Biallelic *B3GALT6* mutations cause spondylodysplastic Ehlers-Danlos syndrome

Supplementary Materials

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Supplementary Note – case reports

Family I

This boy (PI:1) was born to a non-consanguineous couple of Caucasian origin. Part of his clinical history has previously been reported by Brockel *et al* (2017).[1] Family history was unremarkable. Bilateral lower limb anomalies and small cerebellum were detected on prenatal ultrasound, and decreased fetal movements were reported throughout the pregnancy. Labor was induced at 38 weeks of gestation because of oligohydramnios, and the boy was born via normal vaginal delivery. Birth weight was 2905 grams, and length was 47 cm. At birth he presented with multiple congenital anomalies including dysmorphic facial features, laryngeal cleft, lax skin, scoliosis, upper and lower limb contractures, and arachnodactyly. He suffered multiple seizures shortly after birth. These were caused by an intracranial haemorrhage that presumably occurred during vaginal birth, and he was intubated and mechanically ventilated for the first month of life. He underwent right hemidiaphragm plication at eight months of age for a right-sided Morgagni diaphragmatic hernia, and had surgery for bilateral inguinal hernias and hypospadias. During the first 18 months of life, he suffered at least 16 fractures, for which he was started on pamidronate infusions. These were discontinued later because of a clear decrease in the frequency of fractures. At age 6 years, he still needed non-invasive positive pressure ventilation at night and oxygen via nasal cannula while awake because of muscular hypotonia, tracheomalacia and restrictive lung disease. A gastrostomy tube was inserted because of severe swallowing difficulties secondary to muscle hypotonia. Clinical examination at age 6 years showed a pale boy with a jowly, flattened midface, plagiocephaly, mildly posteriorly rotated ears, proptosis, blue sclerae, hypoplastic teeth, and a moderately high arched palate. He had a soft, doughy and fragile skin. He had frequent and severe bruising and platelet-inhibiting medications were avoided. There were contractures of the elbows, knees and the ankles, ulnar deviation of the fingers and wrists, arachnodactyly, and pronounced hypermobility of the distal joints with spontaneous dislocations of the MCP joints and recurrent hip dislocations. Testes had descended bilaterally, but were retractile. He was non-ambulatory and could not sit independently. Given his non-ambulatory status, fractures and joint dislocations were managed conservatively. He was nonverbal, though he communicated via picture-assisted communication on a tablet computer. He received regular physical, occupational and communication therapies, and he attended school with an individualized educational plan in place. Echocardiogram at age 6 showed mitral valve prolapse with moderate regurgitation, mildly dilated aortic annulus (Boston Z-score +2.8), moderate dilation of the sinuses of valsalva (Boston Z-score of +4.7, 2.54 cm), and mild dilation of the ascending aorta (Boston Z-score +3.2); the pulmonary artery was also mildly dilated (Z-score +4.1). Z-scores were stable over time on medical therapy of enalapril. He suffered multiple pneumothoraces, and was successfully treated with chemical pleurodesis. Dilated ophthalmological exam revealed optic nerve atrophy. He has mild visual impairment but no functional deficits. Skeletal radiographs showed gracile and osteopenic bones and evidence of multiple previous fractures, radioulnar synostosis, arachnodactyly, subluxation of the hips, shortened ribs, and a sinistroconvex thoracolumbar scoliosis, which had been progressive until the age of three with a maximum Cobb angle of 62 degrees but was stable now. MRI of the brain

and neck showed invagination of the C1 vertebra into the foramen magnum with secondary hydrocephalus and ventriculomegaly. A vetriculoperitoneal shunt was placed at five months of age, and he wears a collar to stabilize the cervical spine.

Family II

This girl (PII:1) is the first child of a non-consanguineous couple of Caucasian origin. Family history was unremarkable. She was born by caesarean section because of prolonged labor and decelerations at 41 weeks of gestation. Pregnancy was uneventful. Birth weight was 2800 gram. She was born as a floppy infant, and was transferred to the NICU because of multiple congenital anomalies. There was mild respiratory insufficiency and she was noted to have mild hypotonia, contractures of elbows and wrists, windswept fingers, arachnodactyly, adducted thumbs, hypermobile digits, bilateral hip dislocation, club feet, a left femur fracture, and facial dysmorphology. There was an episode of rapid eye movement followed by staring, which was concerning for seizure. She required oxygen via nasal cannula, and received regular physical, and occupational therapies after NICU discharge. She was seen at the cardiac genetics clinic at the ages of four and eight months. The primary concerns had been growth and feeding difficulties, head shape and asymmetry, and bone fragility after two long bone fractures. Weight and height had both fallen off the growth curve, and a nasogastric tube was placed at the age of eight months. She presented with a very thin, soft, and translucent skin. There was hypotonia, generalized joint hypermobility, arachnodactyly, some overlapping toes, and inverted feet. The contractures had been improving with a slow increase in the range of motion. She presented with some dysmorphic features including skull asymmetry, dolichocephaly, blue sclerae, shallow-appearing orbits, a small upturned nose, small mouth, malar hypoplasia, and a very high arched palate. She was smiling, cooing, and babbling, and could sit with assistance at the age of 8 months. Perinatal echocardiogram was notable only for a small patent ductus arteriosus (PDA), a patent foramen ovale (PFO), and a mildly dilated right atrium and right ventricle with mild hypertrophy and normal systolic function. Aortic dimensions were first appreciated to be abnormal at age five months with a Boston Z-score of +4.3 (1.5 cm) at the sinuses of valsalva. Repeat echo at age seven months showed a small residual PFO with left to right shunt and stable aortic dimensions (sinuses of valsalva 1.6 cm, Boston Z-score of +3.7). All other aortic dimensions were within normal range. Skeletal radiographs showed diffuse demineralization, bowing of long bones, bilateral hip dislocation, and a displaced left proximal femoral fracture at birth. At the age of nine months, they additionally showed a mild thoracic levoconvex lumbar scoliosis (Cobb angle of 20 degrees), and unusual posterior tapering of the L5 vertebral body. CT showed asymmetric outward bowing of the left parietal bone with partial premature closure of the coronal sutures inferiorly, and marked anterior atlanto-axial subluxation with impression of the odontoid process upon the cervicomedullary junction.

Family III

PIII:1 is the first child of non-consanguineous Dutch parents. The family history is unremarkable. Prenatal ultrasound showed shortening of the tubular bones, flexion contractures of the wrists, and club feet. She was born at 37 weeks and 4 days of gestation by cesarean section because of breech position. Birth weight, length, and occipitofrontal circumference (OFC) were 2630 g, 45 cm, and 36 cm, respectively. Four days after birth, she started having apneas leading to desaturation, and she required continuous positive airway pressure (CPAP) for seven days and caffeine therapy for several weeks. At the age of two months, she developed respiratory distress with tachypnea, caused by a combination of mild lung hypoplasia, atelectasis, mild distal tracheomalacia with an oval-shaped entrance of the right main bronchus, and compression of the proximal trachea due to the scoliosis. At clinical examination, she presented with short stature, adducted thumbs, bilateral IV-V camptodactyly, subluxation of the left wrist, bilateral hip dislocation, hyperextension of the knees, club feet, and progressive scoliosis. At the age of 7 months she broke her right femur. There was marked hypotonia, and very limited limb movement. Her skin was doughy to the touch with redundant skin folds at the ankles and wrists. There were some facial dysmorphic features such as a long philtrum, depressed nasal bridge, puffy cheeks, and blue-greyish sclerae. Motor development was severely delayed, and because of severe hypotonia, she could only lie flat, and barely lift her arms. Early cognitive development appeared normal, but was difficult to assess. Brain MRI performed at the age of 1 month showed no abnormalities. Echocardiography showed a grade II ASD, but no other cardiovascular abnormalities were reported. Ophtalmic examination at the age of 4 months showed high myopia (OS / OD -5,00 dpt) and peripapillary atrophy. She had bilateral hearing loss of 45 dB, with a conductive component of 20-25 dB at the age of 11 months. Radiographs taken between the age of 7 and 11 months showed an osteopenic aspect of the skeleton, signs of healed rib fractures, scoliosis, flexion contractures of all joints, dislocations of the elbows and knees, hypoplastic iliac bones with a poorly formed acetabulum and delayed ossification of the femoral head, coxa valga, and vertical talus. Bisphosphonate treatment was started at the age of 1 years and 2 months. At the age of 12 months she was admitted to the hospital because of a severe torticollis. MRI showed hydrocephalus and posterior displacement of the vertebral column with atlanto-occipital and atlanto-axial dislocation without compression of the spinal cord. She was treated conservatively. The girl passed away at the age of 18 months due to sputum aspiration and hypoxia.

Family IV

PIV:1 was born via caesarean section as the first daughter and fifth child of a non-consanguineous Congolese-Rwandan couple. She suffered numerous fractures during childhood. At the age of 25 years, height and weight were 130 cm and 40 kg, respectively. She presented with a short stature with short and deformed extremities, joint hypermobility, contractures, severe and progressive scoliosis, and dentinogenesis imperfecta. She had a hyperextensible, velvety skin with multiple atrophic scars, and mild faciale dysmorphic feautures including midafacial hypoplasia, frontal bossing, prominent eyes, blue sclerae, and low-set and posteriorly rotated ears. Cognitive and motor development were normal. Echocardiography was normal. With the exception of microcornea, ophthalmic examination showed no

abnormalities. There was no hearing loss. Radiographs showed scoliosis, flexion contractures, hypoplastic iliac bones, acetabular dysplasia and delayed ossification of the femoral head, metaphyseal flaring, and narrowing and bowing of the long bones. Bone mineral density was severely reduced (T-score lumbar vertebrae -2.2, Z-score -2.1; T-score left femur -3.4, Z-score -3.4).

Her older brother (PIV:2) had a very similar phenotype. He was born by caesarian section. He suffered numerous fractures during childhood, and developed a progressive kyphoscoliosis from the age of 8 years. At the age of 31 years, height, weight, and OFC were 128 cm, 23 kg, and 60 cm respectively. He presented with a short stature with short and deformed extremities, small joint hypermobility, pectus carinatum, severe kyphoscoliosis, flexion contractures of the elbows, dentinogenesis imperfecta, and proptosis. He also had a hyperextensible, velvety skin with multiple atrophic scars, and had a similar facial gestalt. Cognitive and motor development were normal. Echocardiography showed moderate right ventricular hypertrophy and an elevated pulmonary arterial pressure, moderate dilatation of the left atrium, mitral valve prolapse, and mild mitral and aortic insufficiency. Radiographic findings were similar to his sister. Bone mineral density was severely reduced (T-score lumbar vertebrae -7.3, Z-score -7.3; not measurable at the level of the femur because of the severe epiphyseal dysplasia). Pamidronate treatment was started at the age 31 years.

PIV:3 is the second affected son from this family. He was born by caesarian section because of a birth weight upward of 4000 grams. At birth, he was noted to have a hyperextensible skin. He suffered numerous fractures during childhood, and developed a progressive kyphoscoliosis from the age of 5 years. As a consequence, walking has become difficult. At the age of 28 years, he was referred to the departments of genetics and rheumatology with multiple skeletal and cutaneous anomalies. Height, weight, and OFC were 132 cm, 32 kg, and 61 cm respectively. He presented with muscle atrophy, generalized joint hypermobility, flexion contractures of the elbows, a pectus carinatum kyphoscoliosis, short and deformed extremities, and dentinogenesis imperfecta. He had a hyperextensible, velvety skin with multiple atrophic scars. His facial gestalt was similar to his siblings. Cognitive and motor development were normal. Echocardiography showed a mild tricuspid valve insufficiency, but no other abnormalities. Bone mineral density was severely reduced (T-score lumbar vertebrae -6.4, Z-score -6.4; T-score left femur -4.0, Z-score -3.9). Radiographic findings were similar to his siblings.

Family V

This patient (PV:1) is the first child of non-consanguineous Indian parents. He was born with multiple skeletal anomalies including scoliosis, bilateral flexion contractures of the elbows, and wrists, clubfeet, and dislocated hips and knees. His birth weight was 3000 grams. He was referred to a clinical geneticist at the age of 7 years. At clinical examination he was noted to have a short neck, a small thorax with narrow shoulders and short clavicles, severe scoliosis, club feet, adducted thumbs, and a pectus carinatum. There was marked joint hypermobility with easily dislocatable joints (knees, thumbs, shoulder, radial heads). His skin was hyperextensible. He had a long coarse, somewhat progeroid, face with proptosis, downslanting of the palpebral fissures, blue sclerae, a high arched palate and dental crowding. There was a global developmental delay. More specifically, he showed some autistic traits,

and did not speak. Cardiac and abdominal ultrasound were normal. Radiopgraphic examination showed thin and gracile long bones, osteopenia, severe dextroconvex thoracic sinistroconvex lumbar scoliosis, flat femoral heads, and multiple dislocations of the thumbs, finger joints, elbows and knees.

Family VI

This patient (PVI:1) was the first and only child of a non-consanguineous Dutch couple. Pregnancy was normal. She was born at 37 weeks by cesarean section because of breech position. Birth weight was 2580 grams. She needed oxygen for a short period after birth. She was born with club feet for which she got plasters casts, followed by operative correction a few weeks later. In addition, there was ulnar deviation of the fingers, for which she was treated by splints, and bilateral hip luxation, for which she was treated with a Pavlik harness. Because of recurrent dislocations of the left hip she was subsequently casted after surgical reduction. Examination by a clinical geneticist at the age of 3 years showed a happy girl with a short stature and mild facial dysmorphology including frontal bossing, a depressed nasal bridge, thin lips, prominent eyes with long eyelashes, and greyish sclerae. She had severe caries. Joint hypermobility was evident (hyperextension of knees and finger joints), but she had mild contractures of the elbows and the PIP joint of the left index finger. In addition, she had a thoracolumbar dextroconvex torsion-scoliosis. The skin appeared normal, and was not hyperextensible. A very large lump was palpable in her abdomen, which turned out to be a Wilms tumor. The tumor and left kidney were resected, and she received chemotherapy because of lung metastases. She started walking at the age of 2 years, but overall development was normal. Serial radiographs showed a progressive scoliosis in the lower thoracic and lumbar spine, for which she was treated with a brace at the age of 4.5 years. The vertebral bodies were flattened and showed some anterior beaking. The iliac bodies were hypoplastic with a horizontal acetabulum. The femoral necks were short and broad. The left femoral head showed lateralization. The tubular bones of the hands were rather short with narrow shafts. Development of the carpal bones and epiphyses was slightly delayed. The left index finger was angulated at the proximal interphalangeal joint.

Family VII

This patient (PVII:1) is the first and only child of Iranian consanguineous parents. This boy was first examined at the age of three years. Clinical examination showed facial dysmorphic features including midfacial hypoplasia, blue sclerae, downslanting of the palpebral fissures, a short nose with anteverted nares, and a long philtrum. He had a short stature, bilateral club feet, pectus excavatum, severe joint hypermobility in knees, wrists and small joints, and broad thumbs. His skin was soft with poor recoil. Radiographs showed proportionate shortening of the long bones, cervical kyphosis, flattened and anteriorly rounded vertebral bodies, and multiple joint dislocations. His case was submitted to the International Skeletal Dysplasia Registry (ISDR) and SEMD with joint laxity was considered the likely diagnosis. Cognitive development was normal.

Family VIII

PVIII:1 is the second child of young non-consanguineous parents. Pregnancy was uncomplicated. He was born with contraction of the hands, adducted thumbs, and long fingers. He was treated, without satisfying results, with growth hormone for postnatal growth restriction. Final height was 141,5 cm. Inguinal hernia was operated. Thoracolumbar scoliosis was noted at the age of 2-3 years, and required surgery at the age of 15 years. He suffered a perforated diverticulitis at the age of 33 years, which was complicated by difficult cicatrization and necessity of prolongated ileostomy. He suffered a cerebral vascular accident at the age of 35 years. Clinical examination at the age of 37 years showed a man with mild dysmorphic features including downslanting of the palpebral fissures, a long philtrum, microretrognathia, and large, lobulated and posteriorly rotated ears. He presented with short stature, joint hypermobility, kyphoscoliosis, contractures of the elbows and fingers, and arachnodactyly. His skin was thin and bruised easily. There was atrophic scarring. Echocardiography showed a slightly dilated aortic aorta (39 mm at the sinuses of valsalva). Radiographs showed diffuse demineralization, ovoid vertebrae, fracture and compaction of L5, bowing of long bones, hypoplastic ilia, dysplasia of the femoral head, and coxa plana. Bone mineral density confirmed osteoporosis.

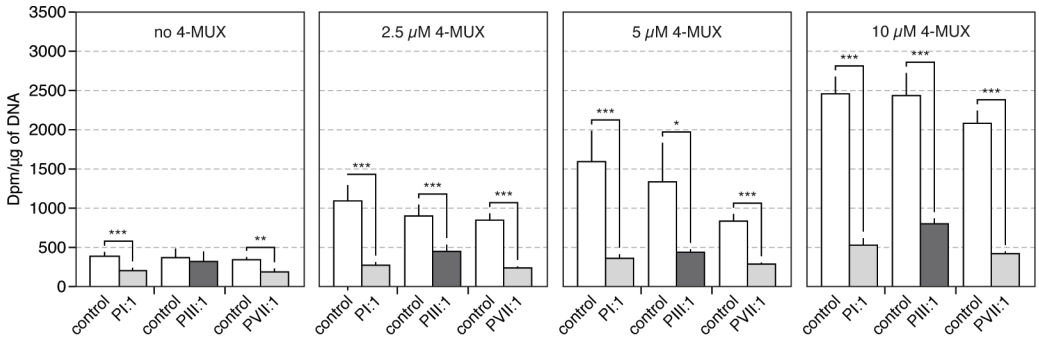
Family IX

A non-consanguineous couple of Caucasian origin had two pregnancies terminated because of severe skeletal dysplasia.

The first pregnancy (PIX:1) was terminated at 22 weeks and 5 days of gestation. Babygrams showed a male fetus with platyspondyly, medial bone spurs, shortening of the long bones with ulnar and radial bowing, club hands, camptodactyly, iliac bone hypoplasia, and rocker bottom feet. There were no organ abnormalities.

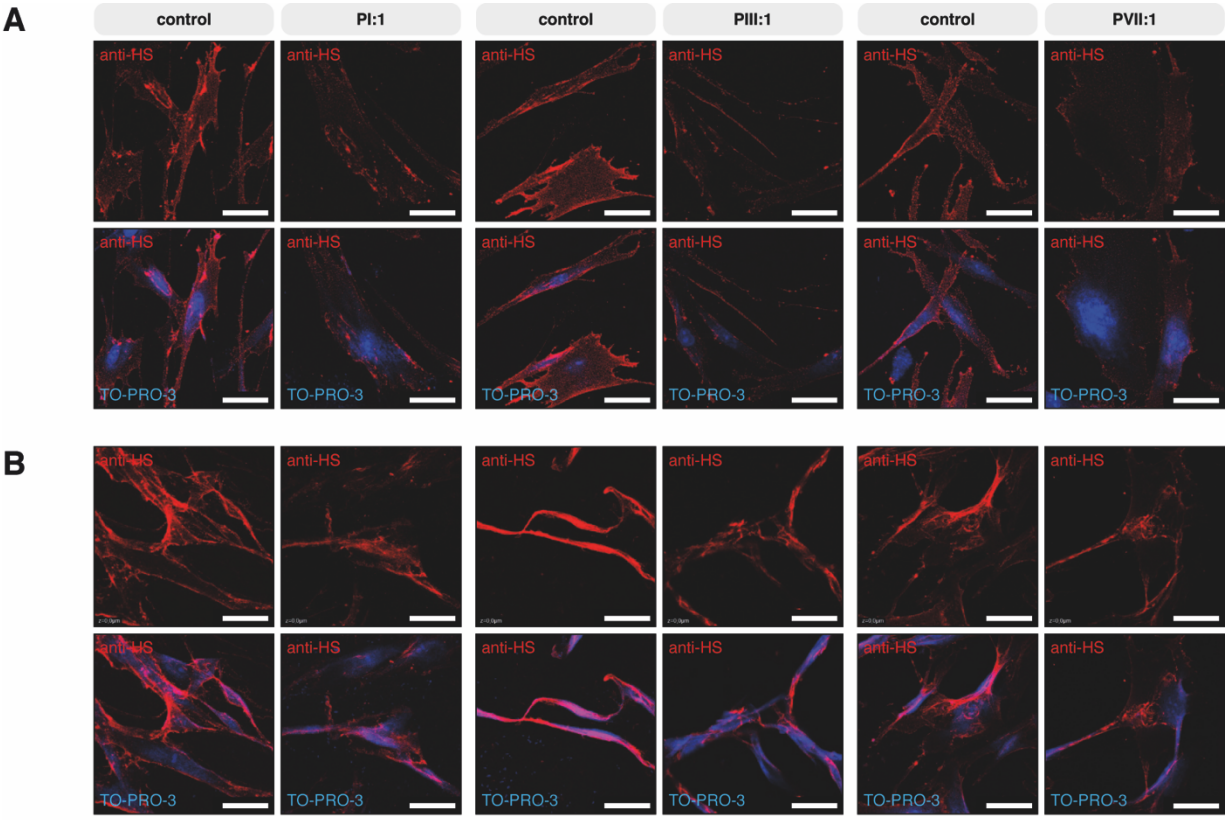
The second pregnancy (PIX:2) was terminated at 22 weeks of gestation because of a similar skeletal dysplasia.

Figure S1. *In cellulo* GAG synthesis assay



GAG synthesis was strongly reduced in patient fibroblast compared to sex- and age-matched controls. At the highest concentration of 4-MUX (10 μM) it was reduced by approximately 70 to 80%. Data are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (Student's *t*-test).

Figure S2. Immunofluorescence staining of HS chains



Immunofluorescence microscopy showed a reduction in the amount of HS chains at the membrane of fibroblasts from patients compared to controls. Nuclei were counterstained with TO-PRO-3. Scale bars represent 10 μm. Panel A and B show pictures taken from two separate experiments.

Table S1. Overview of additional genetic analyses

	PI:1	PII:1	PIII:1	PIV:1	PV:1	PVI:1	PVII:1	PVIII:1	PIX:1
karyotype	NP	NP	NP	NP	NP	NP	NP	NP	46, XY
arrayCGH	normal	normal	NP	NP	NP	NP	NP	NP	NP
single gene sequencing	NP	NP	*B3GALT6* *FLNB*	*B3GALT6*	*B3GALT6*	*B3GALT6* *WT1**	*B3GALT6*	NP	NP
panel sequencing	*EFEMP2* *FBN1* *FBN2* *FKBP10* *FLNA* *PLOD2* *SERPINF1* *SERPINH1* *SP7* *TGFBR1* *TGFBR2*	*B3GALT6* *B4GALT7* *ALPL* *BMP1* *COL1A1* *COL1A2* *CREB3L1* *CRTAP* *FKBP10* *IFITM5* *LEPRE1* *LRP5* *PLOD2* *PLS3* *PPIB* *SERPINF1* *SERPINH1* *SP7* *TMEM38B* *WNT1*						*B3GALT6* *ADAMTS2* *B4GALT7* *CHST14* *COL6A1* *COL3A1* *COL5A1* *COL5A2* *COL1A2* *COL1A1* *DSE* *ELN* *FBLN5* *FKBP14* *PLOD1* *TNXB*	
WES	+	NP	NP	NP	NP	NP	NP	NP	+
SNP array	NP	+	+	NP	NP	NP	NP	NP	NP

* *WT1* sequencing was performed after diagnosis of Wilms tumor. +, performed; NP, not performed.

References

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