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An Anadysplasia-Like, Spontaneously Remitting Spondylometaphyseal Dysplasia Secondary to Lamin B Receptor (*LBR*) Gene Mutations: Further Definition of the Phenotypic Heterogeneity of *LBR*-Bone Dysplasias

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Abstract

We describe a boy who has an anadysplasia-like spondylometaphyseal dysplasia. By whole exome sequencing he was shown to have compound heterozygous mutations of *LBR* that codes for the lamin B receptor. He shares many similarities with a case previously described, but in whom the early natural history could not be established [Borovik et al., 2013]. Thus, in addition to Greenberg dysplasia (a perinatal lethal disorder), homozygosity or compound heterozygosity of mutations in *LBR* can result in a mild, spontaneously regressing bone dysplasia.

Keywords

metaphyseal dysplasia; spondylometaphyseal dysplasia; bone dysplasia; lamin B receptor; Pelger-Huet anomaly; whole exome sequencing; phenotype-genotype correlation; spontaneously remitting bone dysplasias

INTRODUCTION

Recently, Borovik et al. [2013] reported a very mild skeletal dysplasia caused by compound heterozygous mutations in *LBR* that codes for the lamin B receptor and suggested that mutations in *LBR* may result in skeletal dysplasias of markedly differing severity—from lethal Greenberg dysplasia to a mild and seemingly innocuous phenotype primarily characterized by mildly disproportionate small stature. Here we describe a boy who has an anadysplasia-like process with spontaneous regression of radiographic skeletal features but persistent mild, disproportionate small stature. Through whole exome sequencing (WES) he was found to have compound heterozygous mutations in *LBR*. His features and history help to define the phenotypic heterogeneity of *LBR*-bone dysplasias.

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CLINICAL REPORT

The proband is a now 15-year-old male who has been periodically assessed since early childhood. He is the first child born to non-consanguineous 28-year-old mother and 29-year-old father, both healthy and of average stature. An ultrasound at 20 weeks gestation showed disproportionate decremental growth. No specific diagnosis was identified prenatally. He was born at term, with a birth weight of 3968 g (75–90th centile) and length of 48 cm (10–25th centile). At birth, short limbs were noted (Fig. 1a). High-resolution chromosome assessment was normal (46, XY). Radiographs showed rhizomelic shortening of the limbs. The humeri and femora were not only shortened, but also bowed. Less marked shortening of all other long bones was noted (Fig. 2a). The radii were bowed. Metaphyses were widened and irregular, most prominently at the wrist, knees and ankles. Ulnar metaphyses were cupped (Fig. 3a). The ribs were mildly shortened. Platyspondyly and ovoid vertebral bodies were present (Fig. 4a). The pelvis was near normal, with mildly flared ilia. No abnormalities of the hands, feet, or skull were present. A non-specific diagnosis of spondylometaphyseal dysplasia was made.

He was first assessed through the Midwest Regional Bone Dysplasia Clinic at 25 months of age. At that time he was 81.9 cm (–2SD) in height and weighed 11.5 kg. He was generally eumorphic. The upper limbs showed marked rhizomelic shortening while the lower limbs had milder changes. Feet and hands were normal. Motor and mental development was normal. No significant health problems had arisen. Radiographs at 15 months showed marked foreshortening of the long bones, particularly of the humeri. There was metaphyseal flaring with spicules and irregularity (Fig. 2b). However, these changes were less marked than on initial radiographs. Radial bow of the forearms persisted and the distal ulnar metaphyses were divided (Fig. 3b). The previous platyspondyly and ovoid vertebral bodies were far less evident (Fig. 4b). Sequential radiographs showed progressive improvement of the metaphyseal changes and resolution of the spine anomalies (Figs. 2–4). Clinical assessments, likewise, were remarkable for decreasing severity of rhizomelic shortening of the long bones. Resolution of the spine anomalies raised the question of whether findings were compatible with anadysplasia or another metaphyseal dysplasia.

He has had virtually no clinical problems. His height has remained between –2.5 and –3.5 S.D. When last assessed at age 14 years his height was 138.1 cm (–3.4 SD) and arm span was 124.7 cm. The only other features noted included lumbosacral hyperlordosis, mild limitation of shoulder elevation, minor limitation of wrist extension, minimal brachydactyly and minor camptodactyly of the intrinsic joints; he also had minor knee flexion contractures and knee valgus (of 15 degrees bilaterally). None of these was severe enough to require treatment.

Serum calcium, phosphorus, alkaline phosphatase and 1, 25dihydroxy vitamin D levels were all normal.

METHODS AND RESULTS

To capture the target regions, we used the Agilent SureSelect Human All Exon 50 Mb Kit (Agilent Technologies, Santa Clara, CA) following vendor provided protocols. We performed WES (paired end 100 bp reads) on the proband and his parents and an unaffected sibling using the Illumina HiSeq2000 platform (Illumina, Inc. San Diego, CA). We aligned each read to the reference genome (NCBI human genome assembly build 36; Ensembl core database release 50_361) [Hubbard et al., 2009] using the Burrows–Wheeler Alignment (BWA) tool [Marini et al., 2009] and identified single nucleotide variants (SNVs) and small insertion/deletions (indels) using SAM tools [Li et al., 2009]. PCR duplicates were removed using the Picard software. We performed local realignment and base call quality recalibration using GATK [McKenna et al., 2010]. Using the phenoDB analysis tool, we applied a filtering designed to prioritize rare functional variants (missense, nonsense, splice site variants, and indels) that were compound heterozygous in the proband, but not in his parents or unaffected sibling. We identified two mutations in *LBR* in the proband, one in exon 3 (p.R76X) and one in exon 13 (p.N547S). The first is a nonsense mutation and presumably deleterious. The p.N547S mutation has a SIFT score of 0 and a PolyPhen score of 0.999. The mother and an unaffected brother of the proband were heterozygous for the p.N547S mutation and the father did not have either mutation. By Sanger sequencing we validated the variants in the proband, his parents and unaffected brother and genotyped a second unaffected brother for both mutations; this second unaffected sibling was also heterozygous for the p.N547S mutation. Because one mutation was a new mutation, we performed further studies to define their phase. mRNA was extracted from the patient's lymphoblastoid cell line and then reverse-transcribed. cDNA was amplified with a pair of primers flanking both *LBR* mutations, and the PCR product was cloned and then Sanger sequenced, showing that the mutations in the proband were in trans.

Detectable amounts of cholesta-8,14-dien-3-beta-ol were identified in the proband, indicative of partial deficiency of the delta-14-reductase function of *LBR*. Subsequent to learning the results of WES, blood smears were obtained that demonstrated the presence of the Pelger–Huet anomaly in white blood cells, consistent with what is usually presumed to be secondary to a heterozygous mutation; additional record review showed that 7 years previously he had been found to have possible Pelger–Huet anomaly, but that finding was assumed to be irrelevant to his clinical care.

DISCUSSION

Spontaneously regressive bone disorders are few. Not surprisingly, the most common are metabolic disorders amenable to treatment (including various rachitic disorders) [Glorieux, 1991; Wharton and Bishop, 2003]. It is more surprising that certain metabolic bone disorders for which there is no known effective treatment may nonetheless show spontaneous regression. An example is the spontaneous lyregressing form of hypophosphatasia [Moore et al., 1999; Pauli et al., 1999] in which severe osteopenia and bowing in utero results later in life in a non-lethal, relatively benign phenotype. Least intuitive is spontaneous regression of non-metabolic bone dysplasias, as

seen in Weissenbacher–Zweymuller dysplasia [Haller et al., 1975] and some forms of kyphomelic dysplasia [Cisarik et al., 1999].

Metaphyseal anadysplasia is the quintessential example of spontaneously remitting bone dysplasias. First described four decades ago [Wiedemann and Spranger, 1970], two main forms were recognized [LeMerrer and Maroteaux, 1998]. The patient described here shares substantial features with patients with anadysplasia and seems to broadly fall within this diagnostic category. However, early bony changes were more severe and there were quite marked spine changes. Given these differences he might better be described as having a spontaneously remitting spondylometaphyseal dysplasia. Nonetheless, the dramatically regressive course suggested no other obvious diagnosis. Other consultants with experience in diagnosing metaphyseal anadysplasia in the U.S. and Europe differed in their opinions about the appropriateness of assigning the diagnosis of anadysplasia, but were unable to suggest any viable alternative.

Lausch et al. [2009] showed that the dominant and recessive metaphyseal anadysplasia are caused by mutations in the matrix metalloproteinase genes *MMP13* and *MMP9*. This provided an opportunity to confirm whether the proband had a process caused by mutation in either gene. Sanger sequencing of the coding region of *MMP13* and *MMP9* (courtesy of Ekkehart Lausch, Freiburg, Germany) did not show a pathogenic mutation.

By WES, we identified two novel mutations in exon 3 and in exon 13 of *LBR*. The lamin B receptor is a dual-function protein, affecting both cholesterol synthesis and nuclear membrane differentiation [Schuler et al., 1994; Worman and Bonne, 2007]. Compound heterozygosity or homozygosity of mutations in *LBR* sometimes results in a severe, perinatal lethal disorder—Greenberg skeletal dysplasia [Greenberg et al., 1988]. However, there are instances in which far milder skeletal manifestations have been described in individuals with presumed homozygous/compound heterozygous mutations [Haverkamp et al., 1952; Aznar and Vaya, 1981; Gastearna et al., 1982; Erice et al., 1999; Hoffmann et al., 2002; Borovik et al., 2013]. Borovik et al. [2013] showed that two novel mutations in exons 6 and 14 of *LBR* resulted in a subtle bone dysplasia with mild short stature, disproportionately short limbs and mild bowing of the radii. In general, mutations resulting in Greenberg dysplasia are in the C-terminal region (mostly in exons 11–14), while those resulting in Pelger–Huet anomaly were identified everywhere in the gene [Borovik et al., 2013]. Our patient and the girl described by Borovik et al. [2013] had one mutation within the hydrophobic transmembrane domain that results in deficiency of delta-14-reductase [Waterham et al., 2003; Clayton et al., 2010] and a second in or near to the nucleoplasmic domain. Partial loss of delta-14-reductase function is supported by the detectable amounts of cholesta-8,14-dien-3-beta-ol in both cases. Both this boy and the girl described by Borovik et al. [2013]; despite compound heterozygosity of *LBR* mutations, showed the Pelger–Huet phenotype as usually described in “heterozygous” individuals (i.e., dumbbell shaped) [Hoffmann et al., 2002].

These two cases and those of Hoffmann et al. [2002] and Erice et al. [1999] probably constitute a distinct, recognizable entity within the *LBR* spectrum of effects. We suggest that, in these cases, one mutation has a primary effect on bone morphogenesis while the

second allele harbors a mutation having some influence on bone morphogenesis, but insufficient to result in the Greenberg dysplasia. Why mutations in *LBR* can result in a spontaneously remitting process is unknown.

Given these instances of *LBR* mutations causing a mild bone dysplasia, we suggest that a peripheral blood smear to assess for Pelger-Huët anomaly be done as a screening test in an individual with an undelineated metaphyseal or spondylometaphyseal dysplasia.

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FIG. 1.

Clinical photographs at 1 day of age (a, left) and 5 years 4 months of age (b, right). The infant photograph shows dramatic limb shortening as well as an apparently small thorax. At 5 and 4/12 years there is moderate rhizomelic limb shortening, particularly of the arms, without any other obvious unusual features.

FIG. 2.

Lower limb radiographs at (left to right) ages 1 day (a), 15 months (b), 5 years 4 months (c) and 8 years 4 months (d). Initially the long bones are short and broad, the femurs are curved and there is considerable metaphyseal flaring and irregularity consistent with a metaphyseal dysplasia. With increasing age the features become far less marked with much less bowing and far more subtle metaphyseal changes. By 8 4/12 years of age the metaphyseal changes are barely detectable.

FIG. 3.

Upper limb radiographs at ages 1 day (a, upper left), 15 months (b, lower left), 4 years 2 months (c, upper right), 5 years 4 months (d, middle right) and 8 years 4 months (e, f, lower right). As in the legs (Fig. 2) initially there is marked shortening of the long bones with marked metaphyseal cupping of the distal radius and ulna; the ulna is abnormally bowed. There is irregularity of metaphyses. Here, too, changes become far less prominent with increasing age.

FIG. 4.

Lateral spine radiographs at ages 1 day (a, upper left), 15 months (b, upper right), 5 years 4 months (c, lower left) and 8 years 4 months (d, lower right). In infancy there is considerable platyspondyly with ovoid vertebral bodies. The ribs are also mildly shortened. Sequentially there is normalization of all of the vertebral features with only minimal thoracic platyspondyly apparent in (c) and apparent complete normalization in (d); radiograph (d) is somewhat overpenetrated.