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Case Report

Biallelic *CSGALNACT1*-mutations cause a mild skeletal dysplasia

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ABSTRACT

Genetic causes of skeletal disorders are manifold and affect, among others, enzymes of bone and connective tissue synthesis pathways. We present a twelve-year-old boy with a mild skeletal dysplasia, hypermobility of joints and axial malalignment of lower limbs and feet. Exome sequencing revealed a biallelic loss of function mutation in *CSGALNACT1*, which encodes chondroitin sulfate *N*-acetylgalactosaminyltransferase 1 and plays a major role in the chondroitin sulfate chain biosynthesis and therefore in the synthesis of glycosaminoglycans. Recently, the first case of a pediatric patient with a mild skeletal dysplasia due to a compound heterozygous large intragenic deletion and a damaging missense variant in *CSGALNACT1* was reported. We here identify a second case and the first juvenile patient with a homozygous frameshift variant in *CSGALNACT1* which corroborates its role in mild and non-progressive skeletal dysplasia with joint laxity.

1. Introduction

Skeletal dysplasias form a group of genetic disorders mainly characterized by disproportionate short stature and radiographic abnormalities. Besides a generalized manifestation in bones and cartilage, muscles, tendons, and ligaments can be affected as well. Skeletal disorders show a pronounced clinical and genetic heterogeneity with mutations in more than 300 genes [1]. In fact, broad molecular testing approaches increase the mutation detection rate and allow a molecular diagnosis which fosters an accurate clinical management and a precise evaluation of recurrence risks in family members. Still, incomplete mutation detection rates suggest the need for identification of further genes and loci associated with skeletal dysplasias.

Biallelic mutations in *CSGALNACT1* were recently proposed to cause a mild skeletal dysplasia with joint laxity in a single case report of a 3-year-old child [2]. The respective phenotype reflected the skeletal dysplasia of *Csgalnact1*$^{-/-}$ mice [3,4] and resembled the radiographic findings in Desbuquois dysplasia [2]. *CSGALNACT1* encodes chondroitin sulfate *N*-acetylgalactosaminyltransferase-1 (CSGalNAcT-1, ChGn-1) which plays an important role in the biosynthesis of sulfated glycosaminoglycans (GAGs). GAGs are linear polysaccharides that form the side chain of proteoglycans (PGs) and have various roles in a wide range of biological events. CSGalNAcT-1 is particularly important in the synthesis of chondroitin sulfate (CS) and dermatan sulfate (DS) by catalyzing the transfer of a GalNAc residue to the linker region of both proteins [2,5]. In chondrocyte tissue and in the regulation of bone growth and chondrocyte maturation, chondroitin sulfate PGs play a predominant role, but other GAGs are also involved in these processes. Previous studies could show that mutations in genes encoding enzymes for the biosynthesis of chondroitin sulfate cause several disorders with defective bone and connective tissue structure (Fig. 1) [6].

Here we report a case of a twelve-year-old patient with a mild skeletal dysplasia and joint laxity caused by a homozygous loss-of-function mutation in *CSGALNACT1*.

2. Patient and methods

2.1. Case report

Our patient with skeletal dysplasia without cranial dysmorphism (Fig. 2) is the only child of highly consanguineous parents from Turkey.

The family revealed consanguineous partnerships in several generations, with the four grandparents of the patient descending from only two different couples, which were also related to each other. The parents are both healthy and of low normal body height (father 171 cm/8th percentile, mother 157 cm/4th percentile). Family history

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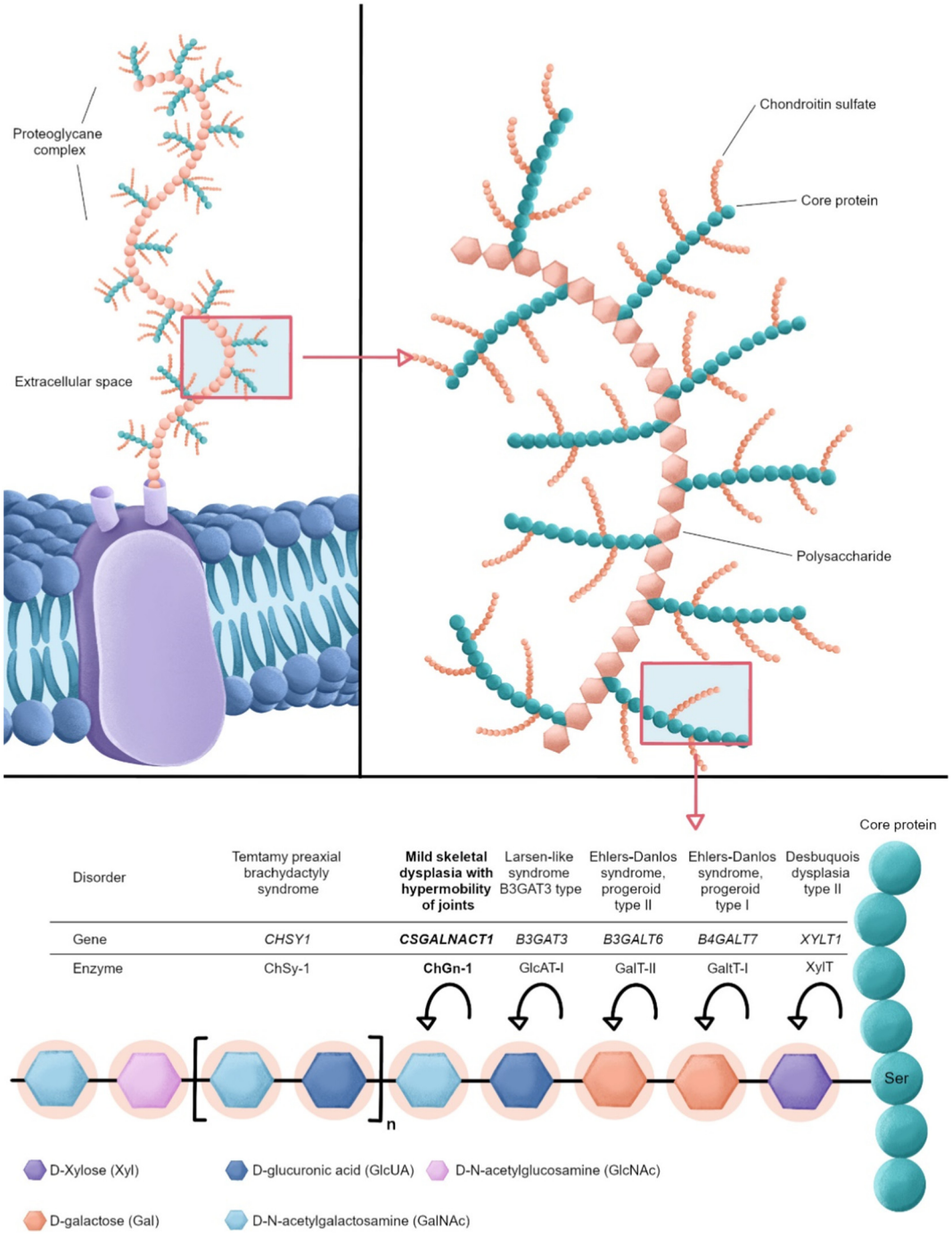

Fig. 1. Overview of the synthesis of the GAG-linker and GAG-repeating disaccharide region of chondroitin sulfate as part of proteoglycan synthesis. Defects of the involved glycosyltransferases are associated with various connective tissue and skeletal disorders. In addition to the synthetic pathway illustrated here, there are alternative and redundant pathways.
Adopted according to [6].

revealed no further skeletal disorders. The patient was born at term after an uneventful pregnancy, however prenatal ultrasound examination revealed a mildly shortened femur bone. Birth measurements were within the normal range (height 50 cm (26th percentile, P26), weight 3100 g (P28) and head circumference 35,5 cm (P69)). In the first months of life growth velocity decreased below the third percentile (Fig. 3), whereas weight and head circumference were normal. With increasing body length, orthopedic problems including genua valga and a bilateral pes planus deformity arose. A mildly accelerated bone age at the age of six years was reported, however, the respective X-rays were not available. At the time of examination at twelve years of age body length was about 10 cm under P3, which corresponds to a significant growth retardation, also when compared to the parent's height. Weight and head circumference were still in the normal range (Fig. 3). The index patient showed hypermobility of joints without obvious muscular hypotonia and profited from physiotherapy. Psychomotor development was normal and the patient currently attends a secondary school with average grades. X-rays of both legs confirmed symmetric genua valga and showed a flat acetabular roof as well as a bilateral concave tibial deviation (Fig. 4). As a consequence of the latest X-ray results different

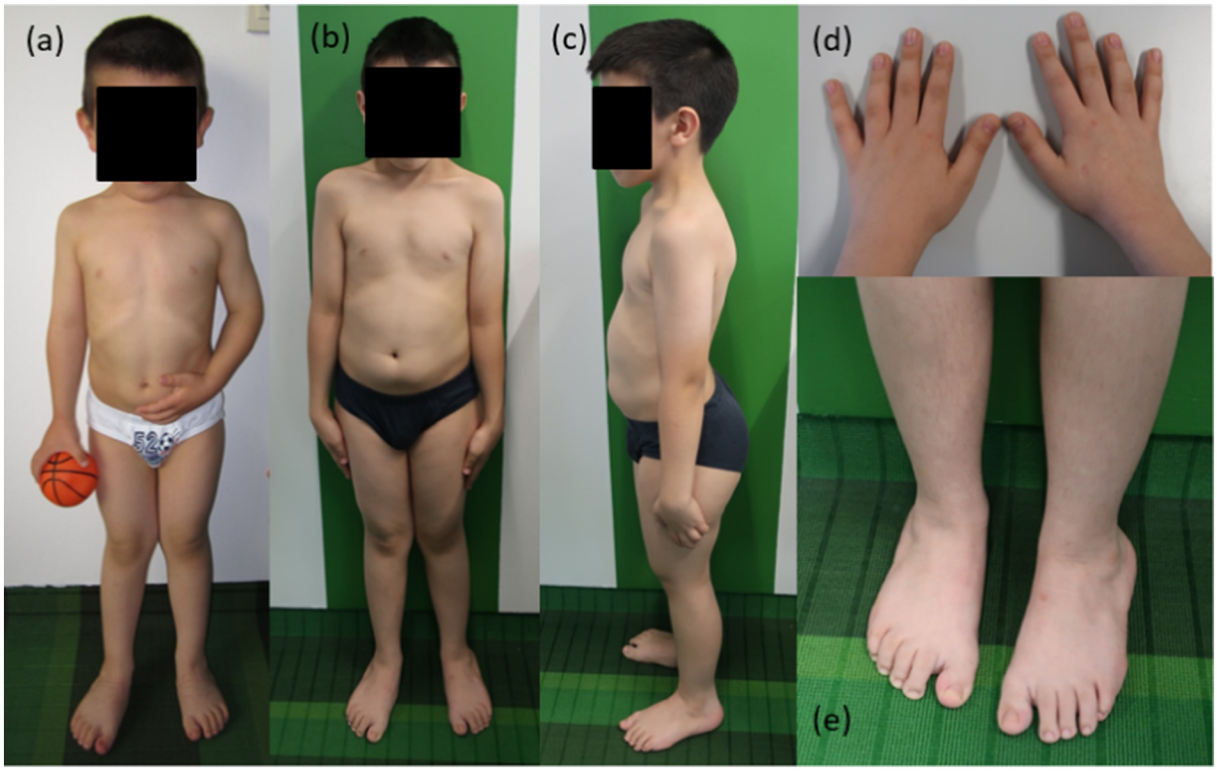

Fig. 2. Patient photographs at age (a) 5 years (b–e) 12 years. The pictures illustrate the mild skeletal dysplasia as well as joint laxity with scapulae alatae, hyperlordosis and planotransversus feet.

conservative and operative therapeutic options were in discussion, e. g. medial distal femoral temporary hemiepiphysiodesis.

2.2. Genetic analysis

For whole exome sequencing a peripheral blood DNA sample of the patient was enriched using the Nextera Rapid Capture Exome (v.1.2) according to the manufacturers protocol (Illumina, San Diego, CA, USA). The exome library was sequenced on a NextSeq500 Sequencer with 2 × 75 cycles on a high-output flow cell. FastQ-files were generated with bcl2fastq2 (Illumina, San Diego, CA, USA). The alignment and variant calling was performed using the automated SeqMule pipeline (v1.2.6) [7]. For variant detection, three different variant callers were used (GATKLite UnifiedGenotyper, SAMtools, FreeBayes consensus) and variants shared by at least one pair of variant callers were written to the final variant file. Variant annotation and bioinformatics prioritization was performed using KGGSeq (v1.0, 20/Jun./2018) [8]. Synonymous variants and variants with a minor allele frequency (MAF) above 0.75% in public databases (i.e. gnomAD, EXAC, 1000 GP, ESP) were excluded. Variants of interest were confirmed and segregation analyses were performed by Sanger-Sequencing on an ABI3500 platform (Applied Biosystems, USA).

3. Results and discussion

Whole exome sequencing revealed a homozygous 1 bp-deletion in *CSGALNACT1* (NM_018371.4:c.372del, p.(His125Thrfs*9)) in the patient. Segregation analysis confirmed both parents as heterozygous carriers. The identified variant was neither reported in large genome databases (gnomAD, dbSNP as of Dec 2018) nor in the literature. The variant causes a frameshift and most probably results in a subsequent premature stop codon in accordance with a disease-causing loss of function mutation. Most likely the gene product will undergo nonsense-mediated mRNA decay (NMD), however, we could not verify this possibility since we found *CSGALNACT1* to be hardly expressed in blood cells from healthy control samples and no other tissue samples from the index case were available for an in-depth evaluation.

To exclude other mutations as cause for the disease, we particularly investigated 325 genes known to cause skeletal disorders as listed in the “Nosology and Classification of Genetic Skeletal Disorders: 2015 Revision” [1], however, we did not identify other potentially disease-causing variants.

Our patient presented with short stature due to a reduced growth of limb bones and axial skeleton. In the growth of the long bones enchondral ossification plays an important role. Therefore, structure, growth and metabolism of cartilage are important factors for a sufficient bone growth and body length. Extracellular matrix (ECM) of cartilage is made up of two major components: the fiber structures consisting mainly of type 2 collagen and proteoglycans such as aggrecan. Chondroitin sulfate is the main component of the aggrecan molecule which consists of an aggrecan core protein and up to 100 attached chondroitin sulfate side chains. Together with linker proteins and hyaluronan, aggrecan forms large multimolecular complexes in the cartilage tissue whose function is the retention of water and the compression resistance of the cartilage [9,10]. Furthermore PGs as CS interact with important signaling pathways and influence developmental processes and cell functions [11]. CSGalNAcT-1 is a key enzyme of the chondroitin sulfate synthesis pathway.

CSGalNAcT-1 transfers GalNAc residues to both the linker tetrasaccharide region and the growing CS chains in proteoglycans (Fig. 1). CS levels in the cartilage of CSGalNAcT-1 deficient mice are reduced by about half in two independent mouse models [3,4]. Microscopic studies of the knockout mice showed shorter and slightly disorganized chondrocyte columns with a reduced extracellular matrix in the overall shorter growth plates of the knock out mice compared to wild type mice.

A reduction of ECM due to the absence of CS was observed in knockout mice, which ultimately leads to an insufficient formation of

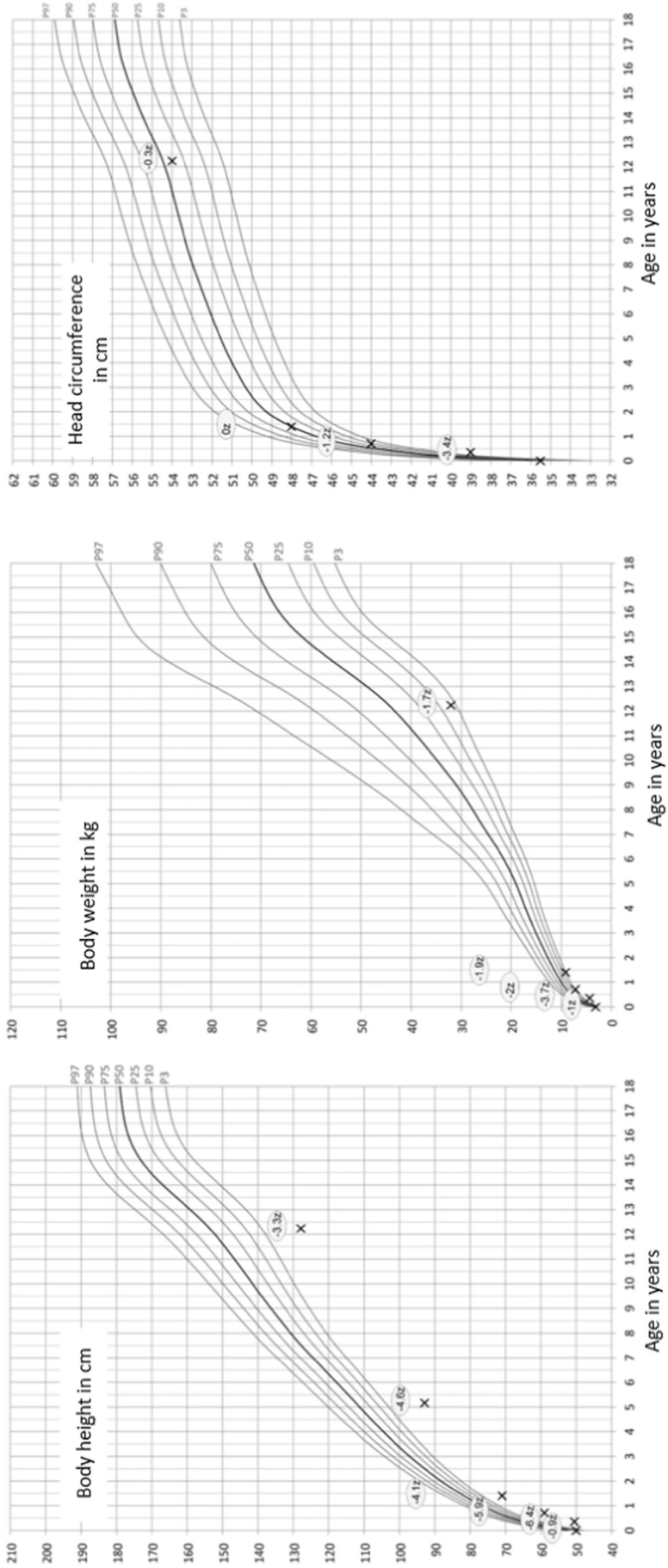

Fig. 3. Percentiles of body measurements show short stature with normal weight and normal head circumference.

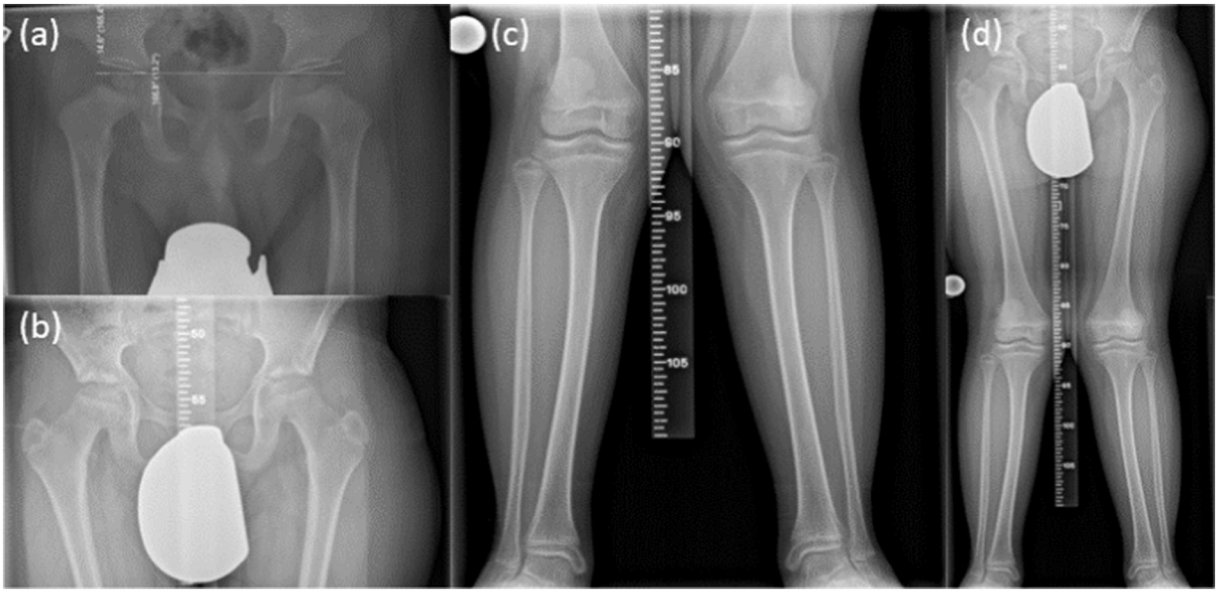

Fig. 4. Radiographs of the pelvis (a), hips (b), knees and lower legs (c) as well as whole legs (d).
AP radiographs of the pelvis performed at the age of 1 year (a) and at the age of 13 years (b) show a flat acetabular roof without enlargement of the lesser trochanter; no "Swedish key" appearance of the proximal femur. AP radiograph of the knees and lower legs at age of 13 years (c) shows no proximal fibular overgrowth, but a symmetric concave tibial deviation to the medial side. The ap radiograph of the whole legs at the age of 13 years (d) shows nearly symmetric genua valga.

proliferative chondrocytes in the growth plate [3]. However, cell proliferation markers showed no difference in the proliferation rate of chondrocytes [10]. In summary, the mouse models show a diminished CS synthesis, which ultimately leads to a disturbed endochondral ossification and thus to a growth disturbance of the bones [3,4].

Csgalnact1$^{-/-}$ mice are viable, fertile, show normal development and have a normal life expectancy, however, the body length of the mice is reduced while the head circumference is of normal size. The examined long bones (humerus and tibia) were significantly shortened compared to WT mice [3,4]. CSGalNAcT-1 deficient mice show a phenotype that reflects the phenotype of our patient and the patient of Vododpuitz et al. [2] (see also Table 1) and therefore supports the conclusion of a causative link of biallelic *CSGALNACT1* mutations and a mild skeletal dysplasia with hypermobility of joints.

Deficiency of other glycosyltranferases of CS synthesis leads to a variety of skeletal and connective tissue diseases (Fig. 1). Vodopuitz et al. report that their patient with CSGalNAcT-1 deficiency meets the radiographic criteria for Desbuquois dysplasia (DD) which can be caused by biallelic mutations in *XLYT1* and *CANT1*. XylT1, which is encoded by *XYLT1*, is also part of the CS synthesis pathway [6].

The major radiological diagnostic criteria for the diagnosis of Desbuquois dysplasia are "Swedish key" appearance of the proximal femur, flat acetabular roof, precocious carpal and tarsal ossification, proximal fibular overgrowth and elevated greater trochanter [12].

In our case only showed a flat acetabular roof as shown in Fig. 4. In our case radiographs of the legs at the age of 12 years showed a symmetric concave tibial deviation to the medial side and nearly symmetric genua valga (Fig. 4c–d). Bone density and trabecular bone structure as well as metaphyseal and epiphyseal configurations appear to be regular in all radiographs (Fig. 2, a–d). In conclusion the radiographic findings (flat acetabular roof, symmetric genua valga, concave tibial deviation) can be compatible with a mild manifestation of Desbuquois dysplasia, however, similarities are marginal.

Overall, the phenotype that is caused by biallelic *CSGALNACT1*

Table 1
Comparison of genetic and clinical data of our patient and the patient from Vodopiutz et al. [2].

	Our patient	Patient of Vodopiutz et al.
Identified mutations		
Maternal allele	c.372del, p.(His125Thrfs*9)	arr[hg19]8p21.3(19269401-19324691)x1, p.(Gly212fs*30)
Paternal allele	c.372del, p.(His125Thrfs*9)	c.1151C > G, p.(Pro384Arg)
Sex	Male	Female
Time of birth	Born at term	Born at term
Length at birth	50 cm (P26)	46,5 cm (P8)
Weight at birth	3100 g (P28)	2826 g (P16)
Head circumference at birth	35,5 cm (P69)	34,0 cm (P54)
Age at last examination	12,0 y	3,5 y
Length	128 cm (8 cm < P1)	95 cm (P13)
Weight	32,0 kg (P8)	14,8 kg (P15)
Head circumference	54,0 cm (P37)	49,5 cm (P55)
Stature	Non-proportionate, micromelic	Non-proportionate, micromelic
Facial dysmorphism	–	Mild
Further findings	Pes planus, hyperlordosis, genua valga	Pes planus, hyperlordosis
Neurological status	Normal	Normal
Psychomotor development	Normal	Normal
Brain-MRI	NA	Normal
Joints	Mild hypermobility	Mild hypermobility

mutations seems to be much milder than that of Desbuquois dysplasia [13,14]. We also found no significant worsening of clinical symptoms of our patient in childhood and early adolescence. This indicates a course of the disease which seems rather mild. So far, there is no clear indication for a specific therapy besides supportive therapy (e.g. physiotherapy).

The identification of the basic molecular mechanism was achieved by whole exome sequencing (WES) which proved as an excellent tool in the diagnostic work-up of this unclear skeletal dysplasia and of heterogeneous genetic disorders in general. Especially in milder and less striking cases of skeletal dysplasias, many different genes have to be taken into account and a step-wise analysis of single genes or restricted gene panels can often be frustrating. In these cases, WES approaches increase the diagnostic yield significantly. The elucidation of the molecular basic mechanism in a patient can improve the clinical management of the patient himself, but it is also the prerequisite for genetic counseling of the patients´ family and the determination of recurrence risks. Siblings of patients with a *CSGALNACT1*-related skeletal dysplasia have a recurrence risk for the disorder of 25% in line with an autosomal recessive inheritance.

In summary, our case is the second report of a patient with biallelic *CSGALNACT1* mutations and provides further evidence for the causative character of *CSGALNACT1* loss in mild skeletal dysplasia with hypermobility of joints. Although the clinical outcome of *CSGALNACT1*-related skeletal dysplasia seems to be favorable, the full spectrum of this new entity has to be fully elucidated with the description of further patients and clinical follow up studies.

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