Diagnosis of Atelosteogenesis Type I suggested by Fetal Ultrasonography and Atypical Paternal Phenotype with Mosaicism

Diagnóstico de atelosteogênese tipo I sugerido por ultrassonografia fetal e fenótipo paterno atípico com mosaicismo

Atelosteogenesis type I (AOI) is an autosomal dominant skeletal dysplasia caused by mutations in the filamin B (FLNB) gene with classic and well-recognizable clinical findings. However, parents affected with a mild phenotype, probably with somatic mosaicism, can generate offspring with a much more severe phenotype of AOI. In the present report, we describe a female newborn with classic AOI leading to early neonatal death, whose diagnostic was based on prenatal radiological findings and on the physical examination of the father. Since her father had limb deformities and corporal asymmetry, suggesting somatic mosaicism, his biological samples were analyzed through a gene panel for skeletal dysplasias. A missense mutation not previously described in the literature was detected in the FLNB gene, affecting ~ 20% of the evaluated cells and, therefore, confirming the diagnosis of mosaic AOI in the father. The molecular analysis of the father was crucial to suggest the diagnosis of AOI in the newborn, since she died early and there were no biological samples available.

A atelosteogênese tipo I (AOI) é uma displasia esquelética autossômica dominante causada por mutações no gene filamina B (FLNB) com achados clínicos clássicos e bem reconhecíveis. No entanto, pais afetados com um fenótipo mais leve, provavelmente com mosaicismo somático, podem gerar uma prole com um fenótipo muito mais grave de AOI. No presente relato, descrevemos um recém-nascido do sexo feminino com AOI...
Introduction

Atelosteogenesis type I (AOI) is a disease of autosomal dominant inheritance associated with mutations in the filamin B (FLNB) gene, located on chromosome 3p14, which encodes the filamin B protein.\(^1\) This syndrome includes a spectrum of phenotypes that may vary from mild, such as Larsen syndrome (LS) and spondylocarpotarsalsynostosis (STC), to severe conditions, such as atelosteogenesis type III (AOIII), and boomerang dysplasia. Atelosteogenesis type I, also known as giant cell chondrodysplasia or spondylohumeroferal hypoplasia, presents as its main manifestation the disordered and incomplete ossification of the skeleton, and it is characterized by severely shortened limbs, displaced hips, knees and elbows, and club feet.\(^2\) Its radiographic features include pelvic hypoplasia; absent, reduced or distally sharpened humerus and femurs; shortened or curved ulna and tibia; absent fibulae; metacarpals and middle and proximal phalanges without ossification or partially ossified; and high perinatal lethality.\(^3\)

Some studies suggest that AOI and AOIII, a chondrodysplasia first described in 1991 with clinical and radiological characteristics similar to AOI, appear to represent a continuous phenotype.\(^3,4\) Unlike AOI, which is highly lethal, AOIII is clinically milder, and usually the affected individual survives after the neonatal period. The clinical picture of AOIII is recognizable from birth, and is characterized by rhizomelic shortening, joint dislocations, club feet, broad nails, polysyndactyly, narrow chest, ocular hypertelorism, flat nasal bridge, micrognathia, and cleft palate. Its radiographic features include distal tapering of the humerus and femurs, short and broad tubular bones in the hands and feet, and mild vertebral hypoplasia. Besides that, the affected children may present respiratory failure due to laryngotraechomalacia and thoracic hypoplasia, indicating that cases of children with AOIII, whose parents had milder phenotypes (similar to Larsen syndrome), occur probably as a result of parental mosaicism, while the children, due to a germline mutation, have all of their cells affected by the mutation and, therefore, present a much more severe phenotype of the disease.\(^5,6\) In the present report, we describe the process of diagnosis of AOI and the variability of the clinical findings in two patients, a father and his child.
Methods

Samples: The DNA sample was extracted from the saliva of the father, which was collected with the Oragene DNA Collection Kits OG-500 and OG-575, and purified following prepIT-L2P manufacturer’s instructions (DNA Genotek, Ottawa, ON, Canada). For the controls, we used our in-house whole exome sequencing data from 609 Brazilians (Online Archive of Brazilian Mutations, ABraOM: http://abraom.ib.usp.br/), as well as public databases (1000 Genomes Project - http://www.internationalgenome.org; Exome Variant Server/NHLBI ESP exomes - http://evs.gs.washington.edu/EVS/; The Genome Aggregation Database (gnomAD) - gnomad.broadinstitute.org/; and Exome Aggregation Consortium (ExAC) http://exac.broadinstitute.org/).

Next-generation sequencing target: An NGS target of a panel of genes associated with skeletal dysplasia (Table 1) was performed with the Illumina MiSeq sequencer (Illumina, San Diego, CA, US), using Illumina’s Nextera kits for library preparation. The KAPA Library Quantification kit (KAPA Biosystems, Wilmington, MA, US) was used to quantify the libraries by real-time quantitative polymerase chain reaction (PCR). The sequence alignment, as well as the data processing, variant calling, and variant annotation were performed with Burrows-Wheeler Aligner (BWA) (http://bio-bwa.sourceforge.net), Picard (http://broadinstitute.github.io/picard/), Genome Analysis Toolkit package (GATK) (https://www.broadinstitute.org/gatk/), and ANNOVAR (http://www.openbioinformatics.org/annovar/) respectively.

In filtering, we have considered only rare mutations with a minor allele frequency (MAF; < 0.5%) in all populations of the public databases analyzed and in our in-house control database. Only variants with > 20 read depths, average quality score > 30, allelic balance > 0.90 for the alternative allele and < 0.10 for the reference allele for variants in homozygous, and allelic balance between 0.2 and 0.8 for variants in heterozygous, and with strand bias < 2 were considered. All loss-of-function variants (LoFs; (mutations...
in splicing sites, frameshifts and stop-gains) were considered pathogenic. Missense variants were considered pathogenic only if predicted to be “possibly damaging” or “probably damaging” by the Polymorphism Phenotyping v2 (PolyPhen-2, Sunyaev lab, Harvard Medical School, Boston, MA, US) software/web server, “deleterious” by the Sorting Intolerant From Tolerant (SIFT) (http://sift.jcvi.org) algorithm, and with the Combined Annotation Dependent Depletion (CADD) (https://cadd.gs.washington.edu/) score reported to be >15. Candidate variants were manually checked on the Integrative Genomics Viewer (IGV) (Broad Institute, Cambridge, MA, US). Synonymous and untranslated region (UTR) mutations were excluded due to the uncertainty of their functional relevance. The genomic position of the mutations is based on the hg19/GRCH37 version of the human reference genome (Genome Reference Consortium – http://www.ncbi.nlm.nih.gov/projects genome/assembly/grc/). All of the remaining variants were checked using the American College of Medical Genetics and Genomics (ACMG) guidelines.

### Results

The sequencing analysis of the NGS-target in the father has led to the identification of 5 rare mutations that were predicted to be damaging (~Table 2). One of the mutations was a splice site-disrupting single nucleotide, and the remaining were four missenses variants in heterozygosis. The splice site mutation was in the BRCA2 (c.8488–1G > A) gene, and it was present in only one control of our Brazilian database (ABraOM). It was described by the Single Nucleotide Polymorphism Database.

### Table 2: List of mutations found after performing the exoma sequencing of the affected father

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>Type of variant</th>
<th>Variant</th>
<th>ACMG guidelines</th>
<th>dbSNP</th>
<th>PolyPhen-2</th>
<th>SIFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr1</td>
<td>LEPRE1</td>
<td>nonsynonymous SNV</td>
<td>NM_001146289:exon10:c.1477G&gt;A</td>
<td>Likely benign</td>
<td>rs201977455</td>
<td>Probably damaging</td>
<td>deleterious</td>
</tr>
<tr>
<td>chr3</td>
<td>FLNB</td>
<td>nonsynonymous SNV</td>
<td>NM_001164317:exon3:c.G596C&gt;p.R199P</td>
<td>Likely pathogenic</td>
<td>NA</td>
<td>Possibly damaging</td>
<td>deleterious</td>
</tr>
<tr>
<td>chr6</td>
<td>LAMA2</td>
<td>nonsynonymous SNV</td>
<td>NM_000426:exon23:c.T3379C&gt;p.C1127R</td>
<td>Uncertain significance</td>
<td>NA</td>
<td>Probably damaging</td>
<td>deleterious</td>
</tr>
<tr>
<td>chr13</td>
<td>BRCA2</td>
<td>splicing</td>
<td>NM_000059:exon20:c.8488–1G &gt; A</td>
<td>Pathogenic</td>
<td>rs397507404</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>chr17</td>
<td>CANT1</td>
<td>nonsynonymous SNV</td>
<td>NM_138793:exon4:c.C896T&gt;p.P299L</td>
<td>Uncertain significance</td>
<td>rs267606700</td>
<td>Probably damaging</td>
<td>deleterious</td>
</tr>
</tbody>
</table>

Abbreviations: ACMG, American College of Medical Genetics and Genomics; Chr, chromosome; dbSNP, The Single Nucleotide Polymorphism Database; PolyPhen-2, Polymorphism Phenotyping v2; NA, not available; SIFT, sorting intolerant from tolerant; SNV, single nucleotide variant.
(dbSNP) and by the ClinVar database, and was classified by
the ACMG guidelines as pathogenic and associated with
breast and ovarian cancers. Despite its probable pathogenicity,
it is not related with our proband phenotype or not relevant to
AOI. Among the missense mutations, only the missense muta-
tion found in heterozygosity in exon 3 of the FLNB gene
(c.596G > C; p.Arg199Pro) appeared to be the causative muta-
tion of the phenotype of the patient. This variant was not
described in any public database; it was predicted as patho-
genic and classified as likely pathogenic by the ACMG. As it
was present in only 20% of the reads sequenced in this
region, we consider that this mutation is presented as mosaic in the patient. The remaining 3 missenses muta-
tions (LEPRE1: exon10:c.1477G > C:p.Ala493Pro; LAMA2:
exon23:c.3379T > C:p.Cys1127Arg; CANT1: exon4:c.896C >
T:p.Pro299Leu) were not considered causal due to a lack of
relevance of the role of the gene for AOI, considering the
phenotype of the patient. Furthermore, these mutations
were not considered pathogenic or likely pathogenic by the
ACMG guidelines, and they are present in our controls, and are
related to recessive diseases. The missense variant in the
LEPRE1 gene was classified as likely benign by the ACMG
guidelines, and it was present in public databases and in our
Brazilian controls. The CANT1 gene variant is present in gno-
mad controls, and was classified as of uncertain significance by
the ACMG guidelines. The LAMA2 gene is associated with
muscular dystrophy, although it is not described in any public
database.

Discussion

The prenatal diagnostic hypothesis of AOI is based on radiolog-
ing imaging tests, such as fetal ultrasonography, which
detects most skeletal anomalies around the second trimester of
pregnancy. As a lethal skeletal dysplasia, this illness is more
amenable to prenatal diagnosis due to an earlier onset
with more severe phenotypic features.

The typical findings include limb shortening, thoracic hypoplasia, and under-
ossification of the long bones. During this investigation, we were not able to obtain X-rays or biological samples of the
newborn, which would have helped with the variant classi-
cification. The clinical investigation of stillbirths presenting
dysmorphism or birth defects, including the performance of
complementary exams and the collection of biological sam-

dles, is still not widespread among health professionals in
Brazil. Therefore, the chance to perform a more accurate
diagnosis is lost, and, in many cases, the genetic counseling of
the parents is not performed. In our report, the fetal ultrasono-
graphy and postmortem photographs served as a basis for the
AOI hypothesis in the father of the newborn, whose
diagnosis was confirmed through molecular analysis of the
FLNB gene. The missense mutation found in exon 3 of the
FLNB gene (c.596G > C; p.Arg199Pro), which has not been
previously described, is present in 20% of the reads, suggest-
ing a case of somatic mosaicism. This finding confirms the
clinical diagnosis of AOI both in the father and in the
daughter. The majority of the mutations reported in AOI
are in exons 2 to 5 of the FLNB gene. Besides that, mutations
in the same protein domain were previously described in
cases of AOI and AOIII, and both skeletal dysplasias are
characterized by overlapping clinical findings. The lethal presenta-
tion of the disease suggests the diagnosis of classic
AOI in the newborn, and the same disease, as a somatic
mosaicism, in the father. This would mean that not all of the
father’s cells are affected by the same mutation, therefore
explaining the milder and asymmetrical expression and his survival beyond the life expectancy of the disease. Since it is a
case of mosaicism, another tissue should be tested, ideally, in
order to obtain more information about the level of mosaici-
sm (such as skin and blood), but we did not have access to
other samples.

Conclusion

The clinical features of the father are characteristic of a
somatic mosaicism. This becomes evident due to the milder
and asymmetrical involvement of his limbs and trunk.
However, his gonadal cells were affected by the mutation,
which explains the birth of an affected newborn with the
complete and lethal phenotype of AOI. In these cases, the
transmission pattern is heterogeneous, depending on the
proportion of gonadal cells affected in the parent, but it can
be considered autosomal dominant. The diagnostic sugges-
tion of AOI in the newborn was only possible through a
molecular analysis of selected genes for skeletal dysplasias in
her father. It is essential to emphasize the important role of
the clinical investigation of a newborn or stillborn with birth
defects to establish a syndromic diagnosis. This will allow
professionals to perform genetic counseling to the parents.
In our case, prenatal and family information were essential to
establish the diagnosis because of the lack of neonatal
information. The present case report reveals the importance
of the prenatal evaluation of the fetus, of the assessment of
the family history, and of the role of NGS and selected panels
for the etiological confirmation of the disease.

Conflict of Interests
The authors have none to declare.

Acknowledgments
The present study was approved by the Ethics Committee of
Instituto de Biociências (Universidade de São Paulo, Brazil).
Biological samples and photographs were obtained after
the patient signed an informed consent form.

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