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ORIGINAL ARTICLE

Exome sequencing reveals two novel compound heterozygous *XYLT1* mutations in a Polish patient with Desbuquois dysplasia type 2 and growth hormone deficiency

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Desbuquois dysplasia type 2 (DBQD2) is a rare recessively inherited skeletal genetic disorder characterized by severe prenatal and postnatal growth retardation, generalized joint laxity with dislocation of large joints and facial dysmorphism. The condition was recently described to result from autosomal recessive mutations in *XYLT1*, encoding the enzyme xylosyltransferase-1. In this paper, we report on a Polish patient with DBQD2 who presented with severe short stature of prenatal onset, joint laxity, psychomotor retardation and multiple radiological abnormalities including short metacarpals, advanced bone age and exaggerated trochanters. Endocrinological examinations revealed that sleep-induced growth hormone (GH) release and GH peak in clonidine- and glucagon-induced provocative tests as well as insulin-like growth factor 1 (IGF-1) and IGF-binding protein-3 levels were all markedly decreased, confirming deficiency of GH secretion. Bone age, unlikely to GH deficiency, was significantly advanced. To establish the diagnosis at a molecular level, we performed whole-exome sequencing and bioinformatic analysis in the index patient, which revealed compound heterozygous *XYLT1* mutations: c.595C>T(p.Gln199*) and c.1651C>T(p.Arg551Cys), both of which are novel. Sanger sequencing showed that the former mutation was inherited from the healthy mother, whereas the latter one most probably occurred *de novo*. Our study describes the first case of DBQD2 resulting from compound heterozygous *XYLT1* mutation, expands the mutational spectrum of the disease and provides evidence that the severe growth retardation and microsomia observed in DBQD2 patients may result not only from the skeletal dysplasia itself but also from GH and IGF-1 deficiency.
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INTRODUCTION

Desbuquois dysplasia (DBQD; MIM 251450) is a severe skeletal genetic condition, inherited in an autosomal recessive manner, belonging to the 'multiple dislocation group' according to the International Nosology of Genetic Skeletal Disorders.[1] Clinical characteristics involve severe prenatal and postnatal growth retardation (−5.0 s.d.), severe joint laxity with facultative dislocations of large joints, flat midface with prominent eyes, micrognathia, cleft palate and progressive scoliosis. Radiologically the syndrome presents with shortening of long bones, enlarged trochanters giving a 'monkey wrench' or 'Swedish key' appearance of the proximal femora, hypoplasia of thorax and ilia, and advanced carpal and tarsal ossification.[2] DBQD has been classified into two subtypes based on the presence (type 1; DBQD1) or absence (type 2; DBQD2) of additional characteristic hand abnormalities, comprising extra ossification center distal to the second metacarpal, delta phalanx or bifid distal phalanx of the thumb and dislocations of the interphalangeal joints.[3,4] Both DBQD type 1 and a subtype of DBQD called 'Kim variant', which is characterized by apparently normal hands, and remarkably advanced carpal age, severe precocious osteoarthritis of the hand and spine, result from mutations in the *CANT1* gene (MIM 613165).[5,6] In addition, patients from two consanguineous Turkish families reported to have DBQD2 described by Furuichi *et al.*[7] were also demonstrated to carry *CANT1* mutations. Very recently, Bui *et al.*[8] showed that mutations in *XYLT1* (MIM 608124), encoding for xylosyltransferase 1 (EC 2.4.2.26)—an enzyme involved in one of the very first steps of proteoglycan synthesis[9,10]—are responsible for a major subset of DBQD2 cases.

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Patients affected by DBQD type 1 and 2 present with features overlapping with several skeletal dysplasias, including diastrophic dysplasia (MIM 222600), spondyloepiphyseal dysplasia with dislocations (MIM 143095), Larsen syndrome (MIM 150250) and chondrodysplasia with joint dislocations (MIM 614078).[11–14] Therefore, determining a specific diagnosis based on the clinical and radiological symptoms may be extremely difficult. Next-generation sequencing (NGS) technology has been recently used as a diagnostic tool to identify the molecular basis of genetically and phenotypically heterogeneous hereditary disorders. In the current study, we applied NGS-based whole-exome sequencing (WES) and bioinformatic tools to establish a diagnosis in a male patient presenting with a severe skeletal dysplasia of prenatal onset associated with developmental delay. We found two novel compound heterozygous *XYLT1* mutations, demonstrating that the proband was affected by DBQD2.

MATERIALS AND METHODS

Patients

We investigated a sporadic index case of Polish ethnicity who presented with a severe skeletal dysplasia of prenatal onset. In addition, we studied an unaffected brother and both healthy parents of the proband. Institutional Review Board at Poznan University of Medical Sciences (Poland) approval was obtained and the family was enrolled with informed consent. DNA was extracted by standard protocols from venous blood samples of the proband, his healthy parents and a healthy brother. Furthermore, parents of the index case gave written consent for presentation of his photographs for publication.

Molecular screening and validation of WES

Before exome analysis, Sanger sequencing-based mutational screening of the *SLC26A2* gene (MIM 606718) was performed as our proband was initially suspected to be affected by diastrophic dysplasia. Validation of NGS results in the proband as well as parental testing was achieved using targeted Sanger sequencing. Primers were designed by means of Primer 3 software (Howard Hughes Medical Institute, Chevy Chase, MD, USA). The sequencing reactions were carried out with dye terminator chemistry (ABI Prism DigDye v.3.1) and run on automated sequencer Applied Biosystems Prism 3700 DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The sequencing results were visualized using Bioedit software (Ibis Biosciences, Carlsbad, CA, USA).

WES and data analysis

DNA sample of the proband was subject to exome capture and high-throughput sequencing. Target enrichment was performed using the Agilent SureSelectXT Human All Exon V4 Kit (Agilent Technologies, Santa Clara, CA, USA) and sequencing of 100 bp paired-end reads was carried out on Illumina HiSeq 1500 (Illumina, San Diego, CA, USA). A variant-discovery pipeline was built based on the GATK Best Practices. The human reference genome (hg 19) was used. Filtering and interpretation of NGS data was carried out using Exomiser running hiPHIVE algorithm. Possibly pathogenic variants were then validated with standard Sanger sequencing in all available family members. Variants were visualized with the use of Integrative Genomics Viewer.

Paternity testing

Paternity analysis was achieved by testing 42 microsatellite markers on 10 different chromosomes with the use of quantitative fluorescent PCR commercially available kit (Devyser Extend v.2; Devyser AB, Stockholm, Sweden), as per the manufacturer's protocol.

RESULTS

Clinical report

The proband was conceived by healthy, nonconsanguineous parents, a 30-year-old mother and a 34-year-old father, who already had a healthy son born from the first pregnancy. The family history was negative for skeletal disorders. The index case was suspected to suffer from a genetic disorder upon routine prenatal ultrasound evaluation performed at 11 weeks and 5 days of gestation (CRL = 53 mm), when increased nuchal translucency (NT = 3.8 mm) was noted. At the same time, measurements of pregnancy-associated plasma protein-A and β-human chorionic gonadotrophin were 3.76 IU l^{-1} (1.4 multiples of the median (MoM) and 151.8 ng ml^{-1} (3.18 MoM), respectively; thus, cumulative risk for trisomy 21 was calculated to be increased to 1 per 50 (PRISCA (Prenatal RISk CAlculation) test). On the basis of these results, amniocentesis was performed at 15 weeks of gestation. Conventional GTG banding carried out on fetal amniocytes showed a normal male karyotype (46,XY). Next, repeated prenatal ultrasound scans in second and third trimesters showed micromelia, generalized long bone shortening and relatively large head (for details about fetal body measurements, see Table 1). Additionally, normal bone mineralization of the skull, but narrow and hypoplastic thorax with relative cardiomegaly (heart area (HA)/chest area (CA) = 0.48), abnormal pinnae and polyhydramnios (amniotic fluid index = 27 cm) were observed. The proband was spontaneously delivered at 39 weeks of gestation (G2P2) with the following birth parameters: weight 2930 g (−0.8 s.d.), length 45 cm (−6.0 s.d.), head circumference (HC) 37 cm (+3.0 s.d.), thoracic circumference 32 cm and Apgar score 8-8 in minutes 1–5, respectively. Upon first physical exam on the day of delivery, the patient presented with severe shortening of all limbs, bowed lower legs, relatively large head, short and bell-shaped thorax, brachydactyly, single palmar creases and facial dysmorphism comprising flat facial profile, broad and low-set nasal bridge, a short nose with anteverted nares and small dysplastic ears (Figure 1a). Babygram confirmed generalized long bone shortening, narrow thorax and large cranium (Figure 1b). Echocardiography performed after birth revealed foramen ovale apertum. Abdominal ultrasound scan was unremarkable, whereas transfontanellar ultrasound showed widening of epidural space and a cyst of the septum pellucidum. At the age of 9 months he presented with severe microsomia with significant limb shortening and relatively large head with the following body parameters: weight 5.5 kg (−5.3 s.d.), length 54 cm (−7.6 s.d.) and HC 45 cm (−1.0 s.d.). Upon repeated clinical genetic evaluations at the age of 17, 22 and 34 months, the clinical phenotype still involved microsomia, whereas facial characteristics comprised large cranium in relation to the face, flat facies, micro- and retrognathia, blue sclerae, short nose with depressed nasal bridge, thin vermilion border and small pinnae with overfolded helices (Figures 1c–e). In addition, generalized joint laxity and hypermobility was noted. Body measurements were as follows: at 22 months of age, height and HC was 61 cm (−10.4 s.d.) and 50 cm

Table 1 Fetal body parameters measured with prenatal ultrasound examinations

Gestational age	*FL*	*HL*	*Ulnar length*	*Radial length*	*BPD*	*HC*
30 weeks+4 days	3.92 cm (–8 weeks)	3.52 cm (–8 weeks)	3.17 cm (–8 weeks)	2.55 cm (–11 weeks)	8.20 cm (+2.5 weeks)	29.56 cm (+2 weeks and 1day)
33 weeks+4 days	4.21 cm (–10 weeks)	3.89 cm (–10 weeks)	3.33 cm (–10 weeks)	3.95 cm (–8 weeks)	8.72 cm (+1.5 weeks)	31.26 cm (+1.5 weeks)

Abbreviations: BPD, biparietal diameter; FL, femoral length; HC, head circumference; HL, humeral length.

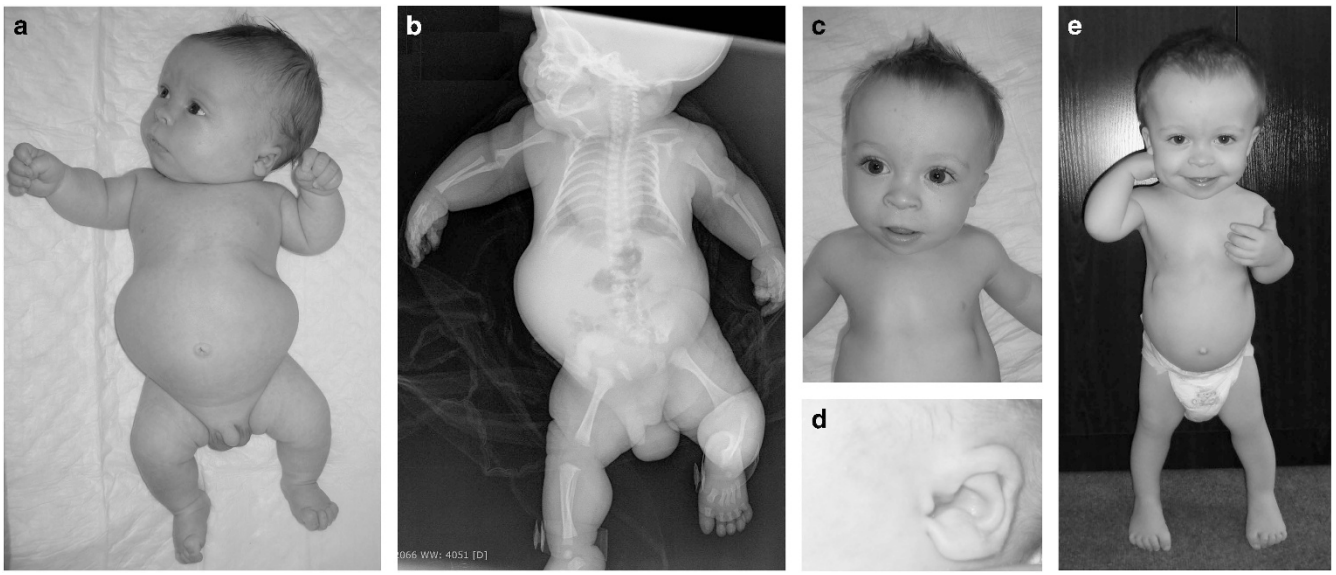

Figure 1 Clinical characteristics including facial view of the proband at the age of 5 weeks (**a**,**b**), 22 months (**c**,**d**) and 34 months (**e**). The patient presented with severe limb shortening, bowed lower legs, relatively large head, short and bell-shaped thorax, brachydactyly and facial dysmorphism comprising flat facial profile, micro- and retrognathia, short nose with depressed nasal bridge, thin upper vermilion border (**a**) and small dysplastic ears with overfolded helix (**d**). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

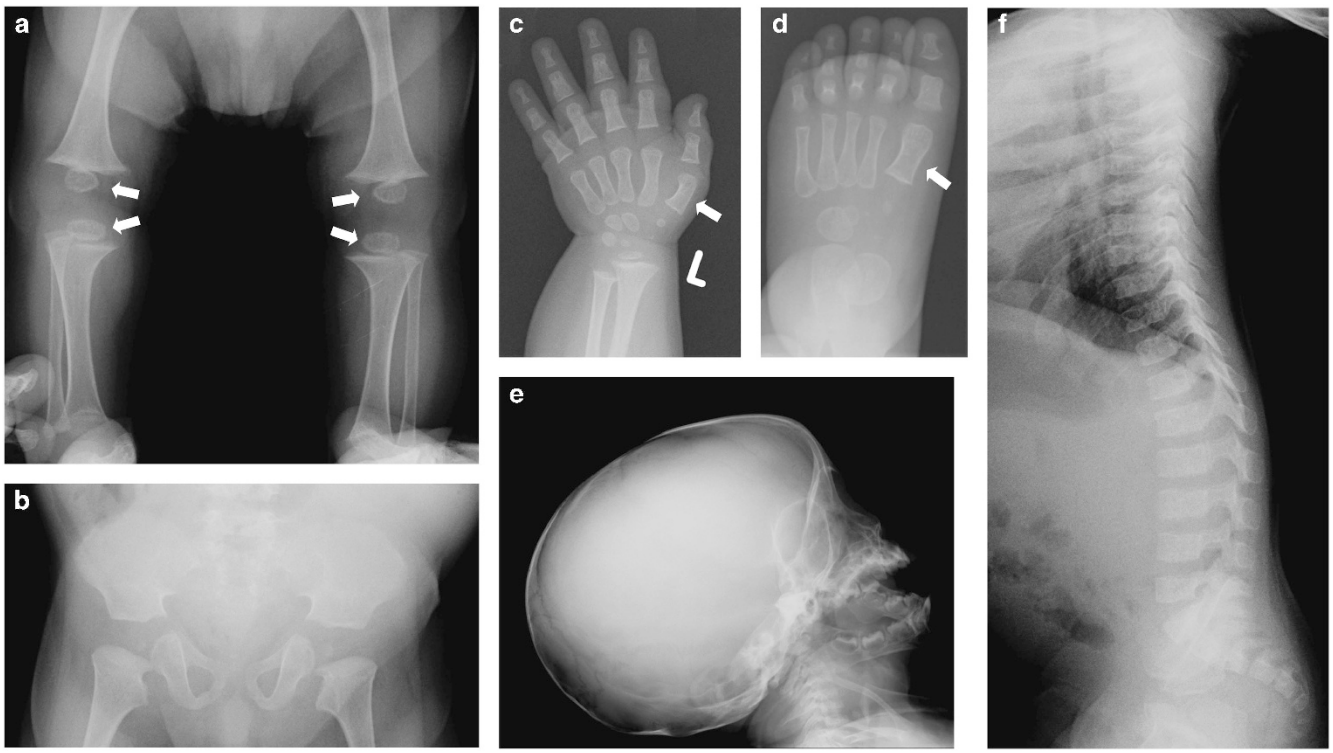

Figure 2 X-ray survey of the skeleton of the index patient. (**a**) Lower extremities. The metaphyses of the long bones are widened and the epiphyseal ossification centers are relatively small. (**b**) Pelvis. The iliac wings are flared and reduced in height. The acetabular roofs are horizontal. The trochanters minors are large and pointed. (**c**) Left hand. Advanced bone age. The radial epiphysis is flattened. The metacarpals are shortened. The first finger is most severely affected. (**d**) Left foot. Advanced tarsal bone age. The metatarsal are shortened. The first toe is most severely affected. (**e**) Skull. Cranium is large in relation to the face. There is also minor hypoplasia of the upper facial bones. (**f**) Spine. Minor kyphosis in the lower thoracic and upper lumbar spine.

(+1.0 s.d.), respectively, whereas at 34 months of age, the height was 65 cm (−10.3 s.d.) and HC 51 cm (+0.4 s.d.), respectively. The panel of radiograms of the skeletal survey at 9 months of age documented generalized osteoporosis of mild to moderate degree accompanied by other skeletal abnormalities presented in detail in Figures 2a–f. Independent sitting and walking were achieved at 10 and 18 months

of age, respectively. At the age of 34 months, the index underwent psychological assessment, which showed mild to moderate psychomotor delay, with the development relevant to 18 months. Speech was delayed at that time and the patient spoke only a few words. Hearing and vision were normal. Tooth eruption was normal; however, at the age of 34 months, the patient manifested advanced caries. Clinical endocrine workup at the age of 37 months evidenced severe short stature, prepubertal status and euthyroidism with nonpalpable thyroid gland. Laboratory tests of the bone metabolism and endocrine evaluation showed normal blood calcium, phosphate, alkaline phosphatase and vitamin D_3 as well as urinary calcium, whereas the PTH level was slightly reduced to 13.5 pg ml^{-1} (reference range 15.0–68.3 pg ml^{-1}). Most importantly, serum GH peak in all tests; i.e., sleep-induced, clonidine-induced and glucagon-induced, as well as insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) serum levels, were all significantly decreased (Table 2), thus confirming deficiency of GH secretion and action. X-ray of the left wrist was performed to evaluate bone age and unexpectedly to growth hormone deficiency (GHD), it was significantly advanced showing heterogeneous bone maturation from 5 years and 6 months to 7 years and 6 months according to Greulich and Pyle atlas.[15] Thyroid gland was normal on ultrasound. Magnetic resonance imaging of the brain with a special attention to pituitary gland was normal.

Table 2 Laboratory results in examined patient

Laboratory tests	*Result*	*Reference range*
Calcium (mmol l^{-1})	2.4	2.2–2.7
Phosphate (mg dl^{-1})	4.95	4.0–7.0
25-OH-D3 (ng ml^{-1})	37.6	30–50
PTH (pg ml^{-1})	**13.5**/26.8	15–68.3
Alkaline phosphatase (IU l^{-1})	161	93–309
TSH (μIU ml^{-1})	2.875	0.600–4.840
fT4 (ng dl^{-1})	1.22	0.96–1.77
fT3 (pg ml^{-1})	3.98	2.41–5.50
IGF-1 (ng ml^{-1})	**31**	103–189
IGFBP-3 (ng ml^{-1})	**2411**	5200–8300
Sleep-induced GH peak (ng ml^{-1})	**4.4**	Standardized data not available
Clonidine-induced GH peak (ng ml^{-1})	**6.9**	>10
Glucagon-induce GH peak (ng ml^{-1})	**4.4**	>10
LH (mIU ml^{-1})	<0.03	Depending on age
FSH (mIU ml^{-1})	0.3	Depending on age
PRL (ng ml^{-1})	5.71	3.46–19.40
Estradiol (pg ml^{-1})	<10	11–44
Testosterone (nmol l^{-1})	0.08	Depending on age
Dihydrotestosterone (pg ml^{-1})	196.8	Depending on age
17-Hydroxyprogesterone (ng ml^{-1})	0.28	0.2–1.6
DHEAS (μmol l^{-1})	**0.58**	1.01–7.16
Cortisol 0800 hours (ng ml^{-1})	81.0	37.0–194.0
ACTH (pg ml^{-1})	19.0	10–60
Glucose (mg dl^{-1})	61	60–101
Insulin (μU ml^{-1})	1.1	<15

Abnormal results are in bold.
Abbreviations: ACTH, adrenocorticotropin; DHEAS, dehydroepiandrosterone sulphate; FSH, follicle-stimulating hormone; fT3, free thyroxin 3; fT4, free thyroxin 4; GH, growth hormone; IGF, insulin-like growth factor; IGFBP-3, insulin-like factor binding protein-3; LH, luteinizing hormone; PRL, prolactin; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone.

Molecular studies

To verify the tentative diagnosis of diastrophic dysplasia variant, we sequenced *SLC26A2* in our proband. The result was normal; therefore, we decided to proceed with WES on a single DNA sample from the index case. After completing the alignment of reads to reference human genome, the mean read depth was 120×. The exome contained 56 936 variant calls with high-quality calls for over 98% of the human coding sequence. We filtered the index patient on the phenotype basis by implementing the hiPHIVE/Exomiser algorithm.[16] The following Human Phenotype Ontology terms[17] were used as an input: severe intrauterine growth retardation (HP: 0008846), short long bones (HP: 0003026), joint laxity (HP: 0001388), advanced ossification of carpal bones (HP: 0004233) and narrow chest (HP: 0000774). We limited our evaluation to the top 20-ranked genes as described in Zemojtel *et al.*[18] Exomiser ranked *XYLT1* in the fifth place (Exomiser Score: 0.887). Two novel compound heterozygous *XYLT1* variants were detected: the nonsense variant c.595C>T (p.Gln199*) and the missense variant c.1651C>T(p.Arg551Cys) (Figures 3a and b). Sanger sequencing confirmed WES findings in the proband and demonstrated that the mother was the carrier of c.595C>T(p.Gln199*) variant, whereas the father and the proband's brother carried none of the variants (Figures 3c and d). Both variants were predicted to be disease causing or damaging by the function prediction algorithms MutationTaster2 (1.00 score), PolyPhen2 (0.97 score) and SIFT (0.0 score), respectively.[19–21] Consistently, a PHRED score calculated by Combined Annotation Dependent Depletion, a bioinformatic tool for *in silico* predictions, was 35, suggesting pathogenicity of both mutations.[22] The variants were not annotated in the 1000 Genomes Project,[23] the dbSNP144,[24] the HGMD[25] and the NHLBI EVS[26] databases; however, the p.Arg551Cys alteration was found in heterozygosity in one healthy control in ExAC database.[27]

As none of the variants was of paternal origin, we performed haplotype analysis using 42 microsatellite markers on 10 different chromosomes, which confirmed paternity and thus *de novo* occurrence of c.1651C>T(p.Arg551Cys) mutation. Alternatively, germline mosaicism cannot be excluded.

DISCUSSION

Our patient presented with prenatally symptomatic skeletal dysplasia characterized by severe limb shortening and relatively large cranium. Based on the postnatal evaluation, babygram, as well as X-ray survey of the skeleton performed at the age of 9 months, we initially suspected diastrophic dysplasia variant, which however was not confirmed by mutational screening of the causative *SLC26A2* gene. Next, to search for pathogenic variations at a molecular level, we carried out WES, which allowed us to revise the initial diagnosis to DBQD2. DBQD2 is an extremely rare skeletal condition belonging to the 'multiple dislocation group'.[1] Apart from severe prenatal and postnatal shortening of long bones, short stature and joint dislocations, the syndrome frequently features developmental delay or intellectual disability.[2–4,8,28] The patient presented here showed all major clinical symptoms of DBQD2, including psychomotor retardation, indicating a possible role of *XYLT1* in brain development (the growth chart of the index is given in Figure 4). The skeletal findings of the presented proband were very similar to the previously reported DBQD2 patients. Radiological examination at the age of 9 months showed large cranium in relation to face, advanced carpal and tarsal bone age, osteoporosis, shortening of all tubular bones with metaphyseal broadening, small epiphyseal ossification centers and short metacarpals and metatarsals, with the first bones being most

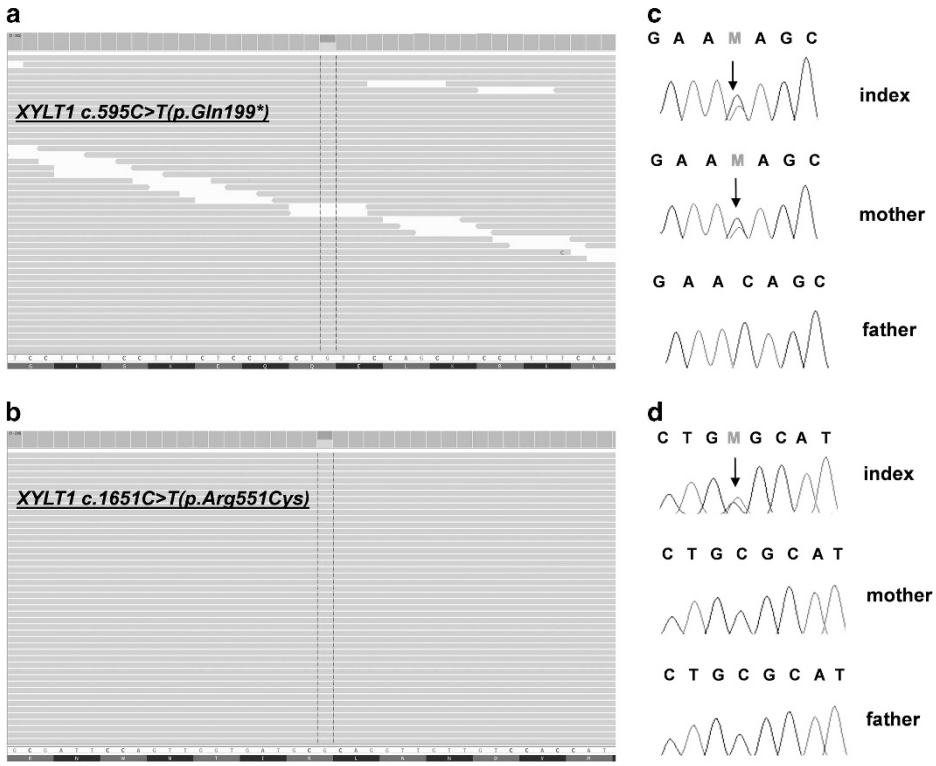

Figure 3 Representation of the compound heterozygous *XYLT1* mutations revealed in the proband by means of whole-exome sequencing. The proband was demonstrated to carry two single-nucleotide variants: c.595C>T(p.Q199*) (**a**) and c.1651C>T(p.R551C) (**b**). Targeted validation studies of the proband and parental testing of *XYLT1* with the use of Sanger sequencing. Mutation c.595C>T(p.Q199*) was shown in heterozygosity in the index case and his unaffected mother (**c**), whereas mutation c.1651C>T(p.R551C) was detected in heterozygosity only in the index but not in his healthy father, mother and brother (**d**). Mutation in (**c** and **d**) was depicted with "M" and indicated by an arrow. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

severely affected. Furthermore, the pelvis demonstrated broad iliac bones with reduced height and horizontal acetabular roofs and the exaggerated, pointed trochanters minors. These features, although typical for DBQD, disappear in the course of the disease and are no longer observed in adult individuals. Importantly, we demonstrated that at the age of 37 months our proband presented with reduced levels of IGF-1 and IGFBP-3 as well as marked GHD in three independently performed sleep-, oral clonidine- and intramuscular glucagon-induced provocative tests. Of note, exome analysis of our patient revealed no pathogenic variations that could account for the reduced GH secretion or activity. Therefore, we hypothesize that the corresponding endocrine GHD may be an additional clinical feature, which contributes to the severe growth retardation and short stature in DBQD2 patients. Owing to *XYLT1* mutations and thereby altered pattern of synthesized proteoglycans, IGFBP-3 may bind to different proteoglycans instead of forming a complex with IGF-1.[8] In such setting, IGF-1 would have a reduced half-life and thus the decreased level in the blood plasma. Nevertheless, further analyses based on a bigger sample are necessary to support the hypothesis that GHD occurs more frequently in DBQD2 patients with reference to the general population. Advanced carpal bone age is a known and frequent clinical symptom of DBQD2.[8] This feature results most likely from the premature chondrocyte maturation and increased Indian hedgehog signaling observed in DBQD2 patients, and occurs secondary to the reduction of xylosyltransferase-1 enzyme activity and aberrant glycosaminoglycan synthesis.[29] On the other hand, GH/IGF-1 deficiency demonstrated in our index should *per se* contribute to the delay of bone age, thereby partially attenuating DBQD2-related bone age advancement. Thus, owing to the severely advanced bone age in DBQD2, the question arises whether treatment with recombinant human GH in this particular bone dysplasia coexisting with GHD may be beneficial. Interestingly, we evaluated our proband since his early prenatal period and demonstrated that both NT (3.8 mm) and β-hCG (3.18 multiples of the median (MoM)) were increased and suggestive for elevated risk of trisomy 21 (1 in 50). Such observation has not been reported thus far; however, the conclusion that DBQD2 may give rise to the abnormal PRISCA test results (e.g. indicative of Down syndrome) is preliminary and further studies of other patients are needed to confirm this assumption.

To date, DBQD2 has been described and molecularly confirmed only in seven consanguineous families, in whom six distinct homozygous mutations were demonstrated in *XYLT1*. Out of the six pathogenic alterations, there were two splicing, two truncating (one frameshift and one nonsense) as well as two missense (p.Arg598Cys and p.Arg481Trp) variants.[8,28] Therefore, our patient is the first case of DBQD2 born from a non-consanguineous couple, in whom the phenotype results from the compound heterozygous *XYLT1* mutations; i.e., c.595C>T(p.Gln199*) and c.1651C>T(p.Arg551Cys). Both mutations are novel and predicted to be pathogenic. While the first mutation introduces a premature termination codon and is expected to give rise to the transcript degradation in a process of nonsense-mediated decay, the second one changes highly conserved arginine at position 551 into cysteine in the protein sequence.

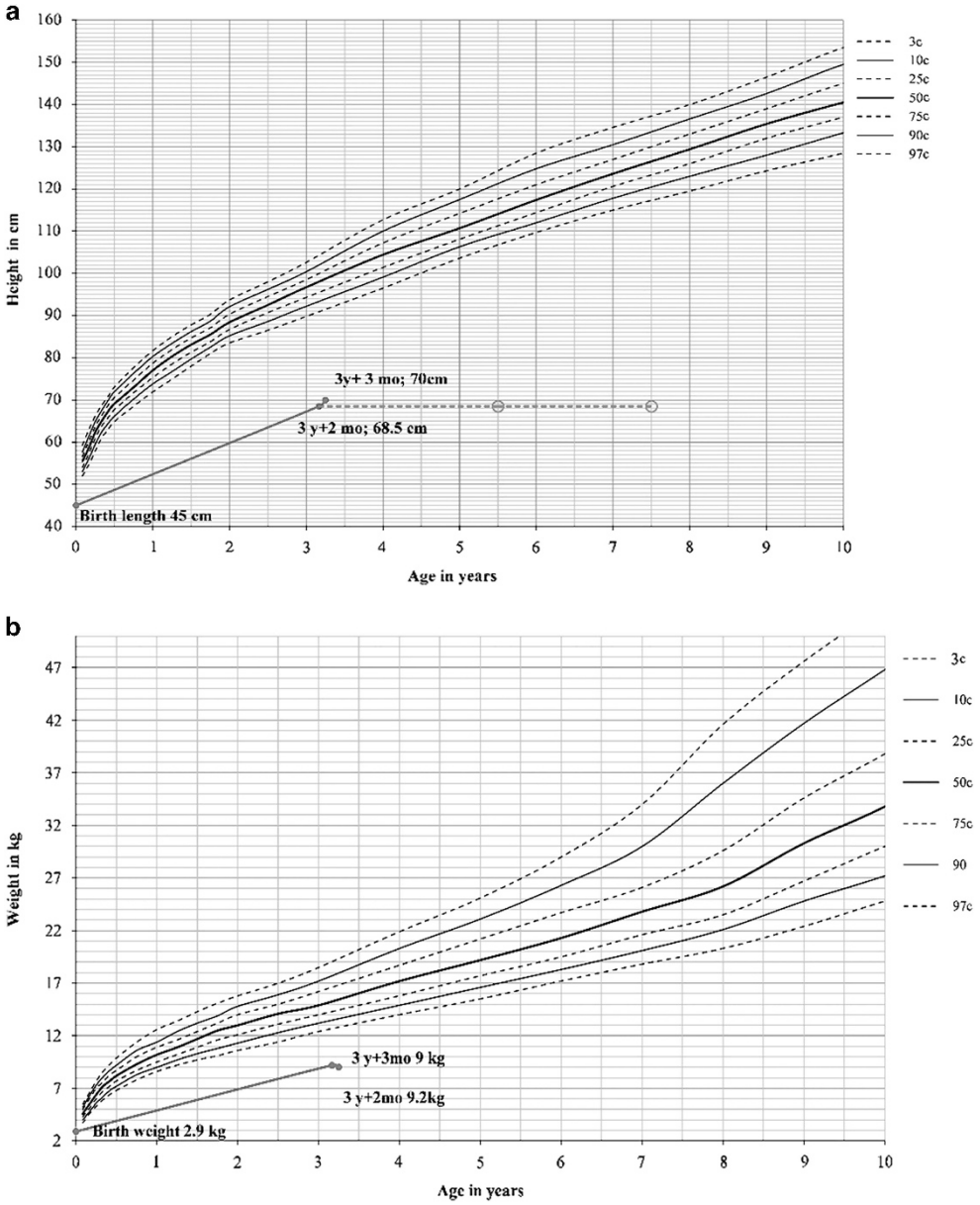

Figure 4 Patient's growth chart (**a**) and weight chart (**b**). Bone age (BA) is accelerated by 2.5–4.5 years (5.5–7.5 years, respectively) compared with chronological age (CA) of 3 years. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

In conclusion, we report on a new DBQD2 patient carrying two novel compound heterozygous mutations—p.Arg551Cys and p.Gln199* in *XYLT1*. Our paper provides a detailed follow-up of the index since his early prenatal period until the end of third year of life. Endocrine evaluation suggested that the severe growth retardation of our proband resulted not only from the skeletal dysplasia itself but also from the marked GH and IGF-1 deficiency that may represent a clinical feature of DBQD2. In such circumstances, the question arises whether growth retardation and severe microsomia associated with DBQD2 would be at least, in part, treatable by GH substitution therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Warman, M. L., Cormier-Daire, V., Hall, C., Krakow, D., Lachman, R., LeMerrer, M. *et al.* Nosology and classification of genetic skeletal disorders: 2010 revision. *Am. J. Med. Genet.* **155A**, 943–968 (2011).
2 Desbuquois, G., Grenier, B., Michel, J. & Rossigno, C. Nanisme chondrodystrophique avec ossification anarchique et polymalformations chez deux soeurs. *Arch. Franc. Pediat.* **23**, 573–587 (1966).
3 Faivre, L., Le Merrer, M., Al-Gazali, L. I., Ausems, M. G., Bitoun, P., Bacq, D. *et al.* Homozygosity mapping of a Desbuquois dysplasia locus to chromosome 17q25.3. *J. Med. Genet.* **40**, 282–284 (2003).

4 Faivre, L., Cormier-Daire, V., Eliott, A. M., Field, F., Munnich, A., Maroteaux, P. *et al.* Desbuquois dysplasia, a reevaluation with abnormal and normal hands: radiographic manifestations. *Am. J. Med. Genet.* **124 A**, 48–53 (2003).
5 Huber, C., Oulès, B., Bertoli, M., Chami, M., Fradin, M., Alanay, Y. *et al.* Identification of CANT1 mutations in desbuquois dysplasia. *Am. J. Med. Genet.* **85**, 706–710 (2009).
6 Kim, O. H., Nishimura, G., Song, H. R., Matsui, Y., Sakazume, S., Yamada, M. *et al.* A variant of Desbuquois dysplasia characterized by advanced carpal bone age, short metacarpals, and elongated phalanges: report of seven cases. *Am. J. Med. Genet. A* **152 A**, 875–885 (2010).
7 Furuichi, T., Dai, J., Cho, T. J., Sakazume, S., Ikema, M., Matsui, Y. *et al.* CANT1 mutation is also responsible for Desbuquois dysplasia, type 2 and Kim variant. *J. Med. Genet.* **48**, 32–37 (2011).
8 Bui, C., Huber, C., Tuysuz, B., Alanay, Y., Bole-Feysot, C., Leroy, J. G. *et al.* XYLT1 mutations in Desbuquois dysplasia type 2. *Am. J. Hum. Genet.* **94**, 405–414 (2014).
9 Prydz, K. & Dalen, K. T. Synthesis and sorting of proteoglycans. *J. Cell. Sci.* **113** (Part 2), 193–205 (2000).
10 Voglmeir, J., Voglauer, R. & Wilson, I. B. XT-II, the second isoform of human peptide-*O*-xylosyltransferase, displays enzymatic activity. *J. Biol. Chem.* **282**, 5984–5990 (2007).
11 Hästbacka, J., de la Chapelle, A., Mahtani, M. M., Clines, G., Reeve-Daly, M. P. & Daly, M. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell* **23**, 1073–1087 (1994).
12 Unger, S., Lausch, E., Rossi, A., Mégarbané, A., Sillence, D., Alcausin, M. *et al.* Phenotypic features of carbohydrate sulfotransferase 3 (CHST3) deficiency in 24 patients: congenital dislocations and vertebral changes as principal diagnostic features. *Am. J. Med. Genet. A* **152 A**, 2543–2549 (2010).
13 Zhang, D., Herring, J. A., Swaney, S. S., McClendon, T. B., Gao, X., Browne, R. H. *et al.* Mutations responsible for Larsen syndrome cluster in the FLNB protein. *J. Med. Genet.* **43**, e24 (2006).
14 Vissers, L. E., Lausch, E., Unger, S., Campos-Xavier, A. B., Gilissen, C., Rossi, A. *et al.* Chondrodysplasia and abnormal joint development associated with mutations in IMPAD1, encoding the Golgi-resident nucleotide phosphatase, gPAPP. *Am. J. Hum. Genet.* **88**, 608–615 (2011).
15 Greulich, W. W. & Pyle, S. I. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, 2n edn (Stanford University Press, Stanford, CA, USA, 1959).
16 Robinson, P. N., Kohler, S., Bauer, S., Seelow, D., Horn, D. & Mundlos, S. The Human Phenotype Ontology: a tool for annotating and analyzing human hereditary disease. *Am. J. Hum. Genet.* **83**, 610–615 (2008).
17 Köhler, S., Doelken, S. C., Mungall, C. J., Bauer, S., Firth, H. V. *et al.* The Human Phenotype Ontology project: linking molecular biology and disease through phenotype data. *Nucleic Acids Res.* **42**, D966–D974 (2014).
18 Zemojtel, T., Köhler, S., Mackenroth, L., Jäger, M., Hecht, J. & Krawitz, P. *et al.* Effective diagnosis of genetic disease by computational phenotype analysis of the disease-associated genome. *Sci Transl Med* **6**, 252ra123 (2014)
19 Schwarz, J. M., Cooper, D. N., Schuelke, M. & Seelow, D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat. Methods.* **11**, 361–362 (2014).
20 Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* **7**, 248–249 (2010).
21 SIFT. Available at: http://sift.jcvi.org/. Accessed 5 January 2016.
22 Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M. & Shendure, J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat. Genet.* **46**, 310–315 (2014).
23 The 1000 Genomes Project. Available at: http://www.1000genomes.org/. Accessed 5 January 2016.
24 The Database of Short Genetic Variation (dbSNP). National Center for Biotechnology Information. Available at: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi. Accessed 5 January 2016.
25 The Human Gene Mutation Database. Available at: http://www.hgmd.cf.ac.uk/ac/index.php. Accessed 5 January 2016.
26 NHLBI Exome Sequencing Project (ESP) Exome Varian Server (EVS). Available at: http://evs.gs.washington.edu/EVS/. Accessed 5 January 2016.
27 The Exome Aggregation Consortium (ExAC). Available at: http://exac.broadinstitute.org/. Accessed 5 January 2016.
28 Schreml, J., Durmaz, B., Cogulu, O., Keupp, K., Beleggia, F., Pohl, E. *et al.* The missing "link": an autosomal recessive short stature syndrome caused by a hypofunctional XYLT1 mutation. *Hum. Genet.* **133**, 29–39 (2014
29 Mis, EK, Liem, KF Jr, Kong, Y, Schwartz, NB, Domowicz, M & Weatherbee, SD Forward genetics defines Xylt1 as a key, conserved regulator of early chondrocyte maturation and skeletal length. *Dev. Biol.* **385**, 67–82 (2014)