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ORIGINAL ARTICLE

Mutational analysis uncovers monogenic bone disorders in women with pregnancy-associated osteoporosis: three novel mutations in *LRP5*, *COL1A1*, and *COL1A2*

S. Butscheidt[1] · A. Delsmann[1] · T. Rolvien[1,2] · F. Barvencik[1] · M. Al-Bughaili[3,4,5] · S. Mundlos[3,4,5] · T. Schinke[1] · M. Amling[1] · U. Kornak[3,4,5] · R. Oheim[1,6]

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Abstract

Summary Pregnancy was found to be a skeletal risk factor promoting the initial onset of previously unrecognized monogenic bone disorders, thus explaining a proportion of cases with pregnancy-associated osteoporosis. Therapeutic measures should focus in particular on the normalization of the disturbed calcium homeostasis in order to enable the partial skeletal recovery.

Introduction Pregnancy-associated osteoporosis (PAO) is a rare skeletal condition, which is characterized by a reduction in bone mineral density (BMD) in the course of pregnancy and lactation. Typical symptoms include vertebral compression fractures and transient osteoporosis of the hip. Since the etiology is not well understood, this prospective study was conducted in order to elucidate the relevance of pathogenic gene variants for the development of PAO.

Methods Seven consecutive cases with the diagnosis of PAO underwent a skeletal assessment (blood tests, DXA, HR-pQCT) and a comprehensive genetic analysis using a custom-designed gene panel.

Results All cases showed a reduced BMD (DXA T-score, lumbar spine − 3.2 ± 1.0; left femur − 2.2 ± 0.5; right femur − 1.9 ± 0.5), while the spine was affected more severely ($p < 0.05$). The trabecular and cortical thickness was overall reduced in HR-pQCT, while the trabecular number showed no alterations in most cases. The genetic analysis revealed three novel mutations in *LRP5*, *COL1A1*, and *COL1A2*.

Conclusion Our data show that previously unrecognized monogenic bone disorders play an important role in PAO. Pregnancy should be considered a skeletal risk factor, which can promote the initial clinical onset of such skeletal disorders. The underlying increased calcium demand is essential in terms of prophylactic and therapeutic measures, which are especially required in individuals with a genetically determined low bone mass. The implementation of this knowledge in clinical practice can enable the partial recovery of the skeleton. Consistent genetic studies are needed to analyze the frequency of pathogenic variants in women with PAO.

Keywords Early-onset osteoporosis · Genetics · Monogenic bone disorders · Pregnancy-associated osteoporosis · Treatment

S. Butscheidt and A. Delsmann contributed equally to this work.

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✉ R. Oheim
r.oheim@uke.de

[1] Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Lottestr. 59, 22529 Hamburg, Germany

[2] Department of Orthopedics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[3] Institute of Medical Genetics and Human Genetics, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

[4] Berlin-Brandenburg Center for Regenerative Therapies, Charité-Universitätsmedizin Berlin, Berlin, Germany

[5] Max Planck Institute for Molecular Genetics, FG Development and Disease, Berlin, Germany

[6] National Bone Board, Martin Zeitz Center for Rare Diseases, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction

Pregnancy-associated osteoporosis (PAO) is a rare skeletal condition with an estimated incidence of 4–8 in 1 million pregnancies, characterized by a significant transient reduction in bone mineral density (BMD) [1]. Related symptoms include severe back or hip pain due to vertebral compression fractures or transient osteoporosis of the hip [2–4], which typically occur during the third trimester or lactation [5]. After delivery, the BMD may increase again to the lower end of the reference range [2, 6]. However, previous studies reported severe cases, characterized by multiple vertebral compression fractures, which show a persistent reduction in BMD [2, 7]. It was hypothesized that a preexisting low bone mass could explain these severe manifestations [2, 8]. Exogenous factors such as malnutrition (i.e., anorexia nervosa [9]) or drug-induced side effects (i.e., corticosteroids [10], heparin [11]) have been discussed as the basis of low bone mass and quality in these cases. Furthermore, Hadji and colleagues identified severe dental problems, lack of exercise in childhood, and immobility to be associated with PAO [7]. As a matter of fact, there are also indications of genetic factors that may promote PAO. For instance, the analysis of BMD in relatives of five PAO patients revealed osteoporosis in 53% indicating a heritable component [12]. Furthermore, single-case reports have already identified monogenic bone disorders as underlying entity in PAO [13–15].

We present a genetic evaluation of seven cases diagnosed with PAO, using a custom-designed bone panel. This study was primarily performed in order to elucidate the role of pathogenic variants in individuals with PAO. Furthermore, we present a comprehensive skeletal assessment including implications for diagnosis and therapy of patients with PAO phenotypes.

Methods

Study design and medical history

The German research association "Detection and Individualized Management of Early Onset Osteoporosis" (DIMEOS) aims to investigate the pathogenesis of EOOP and to develop individualized therapy concepts for affected patients based on both clinical and genetic data. Hereto, all patients of our specialized outpatient clinic, who are younger than 50 years and have osteoporosis, undergo a comprehensive genetic analysis. This prospective approach is legitimated by approval through the local ethics committee (No. PV5364) and all investigations were carried out in accordance with the World Medical Association Declaration of Helsinki.

Between 2015 and 2017, a total of seven women suspected of having PAO presented in our specialized outpatient clinic. For this study, all seven consecutive cases, aged 30–39 years (mean ± SD, 35.1 ± 3.3), were included. The leading symptoms were vertebral compression fractures or transient osteoporosis of the hip, which occurred in a temporal context of pregnancy or lactation. In the framework of the clinical assessment, we focused on type, date, and pathogenesis of pregnancy-associated pathologic events (e.g., vertebral compression fractures). The overall inclusion criteria for such events were atraumatic or low traumatic cause and a temporal connection to the pregnancy and/or lactation. Fractures were classified as vertebral and non-vertebral fractures, respectively. Furthermore, we assessed skeletal risk factors (i.e., historical height loss (HHL), previous fractures, immobility, dental problems, drugs, endocrine disorders, nutrition habits) as well as course of pregnancy and lactation. HHL was assessed based on the comparison of original height (German passports provide this information at time of initial issue) and height at time of presentation. Dental abnormalities were defined as recurrent infections, exceptional toothache, diminished roots, tooth loss, and discoloration such as yellow-brown or blue-gray. Furthermore, ophthalmic abnormalities include, amongst others, blue sclera, amblyopia, and blindness. Drugs included all substances known to have detrimental side effects on bone health such as glucocorticoids, heparine, proton pump inhibitors, or chemotherapeutics. Based on this study's main purpose, we additionally paid special attention to the patient's detailed family history. Pedigrees were drawn for related individuals, if appropriate.

Skeletal characterization

In order to examine bone turnover and to exclude endocrine disorders, blood samples were obtained and biochemical analysis was performed: calcium (Ca), phosphate (P), creatinine (Creat), glomerular filtration rate (GFR), alkaline phosphatase (AP), osteocalcin (OC), vitamin D ($25(OH)D_2$), bone alkaline phosphatase (BAP), parathyroid hormone (PTH), and deoxypyridinoline (DPD). Resulting serum levels were compared to reference data from our local laboratory.

BMD was assessed by dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare; Madison, WI, USA) at the lumbar spine L1–4 (LS), left femur (LF), and right femur (RF). Results are given as absolute values in grams per square centimeter and DXA T-scores for each site, respectively. Baseline measurements were performed at initial presentation in our outpatient clinic. Follow-up scans were available after 12 and 24 months in five cases (A.4, B.3, E.3, G.1, F.1). The development of BMD up to the latest available scan is presented in percent change from baseline for each site. Two cases (D.1, C.4) did not take part in follow-up examinations; thus, data is not available.

High-resolution peripheral quantitative computed tomography (HR-pQCT; XtremeCT®, Scanco Medical, Brüttisellen,

Switzerland) scans were performed in order to examine bone microarchitecture. Measurements were taken out at the distal radius and tibia of the not dominant site, respectively, as described previously by our group [16]. In case of a previous fracture in that area, the contralateral extremity was scanned. The results are given as absolute values and compared to site- and sex-specific normative HR-pQCT data for women aged 30–39 years [17]. HR-pQCT scans were available for five cases (B.3, C.4, E.3, F.1, G.1) and performed at initial contact after delivery.

Genetic analysis

A SureSelect XT gene panel (Agilent, Santa Clara, CA, USA) which was used to enrich the coding exons of 386 genes in which mutations have been associated with changes in bone mass, skeletal dysplasias, dysostoses, or connective tissue diseases (skeletal disorder-associated genome (sDAG)) was applied as described recently [18]. The enriched libraries were sequenced on a MiSeq machine (Illumina, San Diego, CA, USA) with a coverage of more than 10-fold in 98% of the target region. Variant filtering was carried out using the software PhenIX and GeneTalk [19, 20]. PhenIX uses the scores provided by the software MutationTaster, Polyphen, and SIFT to calculate an overall pathogenicity score for each identified variant. Variants are additionally ranked according to phenotype relevance based on the Human Phenotype Ontology. The highest ranking variants were judged in detail using MutationTaster [21]. The variants judged to be causative for the PAO phenotype were validated and segregated by Sanger sequencing using an ABI 3730 sequencer (Thermo Scientific, Waltham, USA).

Statistical analysis

The statistical analyses were carried out using Microsoft® Excel® for Mac 2011 (v.14.0.0) and the sample characteristics are presented as absolute values or mean ± standard deviation (SD). The changes in follow-up BMD measurements were calculated as percent change from baseline. The nonparametric Mann-Whitney *U* test was used to analyze the site-specific differences in DXA T-scores with a level of significance defined as $p < 0.05$.

Results

Seven women with PAO were enrolled in this study. Four cases (A.4, D.1, E.3, F.1, G.1) became symptomatic during their first pregnancy and two cases (B.3, C.4) during their second pregnancy. None of the women had more than two pregnancies. Six out of seven cases reported lactation durations ranging from 2 to 18 months. Only case G.1 did not breastfeed due to agalactia. The predominant clinical symptom, leading to presentation in our outpatient clinic, was pain in the back or hip due to vertebral compression fractures or transient osteoporosis of the hip (Table 1). While neither dental or ophthalmic abnormalities nor relevant drugs were involved in the majority of cases (5/7), immobility has been reported once with a minimum duration of 13 months (G.1). Only case E.3 showed a moderate blue-gray discoloration of the sclera. The genetic analysis revealed novel pathogenic variants (i.e., monogenic bone disorders) in three cases. Case A.4 had a heterozygous missense variant in the *LRP5* gene (c.2377G>A (p.Gly793Arg)) resulting in early-onset osteoporosis. Cases C.4 and E.3 were diagnosed with adult osteogenesis imperfecta (OI) due to heterozygous mutations in the *COL1A2* and *COL1A1* gene (c.874G>A (p.Gly292Ser) and c.1054_1056+2del). The *LRP5* mutation p.Gly793Arg has not been described before, but the exchange of the highly conserved amino acid glycine to arginine is probably not tolerated by the protein so that the mutation was ranked as highly pathogenic [22]. Since two additional siblings that carried the same variant also showed evidence for early-onset osteoporosis (Fig. 1a), the variant was ranked class IV according to ACMG criteria [23] (Table 2). The *COL1A2* mutation p.Gly292Ser is a classic glycine exchange in the triplehelical region of the collagen protein. Exchanges of the flanking amino acids Gly289 and Gly295 are known to be associated with osteogenesis imperfecta, which underline the pathogenicity, resulting in a classification of ACMG class IV (Fig. 1b; Table 2) [24]. c.1054_1056+2del is a novel mutation resulting in defective splicing. Such splicing defects were associated with osteogenesis imperfecta previously [25, 26]. Furthermore, the variant arose de novo (Fig. 1c), which leads to a classification as class V according to ACMG criteria (Table 2). Additional genetic data is given in Online Resource 1.

Biochemical analysis of bone turnover revealed normal PTH levels in 5/7 cases after restoring sufficient vitamin D levels (Online Resource 2). However, cases C.4 and F.1 showed increased BAP and PTH levels indicating a continuing compromised calcium homeostasis. Additionally, the latter case showed hypocalcemia. The results of case A.4 show a teriparatide-induced high turnover. All collected events occurred in the third trimester (antepartum), during birth (peripartum), or within the following 6 months after delivery (postpartum): 37, 33.3, and 29.6%, respectively (Online Resource 3).

The DXA scans demonstrated a consistent reduction of BMD in all seven cases, whereas the decrease was observed predominantly at the lumbar spine (mean ± SD; −3.1 ± 0.9; Fig. 2a). DXA T-scores at the lumbar spine were significantly lower when compared with left and right femur, respectively

Table 1 Clinical overview of the study population

Case	A.4	B.3	C.4	D.1	E.3	F.1	G.1
Age (year)	35	38	30	32	39	36	37
BMI (kg/m^2)	19.9	21.8	20.8	20.4	24.8	18.7	30
Onset of symptoms	Antepartum (para 1)	Postpartum (para 2)	Postpartum (para 2)	Antepartum (para 1)	Peripartum (para 1)	Postpartum (para 1)	Antepartum (para 1)
Fractures/TOH	L1, L3, insufficiency fractures of multiple ribs, MT III left, femoral head right	TOH left	T7, T11, T12, L1, L2, L3; metacarpal	Bilateral TOH	T7, T8, T9, T12, L1, L2, L3, L4, L5	T12	T5, T6, T12, L1
Lactation (month)	+*	3	8	2	12	18	0
Height loss (cm) before/after (Δ)	171/171 (0)	156/155 (− 1)	170/165 (− 5)	173/170 (− 3)	159/157 (− 2)	169/164 (− 5)	160/157 (− 3)
Immobility (month)	–	–	–	–	–	–	13
Dental abnormalities	–	–	–	–	–	–	–
Ophthalmic abnormalities	–	–	–	–	Blue sclera	–	–
Family history	+	+	+	–	+	–	–
Drugs**	–	–	–	–	–	–	–
Basic therapy	+	+	+	+	+	+	+
Specific therapy	T	–	–	D	T	–	T
Mutation	*LRP5*	–	*COL1A2*	–	*COL1A1*	–	–

Basic therapy includes supplementation of vitamin D and calcium

*Unknown duration

**Drugs with known detrimental side effects on bone health (e.g., glucocorticoids, heparine, proton pump inhibitors)

D denosumab (single administration 60 mg s.c.), *T* teriparatide (20 μg/day s.c.)

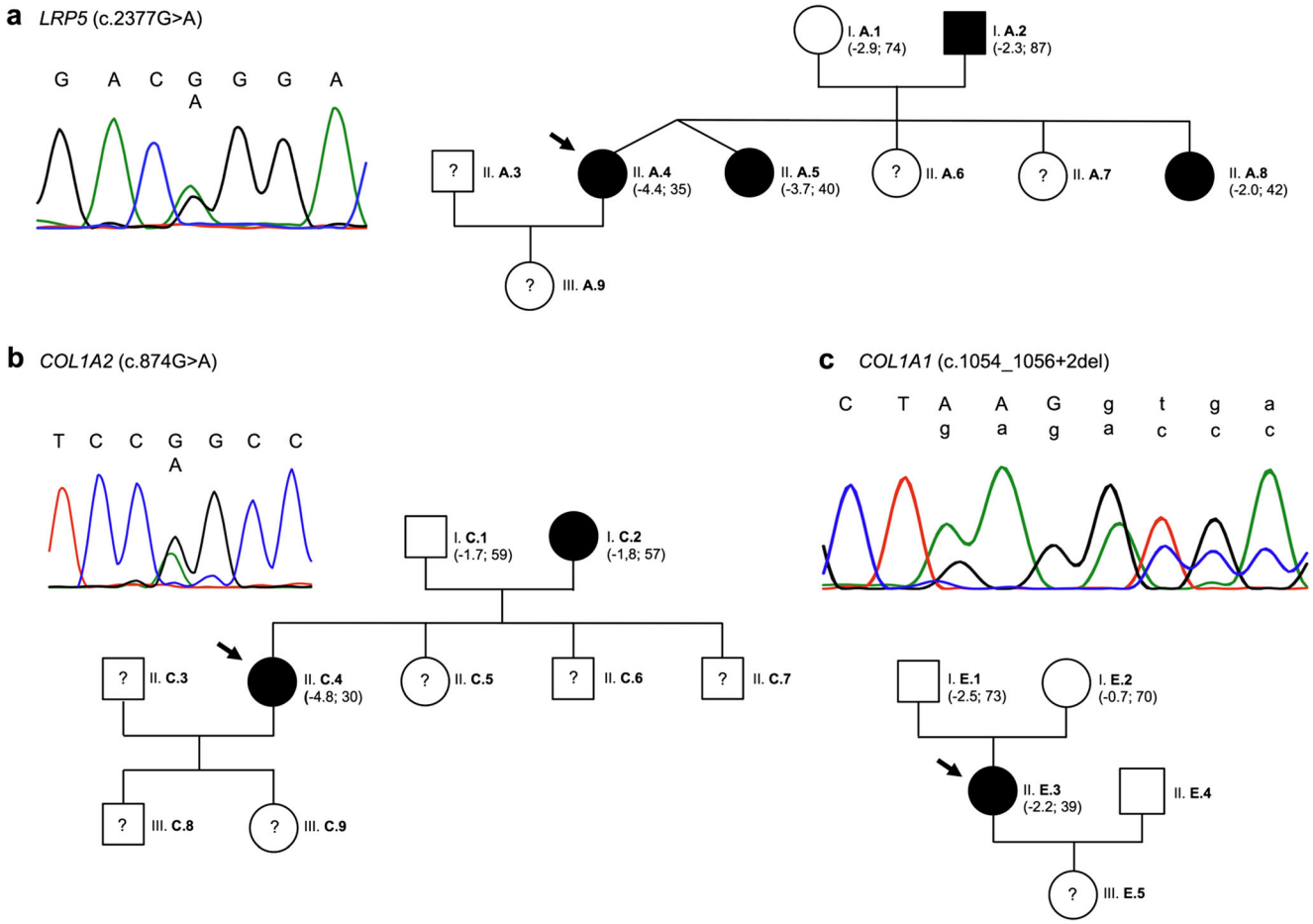


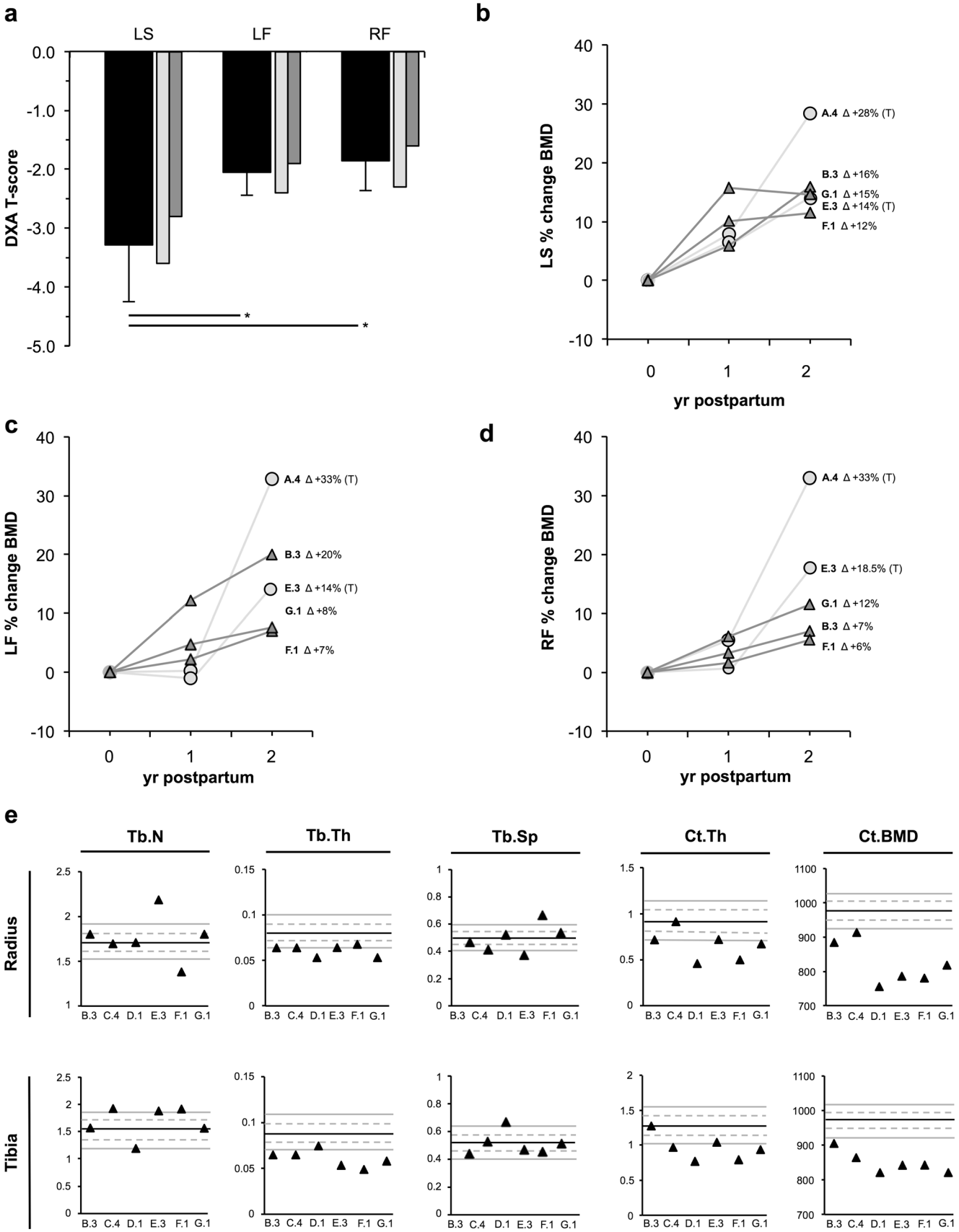
Fig. 1 Family pedigrees and genetic data. **a** Index case A.4 (arrow) with an exchange of glycine to arginine resulting in a missense variant of the *LRP5* gene (ACMG class IV) leading to early-onset osteoporosis. **b** Index case C.4 (arrow) shows a classic glycine exchange in the collagen protein (ACMG class IV) with resulting adult osteogenesis imperfecta; in fact, the index cases A.4 and C.4 show the lowest BMD within the variant carriers, respectively. This indicates that pregnancy is a significant risk factor contributing to the high variability within the families. **c** Index case E.3 (arrow) with a de novo splicing mutation in the *COL1A1* gene (ACMG class V) leading to adult osteogenesis imperfecta. Squares represent male and circles female individuals with black fillings for the detection of the underlying mutation. The question marks indicate family members with unknown genotype. Details in parentheses: DXA T-score; age in years

($p < 0.05$). A more detailed approach revealed that cases A.4, C.4, and E.3, in which genetic mutations were detected, appeared to have a slightly greater decline in BMD, when compared to cases without any variants (B.3, D.1, F.1, and G.1). However, differences were significant for the right femur only ($p < 0.05$). In five cases, follow-up scans were available and showed consistent increases in BMD within 2 years after delivery, ranging from + 1 to + 33% at any site (Fig. 2b, c). Additional DXA data are given in Online Resource 4.

Analysis of bone microarchitecture by HR-pQCT revealed normal results for the trabecular number (Tb.N) and trabecular separation (Tb.Sp). However, trabecular (Tb.Th) and cortical thickness (Ct.Th) was overall reduced and ranged within lower centiles of the normative curve or below it. Additionally, cortical bone mineral density (Ct.BMD) was markedly decreased in all cases (Fig. 2e). Cases with MBD (A.4, C.4, E.3) did not show any significant alterations in the degree of bone loss when compared to non-genetic cases (B.3, D.1, F.1, G.1).

Table 2 Overview of the genetic data

Case	Gene	GenBank accession	Mutation-DNA level	Mutation-protein level	Reference	ACMG class	State	Mode of inheritance
A.4	*LRP5*	NM_002335	c.2377G>A	p.Gly793Arg	Novel	IV	Heterozygous	AD
C.4	*COL1A2*	NM_000089	c.874G>A	p.Gly292Ser	Novel	IV	Heterozygous	AD
E.3	*COL1A1*	NM_000088	c.1054_1056+2del	Splicing	Novel	V	Heterozygous	AD


a
LS
LF
RF
DXA T-score
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
*
*
b
LS % change BMD
A.4 Δ +28% (T)
B.3 Δ +16%
G.1 Δ +15%
E.3 Δ +14% (T)
F.1 Δ +12%
yr postpartum
c
LF % change BMD
A.4 Δ +33% (T)
B.3 Δ +20%
E.3 Δ +14% (T)
G.1 Δ +8%
F.1 Δ +7%
yr postpartum
d
RF % change BMD
A.4 Δ +33% (T)
E.3 Δ +18.5% (T)
G.1 Δ +12%
B.3 Δ +7%
F.1 Δ +6%
yr postpartum
e
Tb.N
Tb.Th
Tb.Sp
Ct.Th
Ct.BMD
Radius
Tibia
B.3 C.4 D.1 E.3 F.1 G.1

◀ **Fig. 2** Bone mineral density and bone microarchitecture. **a** DXA T-score as mean and standard deviation (SD) for any site (black column) as well as its subgroups: mutation (bright-gray) vs. no mutation (dark-gray); asterisks indicate significant differences of mean values for each site, respectively ($p < 0.05$). **b**–**d** BMD follow-up data in percent change from baseline for any site: mutation (bright-gray), no mutation (dark-gray). **e** HR-pQCT data: horizontal lines indicate the normative HR-pQCT data [17]: upper gray solid line (90th centile), upper gray dashed line (75th centile), black solid line (50th centile), lower gray dashed line (25th centile), and lower gray solid line (10th centile). T teriparatide

Discussion

The genetic analysis detected pathogenic variants of bone-specific genes in 3/7 cases, which are known to cause monogenic bone disorders (e.g., osteogenesis imperfecta) with a subsequently compromised skeletal status in terms of bone turnover, mass, and quality. This finding has several implications for (1) the pathogenesis, (2) the diagnostic proceeding, and (3) the general understanding and definition of PAO.

First, these data indicate the relevance of pathogenic variants for the pathogenesis of PAO. This strongly supports the hypothesis of a preexisting low bone mass, which is exposed to additional influences. Especially, the course of pregnancy and lactation is associated with an increasing calcium transfer to the offspring [27], which compromises the maternal calcium homeostasis. Therefore, pregnancy and lactation should be considered a skeletal risk factor, which promotes the initial onset of previously unrecognized monogenic bone disorders. The fact that none of the three women showed any symptoms (e.g., fragility fractures, connective tissue, or tooth anomalies) before the occurrence of the pregnancy further pronounces the impact of pregnancy and lactation on the skeleton. Regarding the other cases, it is within the range of possibilities that other genetic alterations such as single-nucleotide polymorphism are present contributing to the low bone mass phenotype. However, they would not have been detected by this panel analysis, since only coding exons were screened. Besides the impact of genetics, additionally, deteriorating factors include an insufficient enteral calcium resorption (e.g., vitamin D deficiency [28], inflammatory bowel diseases [29]), drug side effects (e.g., steroids [30], proton pump inhibitors [31]), or immobilization [7], which possibly overburden the bone microarchitecture. A similar phenomenon is the first manifestation of a mild maturity-onset diabetes of the young (MODY) type 2 as gestational diabetes [32]. Therefore, screening for mutations causing MODY is recommended in cases of gestational diabetes with positive family history or other indications for a hereditary form of diabetes [33].

Secondly, this knowledge needs to be implemented in the diagnostic proceeding. Given our data, PAO patients have an increased risk to be affected by gene mutations. This is supported by previous case reports [13, 14]. Therefore, they should be examined carefully in order to detect hereditary diseases and to apply preventive measures. Interestingly, Hadji and colleagues identified dental problems and less sport activity in childhood as risk factors for the development of PAO [7]. Especially, dental problems (e.g., tooth loss, recurrent inflammation) might indicate the presence of hereditary disorders in this cohort [18, 34, 35], which would be in line with our findings. This is not only of relevance for the primarily affected individuals but also beneficial for other family members—especially for the offspring. Taken together, consistent genetic analyses are required in those women who present with characteristic symptoms of hereditary disorders before pregnancy and obviously in those with an already manifest PAO phenotype.

Third, we do believe that our presented data make a scientific discussion desirable in order to clarify whether such patients with detected monogenic bone disorders should be referred to as PAO or rather be primarily diagnosed with this underlying disorder and clinical onset in pregnancy. Even though this might be regarded as minor issue, we do propose that the latter approach is rather suitable since it addresses the most likely congenital low bone mass while the term PAO (i.e., secondary osteoporosis) implicates a secondary bone loss of a previously normal status. This knowledge is of major relevance in terms of the expected recovery potential for the patients. However, one might indeed argue that the term PAO should be limited to the clinical description of this rare phenotype and should not be a diagnosis. Thus, continuative diagnostics are required.

Regarding the skeletal assessment of the PAO phenotype, it is noteworthy that the overall BMD reduction in DXA scans was more pronounced at the lumbar spine, contributing to the development of vertebral compression fractures. Furthermore, the HR-pQCT scans revealed a predominant reduction of the trabecular and cortical thickness, while the trabecular number remained within the normal range. This finding is in line with a previous report of bone microarchitecture in PAO [36] and supports the hypothesis of a temporal calcium demand, which might be covered by the maternal skeleton.

Based on the presented data and our extensive clinical experience, we suggest the following two-stage concept in the treatment of PAO phenotypes: the basic therapy aims to improve the maternal calcium supply by timely weaning [8, 37, 38] and supplementation of vitamin D [39] and calcium [38, 40], if indicated by insufficient serum levels. After delivery and weaning, additional measures become available including osteoanabolic (teriparatide [41]) or antiresorptive (denosumab [36]) drugs (specific therapy). The application of bisphosphonates (i.e., neridronate) is not recommendable in women of childbearing age because the active substance is deposited in the bone matrix and detrimental effects in future pregnancies cannot be excluded with any certainty. However, also, the withdrawal of denosumab may be followed by a high-turnover state, which needs to be monitored. Otherwise, a

decreasing BMD with increased fracture risk might be the result. Using this concept, we were able to observe significant increases in DXA follow-up scans (up to 33%), which indicate the ability of the skeleton to recover [42, 43]. Besides this described bone-specific approach, pregnant individuals with known monogenic bone disorders (e.g., osteogenesis imperfecta) may furthermore benefit from a multidisciplinary treatment in a referral center in order to also monitor other complications of connective tissue disorders and the fetal well-being [44]. Whether a cesarean section is associated with fewer complications than vaginal delivery remains controversial but needs to be considered and decided on an individual basis.

As a limitation, the sample size of this study is small. Thus, conclusions concerning the frequency of monogenic bone disorders in patients with PAO are not possible and future studies are required. However, this study represents the first genetic analysis in a series of seven cases and provides new indications for pathophysiologic considerations, diagnostics, and treatment.

In conclusion, the genetic analysis revealed previously unrecognized monogenic bone disorders (e.g., osteogenesis imperfecta) in three women with PAO. This finding suggests that PAO should not be a diagnosis but rather a descriptive term for the phenotype and that continuous diagnostics (i.e., genetic analysis) are required. Pregnancy and lactation should be considered a skeletal risk factor, which can promote the initial onset of hereditary bone diseases. Not only the affected individuals but also the relatives can benefit from this knowledge. The implementation of preventive and therapeutic measures in the clinical management may prevent pregnancy-associated complications and enable the skeletal recovery after delivery. Future genetic studies are needed to determine the frequency of monogenic bone disorders in women with PAO.

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Compliance with ethical standards

This prospective approach is legitimated by approval through the local ethics committee (No. PV5364) and all investigations were carried out in accordance with the World Medical Association Declaration of Helsinki.

Conflicts of interest None.

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