weight of 3.7kg, 87th P). Neonatal period was uneventful, except neonatal jaundice. She began to walk at 30 months and first words were delayed. Because of this global developmental delay, she was referred for clinical genetic evaluation at 3 years of age. Physical examination revealed pigmentary changes on her back on Blaschko's lines and dysmorphic features with coarseness, anteverted nares, short nose, depressed nasal bridge, hypertelorism, thick lower lip and full cheeks. Her weight and head size were on the 50th and 25th centile respectively, while her height was between 0.4th and 2nd centile. Because of her coarse features, metabolic screening (including MPS, OGS, UAA, OA) was performed and showed normal results. At 8 years-old, she was still slow with her learning, communicated via single words and learnt phrases, understood single instructions. Brain MRI was normal. This patient was included in the DDD study. Exome sequencing identified a *de novo* hemizygous splice donor mutation c.780+1G>A (GRCh37) in exon 4 of the *TFE3* gene (NM_006521.5). This has not been previously reported in patient (ClinVar) or control databases (1000Genomes, ExAC, gnomAD).

Patient 10

She is a 22 years old female. Mother's pregnancy prior to Patient 14's was diagnosed with severe Dandy Walker syndrome and agenesis of the corpus callosum among other malformations and was

terminated. Mother took Tegretol during the pregnancy that was otherwise uncomplicated. Labor started at 3 weeks and 1 day early and she had a C section for failure to progress. She was 2900g with no concerns for distress. Mom did not breastfeed due to her own seizure medications. She had a poor suck from birth, but did put on weight. At 6 months, her mother noted that she was delayed, but the pediatrician was not yet concerned. She rolled at 4-5 months. She laughed at 4 months and started to babble around nine months but stopped both of these at nine months. She sat at 11 months. She walked at 7 years, and has had more difficulty walking as she's gotten bigger and older and currently walks with 2 person assistance with a wide based gait. She has near continuous hand movements and rocking back and forth with continuous humming /moaning broken up by yells, does not follow commands. Eye movements are grossly intact, strength is antigravity with resistance. She has increased reflexes throughout, poor coordination with tremor of upper extremities. She has never had spoken words besides mama and yaya. Now mother uses cards to spell out words and ipads for communication. Her seizures started at age 10 shortly after she got her first period at age 10. These were predominately generalized tonic clonic seizures, and were difficult to manage. She has also had abnormal movements and tremors that were not seizures. MRI at age 12 months was noted in previous notes to have delayed myelination prominent CSF spaces in bilateral anterior regions. She was seen by genetics in 2011. At that time a SNP array was sent and was normal. She has had a normal kidney US and ophthalmologic exam. She had a liver US noting "coarse texture". Screening for lysosomal storage disorders was negative and ruled out Salla disease, Wilson's disease, urine organic acids, plasma amino acids, sialic acid, oligosaccharides, lactate, pyruvate, VLCFA, cholesterol, triglycerides, copper, and ceruloplasmin. She reportedly underwent skin biopsy that was negative for GM1, Niemann-Pick, and Gaucher disease. Fragile X syndrome analysis was negative.

Patient 11

She was a female born at term after a normal pregnancy. Birth weight, length and OFC were normal. She had severe intellectual disability, at 9 years she could not walk nor speak. She had one seizure at 5 years old. Brain MRI was normal. On examination, she had mild and asymmetric spasticity as well as hypotonia, skin pigmentation anomalies, unilateral club foot. She had growth retardation on -2.5 SD.

Patient 12

She was a female aged 1 year. She was born at term after an uneventful pregnancy, with normal birth parameters. She developed hypotonia and global developmental delay in the first months of life, at 12 months could sit in tripod. She had no words. On examination, weight was above the 90th centile, she had skin hyperpigmentation and facial dysmorphism. Hearing tests showed conductive hearing loss. Brain MRI is pending.

Patient 13

He was a male of European origin, third child of a 37-year-old mother. The pregnancy was uneventful except for gestational diabetes. He was born at 41 weeks (weight: 2980 g, length: 49 cm, head circumference: 33 cm; APGAR 9/9/9, cord blood pH 7.26) with incomplete release of placenta. At birth was noted an hypotrophic newborn with grey triangle around the mouth and dilated abdomen. At 4 months hepatomegaly was detected. Obesity developed quickly during childhood. Developmental delay was detected at about 12-18 months of age. He could sit at 1,5 years. He had pharmaco-resistant epilepsy, with onset on the first year of life. At 22 years, he could not walk unless supervised or assisted. He was non-verbal. He had severe ID, self-injuring behaviour in childhood, bruxism. Stereotypic behaviour with almost constant (turning head and body from side to side with stereotypic hand movements). He had sleep disturbance, snoring (but no diagnosed obstructive sleep apnoe syndrome). On examination at 22 years, he had spastic tetraplegia, unsteady ataxic gait in orthopaedic shoes (without standing is not possible), strabism, excessive salivation. Brain MRI performed in April 2019 showed unspecific changes in myelinisation paraventricular of 3rd ventricle to thalamus and periventricular to occipital (no PVL-hypoxic pattern), slight generalized cerebral atrophy, and a retro-cerebellar arachnoidal cyst. Facial features were marked by hypertelorism, anteverted nares, short nose, flat nasal bridge, full cheeks, thick beard, synophrys, thick full head of hair, coarseness, almond-shaped eyes, flat face and fleshy earlobes. He had growth retardation, and was reported to be obese in childhood. He had flat feet, hip dysplasia, hyperlordosis, clubbing of thumbs. He had umbilical hernia, splenomegaly, anteriorly displaced anus, asymmetric kidneys.

Patient 14

This patient is a 4.5-year-old boy, born with birth weight 2890 g. after an uneventful pregnancy. His parents are healthy, non-consanguineous, of Ashkenazi-Jewish ancestry. A younger brother is healthy. Since infancy he was noted to have developmental delay as he did not follow objects or smiled by 7 months. However, his motor development has been relatively spared: he started to roll over at 5.5 months and was able to walk by himself at 2.5 years. Currently, he is able to climb upstairs with assistance and his gait is characterized by hypotonia and hyperlordosis. Speech development is severely affected and he does not pronounce any words at the age of 4.5 years. He has severe intellectual disability, his comprehension being consistent with a one year-old child. His behavior is characterized by autistic features and significant hyperactivity and distractibility. He is currently attending a special education program for autistic spectrum disorder. The child needs assistance in all daily activities. In early childhood, following recurrent respiratory infections in the presence of persistent very low oxygen saturation (65-90%), he was diagnosed with interstitial lung disease. Lately, his respiratory function has improved, with oxygen saturation >90% and no need for oxygen supplementation. He also has chronic diarrhea of unknown etiology following workup. On physical examination, facial dysmorphism is noted: coarse face, hypertrichosis, flat nasal bridge, anteverted nares, pink and full cheeks, low set ears and hyperlaxity of joints. Weight is in 75th percentile and length in 90th percentile. Body mass index (BMI) is 16. Head circumference is in 50th percentile. On neurological examination, he has hypotonia with increased deep tendon reflexes and oculomotor apraxia. His visual function is normal, but he is unable to focus his gaze and he has limited eye contact. In the past he was suspected of having cortical visual impairment, which was ruled out. Metabolic workup, brain imaging, echocardiography, hearing testing and ophthalmologic examination, including VER and ERG, were normal. Genetic analysis using WES detected a *de novo* missense mutation in the X-linked *TFE3* gene.

Patient 15

He was a male of European origin with unremarkable family history and neonatal course. He had severe developmental delay, walked at 3 years, and speech delay. He was referred in genetics clinic at 6 years and 6 months. His weight was on +1SD, length -1 SD, OFC +1SD. He had intellectual disability and autistic features. Facial features were remarkable by their coarseness, flat nasal bridge, epicanthus, thick lips and posterior plagiocephaly. He had strabismus. Brain MRI was normal.

Patient 16

He was a male referred to the Genetics Department at 5 years old for developmental delay, chronic lung disease, epilepsy, sensorineural hearing loss, obstructive sleep apnea, gastro-oesophageal reflux, dysphagia and neutropenia. A younger brother had autism. He had a history of premature birth at 33 weeks gestation, and neonatal period was remarkable by jaundice for which he received phototherapy. He sat at 4 years. At 7, he could not walk independently, had no speech. On examination, his BMI was over the 90th centile, he had growth retardation (length on -2.63 SD). His neurologic examination showed autistic features and tight heel cords. He had facial dysmorphism, cutaneous rash and livedo reticularis with pigmentary changes, and a right foot deformity. Brain MRI demonstrated arachnoid cyst and Dandy Walker malformation.

Patient 17

He was born from unrelated parents with no family history. The pregnancy was unremarkable. He was born at term by caesarean section for fetal distress, with all growth parameters around -2 SD and Apgar score at 8 and 9. He was admitted in the neonatal period for seizures on the first day of life, neonatal hypoglycemia due to hyperinsulinism, transient renal insufficiency (acute tubular necrosis), bronchomalacia, and cholestasis. Examination revealed hepatomegaly, hypotonia, umbilical and inguinal hernias. He had severely delayed milestones. Aortapexia was performed for bronchomalacia. A dilated cardiomyopathy was noted that improved over time to a slight left ventricle dilatation at this moment in time. When he was referred at 5 years old, he could not walk or speak, and had severe perceptive hearing loss. He had pigmentation anomalies along Blaschko's lines on trunk and back, ,

his extremities were disproportionally small, flat feet and growth retardation on -4.5 SD. Facial features were coarse, he had a prominent forehead, flat nasal bridge and full cheeks. The EEG showed epileptic activity and brain imaging widened ventricles and peripheral liquor spaces as well as some periventricular white matter hyperintensities. Metabolic screening in blood, urine and liquor did not show abnormalities but a muscle biopsy did show signs of fat- and glycogen accumulation. Also decreased ATP production and substrate oxidation was noted in muscle cells of the patient, although enzyme activity of the mitochondrial complexes was normal. The patient was thought to have an unknown lysosomal storage disorder.