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Supplemental Data

De Novo Mutations in *SLC25A24* Cause a Craniosynostosis Syndrome with Hypertrichosis, Progeroid Appearance, and Mitochondrial Dysfunction

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Supplemental Data

Supplemental Case Reports

Individual 1 is the second daughter of healthy, unrelated parents from Poland and has a healthy older sister. She was born spontaneously after an uneventful pregnancy at 39th weeks of gestation with a weight of 2,200 g (-2.4 SD), a body length of 52 cm (+0.1 SD), and a head circumference (HC) of 28 cm (-4.2 SD). At birth craniofacial malformations were marked. A chromosome analysis, hearing tests, and metabolic screening were normal. At the age of 3.5 years her height was 90.5 cm (-2.5 SD), her weight 9.2 kg (-2.9 SD), and her HC 44.5 cm (-4.1). Despite high caloric nutrition she hardly gained weight. She had turribrachycephaly, a large anterior fontanelle, coarse and sparse parietal scalp hair, a low anterior and posterior hair line, and hypertrichosis of her cheeks, neck, limbs, especially at the extensor sides, and back, especially affecting the midline and lumbosacral region. Her facial abnormalities included depressed supraorbital ridges, laterally upslanting eyebrows, severe midface hypoplasia, downslanting and short palpebral fissures, a small mouth, and a protruding lower lip and tongue. Her subcutaneous adipose tissue seemed reduced and her skin was loose, translucent, and wrinkly, resembling progeroid disorders. She had 5th toe nail hypoplasia, hypoplastic labia majora, an umbilical hernia, and a hypermetropia of +8 dpt. When last seen at the age of 5.5 years, her height was 105 cm (-1.9 SD), her weight 11.6 kg (-3 SD), and her HC 45.5 cm (-4 SD). Hypertrichosis and dystrophy were more pronounced than at earlier examinations and she had valgus deformity of her halluces. Her psychomotor development was delayed with a normal outcome. There was no evidence of teeth anomalies or a congenital heart defect. Her craniofacial malformations, short stature, hypertrichosis, hypermetropia, and genital hypoplasia lead to the diagnosis GCMS at the age of 1.5 years, although coronal craniosynostosis was never radiologically confirmed.

Individual 2 corresponds to the individual reported by Adolphs and coworkers in 2010.[1] She is the firstborn daughter of a healthy, unrelated Hungarian couple. Her younger sister is healthy. She was born at 36 weeks of gestation after an uneventful pregnancy with a weight of 2,225 g (-1 SD) and a body length of 42 cm (-1.5 SD). The HC at birth is unknown. In her first year of life she underwent two craniofacial procedures due to a complex craniofacial malformation with coronal craniosynostosis and severe midface hypoplasia. After a third intervention at the age of 7 years she experienced necrotizing soft tissue infection of the scalp.[1] At clinical examination at the age of 7 years her height was 117 cm (-1.2 SD), her weight 15.5 kg (-2.7 SD), and her HC 50 cm (-1.1 SD). She presented with turricephaly, short palpebral fissures, deeply set eyes, midface hypoplasia, a flat philtrum, prognathia with tongue protrusion, and posteriorly rotated, small, low-set, dysplastic ears. Earlier childhood photographs showed downslanting palpebral fissures. She had rather sparse scalp hair, medially and laterally

sparse eyebrows, but facial and body hypertrichosis, and a low posterior hairline. She had hypoplasia of her labia majora. Her first dentition had been normal, whereas there was oligo- and microdontia of her second teeth. She had deep palmar creases and partial cutaneous syndactyly of the 2nd and 3rd toes. Hand radiographs showed short metacarpals and a retarded bone age, and serum alkaline phosphatase (AP) was slightly reduced. An ophthalmologic examination showed hypermetropia of +10 dpt and a reduction of visual acuity to 0.5. Her psychomotor development and intelligence were normal. 244K Array-CGH and karyotyping displayed normal results, as well as hearing evaluation.

Individual 3 is the fourth child of a healthy, unrelated German couple. There was intrauterine growth retardation, first detected at 34 weeks of gestation, and a prenatal diagnosed skull deformity. The girl was born at 38 weeks of gestation with a weight of 1,600 g (-1.1 SD), a body length of 43 cm (-0.1 SD), and a HC of 29 cm (-3.4 SD). Postnatally, she presented with brachycephaly due to coronal craniosynostosis, which was corrected at the age of 2 years. A persistent ductus arteriosus (PDA), an atrial septum defect type II (ASD II), and pulmonary artery hypertension (PAH) were diagnosed. Due to recurrent aspiration pneumonia she needed tracheostomy. She had a large anterior fontanelle, a depressed nasal root, short palpebral fissures, small, round, and dysplastic ears, and a median chin crease. She had hypoplastic distal phalanges and absent nails of her 4th and 5th fingers, and cutaneous syndactyly 2/3 and 4/5 of her toes. Her hands and feet were short, and all other finger and toe nails were small. Her abdominal wall was translucent and soft with diastasis recti, and she had an umbilical hernia. Her labia majora were hypoplastic. At the age of 1.5 years a hydrocephalus communis was diagnosed and a shunt implanted. When last seen at the age of 5 years, she had a height of 91 cm (-4.4 SD), a weight of 9.8 kg (-2.1 SD), and a HC of 49 cm (-1.3 SD). She presented with thick scalp hair, thick eyebrows, synophris, and hypertrichosis of her arms, legs, and back. Her skin was still translucent and soft, with reduced subcutaneous adipose tissue. She had depressed supraorbital ridges, a prominent glabella, deeply set eyes, epicanthal folds, a depressed nasal bridge, a short nose, a long philtrum, hypodontia, deep palmar creases, and sandal gaps. She had hypermetropia of +9 dpt and mild hearing impairment due to dysplasia of the middle and inner ear. Severe failure to thrive required nasogastric and later percutaneous endoscopic gastrostomy (PEG) tube feeding. Fundoplicatio was performed to treat gastroesophageal reflux. Recently, ectasia of the ascending aorta was noted. She had muscle weekness, reduced endurance, and a motor developmental delay with independent walking at the age of 19 months. She had hemiplegia followed by a craniocerebral injury. Her speech development was first delayed but later in the normal range. Repeated measurements of her body temperature, post-operative measurements of pH, and newborn

metabolic screening were normal. Array CGH as well as analysis for craniosynostosis-related hotspot mutations in *FGFR1*, *FGFR2*, *FGFR3*, and *TWIST1* were normal.

Individual 4 is a one-year-old girl and the fourth child of a healthy, consanguineous Turkish couple. All family members are healthy except one of her brothers who has bilateral brachydactyly of the index fingers. Intrauterine growth retardation (IUGR) was first detected at 20 weeks of gestation, and at 24 weeks of gestation additionally a skull deformity, large intracranial ventricles, a high arched palate, and an umbilical hernia, were noted. The girl was born at 39 weeks of gestation with a weight of 1,700 g (-3.4 SD) and a HC of 29.4 cm (-3 SD). Postnatally, she presented with turrybrachycephaly, a large anterior fontanelle, midface hypoplasia with a depressed nasal root, a small nose, depressed supraorbital ridges, lower eyelid entropion, laterally upslanting eyebrows, downslanting and short palpebral fissures, a small mouth with a protruding lower lip and tongue, small, round, low set, and dysplastic ears, and an umbilical hernia with diastasis recti. Her skin was thin and translucent with nearly absent subcutaneous gluteal fat pads. She had hypoplastic distal phalanges and almost absent nails of her 2^{nd}-5^{th} fingers. Her feet were short and her toe nails were small. Her labia majora were hypoplastic. Sparse parietal scalp hair, a low posterior hair line, and hypertrichosis of her limbs, especially at the extensor sides, and back were marked. Despite high caloric nutrition she hardly gained weight. There was a left ventricular hypertrophy seen at echocardiographic examination, and bilateral nephrolithiasis (4 mm in right renal upper pole; 5 mm and 3.5 mm in left renal pelvis) on renal ultrasound. MRI at 9 months of age showed a diffuse thin corpus callosum, bilateral large lateral ventricles, large posterior fossa, hypoplastic cerebellar vermis, a large retro-cerebellar area suggestive for Dandy Walker anomaly, slowed myelinization at the terminal zone, a 4x3 mm cyst at pineal gland, and a nasal septum deviation. The first step metabolic and biochemical screening tests, including lactic acid, plasma amino acids, as well as urine and plasma organic acids, were unremarkable. Karyotyping and several body temperature measurements were normal. Her eye examination revealed hypermetropia and she failed the hearing test due to narrow external auditory canals. At age 18 months, her weight was 5.7 kg (-0.75 SD), length 62 cm (-7 SD), and HC 43 cm (-4 SD). Her psychomotor development was mildly delayed but was later within the normal range. At the age of 20 months she died after a urinary infection.

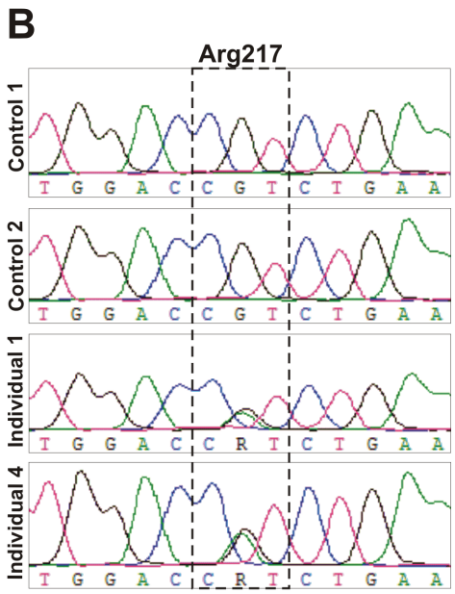
Individual 5 is a 14-year-old female who was born to a 28-year-old G2P2 mother after a pregnancy that was complicated by a vaginal infection during the first trimester and vaginal bleeding from 20-25 weeks of gestation. An ultrasound at five and 22 weeks identified no abnormalities; however, on examination at 32 weeks, a low fundal height was detected, and an ultrasound at 35 weeks showed asymmetric intrauterine growth retardation

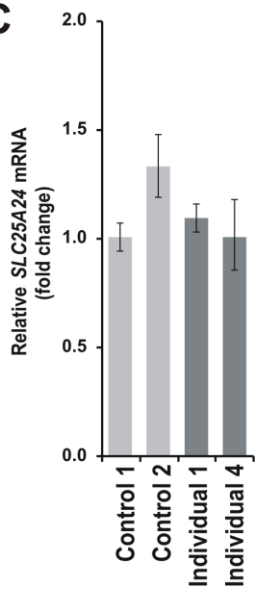
with retention of head size. No placental abnormalities were detected. She was delivered by cesarean section because of prolonged labor at 37 weeks of gestation. Apgars were reportedly 4 at one minute and 9 at five minutes. Birth weight was 1,722 g (-2.58 SD). At 3 months, she was noted to have poor growth and dysmorphic facial appearance including a prominent forehead, increased facial hair, midface hypoplasia, deeply set eyes, mild retrognathia, and slightly posteriorly rotated ears. She had syndactyly and shortened distal phalanges with small nails. Her skin was wrinkled, redundant, and translucent with reduced subcutaneous fat tissue. She had hypoplastic labia majora and an umbilical hernia. The size of her anterior fontanelle was normal. No cranial radiograph was performed regarding a possible craniosynostosis. She continued to have persistent failure to thrive, for which reason she received a gastrostomy tube for supplemental feeding. A patent ductus arteriosus was surgically corrected. She was diagnosed with pulmonary hypertension, which resolved later. She had hydrocephalus with gradually increasing head size and underwent ventriculoperitoneal shunt placement at 6 months of age. Mild unilateral conductive hearing loss and hypermetropia of +13 and +14 were stated. By 10 months of age, gastroesophageal reflux and hydronephrosis were diagnosed. Later, oligo- and microdontia were detected. Her motor development was delayed due to muscular hypotonia, her speech and cognitive development normal. When last seen at the age of 14 years, her school performance was excellent. She required visual supports related to nystagmus, strabismus, blindness in her left eye, and decreased vision in her right eye. Her growth had improved since starting menses and her night time feeding had decreased. She had moderate obstructive sleep apnea and intermittent constipation. Due to her foot abnormalities, braces were recommended and she had scoliosis. Her echocardiogram showed mildly dysmorphic valves, mild insufficiency of all valves, upper mild to moderate tricuspid regurgitation, and aortic ectasia. She had facial and body hypertrichosis as well as coarse and dense scalp hair. At the age of 14 years, her height was 142 cm (-3 SD) and her weight 34 kg (-2.7 SD). Her HC at the age of 13 years was 54 cm (+0.2 SD).

Figure S1. Stable *SLC25A24* mRNA expression in fibroblasts carrying p.Arg217His

A

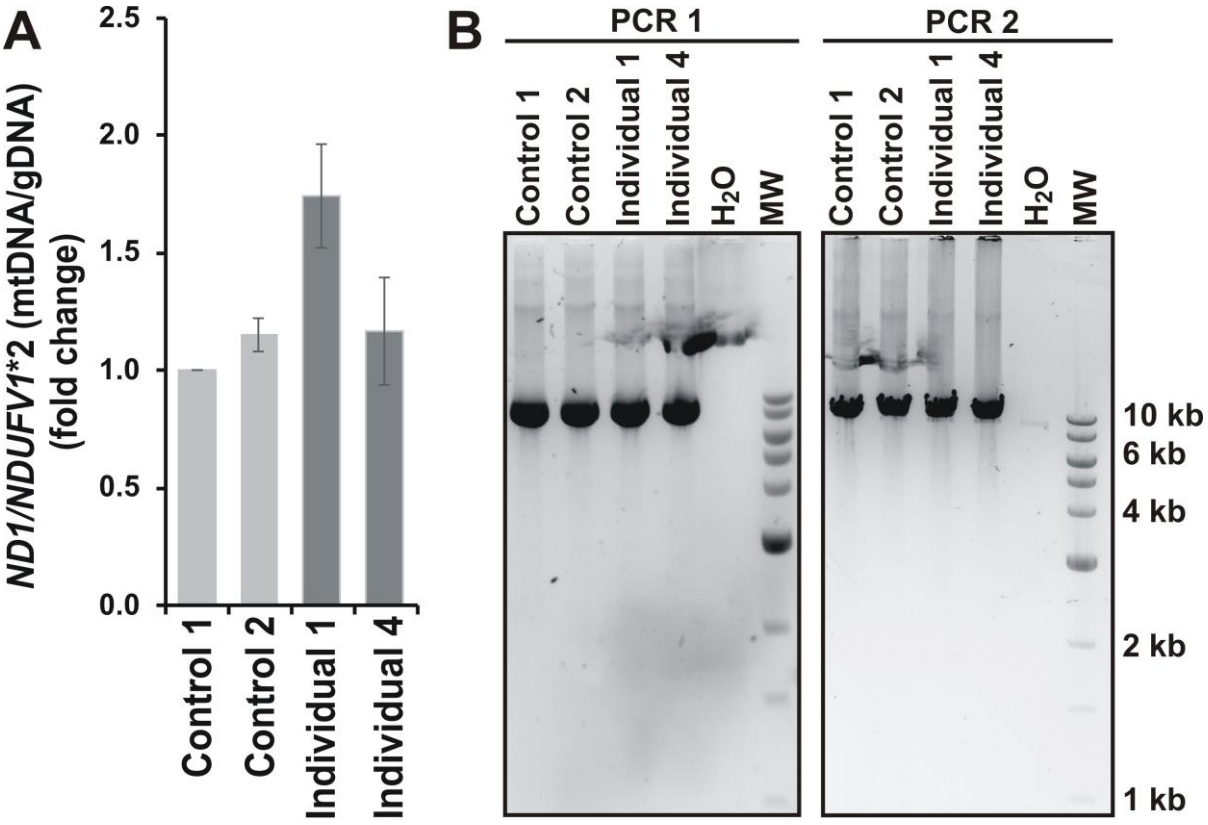
Individual	Coverage
1	31x
2	123x
4	72x
5	30x





(A) Number of reads covering the genomic positons chr1(GRCh37):g.108700103 and chr1(GRCh37):g.108700104 obtained in NGS sequencing studies. **(B)** After RNA isolation from control and probands' fibroblasts and cDNA synthesis the cDNA region containing the codon 217 was amplified and subsequently sequenced. In both fibroblasts lines harboring SLC25A24-p.Arg217His a heterozygous G>A substitution was detectable which was completely absent in the controls. **(C)** Quantitative PCR analysis also revealed no reduction of the affected SLC25A24 mRNA in the fibroblasts from the affected individuals in comparison to controls.

Figure S2. No mitochondrial DNA depletion or common deletions in fibroblasts carrying the SLC25A24 amino acid change p.Arg217His



(A) Quantitative PCR analysis of the nuclear locus containing *NDUFV1* and the mitochondrial DNA localized gene *ND1* in control and probands' fibroblasts revealed no depletion of the mitochondrial genome in cells from the affected individuals. **(B)** Furthermore, common mitochondrial DNA deletions were excluded using two different long-range PCRs.

Supplemental Reference

1. Adolphs, N., Klein, M., Haberl, E.J., Graul-Neumann, L., Menneking, H., and Hoffmeister, B. (2011). Necrotizing soft tissue infection of the scalp after fronto-facial advancement by internal distraction in a 7-year old girl with Gorlin-Chaudhry-Moss syndrome--a case report. J Craniomaxillofac Surg 39, 554-561.