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Lamin B receptor-related disorder is associated with a spectrum of skeletal dysplasia phenotypes

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ABSTRACT

LBR (Lamin B Receptor) encodes a bifunctional protein important for cholesterol biosynthesis and heterochromatin organization on the inner nuclear membrane. Pathogenic variants in *LBR* are associated with marked phenotypic variability, ranging from the benign Pelger-Huët anomaly to lethal Greenberg Dysplasia. We performed trio exome sequencing (ES) on two patients with atypical variants of skeletal dysplasia and their unaffected parents. Patient 1 exhibited frontal bossing, mid-face hypoplasia, short stature with rhizomelic limb shortening, and relative macrocephaly at birth. Although remained short, Patient 1 later showed spontaneous improvement in her skeletal findings. Exome sequencing revealed two novel variants in *LBR*, c.1504C > G (p.Arg502Gly) in exon 12 and c.1748G > T (p.Arg583Leu) in exon 14, which were inherited from her unaffected father and mother, respectively. Sterol analysis revealed an increased level of cholesta-8,14-dien-3β-ol to 2.9% of total sterols, consistent with a functional deficiency of 3β-hydroxysterol Δ14-reductase. Patient 2 presented at birth with short stature and marked rhizomelic limb shortening but later exhibited decreasing severity of shortening of the long bones and improvement in the radiographic skeletal abnormalities although he continued to be significantly short at age 10 years. Exome sequencing revealed that Patient 2 is homozygous for a pathogenic variant c.1534C > T (p.Arg512Trp) in exon 12 of *LBR*, which was inherited from his unaffected consanguineous parents. This report provides further evidence for a phenotypic spectrum of *LBR*-associated disorders and expands the genotypic spectrum by describing 3 novel disease-causing variants that have not been previously associated with a disease. Moreover, our data on Patient 1 demonstrate that variants throughout the gene appear to influence both the sterol reductase and nuclear functions of LBR.

1. Introduction

The Lamin B Receptor (*LBR*) gene (MIM# 600024) encodes a bifunctional protein that serves as the primary C-14 reductase in cholesterol biosynthesis and anchors heterochromatin to the inner nuclear membrane [1,2]. Heterozygous, and rarely homozygous, pathogenic variants in *LBR* are associated with defects in neutrophil segmentation and the Pelger-Huët anomaly (PHA) (MIM# 169400) [3,4], whereas biallelic pathogenic variants are associated with skeletal dysplasia phenotypes with broad phenotypic variability [5–9]. Greenberg dysplasia (MIM# 215140) represents the most severe *LBR*-associated skeletal dysplasia, characterized by fetal hydrops, rhizomelic dwarfism, and abnormal chondro-osseous calcification [5–7]. Rarely reported milder forms have been associated with spontaneously regressing bone dysplasia, spondylometaphyseal dysplasia, and disproportionate short stature [8,9].

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LBR is strongly expressed in many tissues, including brain, skin, cartilage, bone and liver [6]. LBR is encoded by 14 exons located on chromosome 1q42.12 and can be divided into two structurally and functionally distinct portions [2]. The amino portion, also known as the nucleoplasmatic portion, is encoded by exons 1–4 and interacts with diverse components of the nucleus to maintain structural integrity of the lamin network and anchor heterochromatin to the inner nuclear membrane [1,6,16]. As such, this function of LBR has implications on nuclear structure, gene expression, and processes of cell division and death. Pathogenic variants associated with the PHA have been identified in this portion of *LBR,* but also throughout the gene [8]. No other previously reported disease-causing pathogenic variants have been associated solely with the nucleoplasmatic function of LBR [16,17]. The carboxy portion of LBR, encoded by exons 5–14, is termed the transmembrane portion and carries out the metabolic function of LBR through sterol C-14 reductase activity, which is essential for cholesterol synthesis [16]. Most previously reported skeletal dysplasia causing pathogenic variants have been located in this portion of the gene. Pathogenic variants in the transmembrane portion of LBR result in defective sterol metabolism and are thought to contribute to impaired development and membrane dysfunction, contributing to skeletal dysplasia phenotypes and neutrophil hyposegmentation seen in the range of *LBR*-related disorders [3,6]. Debate continues regarding the connection between the structural and metabolic functions of LBR [6,16,17].

Here, we describe two patients with deleterious variants in *LBR* who presented with skeletal abnormalities of moderate severity. Patient 1 presented with spondylometaphyseal dysplasia, rhizomelic short stature, and bi-lobed PHA. Patient 2 similarly presented with short stature associated with spontaneous improvement in his rhizomelic limb shortening. We compare the phenotypes in Patients 1 and 2 with previously reported cases and present potential explanations for the clinical heterogeneity. The patients in this report represent skeletal dysplasia of moderate severity, emphasizing that *LBR*-associated conditions encompass a spectrum of skeletal phenotypes. In addition, Patient 1's biochemical findings and clinical presentation suggest a possible connection between the structural and enzymatic functions of LBR.

2. Materials and methods

2.1. Molecular studies

The Washington University School of Medicine Institutional Review Board approved this study for Patient 1, while study on Patient 2 was performed according to the Research Ethics Guidelines of the Medical Research Institute, University of Alexandria after the approval of the Ethics Committee. Exome sequencing was performed on DNA isolated from peripheral blood leukocytes on patients and their parents using standard protocols. Informed consent was obtained prior to testing. Sequencing was conducted in Patient 1 and her parents using the Illumina HiSeq2000 system (GeneDx, Maryland) with a mean coverage depth of 114× with quality threshold at 95.9%. For *LBR*, 100% of the coding region was covered at a minimum of 10× by XomeDx. For Patient 2 and his parents, fragmented genomic DNA was purified with AMPure XP beads and the quality of the fragmented DNA was assessed with an Agilent Bioanalyzer. Preparation of the exome enriched, barcoded sequencing libraries was performed using Agilent SureSelect Human All Exon v5 kit. The final libraries were quantified with a Qubit Fluorometer (Life Technologies) and the correct size distribution was validated with an Agilent Bioanalyzer. Libraries were sequenced on Illumina HiSeq 2000, generating 100 bp paired-end reads. Raw reads were aligned onto the hg19 reference genome with Novoalign (http://www.novocraft.com) and the data cleanup and variant calling were performed according to GATK Best Practices recommendations. Variant filtering was made with Annovar and with our own perl and bash scripts (available on request). Variants were confirmed using Sanger sequencing. Clinical data was obtained by chart review and direct interaction with patients and their families.

2.2. Biochemical studies

For Patient 1, sterol analysis was performed in cultured lymphoblasts using ion-ratio gas chromatography/mass spectrometry (GC/MS) and gas chromatography-flame ionization detectorGC-FID analyses on an Agilent 6390 N/5973 GC/MS system as previously described [10] with modifications to the GC/MS method to include ions for additional intermediates in the cholesterol biosynthetic pathway between lanosterol and cholesterol, including cholesta-8,14-dien-3β-ol.

3. Results

3.1. Clinical reports

3.1.1. Patient 1

Patient 1 is a female born at 35 2/7 weeks to a 36-year-old G3P3 mother and a 37-year-old father. Both parents are of Caucasian descent and are of typical height. There is no family history of skeletal dysplasia or short stature. Patient 1 was noted to have shortened limbs on 20-week prenatal ultrasound. The condition was assessed to be a lethal skeletal dysplasia. However, Patient 1 did not show any respiratory difficulties postnatally and no oxygen supplementation or resuscitation was required. She was born vaginally at 35 weeks of gestation. Her birth weight was 2.3 kg (1st centile), birth length was 41.3 cm (less than 1st centile) and occipitofrontal circumference (OFC) at 2 weeks of life was 34 cm (75th centile). The neonatal period was complicated only by mild jaundice treated with light for 2 days. Prenatal chromosomal microarray analysis (CMA) and a skeletal dysplasia panel evaluating seven genes (*COL1A1*, *COL1A2*, *COL2A1*, *FGFR3*, *SLC26A2*, *SOX9*, and *TRIP11*) (Connective Tissue Gene Tests, Allentown, PA) were obtained by amniocentesis and revealed no abnormalities. A skeletal survey on the second day of life revealed macrocephaly, narrowing of the thoracic cavity with short, horizontal ribs, platyspondyly, shortening and bowing of both the femurs and humeri, and flaring metaphyses involving multiple long bones (Fig. 2A–D). Head and abdominal ultrasounds as well as an echocardiogram were normal.

Patient 1 was evaluated at 2 weeks of life and was found to be short (height was 42 cm; < 1st centile; −4.5 SD) and relatively macrocephalic (OFC was 34 cm; 16th centile). Her physical exam showed blonde hair, nevus flammeus on forehead and eyelids, a small chest (chest circumference 26 cm; < 3rd centile), inverted nipples, protuberant abdomen, and short limbs (rhizomelic > mesomelic) (Fig. 1A–C).

At 10 months of age, the patient's height was 59.5 cm (< 1st centile; −4.5 SD), weight was 6.7 kg (3rd centile; −2SD) and OFC was 45.5 cm (82nd centile; +1.2 SD). The head was notable for relative macrocephaly, frontal bossing, midface hypoplasia, and a depressed nasal bridge (Fig. 1D, E). The nevus flammeus persisted on forehead and bilateral eyelids. Her chest was small. Neurologic exam revealed mild hypotonia and joint hyperflexibility. Musculoskeletal exam showed rhizomelic > mesomelic shortening of her 4 limbs. Hands and feet were mildly small and digits were normal. Cardiac, pulmonary, abdominal, and genitourinary exams were unremarkable.

Her skeletal survey at 14 months of age revealed that the spondylometaphyseal changes had markedly improved. There were only mild residual metaphyseal and epiphyseal irregularities throughout the long bones. However, there were still shortened, bowed humeri and femora and to a lesser extent shortened, bowed radii and ulnae (Fig. 2A–D). Spine images demonstrated only mild platyspondyly and endplate irregularity (Fig. 2C).

Blood smears from the proband at 14 months of age demonstrated the PHA in approximately 5–10% of the neutrophils (Fig. 1H). Blood smears on parents showed the PHA only in the father (Fig. 1I,J).

Fig. 1. Clinical findings in Patient 1
(A–C) Physical findings at age 4 weeks. Notice nevus flammeus on forehead and eyelids, small chest, protuberant abdomen, and short limbs (rhizomelic > mesomelic).
(D&E) Physical findings at age 10 months. Notice relative macrocephaly, frontal bossing, midface hypoplasia, and depressed nasal bridge.
(F&G) Physical findings at age 2 years 7 months. Notice short stature, relative macrocephaly, and vanishing nevus flammeus on forehead.
(H–J) Blood smears from the proband (H), her mother (I) and father (J) showing hyposegmentation of neutrophil nuclei in the patient and her father.

At 34 months of age, the patient's height was 78.1 cm (< 1st centile, Z score = −3.64), weight was 11.8 kg (13th centile, Z score = −1.13), and OFC was 50 cm (90th centile, Z score = +1.28). Patient 1's overall growth showed continued improvement, as the Z score of her OFC and height continued to increase compared to previous measurements. OFC and height data from 15 days of life until 34 months are summarized in Table 1. Patient 1 continued to exhibit relative macrocephaly with frontal bossing and midface hypoplasia with depressed nasal bridge (Fig. 1F,G). Nevus flammeus on forehead and eyelids continued to vanish. On skeletal survey at 31 months old, Patient 1 again demonstrated shortened humeri and femora as well as minimal platyspondyly and endplate irregularities as seen before and vertebral bodies remained rounded (Fig. 2A–D). However, Patient 1 also demonstrated continued improvement, as previously noted gibbus deformity at C3 had resolved. Spinal MRI revealed mild platybasia of the skull base and mild narrowing of the foramen magnum due to prominent soft tissue in the anterior basion. At 34 months of age, Patient 1 demonstrated good developmental progress and was able to walk, jump, and climb.

3.1.2. Patient 2

Patient 2 is a male who presented with short stature and an abnormal gait. He was the only child of healthy first cousin Egyptian parents (Fig. 3C). He was delivered at 38 weeks of gestation via vaginal delivery, following an uncomplicated pregnancy. At birth, Patient 2 exhibited marked short limb dwarfism associated with striking skeletal abnormalities. X-rays performed after birth showed narrowing of the thoracic cavity, horizontal ribs and bilateral hypoplasia of lateral ends of clavicles, platyspondyly, shortening of all long bones, more severe proximally with bowing of femura, humerii and radii, marked metaphyseal flaring of distal femur and proximal tibia, in addition to malalignment of elbows and wrist joints with dislocation of both proximal and distal radioulnar articulations (Fig. 4A–E). His periodic orthopedic follow-up and radiologic studies demonstrated decreasing severity and spontaneous regression of the skeletal manifestations and the radiographic features (Fig. 4A–E). Apart from delayed walking (at age 18 months), the patient showed normal developmental history and has demonstrated high scholastic achievements.

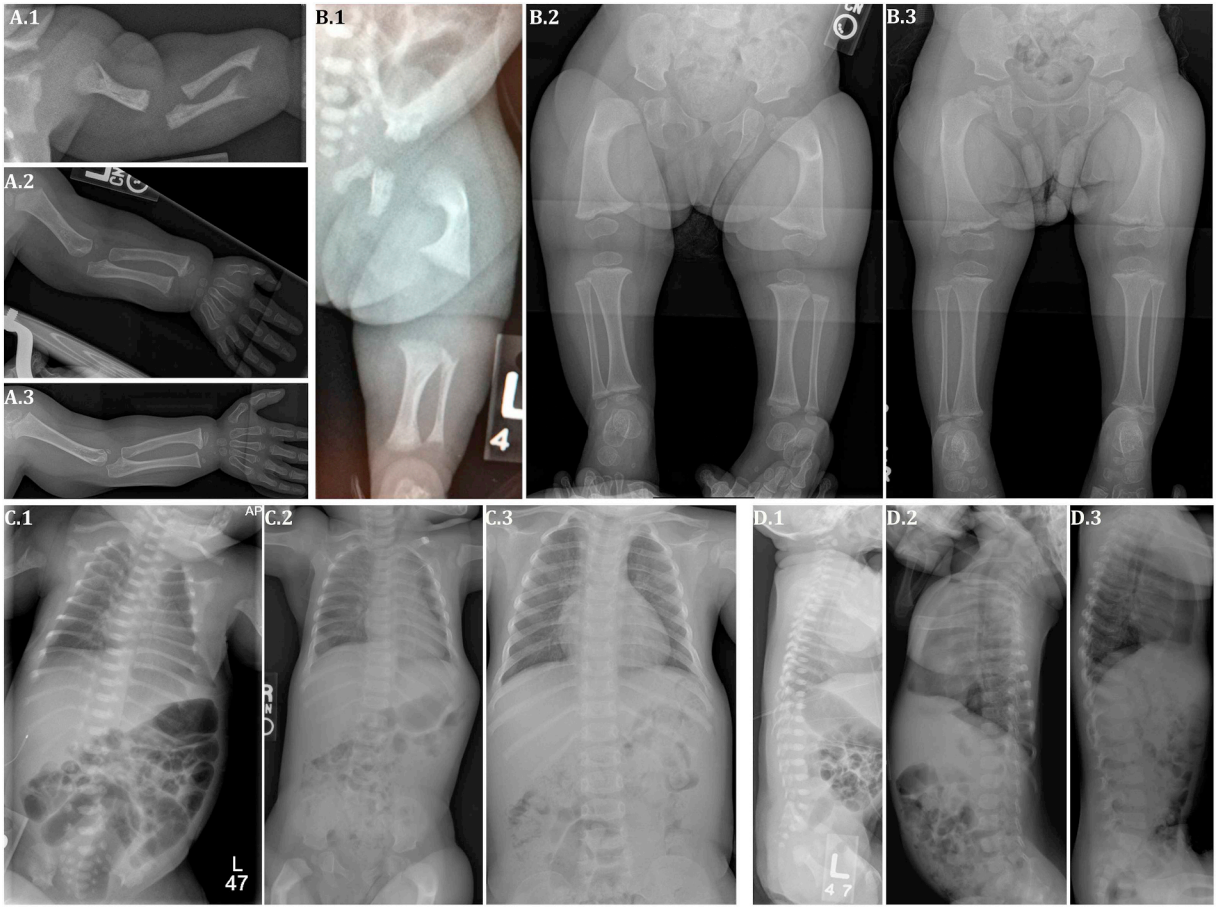

Fig. 2. Radiological findings in Patient 1 at birth (1), 10 months (2), and 2 years 7 months (3) of age.
(A) Left upper extremity showing shortened, bowed humeri and to a lesser extent shortened and bowed radii and ulnae with metaphyseal flaring; all improved with age.
(B) Pelvis and lower limbs. Notice shortened, bowed femura and distal femoral and proximal tibial metaphyseal flaring; these findings gradually improved with age.
(C) Chest and abdomen showing narrowing of the thoracic cavity with short, horizontal ribs. The iliac bones are small with trident appearing acetabular roof.
(D) Lateral Spine. Notice platyspondyly at birth which later markedly improved with only mild platyspondyly and endplate irregularity now identified. At 10 months, the C3 vertebral body was hypoplastic with focal gibbus deformity at that level but later resolved.

Table 1
Change of height and OFC of Patient 1 as a function of her age.

Age (months)	Height			OFC (cm)		
	cm	Percentile	Z score	cm	Percentile	Z score
0.5	42.0	< 1	−5.05	34.0	13	−1.13
3.75	51.0	< 1	−4.70	41.5	82	+0.9
6.5	55.9	< 1	−4.65	N/A	N/A	N/A
10	59.5	< 1	−4.85	45.5	83	+0.94
34	78.1	< 1	−3.64	50	90	+1.28

On physical examination at 10 years of age, Patient 2's height (118 cm) and weight (20.5 kg) were below the 3rd centile, while his OFC (51 cm) was on the 5th centile. He had rhizomelic shortening in his upper limbs, broad joints, thoracolumbar scoliosis, widely spaced nipples, broad big toes and transversely oriented and slit-like umbilicus. The facial features were unremarkable (Fig. 3A, B).

3.2. Molecular and biochemical results

3.2.1. Patient 1

Exome sequencing in Patient 1 revealed compound heterozygous variants in *LBR*. The first genetic variant is designated as c.1504C > G (p.Arg502Gly), located in exon 12, and the second variant is c.1748G > T (p.Arg583Leu) in exon 14. The patient's father is heterozygous for the p.Arg502Gly variant and the mother is heterozygous for the p.Arg583Leu variant. These two variants occur at positions that are conserved across species (Fig. 5A) and are predicted to be deleterious by SIFT and MutationTaster. The variants p.Arg502Gly and p.Arg583Leu were submitted by GeneDx (Gaithersburg, MD) to Clin Var: https://www.ncbi.nlm.nih.gov/clinvar/variation/429297/ and https://www.ncbi.nlm.nih.gov/clinvar/variation/419545/. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

Sterol analysis of cultured lymphoblasts from Patient 1 revealed an increased level of cholesta-8,14-dien-3β-ol relative to total sterols at 2.9% (concurrent control lymphoblasts: 0.03%) (Table 2), confirming a functional deficiency of 3β-hydroxysterol Δ14-reductase.

3.2.2. Patient 2

Exome sequencing in Patient 2 revealed a homozygous pathogenic variant designated as c.1534C > T (p.Arg512Trp) in *LBR*, which was inherited from his first cousin parents (Fig. 3D). This variant impacts a highly conserved amino-acid (Fig. 5A) and is predicted to be deleterious by various *in-silico* software tools (SIFT, MutationTaster). This variant is present in heterozygous state only at very low frequency in the gnomAD database (accessed 8/15/2018): 3.25e-05 and 4.17e-05 (http://gnomad.broadinstitute.org/variant/1-225592358-G-A) among African

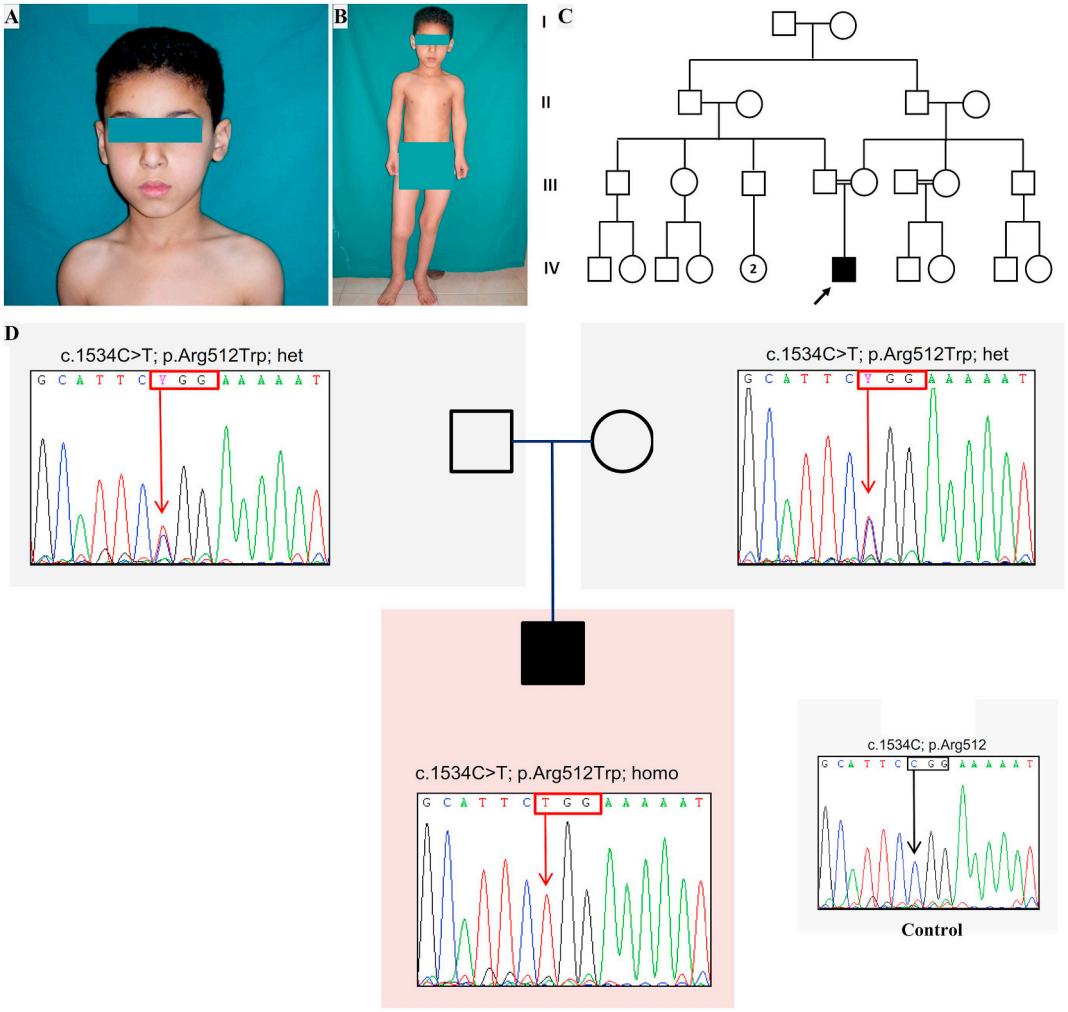

Fig. 3. Clinical and molecular findings in Patient 2.
(A&B) Facial features at age 10 years were unremarkable. Notice rhizomelic shortening in his upper limbs, broad joints, and widely spaced nipples.
(C) Pedigree showing consanguinity between parents.
(D) Electropherogram of the homozygous pathogenic variant, c.1534C > T(p.Arg512Trp), identified in the patient as compared to electropherograms showing heterozygous status in his parents and the wild type allele in a control.

and Asian populations, respectively [11].

4. Discussion

Patients 1 and 2 presented with short stature, spondylometaphyseal dysplasia and spontaneously improving rhizomelic limb shortening associated with compound heterozygous and homozygous pathogenic variants in *LBR,* respectively. While previously reported patients with pathogenic variants in *LBR* presented with lethal Greenberg Dysplasia [6] or mild skeletal dysplasia [8,9], the two patients in this report presented with a skeletal phenotype of moderate severity. The findings in our patients demonstrate that the *LBR*-associated disorder can present with a spectrum of skeletal manifestations with variable severity.

The radiological findings in the two patients described here are most consistent with spondylometaphyseal dysplasia. However, the clinical course in all patients with the milder LBR-related skeletal forms is not compatible with any of the phenotypic entities listed under Group 12 of the Revised Nosology and Classification of Genetic Skeletal Disorders [19]. Improvement of skeletal abnormalities is commonly seen with metabolic bone diseases such as vitamin D responsive rickets or renal osteodystrophy after the correction of the underlying etiology. Nevertheless, spontaneous improvement in skeletal abnormalities was also documented in different forms of genetic skeletal dysplasia such as kyphomelic dysplasia (MIM# 211350), Missouri type of spondyloepimetaphyseal dysplasia (MIM# 602111), and metaphyseal anadysplasia (MIM# 602111). However, close examination of the radiological findings and their interval changes and severity excluded these skeletal dysplasia conditions.

Pathogenic variants in *LBR* are associated with PHA and skeletal dysplasia conditions with broad phenotypic variability. Hoffman et al. reported patients with isolated PHA associated with nonsense, frameshift, and splice site variants distributed throughout the nucleoplasmatic and transmembrane portions of the protein, including one homozygous case with round nuclei, mild retardation, and short stature [3]. Greenberg dysplasia is the most severe *LBR*-related disorder, and is characterized by hydrops, severe shortening of all long bones with a moth-eaten radiographic appearance, platyspondyly, disorganization of chondro-osseous calcification, ectopic ossification center, and prenatal death [5–7]. Five pathogenic variants (p.Val11Glufs*24, p.Tyr468Thrfs*475, p.Leu534*, p.Asn547Asp, p.Arg583Gln) in *LBR* have been reported so far in 5 unrelated families with Greenberg dysplasia [5–7]. Variants causing Greenberg dysplasia are located mainly in the transmembrane domain and predicted to result in deficiency of C-14 reductase activity.

Milder skeletal manifestations associated with *LBR* pathogenic variants have also been described [8,9,14]. Table 3 presents the clinical

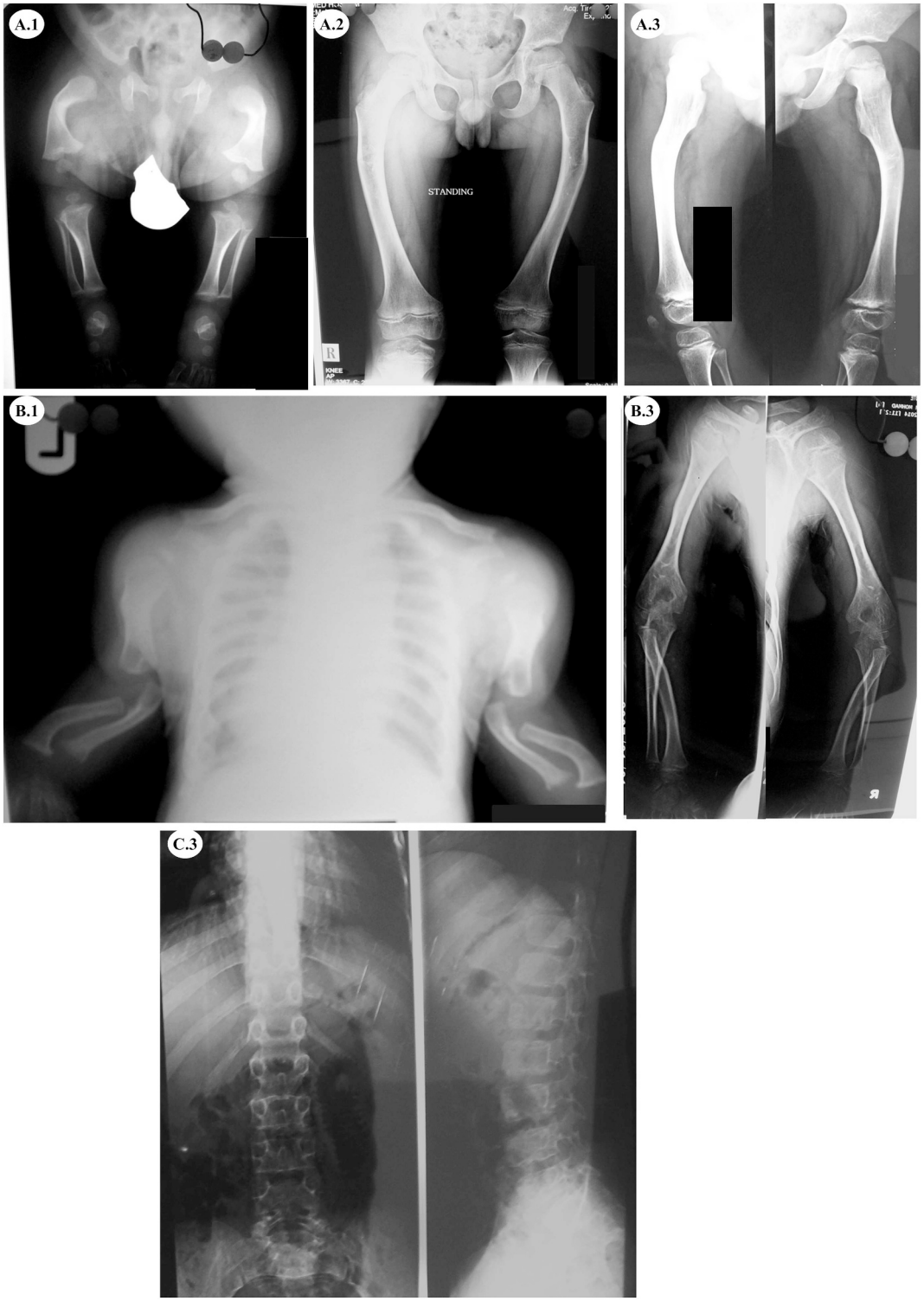

Fig. 4. Radiological findings in Patient 2 at birth (1), at age of 7 (2) and 10 years (3).
(A) Pelvis and lower limbs showing shortened, bowed femura and distal femoral and proximal tibial metaphyseal flaring. Notice the improvement in bowing and shortening of long bones with age.
(B) Chest and upper extremities at birth showing narrowing of the thoracic cavity with short, horizontal ribs and bilateral hypoplasia of lateral ends of clavicles. Notice also shortened and bowed humeri, marked bowing and shortening of radii and to a lesser extent ulnae, with metaphyseal flaring of distal ends, malalignment of elbows and wrist joints with dislocation of both proximal and distal radioulnar articulations; all improved with age.
(C) A–P and lateral spine views at 10 years of age showing mild platyspondyly and endplate irregularity of lumbar spines.

and molecular characteristics of patients with non-lethal LBR-related skeletal disorders. Borovik et al. [8] reported a 12-year-old girl who possesses two variants in *LBR*, p.Ile218Aspfs*19 and a p.Arg586His, and presented with short stature, mild kyphosis and hyperlordosis, brachydactyly, and dumb-bell shaped Pelger-Huët cells. The former variant results in a frame shift in exon 6, which may produce a truncated protein or illicit nonsense-mediated mRNA decay [15]. The p.Arg586His variant is a single amino acid substitution that lies in exon

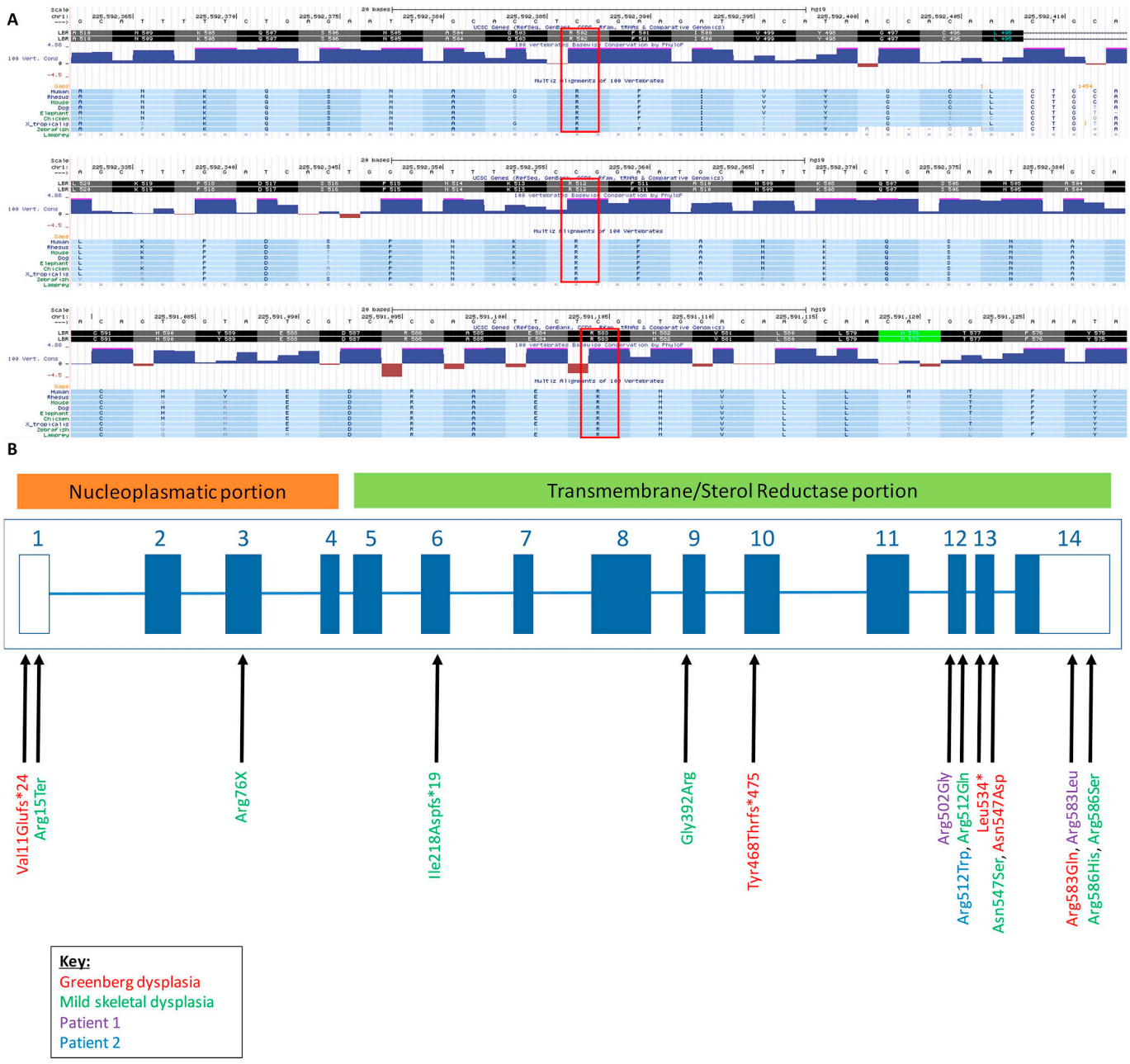

Fig. 5. Evolutionary conservation of *LBR* novel variants and their location in the gene's structure.
(A) Three tracks show multiple alignments of vertebrate species for *LBR* generated by using multiz and other tools in the UCSC (https://genome.ucsc.edu)/Penn State Bioinformatics comparative genomics alignment pipeline. The evolutionary conserved amino acid arginine (R) at positions 502, 512, and 583 is shown in red rectangle.
(B) Exons 1–14 of LBR, based on transcript LBR-201 (ENSG00000143815). The genetic variants described in this paper (Patient 1-purple; Patient 2 blue) and previously reported pathogenic variants associated with Greenberg dysplasia (red) and milder skeletal phenotypes (green) are shown in the diagram. The two structurally and functionally different portions of LBR are depicted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results of sterol analysis of cultured lymphoblasts from Patient 1 and normal control.

	Cholesterol (μg/mg prot) (SD)	cholesta-8,14-dien-3beta-ol total sterol percent (SD)
Patient 1	15.94 (2.67)	2.91 (0.87)
Normal control	20.24 (4.13)	0.03 (0.02)

14, in close proximity to the transmembrane component of the protein [8]. A second patient, reported by Sobreira et al. [9], was found to have spontaneously-regressing rhizomelic limb shortening and platyspondyly with bilobed Pelger-Huët cells on peripheral blood smear. Exome sequencing again revealed compound heterozygous pathogenic variants, p.Arg76* in exon 3 and p.Asn547Ser in exon 13 in *LBR* [9]. Carvalho et al. similarly described a young patient with regressing severity of rhizomelic limb shortening and PHA associated with compound heterozygous variants in *LBR*, p.Arg586Ser and p.Arg15* [14].

Table 3
Clinical, radiological, and genetic characteristics of patients with non-lethal LBR-related skeletal disorder.

	Patient 1	Patient 2	Borovik et al.	Sobreira et al.	Zhang et al.	Carvalho et al.
Variants	p.R502G/p.R583L	p.R512W/p.R512W	p.I218Dfs*19/p.R586H	p.R76*/p.N547S	p.G392R/p.R512Q	p.Arg15*/p.R586S
Location of variants (exons)	12/14	12	6/14	3/13	10/12	1/14
Sex	Female	Male	Female	Male	N/A	Female
Age at last assessment	34 months	10 years	12 years	14 years	2 years, 9 months	8 months
Growth (last exam)						
Height	< 1st centile	< 3rd centile	3-10th centile	3-10th centile	N/A	Normal
OFC	82nd centile	5th centile	2nd centile	2nd centile		
Skeletal findings	Yes	Yes	Yes	Yes	Yes	Yes
	Macrocephaly, frontal bossing, midface hypoplasia, rhizomelic limb shortening, narrow thorax, joint hypermobility	Rhizomelic limb shortening in upper limbs, broad joints, scoliosis, prominent chest	Kyphosis, hyperlordosis, brachydactyly of 4th and 5th metacarpals, rhizomelic limb shortening	Rhizomelic limb shortening, brachydactyly, hyperlordosis	Non-lethal "asphyxiating thoracic dystrophy"	Rhizomelic limb shortening
Radiographic abnormalities	Platyspondyly, short horizontal ribs and narrow chest, metaphyseal flaring, short and bowed long bones	Narrow chest, metaphyseal flaring, short and bowed long bones	Short femoral necks, bowed radii	Platyspondyly, ovoid vertebral bodies, bowed humerii, femora, radii	Moderately short horizontal ribs, short limbs, metaphyseal abnormalities	Mild generalized platyspondyly, possible metaphyseal involvement in shortening of long bones
Improvement of skeletal findings	Yes	Yes	Unknown	Yes	N/A	Yes
Pelger-Huët anomaly	Yes, bi-lobed	N/A	Yes, bi-lobed	Yes, bi-lobed	N/A	Yes
Cholesta-8,14-dien-3β-ol level	2.9%	N/A	"Trace"	"Detectable"	N/A	N/A

N/A, not available.

LBR pathogenic variants associated with milder skeletal phenotypes are missense, nonsense or frameshift distributed throughout the nucleoplasmatic and transmembrane portions of the protein (Table 3). We noticed that in all 6 patients with *LBR*-related mild skeletal phenotype, one of the two pathogenic variants involves exon 12, 13 or 14. However, larger cohorts of patients are needed to elucidate the genotype-phenotype correlation.

Exome analysis in Patients 1 and 2 identified biallelic deleterious variants in *LBR*. The p.Arg502Gly (http://gnomad.broadinstitute.org/variant/1-225592388-G-C), p.Arg512Trp (http://gnomad.broadinstitute.org/variant/1-225592358-G-A), and p.Arg583Leu (http://gnomad.broadinstitute.org/variant/1-225591105-C-A) have allele frequencies of 8.9e-06, 1.57e-05 and 1.79e-05, respectively, among Europeans in the gnomAD database (accessed 8/15/2018) but were never found in homozygous state [11]. Interestingly, the chemically related amino acid glutamine is reported at position p.Arg502 and p.Arg512 in 1.34e-04 and 4.74e-05 European alleles, respectively, and at position p.Arg583 in 8.16e-05 Asian alleles in the gnomAD database (accessed 8/15/2018). While p.Arg583Leu variant has not been previously described in association with skeletal dysplasia, a different amino acid substitution at this residue, c.1748G > A (p.Arg583Gln), has been reported in compound heterozygous state in an individual with Greenberg dysplasia (fetus B) [6]. Furthermore, a nearby missense variant, c.1757G > A (p.Arg586His), was reported as indicated above to be associated with the PHA and a mild form of LBR-related skeletal dysplasia [8], indicating the functional significance of this region of the protein. Also, while p.Arg512Trp in Patient 2 has not been previously reported as pathogenic variant, a different amino acid substitution at this residue, c.1535G > A (p.Arg512Gln), has been reported in compound heterozygous state in an individual with non-lethal asphyxiating thoracic dysplasia (ATD) (MIM# 208500) [12]. The newly discovered *LBR* variants associated with a skeletal phenotype described in this paper and previously reported variants are shown in Fig. 5B.

The hematological and biochemical phenotypes of Patient 1 provide insight on the mechanism of the disease and suggest a connection between the biochemical function of LBR and the resulting phenotype. The deleterious variants in Patients 1 and 2 are located in the sterol reductase domain of LBR. The effect on the sterol reductase function of LBR was expected in Patient 1 and is reflected in increased cholesta-8,14-dien-3β-ol. The two patients reported by Borovik et al. and Sobreira et al. were found to have "trace" or "detectable" levels of cholesta-8,14-dien-3β-ol in cultured lymphoblasts [8,9]. It is possible that there is a correlation between the severity of the skeletal phenotype and the level of cholesta-8,14-dien-3β-ol. While the sterol abnormalities were expected, the PHA in Patient 1 and her father as well as the patient reported by Borovik et al. was notable [8], as the variants in these patients are located only in the transmembrane portion of LBR and are therefore less likely to affect the nuclear structure. Patient 1's mother was heterozygous for the variant p.Arg583Leu and did not demonstrate PHA but the absence of neutrophil hyposegmentation alone is not sufficient evidence to suggest the uncoupling of the two functions of LBR. In fact, these findings demonstrate a connection between the structural and enzymatic activities of LBR, as pathogenic variants in the transmembrane portion of the protein are not expected to cause a change in the nuclear structure if the two functions are entirely distinct [6,8]. A possible explanation for PHA in these instances relies on the importance of LBR sterol reductase activity in cholesterol synthesis. As proposed by Nikolakaki et al., decreased sterol reductase activity caused by variants in the C-terminal domain may influence cholesterol synthesis in the formation of lipid rafts in the inner nuclear membrane, which mediate tethering of heterochromatin and nuclear structure [17].

Oosterwijk et al. [2003] reviewed the literature and found 11 reported patients with biallelic *LBR* variants who exhibited PHA with or without mild skeletal or other congenital anomalies [5]. They argued that the presence of two pathogenic variants can result in distinct mild

(PHA homozygosity) or severe (Greenberg skeletal dysplasia) phenotypes based on allelic heterogeneity [5]. It is possible that these 11 patients have a mild and regressing skeletal phenotype which, was not carefully documented in previous reports. Furthermore, previous reports have predicted biallelic pathogenic variants to result in ovoid nuclei, as expression of *LBR* is thought to influence neutrophil nuclear segmentation in a dose dependent manner [2]. Yet, Patient 1 and the patients described by Borovik et al. [8] and Sobriera et al. [9] exhibit bi-lobed neutrophil nuclei despite having compound heterozygous variants in *LBR*. This suggests that neutrophil nuclear structure is influenced by the location and effect of pathogenic variants in *LBR*, rather than by the homozygous or heterozygous expression alone.

Primary defects of cholesterol biosynthesis can present with skeletal abnormalities, of which rhizomesomelia is the most common and prominent [14]. *LBR* is expressed in chondrocytes, osteoblasts, and osteoclasts [6] and is therefore involved in cartilage and bone development. The mechanism(s) of skeletal dysplasia development is unclear but toxic effects of the accumulated cholesta-8,14-dien-3β-ol (or other upstream metabolites) as well as a deficiency of downstream intermediates in the cholesterol biosynthetic pathway could interfere with other metabolic or signaling pathways (e.g. hedgehog) that are more directly involved in bone development. For example, Zhang et al. suggested that impaired sterol reductase activity of LBR interferes with modification of signaling proteins essential to cilia function in skeletal development [12]. We cannot exclude the possible contribution of the disruption of nuclear envelope integrity [18] that could also affect cytoplasmic [6] and/or nuclear bone-related pathways. The improvement in the skeletal findings in both patients described here as well as in patients described by Sobreira et al. and Cavalcanti et al. is puzzling [9,14]. One explanation would be that the rapid cell growth characteristic of the embryonic period possibly demands faster rates of cholesterol synthesis than in the postnatal period and consequently toxic levels of sterol intermediates might occur in skeletal tissues only during the embryonic period [13]. In fact, when lymphoblast cells from patients with Smith-Lemli-Opitz syndrome, a genetic disease caused by an abnormality in cholesterol biosynthesis, were cultured in cholesterol free medium and induced to grow rapidly, the upstream metabolite, 7 dehydrocholesterol, showed striking accumulation [20]. The low availability of cholesterol in the embryonic period may also explain the fetal lethality of *LBR* variants implicated in Greenberg dysplasia, as well as spontaneous regression of limb shortening during postnatal growth in an environment with increased cholesterol availability [16]. Furthermore, the deleterious effect of impaired sterol modification of signaling molecules (e.g. sonic hedgehog) or cilia [12] during skeletal development could explain the improvement in skeletal findings as growth in the postnatal period does not rely on these same signaling molecules.

In summary, we present a detailed clinical and molecular characterization of two patients with *LBR*-related disorder. The patients in this report exhibit a skeletal dysplasia of moderate and improving severity, illustrating that *LBR*-related conditions encompass a spectrum of skeletal phenotypes. We also expand the genotypic spectrum by describing 3 novel disease-causing variants that have not been previously associated with a disease. We discussed the potential role of cholesterol metabolism in the skeletal phenotype and speculated a potential correlation between the biochemical and clinical phenotypes. The identification of additional affected individuals is necessary to broaden our understanding of the spectrum of LBR-associated disorders and the genotype phenotype correlation. Further study of LBR allelic heterogeneity in affected individuals is needed to clarify the correlation between the enzymatic and structural functions of the protein.

Web resources

- The URLs for online tools and data presented herein are:

  OMIM: http://www.omim/org/
  UCSC: http://genome.ucsc.edu/

- Mutation nomenclature:

  http://www.hgvs.org/mutnomen/recs.html

- The Genome Aggregation Database (gnomAD):

  http://gnomad.broadinstitute.org/

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Conflict of interest disclosure

GD is employed by GeneDx when exome sequencing was performed for patients 1. All other authors declare no conflict of interest.

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