# Novel in-frame *FLNB* deletion causes Larsen syndrome in a three-generation pedigree

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**Abstract** A 4-yr-old female with congenital knee dislocations and joint laxity was noted to have a strong maternal family history comprising multiple individuals with knee problems and clubfeet. As the knee issues were the predominant clinical features, clinical testing included sequencing of *LMX1B*, *TBX2*, and *TBX4*, which identified no significant variants. Research genome sequencing was performed in the proband, parents, and maternal grandfather. A heterozygous in-frame deletion in *FLNB* c. 5468_5470delAGG, which predicts p.(Glu1823del), segregated with the disease. The variant is rare in the gnomAD database, removes a residue that is evolutionarily conserved, and is predicted to alter protein length. Larsen syndrome may present with pathology that primarily involves one joint and thus may be difficult to differentiate clinically from other skeletal dysplasias or arthrogryposis syndromes. The p.(Glu1823del) variant maps to a filamin repeat domain where other disease-causing variants are clustered, consistent with a probable gain-of-function mechanism. It has reportedly been observed in two individuals in the gnomAD database, suggesting that mild presentations of Larsen syndrome, like the individual reported here, may be underdiagnosed in the general population.

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Ontology terms: aplasia/ hypoplasia of the patella; bilateral talipes equinovarus; congenital knee dislocation; joint laxity

[Supplemental material is available for this article.]

## CLINICAL PRESENTATION

The proband was a 4-yr-old European–American female born weighing 7 lbs 3 oz at term by elective cesarean delivery due to breech presentation. She was born with her knees locked in extension and her feet held up against her head. She was subsequently found to have bilateral congenital knee dislocations (Supplemental Fig. 1), which were treated surgically beginning at 6 mo of age with staged open reductions and femoral shortenings. The mother was known to have also had clubfeet and knee dislocations requiring several orthopedic surgeries. Other family members are also noted to be affected as shown in the pedigree (Table 1; Fig. 1). The diagnosis of nail-patella syndrome (NPS) (OMIM 161200) was suspected by her orthopedic physician and agreed on by the initial clinical geneticist who evaluated the proband. *LMX1B* sequencing and deletion/duplication analysis were negative.

The proband was reevaluated by a second geneticist at 2.5 yr of age, and the clinical diagnosis of NPS was questioned because of emerging new clinical and developmental

**Table 1.** Clinical features

| HPO term | Proband | Mother | Maternal uncle | Grandfather |
|---|---|---|---|---|
| Knee dislocation | + | + | + | + |
| Talipes equinovarus | − | + | + | unk |
| Scoliosis | − | + | unk | unk |
| Bowing of the legs | − | − | + | − |
| Cleft palate | − | − | − | − |

Human Phenotype Ontology (HPO) terms are listed with an indication of whether each patient was positive (+), negative (−), or unknown (unk) for each feature.

information. A lateral knee X ray obtained at 20 mo demonstrated soft tissue in the region of the patella, suggesting the presence of a cartilaginous patella. A skeletal survey showed the normal appearance of the elbows, no vertebral segmentation defects or fusions, no iliac horns, and metatarsus adductus. The appearance of the humeri and metacarpals was normal (Supplemental Fig. 2). Bilateral knee ultrasound at 3 yr of age showed normal morphology of bilateral patellae with central ossification centers. Sequencing and deletion/duplication analysis of *TBX2* and *TBX4* were both performed clinically with no clinically significant findings. The family was subsequently enrolled for research genome sequencing.

At 4 yr of age, the proband had normal stature (40th centile) and apparently normal speech and language development. She was noted to have a depressed nasal bridge and root, midface retrusion, mild telecanthus, and a pointed chin. The mother was noted to have similar facial features. The proband did not have cleft palate/bifid uvula, tracheomalacia, spatulate thumbs, abnormally long fingers, or cervical spine dislocation (although her occipital condyles were noted to be relatively large).

## TECHNICAL ANALYSIS AND METHODS

Genome sequencing was performed on genomic DNA from the proband, mother, father, and maternal grandfather using an Illumina NovaSeq6000 instrument according to the manufacturer's protocols. Reads were mapped to the GRCh37 reference sequence and secondary data analysis was performed using Churchill (Kelly et al. 2015). The average sequence depth achieved was ~30.6× for the blood samples (proband, mother, and father) and 23.6× for the saliva sample (grandfather); sequencing metrics are provided in Supplemental Table 1. Our general approach to variant annotation and prioritization has been described (Koboldt et al. 2018); for this evaluation, we prioritized rare nonsynonymous coding variants segregating with disease under a dominant inheritance model because of the family history (Supplemental Table 2).

## VARIANT INTERPRETATION

We identified a heterozygous 3-bp deletion in *FLNB* (Table 2) in the proband and two affected family members (mother and maternal grandfather) that was absent from the unaffected father. It is predicted to cause an in-frame deletion of a single amino acid (glutamic acid) at position 1823 in the protein. Please see Supplemental Material for an explanation of variant classification nomenclature (i.e., PM2, PP1). The variant is present in dbSNP (rs1470699812) and reportedly was observed in two non-Finnish European individuals in the gnomAD database; however, supporting read data are available for only one of these carriers. We
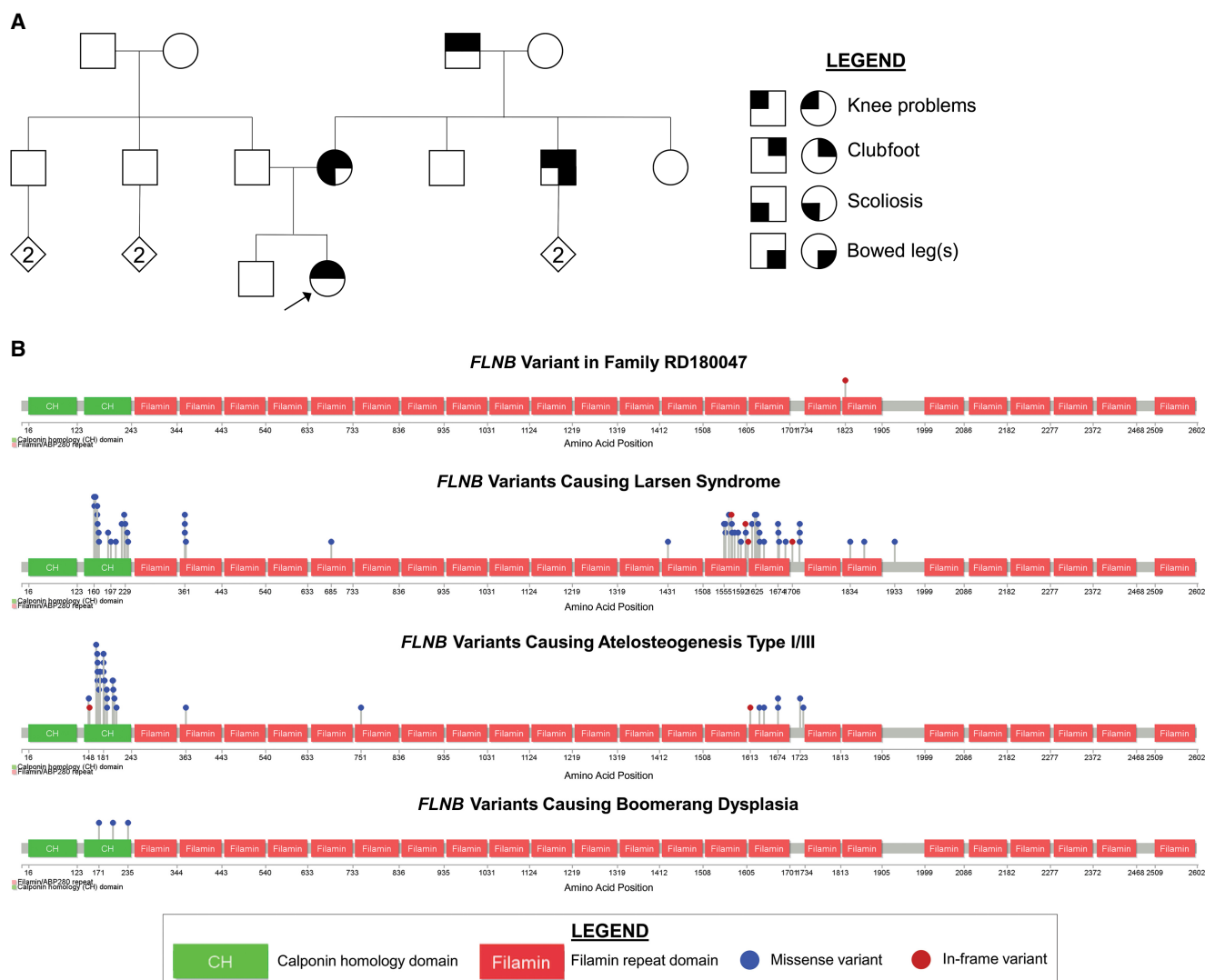
**Figure 1.** Pedigree and *FLNB* variant. (*A*) Pedigree for RD180047 depicting the presence of cardinal features, namely knee problems, clubfoot, scoliosis, and bowed lower leg(s). (*B*) Graphical representation of *FLNB* variants and domain structures. The variant in family RD180057 is shown (*top* plot) in comparison to reported disease-causing variants for Larsen syndrome (*second from top* plot), atelosteogenesis type I/III (*third from top* plot), and boomerang dysplasia (*bottom* plot). Variants are plotted by amino acid position based on the canonical transcript NM_001164317.1. Missense variants are shown in blue; in-frame deletions are shown in red. Disease-causing variants were obtained from ClinVar (20190424), HGMD (20170720), and literature reports. Only variants reported for autosomal dominant OMIM conditions are shown. Plots were generated using Lollipops v1.3.2 using UniProt ID #O75369. Domains were retrieved using the Pfam API.

therefore estimate its allele frequency at 1–2 per 125,000 individuals. It has not been reported in other public cohorts nor has it been submitted to ClinVar. Although the phenotypic status of gnomAD individuals is not available, if they are presumed to be healthy, then a conservative application of ACMG guidelines would not apply PM2 (absent from controls).

Although the variant occurs in a filamin repeat domain, the local sequence context is not repetitive, suggesting that this variant alters protein length and may have a deleterious impact (PM4). It segregates with disease in this family (PP1) and removes a residue that is

**Table 2.** Genomic findings and variant interpretation

| Gene | Genomic location | HGVS cDNA | HGVS protein | Zygosity/parent of origin | Interpretation |
|------|-----------------|-----------|--------------|--------------------------|----------------|
| FLNB | Chr 3:58129288 GAG/- (hg19) | NM_001164317.1:c. 5468_5470delAGG | p.(Glu1823del) | Het/Mat | VUS (PM4, PP1) |

Genomic coordinates reflect build GRCh37 (hg19).

evolutionary conserved across vertebrate species. It also occurs in the 17th filamin repeat of *FLNB* near other pathogenic variants that have been reported in individuals with autosomal dominant Larsen syndrome (OMIM 150250) (Fig. 1B). Under ACMG guidelines, this variant is assessed as a variant of uncertain significance (VUS; Table 2), but we believe it is likely the cause of the patient's disease.

## SUMMARY

Larsen syndrome (OMIM 150250) was first described in 1950 in a clinical series of six patients with multiple congenital joint dislocations in association with particular dysmorphic facial features, equinovarus foot deformities, and occasionally cleft palate or other skeletal anomalies (Larsen et al. 1950). Heterozygous missense variants in *FLNB* were originally described in four simplex affecteds and one family with autosomal dominant inheritance of Larsen syndrome (Krakow et al. 2004). Subsequent authors have described Larsen syndrome as a clinically and radiographically characteristic disorder (Girisha et al. 2016). The distribution of disease-causing variants is nonrandom; most variants reported for Larsen syndrome cluster in the actin-binding domain (ABD) and filamin repeats 13–17 (Bicknell et al. 2007). Filamin proteins are large cytoplasmic actin-binding proteins that regulate the cytoskeletal network (Stossel et al. 2001). Filamins contain an amino-terminal ABD, up to 24 Ig-like folds interrupted by two "hinge" regions, and a filamin repeat at the carboxyl terminus (Girisha et al. 2016). The proteins are highly similar and can interact to form homo- and heterodimers. Mouse *FLNB* knockout models recapitulate the human knockout phenotype (distinct from Larsen syndrome as described below) with skeletal malformations including vertebral anomalies, shortened distal limbs, and spinal curvature (Lu et al. 2007; Zhou et al. 2007).

FLNB-related disorders associated with heterozygous variants and autosomal dominant inheritance represent a continuous spectrum of severity with atelosteogenesis type 1 (OMIM 108720) and boomerang dysplasia (OMIM 112310) representing the severe end and Larsen syndrome the mild end (Robertson 2017). Homozygous or compound heterozygous loss-of-function variants are associated with spondylocarpotarsal synostosis (STS) (OMIM 272460), a skeletal dysplasia characterized by disproportionate short stature and carpal and tarsal synostosis (Fig. 1B, bottom). There is limited understanding of the pathophysiology of abnormal *FLNB* function in Larsen syndrome, and the genotype–phenotype relationship remains unclear. Variants reported to cause Larsen syndrome are predominantly missense variants or, less commonly as in the individual described here, in-frame deletions (Fig. 1B). They appear to cluster in annotated domains near the amino-terminal or in the middle of the protein, consistent with a gain-of-function mechanism (Sawyer et al. 2009). Unlike the loss-of-function variants reported in STS, pathogenic variants associated with Larsen syndrome do not reduce the actin-binding affinity of *FLNB*, suggesting that they may instead exert a more general effect on targeting of filamin B to the appropriate actin structures (Sawyer et al. 2009). Enhanced actin binding and bundling as well as dysregulation the function of hinge 1 are two proposed mechanisms (Daniel et al. 2012).

In summary, a heterozygous 3-bp deletion in *FLNB* was found in a child and her mother with clinical features consistent with Larsen syndrome. The variant also segregated in a maternal grandfather who was described by history to have features consistent with Larsen syndrome. Although classified as a VUS, we argue that the variant is causative of the phenotype in this family based on the fit of the clinical phenotype and the rationale provided in the Variant Interpretation section. As the clinical phenotype may be mild and this variant was reportedly observed in two individuals in the gnomAD database, there may be individuals in the general population with Larsen syndrome related to this variant who go clinically undiagnosed.

## ADDITIONAL INFORMATION

### Data Deposition and Access

The variants and their interpretations have been deposited in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV000599274.

### Ethics Statement

Written consent was obtained enrolling subjects into a research protocol approved by the Institutional Review Board at Nationwide Children's Hospital (IRB11-00215 Study: Using Genome Sequencing to Identify Causes of Rare Birth Defects and Rare Disorders).

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### Author Contributions

All authors contributed to scientific discussion, variant interpretation, and manuscript review.

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