ORIGINAL ARTICLE

PLS3 sequencing in childhood-onset primary osteoporosis identifies two novel disease-causing variants

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Abstract
Summary Altogether 95 children with primary bone fragility were screened for variants in PLS3, the gene underlying X-linked osteoporosis. Two children with multiple peripheral and spinal fractures and low BMD had novel disease-causing PLS3 variants. Children with milder phenotypes had no pathogenic variants. PLS3 screening is indicated in childhood-onset primary osteoporosis.

Introduction The study aimed to determine the role of pathogenic PLS3 variants in children’s bone fragility and to elucidate the associated phenotypic features.

Methods Two cohorts of children with bone fragility were screened for variants in PLS3, the gene underlying X-linked osteoporosis. Cohort I comprised 31 patients with childhood-onset primary osteoporosis of unknown etiology. Cohort II comprised 64 children who had sustained multiple fractures but were otherwise healthy. Clinical and radiological data were reviewed. Peripheral blood DNA was Sanger sequenced for coding exons and flanking intronic regions of PLS3.

Results In two patients of cohort I, where other common genetic causes had been excluded, we identified two novel disease-causing PLS3 variants. Patient 1 was a male with bilateral femoral fractures at 10 years, low BMD (Z-score −4.1; 18 years), and multiple vertebral compression fractures. He had a novel nonsense variant in PLS3. Patient 2 was a girl with multiple long bone and vertebral fractures and low BMD (Z-score −6.6 at 6 years). She had a de novo missense variant in PLS3; whole exome sequencing and array-CGH identified no other genetic causes. Iliac crest bone biopsies confirmed low-turnover osteoporosis in both patients. In cohort II, no pathogenic PLS3 variants were identified in any of the subjects.

Conclusions Two novel disease-causing variants in PLS3 were identified in a boy and a girl with multiple peripheral and spinal fractures and very low BMD while no pathogenic variants were identified in children with less severe skeletal fragility. PLS3 screening is warranted in male and female patients with childhood-onset primary osteoporosis.

Keywords Children · Early-onset osteoporosis · Fractures · Osteogenesis imperfecta · Plastin 3 · X-Linked osteoporosis

Introduction
Childhood-onset primary osteoporosis is a rare but clinically important condition characterized by reduced bone strength and an elevated risk of fractures [1]. Current definition
developing Zebrafish larvae [12, 18]. However, as of yet, the direct

PLS3

with childhood-onset primary osteoporosis due to pathogenic

stature, are usually not present. However, only eight families
classical OI, such as blue sclerae, joint hyperlaxity, and short

features of PLS3 osteoporosis are still undetermined.

In this study, we have attempted (1) to determine the overall
role of pathogenic variants in PLS3 in children with bone
fragility and (2) to increase the understanding of the clinical
features of PLS3 osteoporosis.

Materials and methods

This study involves two patient cohorts assessed for primary
skeletal fragility at the Children’s Hospital, Helsinki
University Hospital. The institutional Ethics Review Board
at Helsinki University Hospital approved the study protocol,
and all patients and/or their guardians gave a written informed
consent before participation.

Cohort characteristics

Cohort I (“primary osteoporosis”) consisted of 31 patients
(17 boys and 14 girls), who had been referred to the
Metabolic Bone Clinic, Children’s Hospital, Helsinki during
the years 2003–2013 for investigation for childhood-onset
primary osteoporosis but where the investigation did not re-
veal a molecular cause of their disease. The inclusion criteria
were (1) exclusion of type I collagen-related OI, either cli-

ically or by genetic testing, and (2) exclusion of secondary
osteoporosis with biochemical and individually determined
clinical evaluations. Further, all had been screened and found
negative for pathogenic variants in WNT1 and LRP5 and sev-
eral had undergone more extensive genetic testing before in-
clusion. At the time of referral, all the patients were between
the ages of 4 and 17 years, displayed clinically an osteoporotic
phenotype, and several of them had similarly affected family
members. Most of the patients (25/31) fulfilled the ISCD
criteria [2] for pediatric osteoporosis. Six patients only had a
history of increased non-spinal fractures and a BMD Z-score
>−2.0 but had other additional features (e.g., osteopenic ap-
pearance on skeletal radiographs, fragile bone on orthopedic
surgery) suggesting osteoporosis.

Cohort II (“fracture prone”) consisted of 64 otherwise
healthy children who had sustained multiple fractures (43
boys and 21 girls), all recruited at the Children’s Hospital,
Helsinki, Finland during a prospective epidemiological study
[20]. Over a 12-month period, all children (n = 1412) aged 4–
15 years who had been treated for an acute and radiographi-
cally confirmed fracture were assessed for fracture history.
The trauma mechanisms and previous medical histories were
available for 1361 (96%) of the children. The inclusion criteria
for cohort II were (1) age 4–15 years, (2) ≥2 low-energy long
bone fractures before age 10 years, (3) ≥3 low-energy long
bone fractures before age 16 years, or (4) ≥1 low-energy ver-
tebrale fracture (loss of ≥20% vertebral height). Children with
a diagnosis or suspicion of OI were excluded, as well as chil-
dren with an underlying disease that could explain their bone
fragility. Altogether 71 patients of the >1400 children fulfilled
these criteria; DNA from peripheral blood was available for
64 of them and they comprised cohort II. Data on blood bio-
chemistry, spinal radiographs, and DXA measurements were
collected for all participants.

PLS3, located on the X chromosome, is one of the most
recently described genes underlying childhood-onset primary os-

toporosis. PLS3 osteoporosis was first described in five families
with apparent X-linked osteoporosis in 2013, and the gene was

diagnosis further requires that other nutritional or medical
causes for the child’s bone fragility have been excluded.

With such a broad definition, it is easy to understand that
childhood-onset primary osteoporosis includes a spectrum of
skeletal diseases with different molecular causes. Most affect-
ed children are classified as having osteogenesis imperfecta
(OI), in which the great majority of cases are due to pathogen-
ic variants in either COLIA1 or COLIA2, the two genes

encoding type I collagen [3, 4]. However, during the last
10 years, studies have shown that pathogenic variants in 20
different genes can cause childhood-onset primary osteopo-
rosis [5–11].

PLS3, located on the X chromosome, is one of the most
recently described genes underlying childhood-onset primary os-
toporosis. PLS3 osteoporosis was first described in five families
with apparent X-linked osteoporosis in 2013, and the gene was
subsequently shown to be of importance in bone metabolism
[12]. PLS3 codes for the protein Plastin3, which is widely
expressed in solid tissues and thought to be involved in cytoskel-
eton remodeling. Plastin3 contains two actin-binding sites and
two calcium-binding sites, and in vitro experiments, it can
form bundles of actin by crosslinking single filaments to each
other [13, 14]. In bone, Plastin3 has been suggested to either be
part of the osteocytes’ mechanosensing apparatus or play a role
in the mineralization process [12, 15]. Plastin3 has also been
reported as a protective modifier in spinal muscular atrophy
(SMA) and to be important in axonogenesis [16, 17]. A PLS3
knockout mouse model shows decreased BMD and knockdown
of PLS3 in Zebrafish results in craniofacial dysplasia in the de-
veloping Zebrafish larvae [12, 18]. However, as of yet, the direct
function of Plastin3, the mechanism of its action, and pathogen-
esis of PLS3 osteoporosis are still undetermined.

Because of PLS3’s location on the X chromosome, males
are in general more severely affected by loss of function vari-
ants than females. PLS3 osteoporosis is foremost character-
ized by vertebral compression fractures, frequent peripheral
fractures, and a low BMD [12, 19]. Other traits common in
classical OI, such as blue sclerae, joint hyperlaxity, and short
stature, are usually not present. However, only eight families
with childhood-onset primary osteoporosis due to pathogenic
variants in PLS3 have been described in the literature, and thus
the features of PLS3 osteoporosis have not been fully charac-
terized [12, 15, 19].

In this study, we have attempted (1) to determine the overall
role of pathogenic variants in PLS3 in children with bone
fragility and (2) to increase the understanding of the clinical
features of PLS3 osteoporosis.
As expected, children in cohort I displayed in general lower BMD Z-scores at the lumbar spine and a higher prevalence of vertebral compression fractures (p < 0.001 and p < 0.004, respectively) than children in cohort II (Fig. 1). Data for BMD at the lumbar spine and femoral neck and information about vertebral compression fractures were not available for four, six, and three children, respectively, in cohort I. In cohort II, 61% (n = 39) of the children had sustained at least three significant fractures, and of them 54% (n = 21) had sustained at least four significant fractures. Twenty percent (n = 13) had sustained at least three significant fractures before the age of 10 years, and of them 38% (n = 5) had sustained at least four significant fractures before the age of 10 years.

Genotyping of PLS3

Genomic DNA was extracted with the Puragene DNA Purification kit (Gentra), Archive Pure DNA Blood Kit (5Prime), or QIAamp DNA Blood Maxi Kit (Qiagen). Primers for PCR amplification were constructed using Primer3Plus and were derived from the canonical ensemble transcript (ENST00000355899). Sanger sequencing was performed using BigDye® technology on a 3730 ABI sequencer, and electropherograms were later interpreted using the Staden package (version 2.0.0b10). Primers are available upon request.

Whole exome sequencing

Patient 2 in cohort I together with her nuclear family was subjected to whole exome sequencing. Sequencing was performed using an Illumina HiSeq 2500 at Science for Life Laboratory, Stockholm, Sweden. The Agilent SureSelect XT All Exon V4 target enrichment kit was used for whole exome capture. All analysis computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) [21]. For a full description of the data processing and analysis, see Supplemental appendix and Supplemental Fig. 1 (Online Resource).

Array comparative genomic hybridization

For patient 2, a customized 2x400K array (Agilent Technologies) was used, with enriched probes in 2269 genes, including over 300 genes known to underlie skeletal diseases. In the specifically targeted genes, this high-resolution array comparative genomic hybridization (array-CGH) has an average coverage of one probe per 100 base pairs in coding regions and one probe per 500 base pairs in introns and UTRs. The experiments were performed using standard procedures, and results were analyzed using Agilent Genomic Workbench 7.0.

Bone histomorphometry

Iliac crest bone biopsies were taken as part of normal diagnostic evaluation for both patients in cohort I who were deemed to have disease-causing variants in PLS3. For bone turnover assessments, the patients were pre-treated with oral tetracycline for 2 × 2 days with a 10-day treatment-free interval. The bone biopsies were analyzed by an experienced histomorphometrist using a semiautomated image analyzer (Bioquant Osteo; Bioquant Image Analysis Corp., Nashville, TN, USA). The recommendations of the American Society for Bone and Mineral Research regarding abbreviations and nomenclature were followed [22]. Age-specific reference values and Z-scores were calculated for all parameters [23, 24].

Statistical analysis

The statistical analyses were performed using R version 3.3.1. Because of the distribution of the data, the Mann-Whitney U test was used to compare BMD between the groups. The chi-square test was used to test categorical data. A p value <0.05 was considered significant.

Results

Genetic findings in cohort I

Sanger sequencing of PLS3 in cohort I identified two patients (6.5%) with novel variants in PLS3 that were deemed to be damaging and causative of their phenotypes (described below). In the remaining 29 patients in cohort I, a total of 7 single nucleotide variants (SNVs) and 1 small deletion were found (Table 1). Three of the SNVs were found in coding regions, all were synonymous and with an allele frequency of at least 4% in the general Finnish population (SISu database); the other four variants were intronic, all previously reported. One of the synonymous variants, rs140121121, has previously been associated with osteoporosis [12], but this SNV was not enriched in our cohort compared with the normal Finnish population (allele frequency 0.067 vs 0.051). Outside the coding region, we found one 13 bp deletion (rs201765481) situated 190 bp upstream of exon 6 of PLS3. Its allele frequency was higher than expected in our cohort (0.043 vs 0.009 (dbSNP)), but when we sequenced this region for 96 healthy Finnish control samples, the frequency was found to be similar (0.059) as in our cohort, suggesting that the deletion is not pathogenic. The other non-coding variants were regarded as not being of significance in this context, either because of their high allele frequencies or location far from canonical splice sites.
Genetic and clinical findings in the two patients with disease-causing variants in PLS3

Patient 1 is a presently 30-year-old Finnish male, who was found to have a novel hemizygous nonsense variant (c.766C>T; pArg256*) in exon 8 of PLS3. This variant is not found in dbSNP, SISu, ExAC, or gnomAD databases and classified as pathogenic according to the American Collage of Medical Genetics and Genomics (ACMG) guidelines for interpreting sequence variants [25]. He has also been sequenced for a core panel of bone fragility genes (Blueprint Genetics, Helsinki), including COL1A1 and COL1A2, without pathogenic findings. The mother of patient 1 was confirmed to be heterozygous for the variant, while the brother, father, and three maternal relatives were negative for the variant. Patient 1 has a history of multiple fractures since early childhood. Between the ages of 9 and 10 years, he fractured both femurs in two separate low-energy traumas. At 10 years, he was diagnosed with multiple vertebral compression fractures (p < 0.005 for both) compared with cohort II. In cohort I, the two patients with disease-causing variants in PLS3 (values encircled) show a markedly low BMD compared to other subjects. (Boys are denoted with a circle and girls with a cross)

Patient 2 whose variant was deemed disease-causing is a 10-year-old Finnish girl with no family history of osteoporosis. She proved to be heterozygous for a novel de novo missense variant in exon 12 (c.1424A>G; p.N446S) that was absent in her parents and healthy sister (Supplemental Fig. 2; Online Resource). The variant was not found in either dbSNP, SISu, ExAC, or gnomAD databases. The amino acid in this position is highly conserved over different species, and the missense variant has a scaled CADD score of 21.5 and is predicted deleterious by both SIFT and MutationTaster.

The patient also displays some extraskeletal features such as slightly blue sclerae, slightly yellow teeth and loss of enamel, generalized joint hyperlaxity, soft skin, minor aortic valve regurgitation, and asthma. In other respects, his pubertal development and adult height (175 cm) were normal. Measurements for calcium, phosphate, and alkaline phosphate were normal and there was no hypercalciuria, but urinary NTX was low (normal creatinine). Taken together, his biochemical profile was normal except for a mild vitamin D deficiency (serum 25-OH-vitamin D 35 nmol/L). The mother, heterozygous for the variant, had osteopenia on DXA scan (total body Z-score −1.4 at 46 years) and had sustained one radius fracture after a fall at 35 years. The mother also had joint hyperlaxity and slightly blue sclerae but was otherwise healthy.

Fig. 1 BMD measurements of the lumbar spine and the proximal femur in cohort I (left panel, n = 31) and cohort II (right panel, n = 64). The bottom two figures show prevalence of vertebral compression fractures in the respective cohorts. Children in cohort I have in general a lower BMD Z-score at the lumbar spine and a higher prevalence of vertebral compression fractures (p < 0.005 for both) compared with cohort II. In cohort I, the two patients with disease-causing variants in PLS3 (values encircled) show a markedly low BMD compared to other subjects. (Boys are denoted with a circle and girls with a cross)
According to the ACMG guidelines [25], this variant is classified as likely pathogenic, which means that the variant is considered to be pathogenic at a level of ≥90% certainty. However, since the phenotype was significantly more severe than anticipated for a female with a heterozygous missense variant in \( \text{PLS3} \), we investigated the possibility of other causative variants. An array-CGH detected no significant gene dosage imbalances; her other \( \text{PLS3} \) allele was also intact. We also performed whole exome sequencing for patient 2 and her nuclear family (healthy parents and healthy sister). Since the parents were completely healthy, the analysis focused on recessively inherited and de novo variants. However, apart from the de novo variant in \( \text{PLS3} \), no other potential disease-causing variants were found. Importantly, no other damaging variants were found in any other genes associated with OI. The genes \( \text{COL1A1} \) and \( \text{COL1A2} \) had also previously been Sanger sequenced without pathogenic findings. Moreover, previously reported female patients heterozygous for pathogenic variants in \( \text{PLS3} \) show variable expressivity [12, 19] (Supplemental Table 2; Online Resource). Based on these findings, the heterozygous \( \text{PLS3} \) variant was regarded as the most likely cause for the phenotype.

**Patient 2** has a history of multiple long bone and vertebral compression fractures and remarkably low BMD. By the age of 6 years, she had sustained three low-energy long bone fractures and one finger fracture and was then referred to the Metabolic Bone Clinic, Children’s Hospital, Helsinki for further investigations. Her BMD Z-scores for the lumbar spine, proximal femur, and total body were −6.6, −4.5, and −3.5, respectively. Spinal radiographs showed three asymptomatic vertebral compression fractures. Extra-skeletal manifestations were seen in the form of joint hyperlaxity with hyperextension in elbows and knees, but she did not have blue sclerae. Secondary causes of osteoporosis were excluded. Serum calcium, phosphate, alkaline phosphatase, PTH, and vitamin D were normal. A bone biopsy confirmed the diagnosis of trabecular osteoporosis, low bone turnover, and

**Table 1** Variations in \( \text{PLS3} \) found in the two different cohorts

| Variants | SNP ID (rs number) | Base substitution | Consequence | Allele frequency (cohort) | Minor allele frequency (population) |
|----------|--------------------|-------------------|-------------|---------------------------|----------------------------------|

**Cohort I**

Coding variants (D)
- c.766C>T Nonsense 0.022
- c.1424A>G Missense 0.022

Coding variants (B)
- rs140121121 c.321T>A Synonymous 0.067 0.0522a
- rs2108099 c.1242T>C Synonymous 0.022 0.0409a
- rs871774 c.1294T>C Synonymous 0.022 0.0402a

Non-coding variants
- rs757124 c.-55C>G 5′ UTR 0.38 0.36b
- rs201765481 c.501-190del(13 bp) Intronic 0.043 0.059c
- rs871773 c.1377+17C>T Intronic 0.022 0.0425a
- rs2301951 c.1511+82T>C Intronic 0.022 0.18b
- rs190387665 c.1-66C>T Intronic 0.022 0.008b

**Cohort II**

Coding variants (B)
- rs140121121 c.321T>A Synonymous 0.035 0.0522a
- rs2108099 c.1242T>C Synonymous 0.071 0.0409a
- rs871774 c.1294T>C Synonymous 0.059 0.0402a
- rs140968059 c.925A>G Missense 0.012 0.003a

Non-coding variants
- rs757124 c.-55C>G 5′ UTR 0.38 0.36b
- rs201765481 c.501-190del(13bp) Intronic 0.082 0.059c
- rs871773 c.1377+17C>T Intronic 0.059 0.0424a
- rs2301951 c.1511+82T>C Intronic 0.071 0.18b
- rs782554235 1635+101G>C Intronic 0.012 0.003b

In total, 12 different allelic variants were found in the two cohorts combined, 7 of them in both cohorts. Two novel variants, both deemed damaging and causative of disease, were found in two different subjects in cohort I. No variants thought to be damaging were found in cohort II.

D damaging, B benign

\( ^{a} \)SISu database

\( ^{b} \)dbSNP (144)

\( ^{c} \)Ninety-six healthy Finnish controls
normal mineralization (Fig. 2 and Supplemental Table 1; Online Resource). Treatment with pamidronate was started at the age of 6 years with a cumulative dose of 9 mg/kg the first year and continued with zoledronic acid 0.025 mg/kg every 6 months thereafter. Follow-up measurements at 17, 29, and 40 months showed a good treatment response and she has not experienced new fractures thereafter (Fig. 4).

Genetic findings in cohort II

In cohort II, Sanger sequencing of the PLS3 gene showed in total 8 SNVs and 1 small deletion in the 64 patients with fractures (Table 1). All variants have been previously described, and most of them corresponded to the findings in cohort I. One coding variant was unique to cohort II, a rare missense variant (rs140968059, p.I309V) found in heterozygous form in one girl. However, the substitution was predicted benign by both SIFT and MutationTaster, and both isoleucine and valine are branched-chain amino acids with very similar chemical structures, making a substitution between the two less likely to be damaging. Thus, none of the variants found in the 64 fracture-prone children were considered causative of their skeletal fragility.

Discussion

In this study, we have tried to answer two questions: (1) Are pathogenic variants in PLS3 responsible for a proportion of bone fragility in children? (2) How to better recognize patients whose bone fragility are caused by pathogenic variants in PLS3? We addressed these questions in the more severely affected children in cohort I and in the seemingly healthy but fracture-prone children in cohort II.

In cohort I, a non-negligible proportion of the patients (6.5%; 2 of 31 screened subjects) had variants in PLS3 that were deemed to be causative of their osteoporosis. In the case of patient 1, the 30-year-old Finnish male was hemizygous for a novel nonsense variant located in the mid-part of the gene. Such a variant, based on what is known, leads to a complete loss of function of the mature protein and the patient will effectively be left without any functioning copy of PLS3 [26, 27]. PLS3 is also recognized as a gene with extremely low tolerance to protein truncating variants (probability of loss of function intolerance (pLI) = 0.99) [28].

In patient 2, the 10-year-old Finnish female, we detected a novel de novo heterozygous missense variant. Other

![Fig. 2](image-url)
potential genetic causes were excluded by array-CGH and whole exome sequencing. \textit{PLS3} is also considered sensitive to missense variants (Z-score 2.51) ranking in the top 15\% of genes most sensitive to missense variants \cite{28}.

Early-onset symptomatic osteoporosis has previously been described in adult females with heterozygous pathogenic variants in \textit{PLS3} \cite{12, 19} but perhaps not to the extent seen in this 10-year-old girl. Our findings therefore extend the phenotypic spectrum of \textit{PLS3} osteoporosis to include also girls with severe primary osteoporosis.

Previously reported patients with severe osteoporosis due to pathogenic variants in \textit{PLS3} have almost all been male, and in general males tend to be more severely affected. In 2015, \textit{Laine et al.} reported on a large Finnish family with osteoporosis due to a pathogenic splice variant in \textit{PLS3}. In this large family, hemizygous males had more severe osteoporosis, but all heterozygous females had low BMD and one affected female had a phenotype more in resemblance with her male relatives, with recurrent peripheral fractures and multiple vertebral compression fractures \cite{19}. \textit{Van Djik et al.} also reported a variable clinical phenotype in females with heterozygous pathogenic variants in \textit{PLS3} \cite{12} (Supplemental Table 2; Online Resource). In our study, variable expressivity can also be seen in females with heterozygous pathogenic variants; the mother of \textit{patient 1} had a mild phenotype while \textit{patient 2} had severe osteoporosis. The cause of this variable expressivity is not known. Some variants could perhaps have a dominant negative effect on the other allele, but since this variable expressivity can be seen also within families where all affected share the same pathogenic variant, other factors are likely to contribute. Skewed X-inactivation could explain why female patients with identical genotypes can display different phenotypic severity, but modifying variants in regulatory elements could also exert an effect on the phenotype. Moreover, lifestyle factors may influence the phenotypic presentation even in monogenic forms of osteoporosis.

Pathogenic variants in \textit{PLS3} seem to have a substantial impact on BMD and involve compression fractures of the vertebrae. In cohort \textit{I}, among the 31 included patients, the

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\textbf{Fig. 3} Radiographs and BMD of \textit{patient 1} with \textit{PLS3} osteoporosis. Spinal radiograph (a) at the age of 12 years shows a kyphosis and a significant spinal osteoporosis with compressed vertebrae. Radiograph (b) at 21 years, after a 1-year zoledronic acid treatment, shows an improvement of kyphosis and the shape of vertebrae, but his BMD remained very low. Long bone radiographs (c) at the age of 21 years show generalized osteopenia and very thin cortices in the lower leg. (d) Lumbar spine BMD from childhood to adulthood (\textit{shaded areas} denote Z-scores \pm 2.0)

\textbf{Fig. 4} Radiographs of \textit{patient 2} with \textit{PLS3} osteoporosis. At 6 years (a), spinal osteoporosis can be seen together with several compressed vertebrae. At this time, bisphosphonate treatment was started. (b) Two years later, an improvement in the radiographic appearance can be seen. (c) Graph of lumbar spine BMD after initiation of bisphosphonate treatment (\textit{shaded areas} denote Z-scores \pm 2.0)
two described patients stood out with the lowest and third lowest BMD Z-score at the lumbar spine and the lowest and second lowest BMD at the femoral neck. Both patients also had a history of multiple vertebral compression fractures as an indication of significant spinal osteoporosis, which seems to be a hallmark for PLS3 osteoporosis. They also had a history of multiple major low-energy long bone fractures at an early age. Bone histomorphometry confirmed in both patients trabecular osteoporosis with low bone turnover and normal mineralization. The low bone turnover in PLS3 osteoporosis stands in contrast to the high bone turnover seen in type I collagen-related osteogenesis imperfecta [29]. These findings are in line with previous reports on patients with osteoporosis due to pathogenic variants in PLS3 [12, 15, 19].

Treatment with bisphosphonates has been evaluated for only a handful of patients with PLS3 osteoporosis, but all these reports suggest that treatment is at least initially beneficial for increasing BMD [12, 15]. In patient 1, the year-long treatment with zoledronic acid, which started as late as at 18 years, increased his BMD only slightly and 1 year after discontinuation his BMD was still very low. In patient 2, bisphosphonate treatment, which started at a much younger age, has significantly improved BMD and prevented further fractures, but long-term treatment results remain yet to be seen.

In cohort II, consisting of seemingly healthy but fracture-prone children, no disease-causing PLS3 sequence variants were found. We also looked for enrichment of both rare and common SNVs that, at least in theory, could be modifiers of protein function and perhaps help to explain the wide range in the number of childhood fractures seen in the general population. However, we did not find any enriched PLS3 SNVs in cohort II and conclude that PLS3 variations do not explain increased bone fragility in this large cohort of children. Based on our findings, and the previously reported cases, it seems that clinically relevant pathogenic variants in PLS3 result not only in increased long bone fractures but are always associated with significantly reduced BMD and vertebral fractures.

We recognize some limitations in our study. Our cohorts were relatively small and this limits our ability to make strong conclusions in the overall pediatric population and may have prevented us from finding significant associations. Furthermore, we only searched for variants that were thought to directly affect protein structure (exonic or splice variants), which means that other possibly important variants in introns or regulatory regions could not be detected. However, because of our fairly stringent inclusion criteria, we believe that our results are representative of patients assessed in pediatric bone clinics for suspected primary osteoporosis. This is also, to our knowledge, the first study that systematically screened for PLS3 variants in a single-hospital based cohort of children with bone fragility, and the finding of two novel pathogenic or likely pathogenic variants in PLS3 supports the relevance of our research approach. We did not perform functional studies to evaluate the mechanisms through which the variants lead to clinical manifestations and it thus remains unknown whether the missense variant leads to protein instability or otherwise interferes with PLS3 function. Such studies were beyond the scope of this study but once more data emerges about the physiological role of PLS3 in skeletal homeostasis, functional evaluation of mutated PLS3 may provide important insights to the pathogenesis of PLS3 osteoporosis.

Conclusions

This study expands the spectrum of disease-causing PLS3 variants and the associated phenotypes; it gives further support to the importance of spinal osteoporosis as a consequence of pathogenic variants in PLS3 and indicates that also females with heterozygous pathogenic variants in PLS3 can develop childhood-onset primary osteoporosis. Based on our findings, PLS3 screening should be considered in children—both boys and girls—with multiple peripheral and spinal fractures and low BMD. Molecular diagnosis is important for appropriate patient management and genetic counseling even if specific treatment for PLS3 osteoporosis is not yet available. In contrast, children who show an increased propensity to fracture but do not fulfill the criteria of osteoporosis (i.e., have BMD within the normal range) are less likely to have disease-causing variants in PLS3, and our study does not provide support that screening for PLS3 variants in these children is meaningful.

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Compliance with ethical standards

Conflicts of interest Anders Kämpe, Alice Costantini, Riikka Mäkitie, Nina Jäntti, Helena Valta, Mervi Mäyränpää, Heikki Kröger, Minna Pekkala, Fulya Taylan, Hong Jiao, and Outi Mäkitie declare that they have no conflict of interest.

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