Molecular Syndromology

Original Article

Mol Syndromol 2022;13:485–495
DOI: 10.1159/000525020

Received: June 11, 2021
Accepted: May 10, 2022
Published online: June 15, 2022

SLC26A2/DTDST Spectrum: A Cohort of 12 Patients Associated with a Comprehensive Review of the Genotype-Phenotype Correlation

Cynthia Silveira[a] Karina da Costa Silveira[a] Maria D. Lacarrubba-Flores[a] Maurício T. Sakata[a] Silvia N. Carbognani[c] Juan Llerena Jr.[d, e] Carolina A. Moreno[b] Denise P. Cavalcanti[a, b]

[a]Skeletal Dysplasia Group, Department of Translational Medicine, Faculty of Medical Sciences, University of Campinas, Campinas, Brazil; [b]Perinatal Genetic Program, Department of Translational Medicine, University of Campinas, Campinas, Brazil; [c]Maternidad Oroño, Rosario, Argentina; [d]Centro de Genética Médica & Centro de Referência para Doenças Raras, IFF/Fiocruz, Rio de Janeiro, Brazil; [e]INAGEMP, Porto Alegre, Brazil

Keywords
SLC26A2/DTDST · Diastrophic dysplasia · rMED · AO2 · ACG1B · Swedish key proximal femur

Abstract
Introduction: Pathogenic variants in the *SLC26A2/DTDST* gene cause the following spectrum of phenotypes: achondrogenesis 1B (ACG1B), atelosteogenesis 2 (AO2), diastrophic dysplasia (DTD), and recessive-multiple epiphyseal dysplasia (rMED), the first 2 being lethal. Here, we report a cohort and a comprehensive literature review on a genotype-phenotype correlation of *SLC26A2/DTDST*-related disorders. ***Methods:*** The local patients were genotyped by Sanger sequencing or next-generation sequencing (NGS). We reviewed data from the literature regarding phenotype, zygosity, and genotype in parallel. ***Results:*** The local cohort enrolled 12 patients, including one with a Desbuquois-like phenotype. All but one showed biallelic mutations, however, only one allele mutated in a fetus presenting ACG1B was identified. The literature review identified 42 articles and the analyses of genotype and zygosity included the 12 local patients. ***Discussion:*** The R279W variant was the most prevalent among the local patients. It was in homozygosity (hmz) in 2 patients with rMED and in compound heterozygosity (chtz) in 9 patients. The genotype and zygosity review of all patients led to the following conclusions: DTD is the most common phenotype in Finland due to a Finnish mutation (c.727–1G>C). Outside of Finland, rMED is the most prevalent phenotype, usually associated with R279W in hmz. In contrast, DTD's genotype is usually in chtz. Despite a large number of variants (38), just 8 are recurrent (R279W, C653S, c.–26+2T>C, R178*, K575Sfs*10, V340del, G663R, T512K). The last 3 in hmz lead to lethal phenotypes. The Finnish mutation is found only in chtz outside of Finland, being associated with all 4 classical phenotypes. The p.R178* and p.K575Sfs*10 variants should be viewed as lethal mutations since both were mainly described with lethal phenotypes and were never reported in hmz. The existence of 9 patients with only one mutated allele suggests that other mutations in the other allele of these patients still need to be unveiled.

Karger@karger.com
www.karger.com/msy

Correspondence to:
Denise Pontes Cavalcanti, denisepcavalcanti@gmail.com

Karger

Introduction

Pathogenic variants in the *SLC26A2*/*DTDST* (solute carrier family 26 member 2/diastrophic dysplasia sulfate transporter) gene lead to a well-recognized spectrum of skeletal dysplasias (SD) ranging from lethal to mild phenotypes. This spectrum classically includes 2 lethal conditions (achondrogenesis type 1B [ACG1B, OMIM #600972] and atelosteogenesis type 2 [AO2, OMIM #256050]), a severe, but not lethal dysplasia (diastrophic dysplasia [DTD, OMIM #222600]), and a mild phenotype (recessive-multiple epiphyseal dysplasia [rMED, OMIM #226900]) [Superti-Furga et al., 1999]. Beyond the clinical-radiological variability of each phenotype, some authors described some patients with overlap between 2 of them into the classic spectrum and named them intermediate phenotypes, e.g., ACG1B/AO2, AO2/DTD, and DTD/rMED [Rossi et al., 1996; Mégarbane et al., 1999; Unger et al., 2001; Maeda et al., 2006; Czarny-Ratajczak et al., 2010; Barbosa et al., 2011; Mattos et al., 2014]. Simultaneously, 2 patients, also having an intermediate phenotype, were described with the Swedish key, a typical feature of Desbuquois dysplasia (DBQD): DTD/DBQD and DTD/rMED/DBQD [Miyake et al., 2008; Panzer et al., 2008].

The gene *SLC26A2/DTDST* (OMIM #606718) is located on 5q32, encodes a solute carrier transporter transmembrane protein of 739 amino acids, and contains 3 exons, but only the last 2 are coding [Hästbacka et al., 1994]. The protein structure of this sulfate transporter is formed by 14 transmembrane domains (TDs), a C-terminal sulfate transporter anti-sigma antagonist (STAS), 3 N-glycosylation sites (2 putative and 1 present if the others are mutated), and a possible extracellular loop disulfide bridge between amino acids 212 and 216 (EC-DB) in addition to extracellular and cytosolic loops [Rapp et al., 2017]. Although noncoding, exon1, or its boundaries, are important because one of the main pathogenic variants, the so-called Finnish mutation associated with DTD when in homozygosity (hmz) (c.–26+2T>C), is located at the 5′ splice donor of intron 1 [Hästbacka et al., 1999].

Along with the *PAPSS2*, *BPNT2*/*IMPAD1*, *CHST3*, *CHST14*, and *DSE* genes, *SLC26A2*/*DTDST* is related to a group of SD with major joint involvement that is classified in the group of sulfation disorders in the last nosology and classification skeletal genetic disorders [Mortier et al., 2019].

A local series of 12 patients with the *SLC26A2/DTDST* spectrum with a predominance of the R279W variant and the presence of a patient with a Desbuquois-like phenotype led us to carry out an extensive literature review for a better understanding of the genotype-phenotype correlation of this gene.

Materials and Methods

Local Cohort

The local cohort included 12 patients from 11 families registered at the Brazilian Skeletal Dysplasia Group of the UNICAMP (Br-OCD). Ten patients were evaluated in 1 of the 2 outpatient clinics: the Skeletal Dysplasia or the Perinatal Genetic outpatient clinic, while 2 were assessed by the website of the Brazilian group of Skeletal Dysplasia (http://ocd.med.br/).

Sequencing Methods

The molecular investigation was carried out by 2 methods: Sanger sequencing (SS) and targeted gene panel (TGP-NGS). For SS, primers were designed including the exon-intron boundaries of the gene. The PCR products were sequenced on an ABI PRISM 3500Xl Genetic Analyser (Applied Biosystems™; Life Technologies, Carlsbad, CA, USA). Additionally, DNA from the patients' parents was Sanger-sequenced for segregation analysis to confirm inheritance.

For NGS, a customized panel (including *SLC26A2*/*DTDST*) was used in Design Studio 1.7 software, Illumina, Nextera™ Rapid Capture Custom Enrichment Kit (Illumina, Inc., San Diego, CA, USA) 150 bp paired-end reads and sequenced on the Illumina MiSeq platform. One patient evaluated by the website had the molecular diagnosis performed by a private laboratory.

Bioinformatic and Analysis

The SS analyses were performed with Codon Code Aligner V9.0.1 (https://www.codoncode.com/aligner/download.htm) using the file with the extension ".ab1". For NGS, the analyses were carried out using BaseSpace™ Sequence Hub Illumina software after aligning the sequences using the hg19/GRCh37 human reference genome. The analyses and identification of variants were performed by inputting the VCF file on the myPhenoDB website [Sobreira et al., 2015]. The IGV (Integrative Genomics Viewer, Broad Institute version 2.8.9, https://software.broadinstitute.org/software/igv/download) software was used for NGS data visual analyses. All variants were reported according to Genome Reference Consortium Human Build 37 (GRCh37).

Variant Classification and in silico Analyses

Novel variants were considered potentially pathogenic if in hmz, were absent in public databases, and predicted to be damaging by in silico algorithms. The population frequency was evaluated in global databases: the Genome Aggregation Database (gnomAD browser, https://gnomad.broadinstitute.org/) and the 1000 Genomes Project (1000 g, https://www.internationalgenome.org/), as well as in 2 Brazilian databases: the Online Archive of Brazilian Mutations (AbraOM, https://abraom.ib.usp.br) and Brazilian Initiative on Precision Medicine (BIPMed, http://bipmed.iqm.unicamp.br/). In silico prediction algorithms were accessed with the following web tools: Polymorphism Phenotyping v2 (Polyphen 2 – prediction of functional effects of human nsSNPs, http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster 2 (http://www.mutationtaster.org/). The classification of novel variants identified was

Table 1. Phenotype and genotype, with the references of the respective variants in the *SLC26A2*/*DTDST* gene, and clinical findings of the 12 patients in the present cohort

Phenotype	Family	Patient	Allele 1	Allele 2	GA, weeks	BW, g	BL, cm	Sex	Con	Malformation	Reference
ACG-1B	1	1	c.1045_1047del p.V340del	Unknown	25	800	21	M	–	Cleft palate	Superti-Furga et al. [1996]
AO-2	2	2	c.862C>T p.R279W	c.1905del p.T627Lfs*23	34	2,125	34	F	–		Hästbacka et al. [1996]; **novel**
	3	3	c.862C>T p.R279W	c.559C>T p.R178*				M	–		Hästbacka et al. [1996]; Superti-Furga et al. [1996]
DTD	4	4	c.862C>T p.R279W	c.727–1G>C p.[?]	39	2,915	39	M	–		Hästbacka et al. [1994, 1996]
	5	5	c.862C>T p.R279W]	c.1751del p.L575Sfs*10	38	3,540	41.5	M	+[a]	Cleft palate	Hästbacka et al. [1994, 1996]
	5	6[b]	c.862C>T p.R279W	c.1751del p.L575Sfs*10	39	3,085	41	M	+[a]	Cleft palate	
	6	7	c.862C>T p.R279W]	c.371G>C p.[115A]	37.5	3,200	38	F	–	Cleft palate tracheomalacia	Hästbacka et al. [1996]; **novel**
	7	8	c.862C>T p.R279W	c.559C>T p.R178*	37.6	2,250		F			Hästbacka et al. [1996]; Superti-Furga et al. [1996]
	8	9	c.862C>T p.R279W	c.559C>T p.R178*	38.4	2,900	41	M	–	Symphalangism of fingers	Hästbacka et al. [1996]; Superti-Furga et al. [1996]
rMED	9	10	c.862C>T p.R279W	c.862C>T p.R279W		3,630	51	M	–		Hästbacka et al. [1996]
	10	11	c.862C>T p.R279W	c.862C>T p.R279W	38	2,885	45.5	F	–	Cleft palate	Hästbacka et al. [1996]
DTD/rMED/ DBQD	11	12	c.862C>T p.R279W	c.2182delG p.A719Qfs*16	35	2,755	41	F	–	Ear with bifid lobe	Hästbacka et al. [1996]; **novel**

GA, gestational age; BW, birth weight; BL, birth length; Con, parental consanguinity; ACG-1B, achondrogenesis 1B; AO-2, atelosteogenesis type 2; DD, diastrophic dysplasia; rMED, recessive multiple epiphyseal dysplasia; DBQD, Desbuquois dysplasia. [a] *F* = 1/256. [b]P6 is brother of P5.

made using Varsome (https://varsome.com/), an automated classification tool based on the ACMG (American College of Medical Genetics and Genomics guidelines). All novel variants included in this study were submitted to the Leiden Open Variation Database (LOVD v.3.0, https://databases.lovd.nl/shared/genes/SLC26A2).

Literature Review

For the literature review, the search included the period 1994 to January 2021 in the following databases: MEDLINE electronic bibliographic database (accessing via PubMED), Scientific Electronic Library Online (SciELO), and EMBASE. The search was based on the following terms: "SLC26A2 DTDST mutation" or "SLC26A2 DTDST variant", "Achondrogenesis 1B", "Atelosteogenesis type 2", "Diastrophic Dysplasia" and "Recessive Multiple Epiphyseal Dysplasia". The inclusion criteria for this review took into account only the articles including both the clinical and molecular diagnosis with the patients' genotype. All reviewed patients were listed on a spreadsheet, including the following information for each patient: phenotype, zygosity, and genotype at both levels of changes in nucleotides and amino acids.

Results

Molecular and Clinical Findings in the Local Cohort

The molecular results of the local cohort according to their respective phenotypes and clinical findings are shown in Table 1.

In short, we identified 8 pathogenic variants, 3 of which are novel: p.G115A (Polyphen-2: probably damaging, score 1.00; Mutation Taster: disease causing; Varsome: likely pathogenic), p.T627Lfs*23 (Mutation Taster: disease causing; Varsome: likely pathogenic), and p.A719Qfs*16 (Mutation Taster: disease causing; Varsome: pathogenic), which have already been deposited in LOVD v.3.0 (#0000132545, #0000132544, and #0000473814). The variant p.R279W was the most frequent and was identified in hmz in 2 patients with rMED and in one of the alleles in all other patients, except in patient 1 (ACG-IB) for whom just

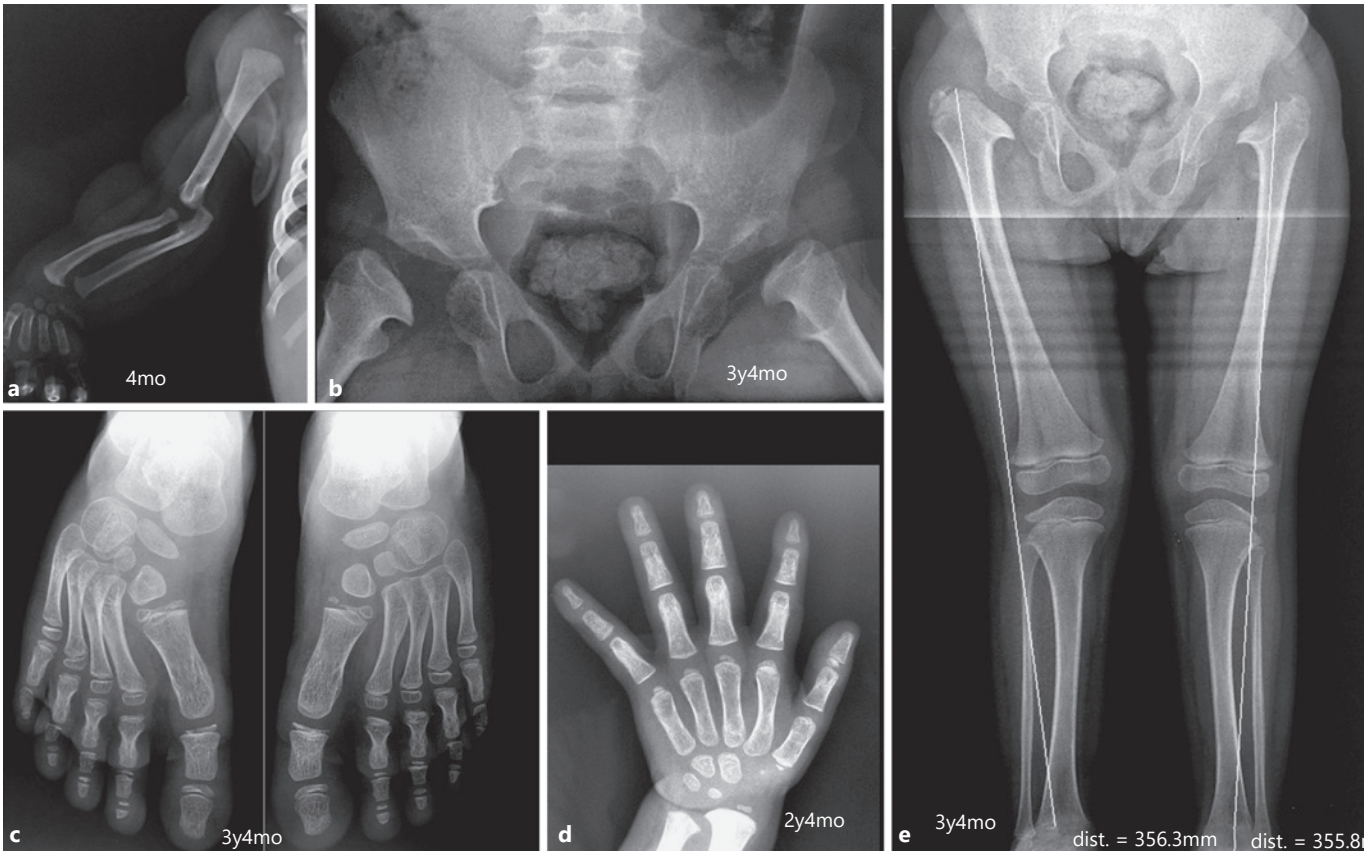

Fig. 1. X-rays of P12 (rMED/DBQD). **a** Short long bones, hypoplasia of the head of the radius, and ulnar deviation at the wrist. **b** Acetabular dysplasia, a Swedish key appearance of the proximal femur, unossified femoral head, and broad femoral necks. **c** Mild advancement of tarsal ossification with hypoplasia of the epiphysis observed mainly in the 1st metacarpal and the 1st phalanx of the hallux. Deviation of the metatarsal bones. **d** Mild advance of carpal ossification, but with delay in epiphyseal ossification of metacarpal and phalanx bones. **e** The long bones of the lower limbs are asymmetrical, right > than left, and there is dislocation of the hip and no ossification of the head of the femur. The distal femur epiphyses are flattened, and the distal fibulae are longer than the tibia. Genu varum.

one variant (c.1045_1047del, p.V340del) in heterozygosity (htz) has been detected so far. The second allele of the remaining patients had the following variant types: frameshift (P2, P5, P6, and P12), nonsense (P3, P8, and P9), missense (P7), and splice mutations (P4). Segregation analysis confirmed the inheritance of the variants in 5 patients (P3, P4, P5, P6, and P12). However, it was incomplete in 3 patients (P1: only paternal DNA was available and did not carry the only variant found in the proband; P7 and P10: maternal segregation confirmed the inheritance of one of the alleles). There was no DNA available from either parent for the other patients (P2, P8, P9, and P11).

Regarding clinical findings, 7 patients were male, 5 were female, and parental consanguinity was observed in just 1 family (family 5). However, the genotypes were in compound heterozygosity (chtz) but not hmz. Associated malformations were observed in 6 patients, 5 with posterior cleft palate and 1 with symphalangism in the hands.

Short Clinical Report of Patient 12 with a Desbuquois-like Phenotype

Patient 12 (P12) was initially evaluated at 27 months due to a presumptive diagnosis of hypochondroplasia. She is the 2nd child of healthy and nonconsanguineous parents. Birth measurements are shown in Table 1. At the age of 35 months, she had disproportionate short stature with short limbs. Height was 83.5 cm (−3 SD) and OFC was 50 cm (p3). The main features included a bifid lobe on the left ear, elbow stiffness, flat feet with prominent calcaneus, an abnormal gait, and good neuropsychomotor development. She complained of pain in the hips and

knees after daily activities and had a lateral deviation of the left foot that hindered the use of ordinary shoes.

Skeletal surveys at different ages showed shortening of long bones, mild scoliosis, and a mild advance of carpal and tarsal ossification but with a delay of epiphyseal ossification of the tubular long bones of hands and feet. Hypoplasia and mild dislocation of the head of the radius, ulnar deviation of the wrists, acetabular dysplasia, Swedish-key of the proximal femur, absence of ossification of the head of the femur and flattening of the distal femoral epiphysis, tibial deviation of metatarsal bones, and genu varum were also observed (Fig. 1).

The Swedish key appearance of the femora led us to initiate the molecular investigation by SS of *CANT1*. Since no pathogenic variant was found, she was then investigated by targeted NGS, and a chtz genotype was identified in *SLC26A2/DTDST*: p.R279W and a novel frameshift variant, c.2182delG producing an alanine to glutamine change with a stop codon after 16 amino acids (p.A719Qfs*16) (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/525020). The segregation analysis showed that p.R279W was inherited from the mother, while p.A719Qfs*16 was from the father.

A comparison of this patient's clinical and radiological findings with those previously reported [Miyake et al., 2008; Panzer et al., 2008] is shown in online supplementary Table 1.

Literature Review

To follow the criteria of inclusion, patients without a known genotype [Rossi and Superti-Furga, 2001] or good clinical-radiological descriptions available [Yang et al., 2019; Li et al., 2020; Ruault et al., 2020] were excluded from the analysis. Patients with DTD from Finland (179 patients from 147 families) were not included in the allele frequency analysis or in the evaluation of genotypes to avoid ascertainment bias [Hästbacka et al., 1999; Remes et al., 2002; Bonafé et al., 2008; Mäkitie et al., 2015]. Patients described as having an intermediate phenotype within the *SLC26A2/DTDST* spectrum [Rossi et al., 1996; Mégarbane et al., 1999; Unger et al., 2001; Maeda et al., 2006; Barbosa et al., 2011; Mattos et al., 2014] were classified here as having a more severe phenotype, except the patients reported by Macías-Gómez et al. [2004] and Mégarbane et al. [1999], for whom a milder diagnosis was considered (online suppl. Table 2). The intermediate phenotypes considered here were those that present characteristics of both phenotypes, *SLC26A2/DTDST* spectrum and Desbuquois dysplasia [Miyake et al., 2008; Panzer et al., 2008].

According to the criteria of inclusion, 42 of the 46 articles reported until January 2021 were included in the analysis. In addition, the 12 patients in the present cohort were also included in this analysis. Therefore, this review was based on 320 patients from 266 families. Because of a great number of Finnish individuals with DTD due to a founder effect, these patients were reviewed separately. In online supplementary Table 2, all the reviews are summarized in a spreadsheet with 12 sheets, with the first 5 sheets related to the genotype and phenotype as well as the allele frequency and the most common genotypes.

Most reported patients belonging to the *SLC26A2/DTDST* classical spectrum are represented by the DTD phenotype (more than 65%); however, if we exclude Finnish patients with DTD, this frequency drops to 52% (Fig. 2a).

Figure 2b shows the zygosity among the 4 classic phenotypes. Most patients with DTD are in hmz. However, this is true only among Finnish. For non-Finnish patients, 76% are in chtz. Concerning the other classic phenotypes, while for AO2, most patients are also in chtz, 69% of patients with rMED and a half with ACG1B are in hmz.

A total of 56 pathogenic variants in *SLC26A2/DTDST* have been reported so far. However, for the principal analysis of this review (allelic frequency, zygosity, and genotype-phenotype correlation), only 38 pathogenic variants were considered, because the series of patients presented by Rossi and Superti-Furga [2001] did not show the genotypes. The location of all known variants throughout the gene is shown in Figure 3. Most of the variants are in the transmembrane domain (TD) (14 missense, 3 frameshifts, and 1 in-frame indel), and the STAS domain (5 missense and 5 frameshifts). The other variants are in the following protein domains: extracellular loops (1 nonsense and 2 missense), cytosolic loops (2 missense and 1 frameshift), and N-terminal (1 frameshift and 1 missense). The last 2 variants are at splice sites, with the c.−26+2T>C variant being at the 5′ splice donor of intron 1 [Hästbacka et al., 1999]. Based on the protein location of the variant, no correlation with severity can be ascertained (online suppl. Table 2).

Functional consequences of several variants were elucidated based on functional studies (online suppl. Table 2) [Karniski, 2001, 2004; Maeda et al., 2006].

Among the 38 variants with known genotypes, most were associated with a single phenotype, and 8 were considered more frequent for presenting a frequency of approximately or above 3%: p.R279W (50.2%); p.C653S (8.1%); c.−26+2T>C (6.2%); p.R178* (5.9%); p.K575S-

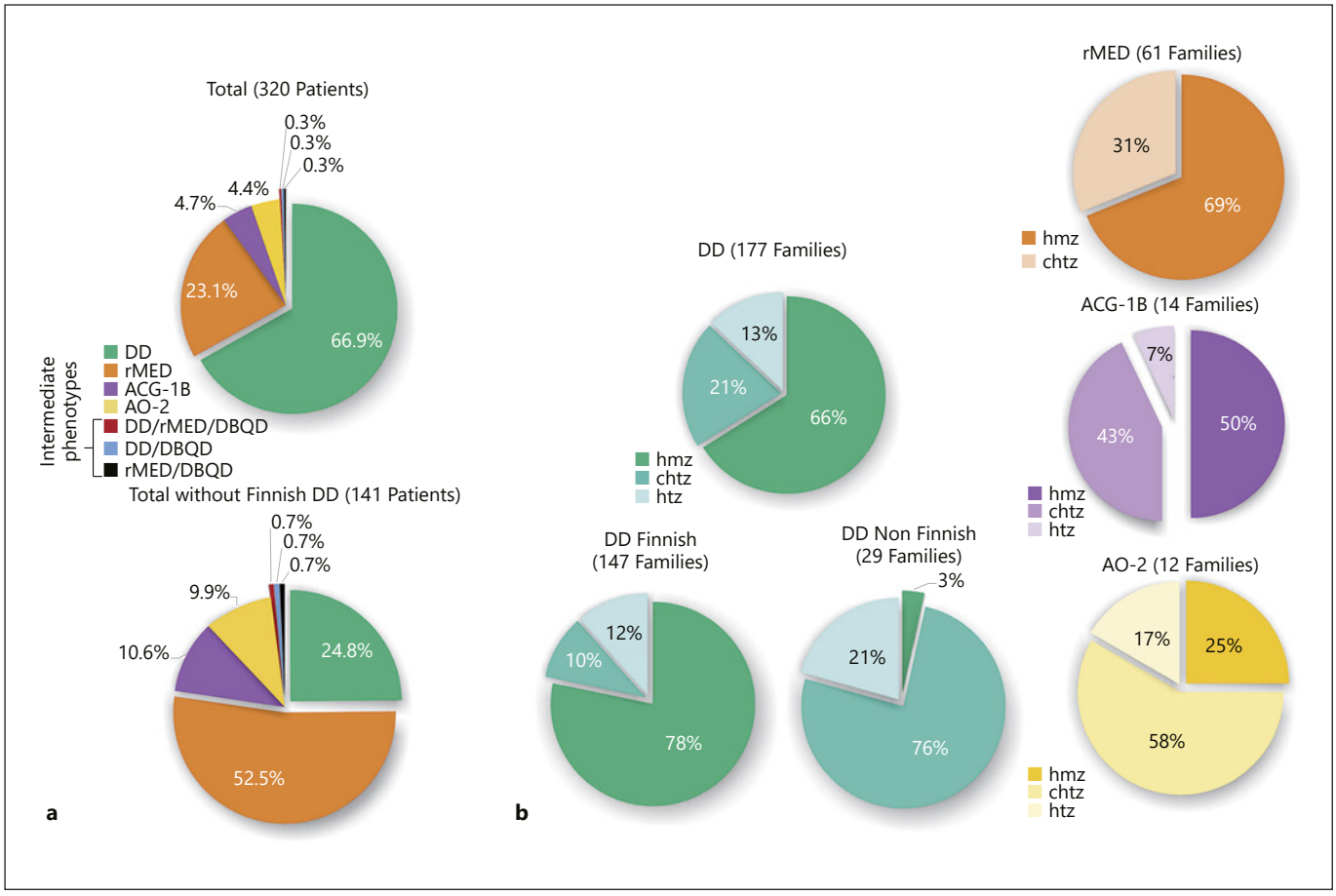

Fig. 2. Pie charts showing the proportions of the different phenotypes (**a**) and the zygosity of each phenotype in the *SLC26A2*/*DTDST* spectrum (**b**). **b** The criterion for differentiating homozygotes (hmz), compound heterozygotes (chtz), and heterozygotes (htz) was color intensity, with the darkest color for homozygotes and the lightest for heterozygotes.

fs*10 (2.9%); p.V340del (2.9%); p.G663R (2.6%); and p.T512K (2.6%) (Fig. 3 ;online suppl. Table 2). Ten variants (p.V340del, p.R178*, p.R279W, p.N425D, p.T512K, p.K575Sfs*10, p.C653S, p.G663R, p.[715V, c.−26+2T>C), were described in more than one phenotype (online suppl. Table 2).

The Finnish mutation c.−26+2T>C, in chtz has been found to be associated with all phenotypes in non-Finnish individuals. When in hmz, this variant has always been associated with the DTD phenotype and has only been reported in Finnish patients so far.

The most common variant p.R279W appeared mainly in hmz (46/141 patients, 32.62%) and was associated with the rMED phenotype. It has also been reported in chtz with 16 different variants in the second allele producing the same phenotype, rMED, or any of the other phenotypes of the spectrum, except ACG1B (online suppl. Table 2).

Homozygosity was also reported with the following variants with their respective phenotypes: p.C653S (rMED), p.V340del (ACG1B), p.G663R (ACG1B), and p.T512K (AO2). The variants p.R178* and p.K575Sfs*10 were never reported in hmz. In addition, these last 2 variants were never associated with the milder phenotype (rMED) (online suppl. Table 2).

Among the chtz genotypes, some have been found to be associated with variable phenotypes. Thus, the combinations p.[R279W];[R178*], p.[R279W];[K575Sfs*10], and p.[R279W];[N425D] were all reported with both DTD [Macías-Gómez et al., 2004; Barbosa et al., 2011; present cohort] and AO2 [Hästbacka et al., 1996; Rossi et al., 1997; Mattos et al., 2014]. Similarly, the following 2 genotypes p.[R279W];c.−26+2T>C, and p.[C653S];(A715V) have been reported with both rMED [Balhausen et al., 2003; Jackson et al., 2011; Makitie et al., 2015] and DTD pheno-

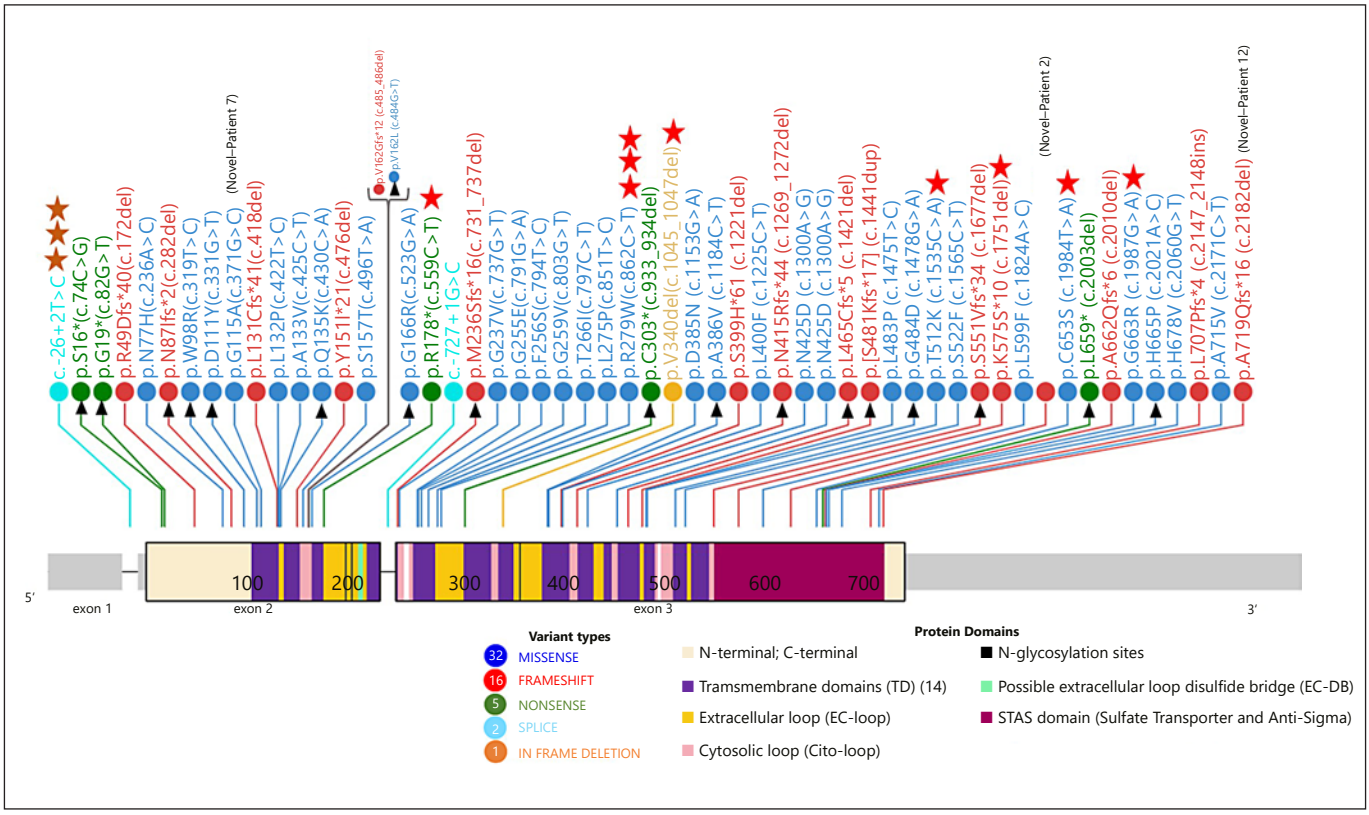

Fig. 3. Schematic of the *SLC26A2/DTDST* (GRCh37 NM_000112.4/NP_000103) gene showing the 3 exons (including noncoding sequences as gray boxes), separated by introns (horizontal black line). The transmembrane domains are shown in dark purple, STAS domain in burgundy, N-glycosylation sites are narrow black bars, a light green bar represents a possible extracellular loop disulfide bridge between amino acids 212 and 216 (EC-DB), extracellular loop (EC-loop) are mustard bars, N-terminal and C-terminal parts are colored in cream at the extremities. Pathogenic variants related to the skeletal dysplasia spectrum are colored by each amino acid consequence as indicated by the legend below. The black triangles represent variants from the literature removed from the analysis due to insufficient genotype information. The stars above the variants indicate the 8 more frequent variants (in orange the Finnish mutation and in red other ones). The plot was generated with Protein Paint and the protein domain localizations were taken from Rapp et al. [2017].

types [Dwyer et al., 2010; Czarny-Ratajczak et al., 2010; Zechi-Ceide et al., 2013].

Patients with only one mutated allele (htz) have been reported to be associated with all phenotypes, except for rMED. Hästbacka et al. [1994] and Rossi et al. [1996] reported 6 patients with DTD showing only one mutated allele. Hästbacka et al. [1996] and Rossi et al. [1996] described 2 htz babies with AO2. Finally, in the present cohort, we report a fetus with typical ACG1B for whom only one allele was found to be mutated.

The 3 patients reported with the intermediate phenotype, DTD/DBQD [Panzer et al. 2008], DTD/rMED/DBQD [Miyake et al. 2008], and P12 described here showed the following genotypes: p.[A133V];[R178*], p.[T266I];[V340del], and p.[R279W];[A719Qfs*16], respectively.

Discussion

We presented the genotype-phenotype relationship of the *SLC26A2/DTDST* mutation spectrum based on a local Brazilian cohort and an extensive literature review. In the local cohort, results recapitulated those in the previous reports on the non-Finnish population. A mild pathogenic allele, p.R279W was the most common, and a severe one, p.R178*, was the second most common. The local cohort included 3 novel pathogenic variants (Table 1).

Diastrophic dysplasia is the main phenotype reported thus far; however, most of them are from Finland and are most frequently associated with the variant c.–26+2T>C, also called the Finnish variant, which is well known to be associated with a founder effect [Hästbacka et al., 1999; Remes et al., 2002; Bonafé et al., 2008; Makitie et al., 2015].

Unlike what is known in Finland, the main phenotype related to the *SLC26A2*/*DTDST* spectrum in the literature is rMED (74 patients or 52.5%), most of them in hmz for the variant c.862C>T, p.R279W (Fig. 2b).

The variant c.862C>T, p.R279W is the most common variant observed in the *SLC26A2*/*DTDST* gene when the Finland population is excluded from the analysis. The c.862C>T position is located at a CpG dinucleotide, and the C>T (CGG to TGG) substitution is a common change in genes justifying the high frequency. In addition, this variant is associated with a significant sulfate transport function in HEK 293 cells. In hmz, it is related to the mildest phenotype of the spectrum and probably contributes to phenotype rescue when associated with more severe variants, such as some frameshift, missense, nonsense, splice site, and 5′ splice donor of intron 1 [Rossi et al., 2001; Karniski 2004].

Out of Finland, patients with DTD (35 or 24.8%) were mainly chtz (Fig. 2b) without a preferential genotype (online suppl. Table 2). When severe variants, for instance p.R178*, c.–26+2T>C, p.K575Sfs*10, c.727–1G>C, p.N425D, p.G115A, p.S157T, p.G663R, and p.L707Pfs*4, were observed in combination with the p.R279W in DTD patients, this last variant has been regarded as a change rescuing the phenotype [Macías-Gómez et al., 2004; Maeda et al., 2006; Dwyer et al., 2010; Barbosa et al., 2011; Honório et al., 2013; Pineda et al., 2013; Zechi-Ceide et al., 2013; present cohort]. Homozygosity in DTD was reported in only one patient with unusual features and the variant p.Q454P, located at TD11 and with a sulfate transport activity of approximately 39% compared to the wild type [Mégarbané et al., 1999; Karninski 2004].

The 2 lethal phenotypes were observed in less than 25% of all patients: AO2 (14 or 9.9%) and ACG1B (15 or 10.6%) (Fig. 2a). While patients with AO2 were predominantly in chtz (58%) without a common genotype, 50% of patients with ACG1B were in hmz. The main mutation in hmz associated with ACG1B was p.V340del at the TD and considered a null variant either by its poor expression in HEK 293 cells or by its rapid protein degradation. This mutation could also induce a shift in the 3D helix structure [Karniski, 2004; Rapp et al., 2017]. The homozygosity observed in patients with AO2 involved 3 consanguineous families from specific populations, the p.L599F variant reported in India and Bangladesh and p.T512K in a family from Finland [Miller et al., 2008; Bonafé et al., 2008; Vikraman et al., 2016]. Interestingly the p.T512K variant when in combination with p.R279W produces the mildest phenotype, i.e., rMED [Syvänen et al., 2013; Mäkitie et al., 2015; Kausar et al., 2019].

As shown in online supplementary Table 2, p.C653S is the second most common variant following p.R279W and has also been reported in hmz associated with the rMED phenotype [Balhausen et al., 2003; Matikie et al., 2003; Hinrichs et al., 2010]. This variant also led to the rMED phenotype in chtz with the Finnish mutation (c.–26+2T>C) or with the p.A715V [Czarny-Ratajczak et al., 2010; Jackson et al., 2011; Kausar et al., 2019]. This last variant, at the STAS domain, is predicted to force the 3D helix causing conformational changes where proteins interact [Rapp et al., 2017].

For the genotypes associated with the other 6 most frequent variants (online suppl. Table 2), most are related to severe phenotypes.

Nevertheless, regarding the genotype-phenotype correlation, the specific type of mutation and their combinations are more determinant of the severity than the localization of the variant.

It is worth emphasizing that the same genotype was associated with different conditions AO2 and DTD [Hästbacka et al., 1996; Rossi et al., 1996, 1997; Macías-Gómez et al., 2004; Barbosa et al., 2011; Mattos et al., 2014; present cohort] and DTD and rMED [Remes et al., 2002; Ballhausen et al., 2003; Dwyer et al., 2010; Zechi-Ceide et al., 2013; Makitie et al., 2015], reinforcing both the absence of a precise genotype-phenotype correlation for most of the known variants and the interfamilial variability among patients with identical genotypes (rMED and DTD) [Ballhausen et al., 2003; Mäkitie et al., 2003].

Finally, except for rMED, in the other phenotypes of the spectrum only one mutated allele was reported (ACG1B, DTD, and AO2) [Hästbacka et al., 1994, 1996, 1999; Rossi et al. 1996; present cohort], suggesting that other variants in regions usually not evaluated by gene sequencing, either SS or NGS, are lost in the analysis or that other unknown mechanisms need to be unveiled.

Although previously reported as an intermediate phenotype because of the association between a milder phenotype and a Swedish key appearance of the proximal femur [Miyake et al., 2008; Panzer et al., 2008; P12 in the local cohort], currently, this phenotype is considered just a variation of DTD. However, it is worth calling attention to the particular combination of variants in chtz with at least one severe (premature stop codon or in-frame deletion) plus one missense at TD or EC-loop. P12 has an in-frame deletion (p.A719Qfs*16) at the STAS domain that led to a shortening of the final part of the protein, associated with the mild variant (p.R279W), presenting a residual sulfate uptake rate of ~50% in HEK 293 cells when compared with the wild-type protein in a study by Karni-

ski [2004] that probably rescued the phenotype as proposed by Rossi and Superti-Furga [2001].

The results of the present study allow for the following conclusions:

1. All but 1 of the patients in the cohort studied here presented one allele with the p.R279W variant, reinforcing the high prevalence of this variant. Beyond that, the present cohort adds 3 novel variants in the *SLC26A2*/*DTDST* gene (p.G115A, p.T627Lfs*23, and p.A719Qfs*16).
2. Like the 2 previously described patients with an intermediate phenotype, P12 described here first led to the diagnosis of DBQD because of the Swedish key signal on radiological evaluation. The severity of the variant of one of the alleles in this patient (p.A719Qfs*16) seems to be attenuated by the presence of p.R279W, rescuing the phenotype.
3. The main phenotype within the classic *SLC26A2*/*DTDST* spectrum, outside of Finland, is rMED. A quarter of patients have DTD, followed by the 2 lethal phenotypes (AO2 and ACG1B), both with almost the same proportion.
4. Excluding Finnish patients with DTD, while 69% of rMED and 50% of ACG1B patients are in hmz, most DTD and AO2 patients have a chtz genotype.
5. Despite a large number of pathogenic variants (38) in the *SLC26A2*/*DTDST* gene among the genotypes known so far, only 8 are recurrent with a frequency of approximately 3% or more (p.R279W, p.C653S, c.−26+2T>C, p.R178*, p.K575Sfs*10, p.V340del, p.G663R, p.T512K).
6. The p.R279W variant is the most frequent (50.2%) outside of Finland, and it occurs more frequently in homozygotes (41/91) associated with rMED.
7. The Finnish variant (c.−26+2T>C), so far in chtz outside of Finland, has been associated with all 4 classical phenotypes.
8. Homozygosity of the p.C653S variant has also been associated with rMED; however, other frequent variants when in homozygosity lead to lethal phenotypes: p.V340del, and p.G663R (ACG1B), and p.T512K (AO2).
9. The p.R178* and p.K575Sfs*10 variants must be seen as lethal variants since both were mainly described with lethal phenotypes and were never reported in hmz.
10. Although phenotypic variability is commonly observed in genetic diseases, the same genotype in *SLC26A2/DTDST* has been described in more than one phenotype, for instance, the genotype p.[R279W];p.[R178*] has been described as associated with both DTD and AO2, suggesting that unknown mechanisms might modulate the effect of some pathogenic variants on this gene.
11. Finally, the existence of 9 patients presenting the typical phenotypes, except rMED, with only one mutated allele suggests that other variants in regions not usually covered by standard sequencing (SS or NGS) or that different mutations in regulatory sites outside the gene must be present in the other allele of these patients.

Acknowledgement

We thank the patient's families who agreed to participate in the study contributing to this research.

Statement of Ethics

This study was approved by the Institutional Committee for Ethics in Research of the Faculty of Medical Sciences at the State University of Campinas (FCM - UNICAMP). Subject's parents or guardians provided written informed consent to participate in this research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources Statement

This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Financial Code 001; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant/Award Number: 590148/2011-7; Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Grant/Award Numbers: 2015/22145-6 and 1998/16006-6.

Author Contributions

The study was designed by D.P.C. and C.S. Molecular analysis was performed by C.S. and K.d.C.S. Patients were selected by M.D.L.-F., M.T.S., S.N.C., J.L.Jr, C.A.M., and D.P.C. The manuscript was prepared by C.S. and M.D.L.-F. and revised by D.P.C.

Data Availability Statement

All data generated or analysed during this study are included in this article and/or its supplementary material files. Further inquiries can be directed to the corresponding author.

References

Ballhausen D, Bonafé L, Terhal P, Unger SL, Bellus G, Classen M, et al. Recessive multiple epiphyseal dysplasia (rMED): phenotype delineation in eighteen homozygotes for DTDST mutation R279W. J Med Genet. 2003; 40(1):65–71.

Barbosa M, Sousa AB, Medeira A, Lourenço T, Saraiva J, Pinto-Basto J, et al. Clinical and molecular characterization of Diastrophic Dysplasia in the Portuguese population. Clin Genet. 2011;80(6):550–7.

Bonafé L, Hästbacka J, de la Chapelle A, Campos-Xavier AB, Chiesa C, Superti-Furga A, et al. A novel mutation in the sulfate transporter gene SLC26A2 (DTDST) specific to the Finnish population causes de la Chapelle dysplasia. J Med Genet. 2008;45(12):827–1.

Czarny-Ratajczak M, Bieganski T, Rogala P, Glowacki M, Trzeciak T, Kozlowski K. New intermediate phenotype between MED and DD caused by compound heterozygous mutations in the DTDST gene. Am J Med Genet A. 2010;152A(12):3036–42.

Dwyer E, Hyland J, Modaff P, Pauli RM. Genotype-phenotype correlation in DTDST dysplasias: Atelosteogenesis type II and diastrophic dysplasia variant in one family. Am J Med Genet A. 2010;152A(12):3043–50.

Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, et al. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell. 1994;78(6):1073–87.

Hästbacka J, Superti-Furga A, Wilcox WR, Rimoin DL, Cohn DH, Lander ES . Atelosteogenesis type II is caused by mutations in the diastrophic dysplasia sulfate-transporter gene (DTDST): evidence for a phenotypic series involving three chondrodysplasias. Am J Hum Genet. 1996;58(2):255–62.

Hästbacka J, Kerrebrock A, Mokkala K, Clines G, Lovett M, Kaitila I, et al. Identification of the Finnish founder mutation for diastrophic dysplasia (DTD). Eur J Hum Genet. 1999; 7(6):664–70.

Hinrichs T, Superti-Furga A, Scheiderer WD, Bonafé L, Brenner RE. Recessive multiple epiphyseal dysplasia (rMED) with homozygosity for C653S mutation in the DTDST gene – Phenotype, molecular diagnosis and surgical treatment of habitual dislocation of multilayered patella: Case report. BMC Musculoskelet Disord. 2010;11:110.

Honório JC, Bruns RF, Gründtner LF, Raskin S, Ferrari LP, Araujo Júnior E, et al. Diastrophic dysplasia: prenatal diagnosis and review of the literature. Sao Paulo Med J. 2013;131(2): 127–32.

Jackson GC, Mittaz-Crettol L, Taylor JA, Mortier GR, Spranger J. Pseudoachondroplasia and multiple epiphyseal dysplasia: a 7-year comprehensive analysis of the known disease genes identify novel and recurrent mutations and provides an accurate assessment of their relative contribution. Hum Mutat. 2011; 33(1):144–57.

Karniski LP. Mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene: correlation between sulfate transport activity and chondrodysplasia phenotype. Hum Mol Genet. 2001;10(14):1485–90.

Karniski LP. Functional expression and cellular distribution of diastrophic dysplasia sulfate transporter (DTDST) gene mutations in HEK cells. Hum Mol Genet. 2004;13(19):2165–71.

Kausar M, Mäkitie RE, Toiviainen-Salo S, Ignatius J, Anees M, Mäkitie O. Recessive Multiple Epiphyseal Dysplasia - clinical characteristics caused by rare compound heterozygous SLC26A2 genotypes. Eur J Med Genet. 2019; 62(11):103573.

Li J, Meng Y, Li M, Liu C, Li-Ling J, Lyu Y. [Diagnosis of a fetus with atelosteogenesis type 2 through combined prenatal ultrasonography and whole exome sequencing]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2020;37(7):767–70.

Maeda K, Miyamoto Y, Sawaki H, Karniski LP, Nakashima E, Nishimura G, et al. A compound heterozygote harboring novel and recurrent DTDST mutations with intermediate phenotype between atelosteogenesis type II and diastrophic dysplasia. Am J Med Genet A. 2006;140(11):1143–7.

Mäkitie O, Savarirayan R, Bonafe L, Robertson S, Susic M, Superti-Furga A, et al. Autosomal recessive multiple epiphyseal dysplasia with homozygosity for C653S in the DTDST gene: Double-Layer Patella as a Reliable Sign. Am J Med Genet 122A. 2003(3):187–92.

Mäkitie O, Geiberger S, Horemuzova E, Hagenäs L, Moström E, Nordenskjöld M, et al. SLC26A2 disease spectrum in Sweden – high frequency of recessive multiple epiphyseal dysplasia (rMED). Clin Genet. 2015;87(3): 273–8.

Martínez García M, Velez C, Fenollar-Cortés M, Bustamante A, Lorda-Sanchez I, Soriano-Guillén L, et al. Paternal isodisomy of chromosome 5 in a Patient with Recessive multiple Epiphyseal dysplasia. Am J Med Genet A. 2014;164A(4):1075–8.

Mattos EP, Magalhães JAA, Mittaz-Crettol L, Azambuja R, Okada L, Cavalcanti DP, et al. Atelosteogenesis Type 2/Diastrophic dysplasia phenotypic spectrum: from prenatal to preimplantation genetic diagnosis. Open J Obstet Gynecol 2014(4):399–404.

Mégarbané A, Haddad FA, Haddad-Zebouni S, Achram M, Eich G, Le Merrer M, et al. Homozygosity for a novel DTDST mutation in a child with a 'broad bone-platyspondylic' variant of diastrophic dysplasia. Clin Genet. 1999; 56(1):71–6.

Miller E, Blaser S, Miller S, Keating S, Thompson M, Unger S, et al. Fetal MR imaging of atelosteogenesis type II (AO-II). Pediatr Radiol. 2008;38(12):1345–9.

Miyake A, Nishimura G, Futami T, Ohashi H, Chiba K, Toyama Y, et al. A compound heterozygote of novel and recurrent DTDST mutations results in a novel intermediate phenotype of Desbuquois dysplasia, diastrophic dysplasia, and recessive form of multiple epiphyseal dysplasia. J Hum Genet. 2008; 53(8):764–8.

Mortier GR, Cohn DH, Cormier-Daire V, Hall C, Krakow D, Mundlos S, et al. Nosology and classification of genetic skeletal disorders: 2019 revision. Am J Genet A. 2019;179A(12): 2393–419.

Panzer KM, Lachman R, Modaff P, Pauli RM. A phenotype intermediate between Desbuquois dysplasia and diastrophic dysplasia secondary to mutations in DTDST. Am J Med Genet A. 2008;146A(22):2920–4.

Pineda T, Rossi A, Bonafè L, Superti-Furga A, Velasco HM. Report of a novel mutation in the SLC26A2 gene found in a Colombian adult patient with diastrophic dysplasia. Rev Fac Med. 2013;61(3):255–9.

Rapp C, Bai X, Reithmeier RAF. Molecular analysis of human solute carrier SLC26 anion transporter disease-causing mutations using 3-dimensional homology modeling. Biochim Biophys Acta Biomembr. 2017;1859(12):2420–34.

Remes VM, Hästbacka JR, Poussa MS, Peltonen JI. Does genotype predict development of the spinal deformity in patients with diastrophic dysplasia? Eur Spine J. 2002;11(4):327–31.

Rossi A, Bonaventure J, Delezoide AL, Cetta G, Superti-Furga A. Undersulfation of proteoglycans synthesized by chondrocytes from a patient with achondrogenesis type 1B homozygous for an L483P substitution in the diastrophic dysplasia sulfate transporter. J Biol Chem. 1996a;271(31):18456–64.

Rossi A, van der Harten HJ, Beemer FA, Kleijer WJ, Gitzelmann R, Steinmann B, et al. Phenotypic and genotypic overlap between atelosteogenesis type 2 and diastrophic dysplasia. Hum Genet. 1996b;98(6):657–61.

Rossi A, Bonaventure J, Delezoide AL, Superti-Furga A, Cetta G. Undersulfation of cartilage proteoglycans ex vivo and increased contribution of amino acid sulfur to sulfation in vitro in McAlister dysplasia/atelosteogenesis type 2. Eur J Biochem. 1997;248(3):741–7.

Rossi A, Superti-Furga A. Mutations in the diastrophic Dysplasia Sulfate Transporter (DTDST) Gene (SLC26A2): 22 Novel Mutations, Mutation Review, Associated Skeletal Phenotypes, and Diagnostic Relevance. Hum Mutat. 2001;17(3):159–71.

Ruault V, Yauy K, Fabre A, Fradin M, Van-Gils J, Angelini C, et al. Clinical and molecular spectrum of nonsyndromic early-onset osteoarthritis. Arthritis Rheumatol. 2020;72(10): 1689–93.

Sobreira N, Schiettecatte F, Boehm C, Valle D, Hamosh A. New tools for Mendelian disease gene identification: PhenoDB variant analysis module and GeneMatcher, a web-based tool for linking investigators with an interest in the same gene. Hum Mutat. 2015;36(4):425–31.

Superti-Furga A, Hästbacka J, Wilcox WR, Cohn DH, van der Harten HJ, Rossi A, et al. Achondrogenesis type IB is caused by mutations in the diastrophic dysplasia sulphate transporter gene. Nat Genet. 1996;12(1):100–2.
Superti-Furga A, Neumann L, Riebel T, Eich G, Steinmann B, Spranger J, et al. Recessively inherited multiple epiphyseal dysplasia with normal stature, club foot, and double layered patella caused by a DTDST mutation. J Med Genet. 1999;36(8):621–4.
Syvänen J, Helenius I, Hero M, Mäkitie O, Ignatius J. Recessive MED with auricular swelling due to compound heterozygosity Arg279Tpr/Thr512Lys in the SLC26A2 gene. Am J Med Genet A. 2013;161A(6):1491–4.
Unger S, Le Merrer M, Meinecke P, Chitayat D, Rossi A, Superti-Furga A. A new dysplasia or achondrogenesis type 1B? The importance of histology and molecular biology in delineating skeletal dysplasias. Pediatr Radiol. 2001;31(12):893–4.
Vikraman SK, Balakrishnan B, Chandra V, Batra M, Kuriakose R, Kannoly G. A case of hereditary novel mutation in SLC26A2 gene (c.1796A>C) identified in a couple with a fetus affected with atelosteogenesis type 2 phenotype in an antecedent pregnancy. Case Rep Perinat Med. 2016;5(1):65–7.
Yang K, Shen M, Yan Y, Tan Y, Zhang J, Wu J, et al. Genetic Analysis in Fetal Skeletal Dysplasias by Trio Whole-Exome Sequencing. Biomed Res Int. 2019;14:2492590.
Zechi-Ceide RM, Moura PP, Raskin S, Richieri-Costa A, Guion-Almeida ML. A compound heterozygote SLC26A2 mutation resulting in robin sequence, mild limbs shortness, accelerated carpal ossification, and multiple epiphysial dysplasia in two Brazilian sisters. A new intermediate phenotype between diastrophic dysplasia and recessive multiple epiphyseal dysplasia. Am J Med Genet A. 2013;161A(8):2088–94.