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Atelosteogenesis Type 2/Diastrophic Dysplasia Phenotypic Spectrum: From Prenatal to Preimplantation Genetic Diagnosis

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Abstract

Atelosteogenesis type II (AO2) and diastrophic dysplasia (DTD) are two recessively inherited, severe skeletal dysplasias caused by mutations in the *SLC26A2* gene. AO2 is an invariably lethal condition, while DTD patients may reach adult life, although both diseases have overlapping diagnostic features. Here we report a patient with an intermediate phenotype between AO2 and DTD and present the successful application of preimplantation genetic diagnosis (PGD) in this situation. Sequencing of *SLC26A2* alleles in the infant identified two compound heterozygous mutations, p.Arg178Ter and p.Arg279Trp, of paternal and maternal origin, respectively. At request from the parents, PGD was developed by haplotype mapping of parental *SLC26A2* alleles in eleven five-day embryos. Transference to the mother was attempted twice, finally resulting in pregnancy and delivery of a healthy baby. This exemplifies the utility of PGD for inherited lethal conditions with a significant risk of recurrence, and highlights the importance of accurate diagnosis of skeletal dys-

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plasias with prenatal manifestation.

Keywords

Atelosteogenesis Type 2, Diastrophic Dysplasia, Preimplantation Genetic Diagnosis, Prenatal Diagnosis, Skeletal Dysplasia

1. Introduction

Skeletal dysplasias, or osteochondrodysplasias (OCDs), comprise a vast group of genetic diseases affecting the formation of the skeleton [1]. About 40% of OCDs can be recognized in the prenatal period [2], and combined; OCDs are frequent malformations that significantly contribute to perinatal death [3]. An interesting group of recessive OCDs is due to mutations in the diastrophic dysplasia sulfate transporter gene (*DTDST*), also known as *SLC*26*A*2. Conditions with this genetic basis range from severe achondrogenesis type 1B (ACG1B) and atelosteogenesis type II (AO2), to diastrophic dysplasia (DTD), and milder recessively inherited multiple epiphyseal dysplasia (rMED) and diastrophic dysplasia variant (mDTD) [4]-[6]. ACG1B and AO2 are lethal conditions [7], while individuals with DTD and rMED reach adult life [8].

AO2 comprises micromelia with adducted ("hitchhiker") thumbs and *talipes equinovarus* with a wide gap between the first and second toes. Micrognathia, cleft palate, and small chest can also be present [9]. Shortening of tubular bones, humeri with U- or V-shaped distal ends, bowed radiuses and ulnae, and unossified pubic bones are also observed [9]. DTD usually has a similar phenotype, which may hinder the antenatal distinction between AO2 and severe DTD [8] [9]. This points to a transitional phenotype between these conditions, in which different *SLC*26*A*2 alleles contribute to a spectrum of OCDs [10] [11].

We present a Brazilian fetus with a severe OCD diagnosed as an intermediate phenotype between AO2 and severe DTD by *post mortem* radiological investigation, the molecular diagnosis and subsequent preimplantation genetic diagnosis (PGD) offered to the family, leading to pregnancy and delivery of a healthy infant.

2. Methods

2.1. Detection of Mutations

The *SLC*26*A*2 gene was amplified by PCR and screened for the four most common *SLC*26*A*2 mutations by restriction enzyme digestion and gel electrophoresis with positive and negative controls. Subsequently, selective fragments of the gene were analyzed by bidirectional fluorescent direct sequencing. Results have been confirmed in a second amplification product. Primer sequences and restriction enzymes used are listed on **Table 1**.

2.2. Preimplantation Genetic Diagnosis

The established protocol followed the guidelines of the Preimplantation Genetic Diagnosis (PGD) International

Table 1. Polymersase chain reaction primers and restriction enzymes used for *SLC26A2* screening.

SLC26A2 utation[a]	Reagents		
	Forward primer	**Reverse primer**	**Restriction enzyme**
c.−26 + 2T > C	5'-CTTCGGAGTC CGAGCGATGG-3'	5'-GACCCCTGAT CTGGGATTCT-3'	Hph I
c.559 C > T	5'-AGGAAGCTGA ACCATCTATC-3'	5'-GATTCCTCAG ATCCCTTAGAG-3'	Dde I
c.862 C > T	5'-CTCCATGCAA GAAATGTCAGG-3'	5'-TGATACAGTG ATAGCAAAACC-3'	Sty I
c.1984 T > A	5'-CAACCCAATC TTAATAAAGGTG-3'	5'-CTAGACATTC TTCTATCTACC-3'	-

a. Sequencing primer: 5'-CAACCCAATCTTAATAAAGGTG-3'.

Society [12]. Twenty-four oocytes were fertilized and 19 embryos were successfully produced. DNA was extracted from trophectoderm cells of eleven viable embryos on the fifth day post-fertilization. All biopsied embryos were cryopreserved until transference to the mother. Whole-genome amplification was performed and samples were screened for *SLC*26*A*2 alleles by parental haplotype analysis, based on the mutations identified. As requested by the parents, 24-chromosome array-comparative genomic hybridization (aCGH) was also employed for detection of chromosomal abnormalities.

2.3. Ethical Approval

The individuals reported here were informed and consented with the anonymous disclosure of their medical information. This work was ethically reviewed and approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre.

3. Results

Recently, a non-consanguineous couple (32-year-old father and 30-year-old mother) of Portuguese ancestry was referred to our Institution during their second pregnancy due to recurrence of detection of a severe OCD. Their previous pregnancy ended at 16 weeks of gestation, after short fetal long bones and trunk constriction were detected. During the second pregnancy, ultrasonography identified again very short long bones and pregnancy ended at 17 weeks. Postnatal physical examination revealed a female fetus with severely shortened limbs and fingers with hitchhiker thumbs, *varus* deformity of lower limbs, *talipes equinovarus* with a wide gap between first and second toes, relative macrocephaly, midface hypoplasia, micrognathia, and short trunk (**Figure 1(a)**). Babygram showed narrowing of upper thorax, thin ribs, round ilia, and short long bones and metacarpals (**Figure 1(b)**). These findings were very suggestive of either AO2 or DTD.

Sequencing of the mother-father-child trio characterized the fetus as a compound heterozygote for two known pathogenic *SLC*26*A*2 point mutations, c.559C > T (p.Arg178Ter) of paternal, and c.862 C > T (p.Arg279Trp) of maternal origins (**Figure 1(c)** and **Figure 2**). This genotype has been previously observed and associated with AO2 and DTD [9], and the 17-week fetus presented indeed characteristic radiographic findings of both disorders.

A PGD protocol was elected by the family for a subsequent pregnancy. Upon the establishment of a PGD protocol, haplotype reconstructions identified only two embryos carrying both wild type *SLC*26*A*2 alleles (**Table 2**). These were firstly transferred to the mother, but no pregnancy developed. Of the other 9 samples, seven carried just one of the two pathogenic alleles, and only five were chromosomally balanced. The parents opted for the implantation of two heterozygous carrier embryos, and this second attempt resulted in pregnancy and delivery of a healthy infant, although the baby was not postnatally screened for *SLC*26*A*2 mutations.

4. Discussion

In the present study, the evaluated fetus had clinical findings compatible with both AO2 and severe DTD. In fact, the skeletal abnormalities reported here are strikingly similar to those identified in patients reported by Macías-Gomez and colleagues (2004) [10] and Maeda and colleagues [11], who were also diagnosed with an intermediate AO2/DTD condition.

In *SLC*26*A*2, the p.Arg279Trp mutation impairs protein activity by approximately 50%, when compared to the wild-type transporter [13]. Additionally, p.Arg178Ter is the second most common mutation found in patients of the *DTDST* spectrum [4]. In *Xenopus laevis* oocytes, it was shown that p.Arg178Ter behaves almost as a null allele, practically abolishing the protein's sulfate transport activity [14].

The substitution p.Arg279Trp has been found in AO2 mostly in compound heterozygosity with p.Arg178Ter or with another null allele [7] [10] [13] [15], and the p.Arg178Ter + p.Arg279Trp genotype has been identified in almost half of the patients with AO2, as well as in 20% of patients with DTD diagnosed at the Lausanne Molecular Pediatrics laboratory (L. Mittaz-Crettol, personal communication). Barbosa and colleagues (2011) identified compound heterozygosity for p.Arg178Ter and p.Arg279Trp in seven Portuguese patients diagnosed with classical DTD, and commented on the phenotypic overlap due to *SLC*26*A*2 mutations [8]. Another group reported both mutations in an infant with clinical and skeletal findings suggestive of a severe variant of DTD or a mild form of AO2 [10]. These observations reinforce the notion that the same *SLC*26*A*2 mutations may not

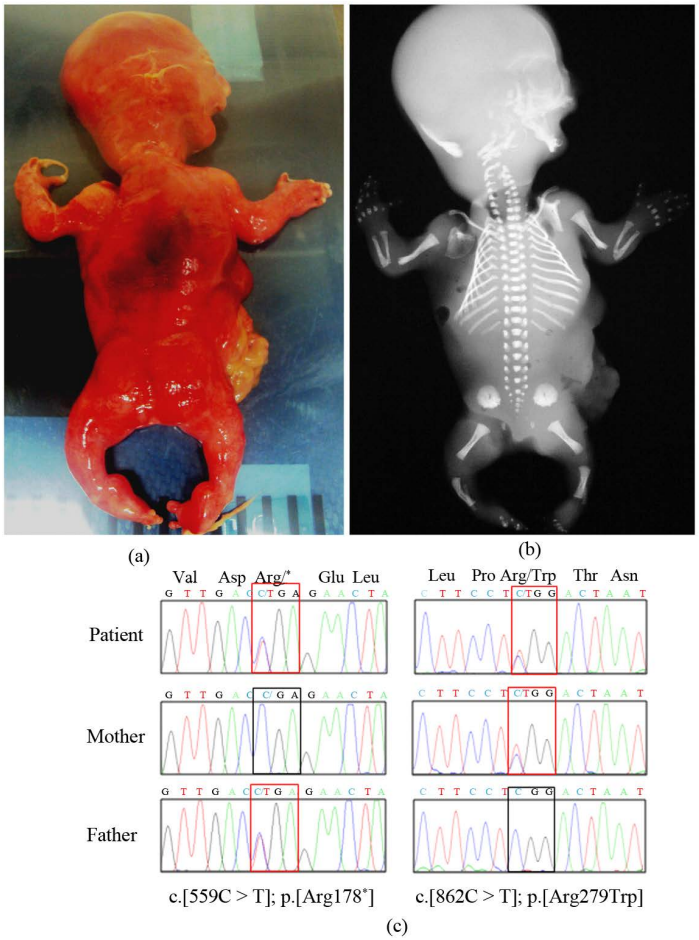

Figure 1. Phenotype of the fetus at 17 weeks of gestation. A: Fetus appearance upon necropsy. Note the short trunk and limbs and the bowing of lower limbs with club feet. Hands with the characteristic "hitchhiker" thumb and feet with a wide gap between the first and second toes are also present. B: Whole-body radiography of the fetus, revealing short long bones, thoracic constriction, thin ribs, and round ilia with unossified pubis. C: *SLC*26A2 sequencing of the mother-father-child trio. Electropherograms depict heterozygous peaks corresponding to the c.559 C > T substitution (p.Arg178Ter) of paternal origin, and to the c.882 C > T substitution (p.Arg279Trp) of maternal origin.

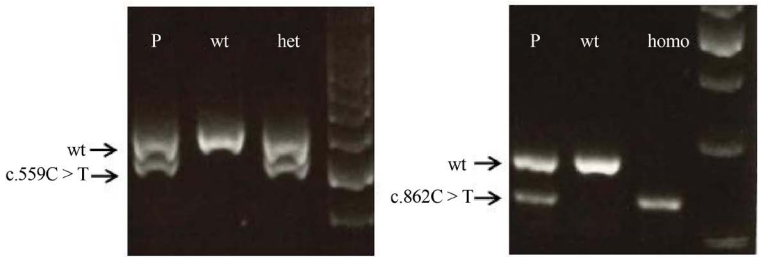

Figure 2. Restriction enzyme digestion of PCR products amplified from the DNA of the fetus. Top legend: P stands for patient, wt for a wild type control, het for a heterozygous control sample and homo for a homozygous control sample for the given mutation. Side legend: wt stands for the non-digested allele. The allele being digested is presented with the description of the mutation that gives rise to the new restriction site.

Table 2. Preimplantation genetic diagnosis for AO2/DTD and embryo selection.

Sample	Embryo characteristics			
	Inferred alleles[a]	aCGH result	Interpretation	Fate of embryo
2	p.Arg178Ter wt[c]	45, XX; -2	Heterozygous carrier (paternal mutant allele); chromosome 2 monosomy	Not used
5	p.Arg178Ter/wt	45, XX; -17	Heterozygous carrier (paternal mutant allele); chromosome 17 monosomy	Not used
6	wt/p.Arg279Trp	46, XY	Heterozygous carrier (maternal mutant allele)	Transferred attempt #2, healthy infant born
7	p.Arg178Ter/wt	46, XX	Heterozygous carrier (paternal mutant allele)	Transferred attempt #2
9	wt/p.Arg279Trp	46, XY	Heterozygous carrier (maternal mutant allele)	Cryopreserved
10	wt/wt	46, XX	Wild type at the *SLC26A2 locus*	Transferred attempt #1
11	wt/p.Arg279Trp	46, XY	Heterozygous carrier (maternal mutant allele)	Cryopreserved
13	wt/p.Arg279Trp	46, XX	Heterozygous carrier (maternal mutant allele)	Cryopreserved
14	p.Arg178Ter/ p.Arg279Trp	NT[b]	Compound heterozygous mutant (maternal and paternal mutant alleles)	Not used
17	p.Arg178Ter/ p.Arg279Trp	NT	Compound heterozygous mutant (maternal and paternal mutant alleles)	Not used
19	wt/wt	46, XY	Wild type at the *SLC26A2 locus*	Transferred attempt #1

a. Alleles from embryos were imputed from parental haplotypes. b. NT: not tested. c. wt: wildtype allele, without any of the parental mutations, as inferred by haplotype analysis.

always translate into a single, characteristic phenotype, implying genetic and/or environmental factors influencing sulfate transport activity and clinical outcome.

5. Conclusion

This report exemplifies the growing application of PGD for severe monogenic diseases [16]. To the best of our knowledge, this is one of the first published descriptions on the application of PGD for a recessive lethal skeletal dysplasia with phenotypic characteristics between AO2 and DTD, leading to the successful live birth of a healthy infant. This exemplifies a growing trend in the medical genetics field, especially for the management of lethal recurrent Mendelian traits with substantial risk of recurrence.

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