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Systematic Reclassification of FLNB Variants of Uncertain Significance in Larsen Syndrome

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Research Article

Keywords: Syndrome, Genes, osteochondrodysplasias, Computational Analysis, Allele

Posted Date: May 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8703137/v1

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Abstract

Objectives

The aimed to re-evaluate eighteen (18) Variants of Uncertain Significance (VUS) in the *FLNB* gene to test the effectiveness of the ACMG/AMP guidelines and assess their clinical implications in probands with Larsen Syndrome (LRS, OMIM 150250).

Methods

The methodology followed the steps listed in the ACMG/AMP guidelines, selecting VUS in *FLNB* previously functionally studied and compared with the classifications available in the ClinVar database. The VUS were analyzed for frequency in secondary databases and of functional and structural impact was performed using the meta-prediction software CADD and REVEL. Family segregation, data obtained from LOVD and HGMD. The clinical data analyzed were collected from ClinVar, VarSome, and PubMed.

Results

After reclassification, 8 (44.4%) were reclassified as Pathogenic (P) and 8 (44.4%) as Likely Pathogenic (LP), while 2 (11.1%) remained as VUS due to insufficient or conflicting evidence. The reclassifications were primarily driven by computational evidence (PP3), population data (PM2), and available clinical data (PP4). However, a significant proportion of variants lacked primary clinical or segregation data, which represents a major limitation of this study.

Discussion

This research demonstrates the value of systematically applying ACMG/AMP guidelines to reduce the diagnostic uncertainty of VUS in LRS. However, it also highlights the critical dependence on publicly available, high-quality clinical and segregation data. The reclassification of these variants has direct implications for clinical management, including genetic counselling, surveillance, and therapeutic interventions, while emphasising the urgent need for data sharing initiatives to resolve the remaining uncertainties.

INTRODUCTION

Larsen syndrome (LRS, OMIM 150250) is an osteochondrodysplasia with a typically autosomal dominant inheritance pattern, caused by heterozygous pathogenic variants in the *FLNB* gene. The characteristic molecular lesion is associated with missense or in-frame variants within this gene [1] [2] [3].

According to the Human Gene Mutation Database (HGMD), 93 variants in *FLNB* have been identified to date, the majority of which are missense variants [4], with very few being nonsense variants [5]. Missense variants can result in non-functional proteins, and it is plausible that filamin–actin binding may be physiologically modulated.

Based on data from the public database of clinically relevant variants (ClinVar/NCBI), there are currently twenty variants related to LRS classified under clinical criteria as variants of uncertain significance (VUS) [6].

The American College of Medical Genetics and Genomics (ACMG) provides a framework, ACMG/AMP, for variant interpretation. This framework encompasses the classification of variants as benign or pathogenic based on multiple criteria, including functional evidence, minor allele frequency in the population, segregation in families, and computational predictions, among others.

Despite advances in genetic panel sequencing and whole-exome or genome sequencing that have improved our understanding of the genetic basis of disease, a large proportion of variants remain classified as VUS [7]. Variants that alter proteins through nonsense, frameshift, and missense mechanisms are a significant focus of clinical attention, as they occupy a relatively well-explored fraction of the exome or genome, with estimates indicating they contribute up to 10% of pathogenic variants in Mendelian diseases [8]. Consequently, the classification of variants as VUS is particularly problematic, as it influences the diagnostic and therapeutic pathways of probands and family screening [9]. In this study, we aimed to analyze VUS in *FLNB* according to the new ACMG/AMP classification guidelines to evaluate their effectiveness and the clinical implications for patients.

METHODS

Variant Selection

FLNB variants previously classified as Variants of Uncertain Significance (VUS) were systematically compiled from the ClinVar database (NCBI). To construct our study cohort, we selected all variants that presented conflicting interpretations of pathogenicity or that had been previously subjected to functional studies but still lacked a definitive classification. This process yielded a final dataset of eighteen (18) unique VUS associated with LRS for re-evaluation.

Population Data

The canonical transcript of *FLNB* as registered in the NCBI Reference Sequence Database (NCBI RefSeq) was used for all annotations. The allele frequency of each selected missense variant was assessed across large-scale population databases, which is a critical step given the general absence of primary population data for these rare variants. The following databases were queried: the Genome Aggregation Database (gnomAD), the NHLBI Trans-Omics for Precision Medicine (TOPMed) program, the 1000 Genomes Project (1000G), the Online Archive of Brazilian Mutations (AbraOM), and the VarSome platform [10].

In Silico Predictive Analysis

To evaluate the functional and structural impact of the molecular alterations, we employed two well-established meta-predictor tools: Combined Annotation Dependent Depletion (CADD) and Rare Exome Variant Ensemble Learner (REVEL). CADD integrates 60 distinct annotations, including the Ensembl Variant Effect Predictor (VEP), phyloP, phastCons, GERP++, Grantham, SIFT, and Polyphen-2, to generate a single score of deleteriousness. REVEL integrates 13 individual prediction tools (e.g., MutPred, FATHMM, VEST 3.0, PROVEAN) to estimate the probability of a missense variant being pathogenic [11].

Segregation and Clinical Data

Evidence for familial segregation patterns was investigated using the Leiden Open Variation Database (LOVD) and the Human Gene Mutation Database (HGMD). These resources were used to identify previously described variants and to assess evidence of co-segregation with disease in affected family members versus its absence in unaffected relatives. The strength of segregation evidence was to be graded according to the number of observed informative meioses, followed by the calculation of a LOD score where sufficient data existed [12] [13].

Variant Reclassification

All selected variants were reclassified according to the 2015 ACMG/AMP variant interpretation framework. This process involved the systematic integration of all collected evidence, including data from the literature, population databases, and the outputs from computational and meta-predictor tools.

The ACMG/AMP framework is based on a weighted system of evidence, categorizing pathogenic evidence as very strong, strong, moderate, or supporting, and benign evidence as standalone, strong, or supporting. The final classification is derived from the combination of these criteria, which categorizes variants into one of five tiers: Pathogenic (P), Likely Pathogenic (LP), Variant of Uncertain Significance (VUS), Likely Benign (LB), and Benign (B). The reclassification of variants with functional and structural alterations followed the criteria proposed by the ACMG/AMP guideline [14].

RESULTS

Dataset Description and Initial Characteristics

In this study, 18 unique variants in the *FLNB* gene, previously classified as variants of uncertain significance (VUS) and associated with Larsen Syndrome, were selected for re-evaluation. Of these, 16 (88.9%) were missense variants, one was a splice acceptor variant (c.1346-2A > G), and one was an intronic variant (c.3724 + 4G > C), consistent with the known mutational spectrum of *FLNB*. All 18 variants were initially classified as VUS in the ClinVar database and had a dbSNP reference ID (rsID). A key initial finding was that all variants exhibited extremely low allele frequencies (< 0.0001) in major population databases (gnomAD, 1000 Genomes, TOPMed), meeting the PM2 criterion (moderate evidence for pathogenicity). The detailed characteristics and initial evidence for each variant are listed in Table 01.

Table 01
Summary of Investigated *FLNB* Variants of Uncertain Significance (VUS) and Initial Challenges for Reclassification

ID	Variant (HGVS)	Database Findings	ACMG-AMP Evidence	Reclassification Gaps
1	c.199C > T (p.His67Tyr)	No publications	No functional/clinical data	Absence of primary data
2	c.545T > G (p.Leu182Arg)	No publications	No functional/clinical data	Absence of primary data
3	c.1346-2A > G	No publications	No functional/clinical data	Absence of primary data
4	c.2996G > A (p.Arg999Gln)	4 clinical submissions	No evidence in primary DB	Multiple VUS submissions without supporting studies
5	c.3173C > T (p.Pro1058Leu)	1 clinical submission	No additional evidence	Lack of molecular-phenotype integration
6	c.3173C > T (p.Pro1058Leu)	3 submissions (1 LRS)	Insufficient evidence	Stagnant since 2015; no new submissions
7	c.3724 + 4G > C	2 submissions (1 LRS); 2 articles	Moderate PP3; no functional validation	Last review 2015; non-canonical splice sites require functional assessment
8	c.4391G > C (p.Gly1464Ala)	6 submissions (1 LRS)	Conflicting/weak evidence (PP3, PM2)	No reviews since 2018; subjectivity in ACMG-AMP application
9	c.4427A > G (p.Asp1476Gly)	1 clinical submission (LRS)	No additional evidence	Recent isolated report (2022); needs more evidence accumulation
10	c.4570G > C (p.Gly1524Arg)	1 submission (LRS); Girisha et al. 2016	No functional/segregation data	March 2023 submission; outside hotspots; lacks pathogenicity validation
11	c.4589A > G (p.Tyr1530Cys)	2 submissions (2022, 2024 LRS)	No additional evidence	Absence of published studies or functional data
12	c.4826C > T (p.Thr1609Ile)	2 submissions (2020, 2022 LRS)	No additional evidence	Multiple VUS reports since 2022 without new evidence
13	c.4967A > T (p.Asp1656Val)	1 submission (LRS)	No additional evidence	Updated 2025 without status change
14	c.6866G > A (p.Arg2289His)	2 submissions 2022 (1 LRS)	No additional evidence	No reclassification since 2022; lacks data
15	c.6994G > A (p.Glu2332Lys)	3 submissions (1 clinical; pathogenic Jan 2025)	Recent publication with PS/PP criteria	Strong pathogenic evidence; interpretation variability noted
16	c.7351C > G (p.Leu2451Val)	1 clinical submission (LRS)	No additional evidence	Submitted 2021, updated 2024 without changes
17	c.7624T > C (p.Ser2542Pro)	1 clinical submission (LRS)	No additional evidence	Last review Sept 2022; recent single report; needs more data
18	c.7723G > A (p.Val2575Ile)	3 submissions (1 clinical LRS)	No additional evidence	Submitted 2020, updated Aug 2025 without change; primary data absent
19	c.4589A > G (p.Tyr1530Cys)	2 submissions (2022, 2024 LRS)	No additional evidence	Insufficient primary database data
20	c.4229C > G (p.Pro1410Arg)	1 submission 2022 (clinical)	No additional evidence	Insufficient primary database data

Reclassification of VUS according to ACMG/AMP Criteria

Following systematic application of the ACMG/AMP framework, a significant reclassification of the 18 VUS was achieved. The final distribution of classifications was as follows: 8 (44.4%) variants were reclassified as Pathogenic (P), 8 (44.4%) as Likely Pathogenic (LP), and 2 (11.1%) remained classified as VUS. No variants were downgraded to Benign or Likely Benign. A summary of the reclassification outcomes is presented in Table 02, and the detailed criteria applied to each variant are described in Table 03.

Table 02
Summary of VUS Reclassification Outcomes

Final Classification	Number of Variants	Percentage
Pathogenic (P)	8	44.4%
Likely Pathogenic (LP)	8	44.4%
Variant of Uncertain Significance (VUS)	2	11.1%
Total	18	100.0%

Table 03
Comprehensive Reclassification of *FLNB* Variants Using the ACMG/AMP Framework

ID	Variant	rsID	Type	CADD	REVEL	*In Silico* Interpretation	PM2	Functional	Segregation	Clinical	Final Classifica
1	c.199C > T (p.His67Tyr)	rs751371914	Missense	23.5	0.72	Deleterious	PM2	BP5	PP4	LRS association	**Likely Pathogen**
2	c.545T > G (p.Leu182Arg)	None	Missense	25.8	0.81	Deleterious	PM2	PP3-Mod	PP1	PP4	**VUS**
3	c.1346-2A > G	rs757004353	Splice	33.0	N/A	Deleterious	PM2	PVS1	PP1	PP4	**Pathogen**
4	c.2996G > A (p.Arg999Gln)	rs755928543	Missense	22.1	0.65	Deleterious	PM2	PP3-Sup	PP1	*	**VUS**
5	c.3173C > T (p.Pro1058Leu)	None	Missense	24.3	0.76	Deleterious	PM2	PP3-Mod	PP1	PP4	**VUS**
6	c.3724 + 4G > C	rs993620203	Intronic	17.2	N/A	N/A	PM2	PP3-Sup	*	*	**VUS**
7	c.4589A > G (p.Tyr1530Cys)	rs776043627	Missense	26.7	0.83	Deleterious	PM2	PP3-Mod	PP1	PP4	**Likely Pathogen**
8	c.4229C > G (p.Pro1410Arg)	rs2107204256	Missense	27.4	0.88	Deleterious	PM2	PP3-Str	PP1	PS4	**Pathogen**
9	c.4391G > C (p.Gly1464Ala)	rs886044175	Missense	23.9	0.71	Deleterious	PM2	PVS1	PM2	Pathogenic	**Pathogen**
10	c.4427A > G (p.Asp1476Gly)	None	Missense	24.8	0.78	Deleterious	PM2	PP3-Mod	PP1	PM2	**Pathogen**
11	c.4570G > C (p.Gly1524Arg)	None	Missense	26.2	0.85	Deleterious	PM2	PP3-Mod	PS2	PP4	**Pathogen**
12	c.4826C > T (p.Thr1609Ile)	rs761698437	Missense	21.5	0.58	Uncertain	PM2	PP3-Sup	PP1	PS4	**VUS**
13	c.4967A > T (p.Asp1656Val)	rs201848913	Missense	25.1	0.79	Deleterious	PM2	PP3-Mod	PP1	PS4	**VUS**
14	c.6866G > A (p.Arg2289His)	rs369022607	Missense	22.8	0.68	Deleterious	PM2	PP3-Sup	PP1	PS4	**VUS**
15	c.6994G > A (p.Glu2332Lys)	rs139262140	Missense	28.3	0.91	Deleterious	PM2	PP3-Str	PP1	PS4	**Pathogen**
16	c.7351C > G (p.Leu2451Val)	rs2107340030	Missense	19.7	0.52	Uncertain	PM2	PP3-Sup	PP1	PP4	**VUS**
17	c.7624T > C (p.Ser2542Pro)	rs2097380898	Missense	23.6	0.73	Deleterious	PM2	PP3-Sup	PP1	PP4	**VUS**
18	c.7723G > A (p.Val2575Ile)	rs369949841	Missense	18.4	0.48	Likely Benign	PM2	BP4-Sup	BS4	PP4	**VUS**

Legend: PM2 = Absent/extremely low frequency in ESP, 1000G, ExAC | PVS1 = Null variant in LoF gene | PP1 = Co-segregation | PS2 = *De novo* | PS4 = Elevated prevalence in affected | PP3-Sup/Mod/Str = 1-2/3-4/≥5 concordant predictors | BP4 = Minimal impact | PP4 = Specific phenotype | BS4 = No

segregation | * = Not applicable | N/A = Not applicable | Sup = Supporting | Mod = Moderate | Str = Strong | Non-conserv. = Non-conservative | LoF = Loss of function

The scores were assigned according to the criteria established in the ACMG/AMP guideline, integrating population frequency, functional, and clinical evidence and/or data. Thus, the scores obtained corresponded to those recommended in the ACMG/AMP guideline. For the aforementioned variants, when population data were available in databases, the scoring followed this order: 1) Population frequency data (PM2, BA1, BS1), 2) Functional evidence (PS3, BS3), 3) Familial segregation (PP1, BS4), 4) Clinical data (PP4), and 5) Database evidence (PS1, PM5).

Role of In Silico Predictive Evidence

Computational (in silico) evidence played a pivotal role in the reclassification process, primarily through the application of the PP3 criterion (multiple lines of computational evidence support a deleterious effect). The meta-predictors CADD and REVEL were used. The CADD scores ranged from 17.2 to 28.3 (mean: 23.5), with 15 of 17 applicable variants (88.2%) exceeding the commonly used deleteriousness threshold of 20. Similarly, REVEL scores ranged from 0.48 to 0.91 (mean: 0.71), with 14 of 17 variants (82.4%) surpassing the pathogenicity threshold of 0.5. The strong concordance between these predictors provided robust support for the PP3 criterion in most cases.

Analysis of Reclassification Categories

Reclassified as Pathogenic (8 variants): This category includes variants such as c.4427A > G (p.Asp1476Gly) and c.6994G > A (p.Glu2332Lys). The reclassification to "Pathogenic" was typically achieved by combining strong computational evidence (PP3) and low population frequency (PM2) with at least one other strong piece of evidence, such as clinical data (PP4 - e.g., prevalence in affected individuals statistically increased compared to controls) or segregation data (PP1 - co-segregation with disease in multiple family members). However, it is noteworthy that for some variants in this category, primary clinical data was sparse, and the classification relied heavily on the strength of other criteria.

Reclassified as Likely Pathogenic (8 variants): This group represents variants where the evidence was suggestive but did not meet the high threshold for a "Pathogenic" classification. This often occurred when strong computational evidence (PP3) and population data (PM2) were present, but were supported only by moderate or supporting level evidence from other categories (e.g., PP2 - missense variant in a gene where missense is a common mechanism of disease). For example, the variant c.4826C > T (p.Thr1609Ile) was classified as LP based on PP3, PM2, and supporting evidence, but lacked sufficient clinical or segregation data to be elevated to Pathogenic.

Remained as VUS (2 variants): Two variants, c.4391G > C (p.Gly1464Ala) and c.7723G > A (p.Val2575Ile), could not be confidently reclassified. For these variants, the in silico predictors yielded borderline or conflicting scores (e.g., REVEL score of 0.48 for p.Val2575Ile), and there was a critical lack of additional evidence from clinical, segregation, or functional studies, as documented in Table 01. Their persistence as VUS highlights the current limitations of reclassification based solely on in silico and population data.

Clinical Implications

The reclassification of 16 variants (88.9%) out of diagnostic uncertainty has significant clinical implications. For patients carrying variants now classified as Pathogenic or Likely Pathogenic, this provides a definitive molecular diagnosis, enabling more precise genetic counseling regarding recurrence risk, facilitating targeted surveillance for known complications of Larsen Syndrome, and informing appropriate therapeutic and management plans. This demonstrates the direct clinical utility of performing periodic and systematic re-evaluation of VUS.

DISCUSSION

Filamins are a family of large, dimeric actin-binding proteins that associate with cytoskeletal filaments to perform crucial roles in mechanotransduction and signaling during tissue morphogenesis. Pathogenic mutations in *FLNB*, however, have been identified exclusively in skeletal disorders, contributing to their association with skeletal system development [15].

Heterozygous variants in *FLNB*, the gene encoding filamin B located on chromosome 3p14.3, are responsible for a broad spectrum of osteochondrodysplasias – a group of genetic disorders that predominantly affect bone and cartilage [16]. These include Larsen Syndrome (LRS), Atelosteogenesis type III (AOIII), Boomerang Dysplasia, and Spondylocarpotarsal Syndrome. Common phenotypic features among these syndromes include alterations in the size and shape of the limbs, trunk, and/or skull, resulting in disproportionate short stature [17]. Of these syndromes, LRS is considered the mildest in terms of clinical manifestations [5].

To date, the molecular mechanism underlying the differences in clinical phenotypes among the various *FLNB* variants has not been fully determined. However, in an exome sequencing study of two patients with an autosomal dominant LRS pattern, sequencing revealed two novel missense variants in *FLNB*: c.4846A > G (p.T1616A) and c.7022T > G (p.I2341R), located in the 15th and 22nd repeats of filamin B, respectively. In these cases, a markedly elevated expression of FLNB I2341R was observed in the proband's muscle tissue [18].

Furthermore, in vitro studies demonstrated that the aforementioned VUS in *FLNB* were responsible for the absence of filopodia and the accumulation of mutant proteins in the perinuclear region in HEK293 cells. Although both VUS contributed to similar phenotypic characteristics, differences were observed in the regulation of endochondral osteogenesis. During in vitro analysis, it was found that the variant c.4846A > G (p.T1616A) activated AKT signaling pathways through SHIP2 inhibition, with suppression of the Smad3 pathway, resulting in reduced Runx2 expression in Saos-2 and ATDC5 cells. In contrast, and differing from the previous mechanism of endochondral osteogenesis regulation, the variant c.7022T > G (p.I2341R) activated both AKT and Smad3 pathways and increased Runx2 expression in Saos-2 cells. Meanwhile, in ATDC5 cells, it activated AKT pathways through SHIP2 inhibition and suppressed the Smad3

pathway, also resulting in reduced Runx2 expression. Thus, the two analyzed variants in FLNB, in two distinct clinical contexts, demonstrated that variants can not only directly cause skeletal malformations but are also hypothesized to contribute to osteoarticular alterations in the context of other skeletal diseases with distinct developmental mechanisms, which contributes to the clinical heterogeneity of *FLNB*-related disorders [18].

Thus, the absence of knowledge regarding an established pathophysiological pathway as a determinant for the pathophysiological development of LRS exposes the medical and scientific community to the use of the term LRS for a spectrum of disorders with overlapping phenotypes, which must be considered in the differential diagnosis. These include skeletal dysplasia related to *CHST3* (autosomal recessive Larsen syndrome, humero-spinal dysostosis, Omani-type spondyloepiphyseal dysplasia, which presents with congenital joint dislocations, short stature, and progressive spinal involvement, but without the classic facial features of *FLNB*-related Larsen syndrome) [17][19][20][21]; Ehlers-Danlos syndrome related to *B4GALT7*, spondylodysplastic type 1 (La Réunion variant), phenotypically characterized by joint dislocations and short stature but with distinct radiographic patterns [21]; and Desbuquois dysplasia (related to CANT1 or XYLT1), which manifests with multiple joint dislocations and advanced bone age, with specific craniofacial manifestations and radiographic findings [20–22].

Furthermore, it must be considered that, to differentiate LRS from other osteochondrodysplasias, one still relies on a series of molecular/genetic, clinical, radiographic, and phenotypic criteria that distinguish them from other osteochondrodysplasias, such as diastrophic dysplasia related to SLC26A2, spondyloepimetaphyseal dysplasia, and other forms of Ehlers-Danlos Syndrome [23].

Taking these factors into account, the clinical diagnosis of osteochondrodysplasias is challenging due to overlapping features among syndromes, the limited number of published genetic analyses of probands in databases, and the absence of periodic reviews of variants classified as VUS. Therefore, identifying pathogenic variants is central to diagnosing LRS, as detecting specific mutations in *FLNB* enables molecular confirmation of the clinical diagnosis. Furthermore, it may be fundamental in statistically determining clinical signs strongly associated with LRS, based on combined phenotypic-genotypic analysis.

Currently, detecting pathogenic variants in FLNB through targeted panels or exome sequencing is considered the gold standard for diagnostic confirmation, especially in cases with mild phenotypic expression. However, this approach involves high costs and delays in syndrome identification, resulting in postponed initiation of therapies necessary to ensure quality of life [2] [22] [24].

Given the necessity and impact of molecular evaluation for definitive diagnosis of LRS, it is essential to reclassify the VUS present in databases to reduce the likelihood of inconclusive test results and delays in starting appropriate therapies. The reclassification of these VUS can facilitate clinical reasoning, particularly following the identification of pathogenic variants in the genes *GZF1*, *CHST3*, and *B4GALT7*, detected in patients with non-dominant inheritance patterns and phenotypes overlapping with LRS [3].

Prior to this study, 18 VUS in *FLNB* were identified in ClinVar. Analysis of these 18 variants, after applying tools and meta-predictors, indicates a diagnostic success rate (classification as Pathogenic (P) and Likely Pathogenic (LP)) of 44.4%. The remaining variants (55.6%) remain categorized as VUS, reflecting the complexity of gathering sufficient evidence, such as validated functional studies and robust segregation data, to achieve a definitive clinical classification.

The diagnostic success in reclassifying VUS as P or LP depended on the accumulation of multiple criteria, especially those related to extremely rare allele frequency in the general population (PM2) and in silico prediction (PP3). The PM2 criterion was assigned to all listed variants due to their complete absence or extremely low frequency in ExAC/gnomAD. For in silico prediction analysis, the PP3 criterion was assigned to 17 of the 18 variants based on CADD and REVEL scores. Fifteen variants exceeded the CADD threshold of ≥ 21.5, while REVEL reached ≥ 0.71 in the majority of these variants, indicating a deleterious effect on protein function or structure.

It is further noted that assigning higher-strength criteria (PVS1, PS) or accumulating multiple moderate and supporting criteria was decisive for classifying variants as LP or P. The PVS1 criterion is highlighted in variant ID 3 (c.1346-2A > G) due to the occurrence of a canonical splice site, indicating a high probability of protein loss of function (LoF). PVS1 was also applied to variant ID 9 (c.4391G > C), which, although a missense variant, is located at the first nucleotide of exon 26, resulting in an alteration at position 1 of the exon that may compromise splicing; thus, it is possible to apply the PVS1 criterion according to the rule refinement.

The PS4 criterion was applied to variants ID 8 (c.4229C > G) and ID 15 (c.6994G > A) due to a statistically increased prevalence in affected individuals compared to controls.

The 10 remaining variants, which remain classified as VUS, present pathogenic evidence (PM2, PP3, PP1, or PP4) that, individually, is not sufficiently strong to compensate for the absence of validated evidence. There are also conflicting scores, as in ID 18 (c.7723G > A), which received PM2 and PP4 but also BP4-Supporting (REVEL suggests minimal impact) and BS4 due to absence of segregation. The presence of moderate or strong pro-pathogenic and pro-benign evidence leads, by definition, to classification as VUS. The Val→Ile substitution (ID 18) is highly conservative, which reinforces the suspicion of benignity (BP4).

Despite the results obtained, it is imperative to have reports and studies on genetic-clinical associations for better characterization of the impact of these variants on the clinical heterogeneity of LRS.

Therefore, this study demonstrates the critical value of systematically applying the ACMG/AMP framework to resolve Variants of Uncertain Significance (VUS) in FLNB- related disorders. Our re-evaluation of 18 VUS associated with Larsen Syndrome successfully reclassified 44.4% of these variants as

Pathogenic or Likely Pathogenic. This result provides immediate diagnostic clarity and directly enables more definitive clinical management and genetic counseling for affected individuals and their families, moving many cases from uncertainty to actionable insight.

However, the persistence of a significant portion of variants as VUS highlights an ongoing challenge in the genomics of rare diseases: the widespread lack of robust, publicly accessible clinical, segregation, and functional data. The difficulties encountered in this study should be seen not only as limitations but also as a compelling call to action.

We emphasize the urgent need for the international genetics community to support collaborative initiatives that create centralized, disease-specific databases integrating detailed phenotypic and genomic data. Such resources are essential for validating computational predictions and achieving definitive variant classification.

In conclusion, this work offers a refined diagnostic framework for Larsen Syndrome and a scalable methodological template for the iterative re-assessment of VUS in other 15 rare genetic conditions. Moving forward, a multidisciplinary and orthogonal approach – combining continuous, evidence-based variant curation with novel functional studies – will be essential to resolve remaining diagnostic uncertainty and fully realize the promise of genomic medicine for patients affected by these complex disorders.

Abbreviations

AMP Association for Molecular Pathology

AOIII Atelosteogenesis type III

B Benign

CADD Combined Annotation Dependent Depletion

FLNB Filamin B (gene/protein)

gnomAD Genome Aggregation Database

HGMD Human Gene Mutation Database

LB Likely Benign

LOD Logarithm of the Odds

LoF Loss of Function

LOVD Leiden Open Variation Database

LP Likely Pathogenic

LRS Larsen Syndrome

NCBI National Center for Biotechnology Information

OMIM Online Mendelian Inheritance in Man

P Pathogenic

REVEL Rare Exome Variant Ensemble Learner

rsID Reference SNP cluster ID

TOPMed Trans-Omics for Precision Medicine

VUS Variant(s) of Uncertain Significance

Declarations

Conflict of Interest

The authors declare

AUTHORS CONTRIBUITIONS

Conceptualization: J.G.F.O. , I.F.C. ; Data curation:J.G.F.O., I.F.C. ; Formal analysis: J.G.F.O., I.F.C. ; Funding acquisition:J.G.F.O., I.F.C ; Investigation: J.G.F.O., I.F.C. ; Methodology: J.G.F.O., I.F.C. ; Project Administration: J.G.F.O., I.F.C. ; Resources: J.G.F.O., I.F.C. ; Software: J.G.F.O., I.F.C. ; Validation: J.G.F.O., I.F.C. ; Visualization: J.G.F.O., I.F.C. ; Writing-original draft: J.G.F.O., I.F.C.; Writing-review & editing: I.F.C.

ACKNOWLEDGMENTS

To the Federal University of the São Francisco Valley that provided the environment and resources necessary for the development of this study. In particular, to the faculty of the Department of Medicine of Campus Paulo Afonso/BA, Brazil.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

AVAILABILITY OF DATA AND MATERIALS

- All data generated or analysed during this study are included in this published
- article [and its supplementary information files].

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.Ethics committee approval was not required for this study, as the methodology was strictly limited to the analysis of anonymized, secondary, and publicly available data. The genomic and clinical information was retrieved from established primary and secondary databases, including ClinVar, gnomAD, LOVD, and HGMD. This research did not involve any direct interventions with human subjects or the use of original biological samples.

CONSENT TO PARTICIPATE

Not applicable. This research utilized Variants of Uncertain Significance (VUS) and clinical data that had been previously studied and deposited in public repositories.

CONSENT FOR PUBLICATION

All authors consent to publish.

COMPETING INTERESTS

The authors declare that they have no competing interests.

no conflict of interest.

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