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Research Article

Primary hypertrophic osteoarthropathy: phenotypic variability and penetrance rate in heterozygotes for *SLCO2A1* variants

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Abstract

Primary hypertrophic osteoarthropathy (PHO) is a rare autosomal recessive disease caused by pathogenic variants (PVs) in *HPGD* and *SLCO2A1* genes whose phenotypes are, respectively, designated as PHOAR1 and PHOAR2. Recently, a dominant inherited form (PHOAD) was identified in *SLCO2A1* heterozygotes whose PHO penetrance is widely unknown, and data on phenotype are markedly limited. Our aim was to reveal the penetrance and extend/refine data on phenotype of *SLCO2A1* heterozygotes. Both genes were sequenced using Sanger sequencing. The 4 probands had a typical complete form (CF) of PHO. Mean ages at symptom onset and clinical diagnosis were, respectively, 18.5 ± 2.7 (16–22) years and 22 ± 3.4 (18–26) years. They were homozygotes for *SLCO2A1* (p.Q188R, p.C420F, p.A176T; p.G104*) PVs; 2 were novel variants. We focused on 14 *SLCO2A1* heterozygous screened relatives from 3 families: 5 elderly individuals (mean age: 78 ± 6.7 [72–86] years) of the parental generation were affected, 2 by incomplete form (IF) and 3 with isolated digital clubbing (IDC). Combining our 14 carriers and 33 reported so far, the estimated overall PHO penetrance was 70%, being significantly higher in men (83% vs 50%; $p = .024$) and individuals carrying truncated *SLCO2A1* PVs (88% vs 53%; $p = .053$). In turn, the periostosis penetrance rate in women was 28% (5/18), including our oldest patient (86 years). In the probands, the predominant phenotypes were CF (64%) and IF (36%). Among screened carriers, phenotypes were IDC (41%) followed by IF and fruste form (FF) (28%, each), whereas IDC and FF were the predominant phenotypes in screened men and women, respectively. As a novelty, we uncovered an incomplete penetrance of PHO in *SLCO2A1* heterozygotes, with higher rates in elderly individuals, males, and those with truncated PVs. Regarding phenotype, PHO is more pronounced in males, periostosis is likely more frequent in females than previously documented in PHOAR2, and IDC may represent a distinct clinical feature in *SLCO2A1* heterozygotes.

Keywords: molecular diagnosis, pachydermoperiostosis, primary hypertrophic osteoarthropathy, *cutis verticis gyrata, SLCO2A1* gene, penetrance

Lay summary

Primary hypertrophic osteoarthropathy (PHO) is a rare genetic disorder affecting bones and skin. It causes bone growth around the edges of bones, thickened skin, widening of fingers and toes, and wrinkling on the scalp. PHO has 2 forms: the recessive form (PHOAR1 and PHOAR2), which occurs when both parents pass on faulty genes, and the dominant form (PHOAD), where only 1 parent passes on the faulty gene. Our study found that PHO in the dominant form does not appear in all carriers, but is more common in older individuals, men, and those with specific genetic changes.

Introduction

Primary hypertrophic osteoarthropathy (PHO [OMIM 167100]), also known as pachydermoperiostosis (PDP), is a rare inherited genetic disease. Its clinical manifestations primarily involve bone and skin. The main features are represented by the triad periostosis, pachydermia, and digital clubbing, including *cutis verticis gyrata* (CVG).[1–8] PHO is usually classified into complete, incomplete, and fruste forms. In the complete form, CVG is always associated with the 3 main symptoms (periostosis, pachydermia, digital clubbing), whereas the incomplete form is characterized by absence of CVG. In turn, isolated pachydermia or mild degrees of periostosis identify the fruste form.[1,3–8]

Several other bone and rheumatologic manifestations may be present in PHO such as acro-osteolysis, painful joint enlargement and deformity, facial skin and/or scalp thickening, and coarse facial features with oily skin, seborrhea, acne, and hyperhidrosis resembling acromegaly.[1,4,5,9–11] Moreover, in early childhood, delayed closure of cranial sutures and fontanelles has been observed along with persistence

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of the ductus arteriosus in 25% of cases. It is noteworthy that, except for the cranial and cardiac manifestations, all other PHO symptoms may be found in patients with secondary hypertrophic osteoarthropathy (SHO).[4,9] SHO is mainly related to chronic hypoxia resulting from neoplastic conditions and cardiopulmonary diseases such as cystic fibrosis and uncorrected congenital cyanotic heart disease. Importantly, the diagnosis of PHO should be established after secondary causes of hypertrophic osteoarthropathy are excluded.[1–3,6,7,9]

PHO was first described in 2 affected brothers[12] and subsequently was distinguished from SHO.[13,14] Before discovery of mutations in 2 PHO-causing genes (2008–2012), early reports recognized the familial nature and proposed both autosomal recessive and dominant inheritance patterns.[1–3] Thus, Castori et al.[1] reviewed clinical and genealogical data of 204 patients with PHO from 68 PHO families and associated the dominant inheritance with 54.4% of them and recessive inheritance for the remaining families.

Genomic studies documented the discovery of the 2 genes associated with the PHO phenotype.[2–5,9] Thus, homozygous mutations in the hydroxy-prostaglandin dehydrogenase gene (*HPGD*) were described, consistent with the previously suggested autosomal recessive inheritance in PHO.[2] The *HPGD* gene encodes the 15-HPGD enzyme, which is responsible for prostaglandin degradation at the cellular level.[2–9] In 2012, a homozygous germline mutation in the solute carrier organic anion transporter family member 2A1 gene (*SLCO2A1*) was identified by exome sequencing in a PHO case from a consanguineous family, and its complicity was validated by detection of compound heterozygous *SLCO2A1* mutations in 2 PHO cases in nonconsanguineous families.[3] Other studies associated germline *SLCO2A1* mutations with PHO, with or without myelofibrosis, and isolated familial digital clubbing[4–7,9,15] and, more recently, with enteropathy.[16,17] The *SLCO2A1* gene encodes the prostaglandin transporter protein (PGT), responsible for transporting prostaglandin across the plasma membrane.[3–9] These genetic findings reinforced the previously postulated existence of an autosomal recessive inheritance pattern in PHO.[1,18] Thus, mutations in both *HPGD* and *SLCO2A1* genes result in loss-of-function proteins, directly involved in prostaglandin metabolism.[2–9] Concordantly, patients with PHO have chronic elevated circulating levels of prostaglandins, which are responsible for the pathogenesis of this rare disorder.[2–9]

Based on this genetic background, PHO has been categorized as follows: (1) primary hypertrophic osteoarthropathy, autosomal recessive 1 (PHOAR1; OMIM 259100), caused by *HPGD* gene deficiency, and (2) primary hypertrophic osteoarthropathy, autosomal recessive 2 (PHOAR2; OMIM 614441), caused by *SLCO2A1* deficiency. However, early reported patients and families with phenotypes similar to those reported in association with *SLCO2A1* mutations showed genealogies suggesting the existence of an autosomal dominant inheritance pattern.[1,7] Recently, Xu et al.[19] published the first study, including 12 Chinese families, relating the *SLCO2A1* gene to a dominant autosomal form defined as PHOAD (OMIM: 167100) by the finding of monoallelic mutations associated with the PHO phenotype.

Since the discovery of *HPGD* and *SLCO2A1* mutations, and even before, case reports and small PHO series have been described worldwide, while large cohorts have been mostly documented on the Asian continent.[2,3,5–7,19–22] Overall, 13 case reports of Brazilian patients with PHO have been reported, most of them as brief communications, with no supported genetic diagnosis.[23–35]

Notably, heterozygous PHO cases showed high phenotypic variability, which was usually associated with milder later-onset phenotypes and lower penetrance than those documented in homozygous or compound heterozygote cases of *SLCO2A1* mutation.[19] Recently, monoallelic *SLCO2A1* mutations were reported by deep sequencing in 4 heterozygous probands with PHO, excluding a second *SLCO2A1* pathogenic variant and strengthening the existence of autosomal dominant inheritance.[8] However, the phenotype description of heterozygous *SLCO2A1* carriers is scarcely known as it is still limited to a few cases.[8,19] In the present study, we identified 4 unrelated PHO probands associated with *SLCO2A1* germline mutations. Additionally, we expanded the phenotypic landscape of heterozygous relatives, validating/ratifying the combined presence of both autosomal dominant and recessive inheritance models associated with *SLCO2A1* mutations. Finally, we documented variable *SLCO2A1*-related PHO penetrance patterns, modulated by gender, age, and genotype.

Materials and methods

The study was conducted at the Hospital das Clinicas, University of São Paulo School of Medicine, after approval by the local (CAPPesq) and national (CONEP) ethics committees. All 4 unrelated index cases were clinically diagnosed with the complete form of PHO. These probands and their at-risk family members provided written informed consent for clinical examination, genetic testing, genetic counseling, and photos.

Genomic DNA was extracted from peripheral blood samples. Primers, used to amplify genomic material by PCR, were designed from tools accessible on the NCBI website (Primer designing tool) to cover the entire coding regions and intron/exon boundaries of the 7 *HPGD* exons and the 14 *SLCO2A1* exons (Tables S1 and S2). PCR reactions and thermocycling conditions were optimized for each gene (**Supplementary data**; Figures S1–S3). Sequencing reactions were performed from purified PCR products using primers and Big Dye Terminator v3.1 (Applied Biosystems, Foster City, CA). According to the manufacturer's recommendation, analyses were carried out on an automated sequencer (ABI Prism 3130XL; Applied Biosystems).

The classification of genetic variants was performed following the American College of Medical Genetics and Genomics and the Association for Molecular Pathologists (ACMG-AMP) recommendations.[36] The following databases were used to analyze the impact and effects of variants: the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php), GnomAD (http://gnomad.broadinstitute.org), ABraOM (http://abraom.ib.usp.br/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), Varsome https://varsome.com/, ClinGen (https://clinicalgenome.org/), and Franklin (https://franklin.genoox.com/clinical-db/home). Overall, aggregated meta-predictive algorithms were applied to analyze the impact and effects of variants.

Results

Overall, the genetic and clinical data of all 4 unrelated index cases diagnosed with a complete form of PHO are presented (Table 1). The mean ages at symptom onset

Table 1. Overall data, clinical manifestations, and *SLCO2A1* pathogenic variants of 4 unrelated probands presenting the complete form of primary hypertrophic osteoarthropathy (PHO).

	Cases			
	1	**2**	**3**	**4**
Gender	Male	Male	Male	Male
Age at onset of symptoms/signs, yr	16	17	19	22
Age at clinical diagnosis, yr	21	18	23	26
Age at molecular diagnosis, yr	57	48	38	47
Actual age, yr	63	55	38	47
Time elapsed between the first symptoms and clinical diagnosis, yr	5	1	4	4
Consanguineous (parental generation)	No	No	Yes	Yes
Familial history of PHO	Yes	No	No	No
Skin features				
Pachydermia	Yes	Yes	Yes	Yes
Blepharoptosis	Yes	Yes	No	Yes
Cutis verticis gyrate	Yes	Yes	Yes	Yes
Eczema	No	No	No	No
Hyperhidrosis palmar and plantar	Yes	Yes	Yes	Yes
Acne	No	No	No	No
Seborrhea	Yes	Yes	Yes	Yes
Osteoarticular features				
Acro-osteolysis	No	Yes	Yes	Yes
Arthritis/arthralgia	Yes	Yes	Yes	Yes
Digital clubbing/periostosis	Yes	Yes	Yes	Yes
Arthrosis/hydrarthrosis/hemarthrosis	Yes	No	Yes	Yes
Wide cranial sutures	No	No	No	No
Other manifestations				
Gastrointestinal involvement (gastritis, peptic ulcer)	No	Yes	No	No
Myelofibrosis/BMH	No	No	No	No
Patent ductus arteriosus	No	No	No	No
Associated enteropathy (diarrhea)	No	Yes	No	No
Anemia	No	Yes	No	No
Cardiovascular anomalies	Patent oval foramen	No	No	No
***SLCO2A1* variants**				
Exon	4	9	4	3
Nucleotide	c.563 A > G	c.1259G > T	c.526G > A	c.310 G > T
Protein	p.Q188R	p.C420F	p.A176T	p.G104*
Type	MS	MS	MS	NS
Homozygote	Yes	Yes	Yes	Yes
MAF (GnomAD) (%) #	0.00	0.0012	0.00	0.000479
ACMG/AMP criteria ##	PM2, S PM3, S PP4, VS	PS3, S PM2, M PM3, S PP3, M PP4, VS	PM2, S PM3, M PP4, VS	PVS1, VS PS4, M PM3, M PM2, S PP4, VS
ACMG/AMP classification	PV	PV	PV	PV
First report	Novel	Diggle et al.[9]	Novel	Diggle et al.[9]

Transcript, NM_005630.3. Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics/Association for Molecular Pathologists; M, moderate pathogenicity; MAF, minor allele frequency; MS, missense; NS, nonsense; PV, pathogenic variant; S, supporting pathogenicity; S, strong pathogenicity; *SLCO2A1*, solute carrier organic anion transporter family member 2A1; VS, very strong pathogenicity.

and clinical and genetic diagnosis were, respectively, 18.5 ± 2.7 years (range: 16–22 years), 22 ± 3.4 years (range: 18–26 years), and 47.5 ± 7.8 years (range: 38–57 years). The mean time elapsed between the first symptoms and clinical diagnosis was 3.5 ± 1.7 years (range: 1–5 years). A detailed clinical description of each of the index cases is presented in the **Supplementary data.**

Investigation and analysis of allelic variants

Case 1

The novel missense allelic variant c.563A > G (p.Q188R) (NM_005630.3) was identified in case 1. This variant, located in *SLCO2A1* gene exon 4, results from the homozygous substitution of adenine with guanine at position 563 of the DNA, leading to a glutamine (Q) to arginine (R) change at codon 188 of the protein. Figure 1A demonstrates this variant with the nucleotide exchange from A > G (arrow). According to ACMG/AMP criteria, this variant was classified as pathogenic: PM2, supporting; PM3, supporting; and PP4, very strong (Table 1). This variant, even in heterozygotes, was absent in all accessed major public genomic databases (gnomAD, ABraOM) as well as in variant repositories such as ClinVar (ncbi.nlm.gov/projetcs/snp), OMIM (ncbi.nlm.gov/projetcs/snp), and HGMD (Human Gene Mutation Database; hgmd.fac.uflac/index.php). Its pathogenicity was reinforced by in silico programs such as BayesDel addAF metaRNN, and AlphaMissense, among others, classifying this variant as deleterious.

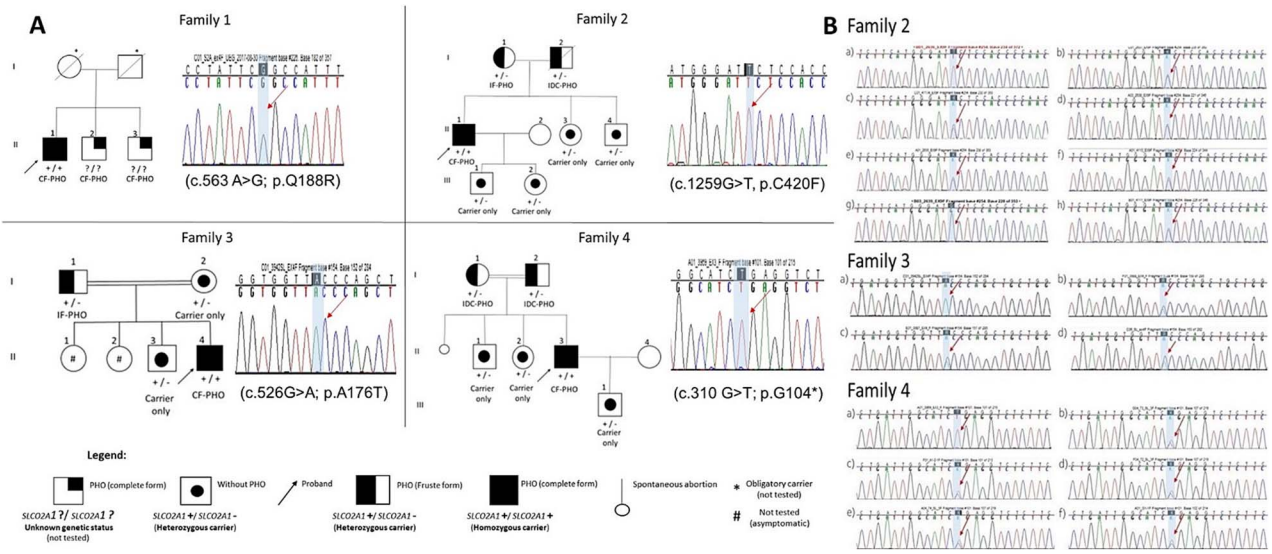

Figure 1. A: Genealogies of the 4 index cases with clinical diagnosis of the complete form of primary hypertrophic osteoarthropathy (CF-PHO) and electropherograms documenting the pathogenic allelic variants (PVs), in homozygosity, found in these index cases. *Obligate carrier of PV; family members carrying PVs in the solute carrier organic anion transporter family member 2A1 (*SLCO2A1*) gene, in heterozygosity, presenting with the incomplete (IF-PHO)/fruste (FF-PHO) form or without PHO. B: Electropherograms showing the PVs of the 3 pedigrees whose at-risk family members were genetically screened—Family 2: electropherogram showing the PV c.1259G > T (p.C420F, rs387907295), in homozygosity, in the index case and, in heterozygosity, in the screened at-risk family members: a) index case, b) mother, c) father, d) wife (normal control), e) son, f) daughter, g) brother, h) sister; family 3: electropherogram of PV c.526G > a (p.A176T), in homozygosity, in the index case and, in heterozygosity, in the screened at-risk relatives: a) index case, b) father, c) mother, d) brother; family 4: electropherogram of PV c.310G > T (p.G104*), in homozygosity, in the index case and, in heterozygosity, in the screened at-risk relatives: a) index case, b) mother, c) father, d) sister, e) brother, f) son.

Case 2

The previously described pathogenic allelic variant,[9,37] in homozygosity, was identified (c.1259G > T, p.C420F, rs 387 907 295; ClinVar, variation ID: 37169) (NM_005630.3) in exon 9 of the *SLCO2A1* gene. The substitution of guanine with thymine at DNA position 1259 results in the substitution of the amino acid cysteine (C) with phenylalanine (F) at codon 420 of the protein. Figure 1A shows the gene variant found in case 2 with PHO (arrow). This variant was classified by ACMG/AMP as pathogenic, based on the following criteria: PS3, strong; PM2, moderate; PM3, strong; PP3, moderate; and PP4, very strong (Table 1). This variant is rarely found in population genomic databanks: gnomAD (aggregated) and gnomAD (exome) with a minor allele frequency (MAF) of 0.0012%, being 3 alleles, in heterozygosity, from 251 432 and coming from an admixed American subpopulation (MAF, 0.0087%). It was absent in ABraOM and all other databanks. The aggregated prediction score (0.85) classified it as a deleterious resultant of the most in silico tools predicting pathogenicity. In addition, a previous functional study demonstrated that the C420F construct stimulated only 10% or less of the uptake of radiolabeled PGE2 compared with that stimulated by a WT construct.[9]

Case 3

A novel pathogenic variant was found in exon 4 of the *SLCO2A1* gene, in homozygosity, at position 526 of the complementary DNA (c.526G > A; p.A176T) (NM_005630.3) (Figure 1A). The substitution of guanine with adenine at position 526 of the complementary DNA results in the substitution of the amino acid alanine (A) with threonine (T) at codon 176 of the SLCO2A1 protein. The variant found (variant location 3-133 955 065-C-T) has not thus far been described in gnomAD, BRAVO, and ABraOM population databases as well as in variant databases such as HGMD, ClinVar, and dbSNP. According to ACMG/AMP, this variant was classified as pathogenic: PM2, supporting; PM3, moderate; and PP4, very strong (Table 1).

Case 4

A known pathogenic nonsense variant (c.310 G > T; p.G104*; p.Gly104Ter; rs387907297) (NM_005630.3) was found, in homozygosity, in *SLCO2A1* exon 3 (Figure 1A). The substitution of a guanine with thymine at position 310 of the complementary DNA (c.310 G > T) generated a premature stop codon (nonsense variant) corresponding to the amino acid glycine, located at codon 104, resulting in a truncated protein p.G104* (p.Gly104Ter). This variant was previously reported, in heterozygosity, in the compilation of exomes from the gnomAD population database (variant location 3-133 973 750-C-A) in an admixed American individual, resulting in an MAF of 0.00224% (1:44 712 alleles evaluated) and in 6 individuals from a non-Finnish European subpopulation (MAF, 0.000539%; 1:185 330 alleles). Based on all evaluated populations, the variant prevalence was 0.000479% (1:208 821). In the BRAVO population database, 4 individuals carrying this variant were found, all in heterozygosity, resulting in an estimated prevalence of 0.001511% (1:66 172). It was absent in ABraOM, and it was not found in the gnomAD genome, where the percentage of samples with over 20× coverage was 94.99%. Furthermore, this same variant was associated, in homozygosity, with both complete and incomplete forms of PHO and, in heterozygosity, with isolated digital clubbing (IDC).[1,5,9,15] Other PVs, found in the same position (c.310G > A; p.G104R), were reported by Xiao et al.,[22] strengthening the pathogenicity of the variant p.G104*. Thus, by ACMG/AMP, this variant was classified as

Table 2. Genetic diagnosis and phenotype of index cases and their at-risk relatives.

Individual	Relatives	Variant	Phenotype
Index case 1 (II-1)			
II-1	Proband, 63 yr	c.563 A > G (p.Q188R) homozygosis	PHO (complete form)
II-2	Brother, 53 yr	Unknown	PHO (complete form)
II-3	Brother, 50 yr	Unknown	PHO (complete form)
Index case 2 (II-1)			
II-1	Proband, 55 yr	c.1259G > T (p.C420F) homozygosis	PHO (complete form)
I-1	Father, 81 yr	c.1259G > T (p.C420F) heterozygosis	Digital clubbing
I-2	Mother, 86 yr	c.1259G > T (p.C420F) heterozygosis	Incomplete form: periostosis, pachydermia
II-3	Sister, 52 yr	c.1259G > T (p.C420F) heterozygosis	Normal
II-4	Brother, 50 yr	c.1259G > T (p.C420F) heterozygosis	Normal
III-1	Son, 17 yr	c.1259G > T (p.C420F) heterozygosis	Normal
III-2	Daughter, 13 yr	c.1259G > T (p.C420F) heterozygosis	Normal
Index case 3 (IV-6)			
IV-6	Proband, 38 yr	c.526G > A (p.A176T) homozygosis	PHO (complete form)
III-1	Father, 73 yr	c.526G > A (p.A176T) heterozygosis	Incomplete form: digital clubbing, periostosis
III-2	Mother, 65 yr	c.526G > A (p.A176T) heterozygosis	Normal
IV-5	Brother, 45 yr	c.526G > A (p.A176T) heterozygosis	Normal
Index case 4 (II-3)			
II-3	Proband, 47 yr	c.310G > T (p.G104*) homozygosis	PHO (complete form)
I-1	Father, 74 yr	c.310G > T (p.Gly104T*) heterozygosis	Digital clubbing
I-2	Mother, 72 yr	c.310G > T (p.Gly104T*) heterozygosis	Digital clubbing
II-1	Brother, 39 yr	c.310G > T (p.Gly104T*) heterozygosis	Normal
II-2	Sister, 52 yr	c.310G > T (p.Gly104T*) heterozygosis	Normal
III-1	Son, 22 yr	c.310G > T (p.Gly104T*) heterozygosis	Normal

I-IV, correspond to position of each patient in the genealogy (see Figure 1 for localization; eg, II-2 refers to the second patient represented from left to right in the first generation). Pathogenic germline allelic variants in the *SLCO2A1* gene were found in index cases 1 to 4, in homozygosity, and in at-risk relatives, in heterozygosity. Abbreviations: PHO, primary hypertrophic osteoarthropathy; *SLCO2A1*, solute carrier organic anion transporter family member 2A1.

pathogenic based on the following criteria: PVS1, very strong; PS4, moderate; PM3, moderate; PM2, supporting; PP4, very strong (Table 1).

Familial segregation of pathogenic variants

Case 1

Case 1 denied consanguinity in his history of the parents who showed no apparent symptoms and signs of the disease. His father died from diabetes at the age of 77 and his mother at 78 of unknown cause (Figure 1A). His father had 3 healthy daughters from his first marriage with a Spanish woman, and also 9 sons in his second marriage with a Palestinian woman. He was the first family member to be diagnosed with PHO at the age of 21. Subsequently, 2 brothers living in the West Bank developed progressive symptoms and signs associated with PHO, and the PHO diagnosis was clinically confirmed at the ages of 19 and 21, respectively. The 2 brothers with PHO are currently 53 and 50 years old (Figure 1A) (Table 2). One of them underwent facial plastic surgery, while the other has never undergone treatments for PHO. From the information obtained, all other siblings are healthy and with no apparent signs or symptoms related to PHO.

Case 2

There was no apparent consanguinity of the parents who were born in small neighboring cities in the southern mountainous area of Minas Gerais State (Figure 1A). At genetic screening, all 6 relatives at risk were characterized as carriers of the pathogenic variant c.1259G > T (p.C420F, rs 387 907 295), in heterozygosity (Figure 1B) (Table 2).

Based on clinical history, photographic analysis, and comparison with the patient, a milder degree of PHO was suspected in his deceased paternal grandfather. This suspicion was due to (1) the disproportionate size of his feet (photograph not provided due to its low quality), (2) the presence of mild/moderate digital clubbing of the third, fourth, and fifth digits of the right hand, and (3) mild clubbing of the second and third digits of the left hand observed in his father (Figure 2A). The death of the index case's father in 2022 (81 years) prevented us from performing radiological examinations to investigate periostosis. Clinically, 2 brothers, the mother, and 2 children of the index case had no complaints or clinical history suggestive of PHO (Table 2). Thus, the familial history initially suggested an autosomal dominant inheritance pattern with variable phenotypic presentation across 3 generations. Evaluation of photographs of the index case's mother, 86 years old, indicated the absence of digital clubbing. There was also no *cutis verticis gyrata*. However, radiological examination revealed mild periostosis in the hands and feet and mild skin thickening suggestive of pachydermia (Figure 2B and C). Radiological examinations of the index case's son (17 years old), sister (52 years old), and brother (50 years old) were normal, with no periostosis, and photographic analysis was negative for digital clubbing. None of them had *cutis verticis gyrata*. The physical examination of the index case's daughter, aged 13 years, was normal. All of the mentioned relatives (father, mother, son, daughter, brother, and sister) were heterozygous for the pathogenic variant c.1259G > T (p.C420F, rs 387 907 295).

Case 3

With the detection of the pathogenic variant c.526G > A (p.A176T) in index case 3, genetic counseling was offered to at-risk relatives. This variant, in heterozygosity, was

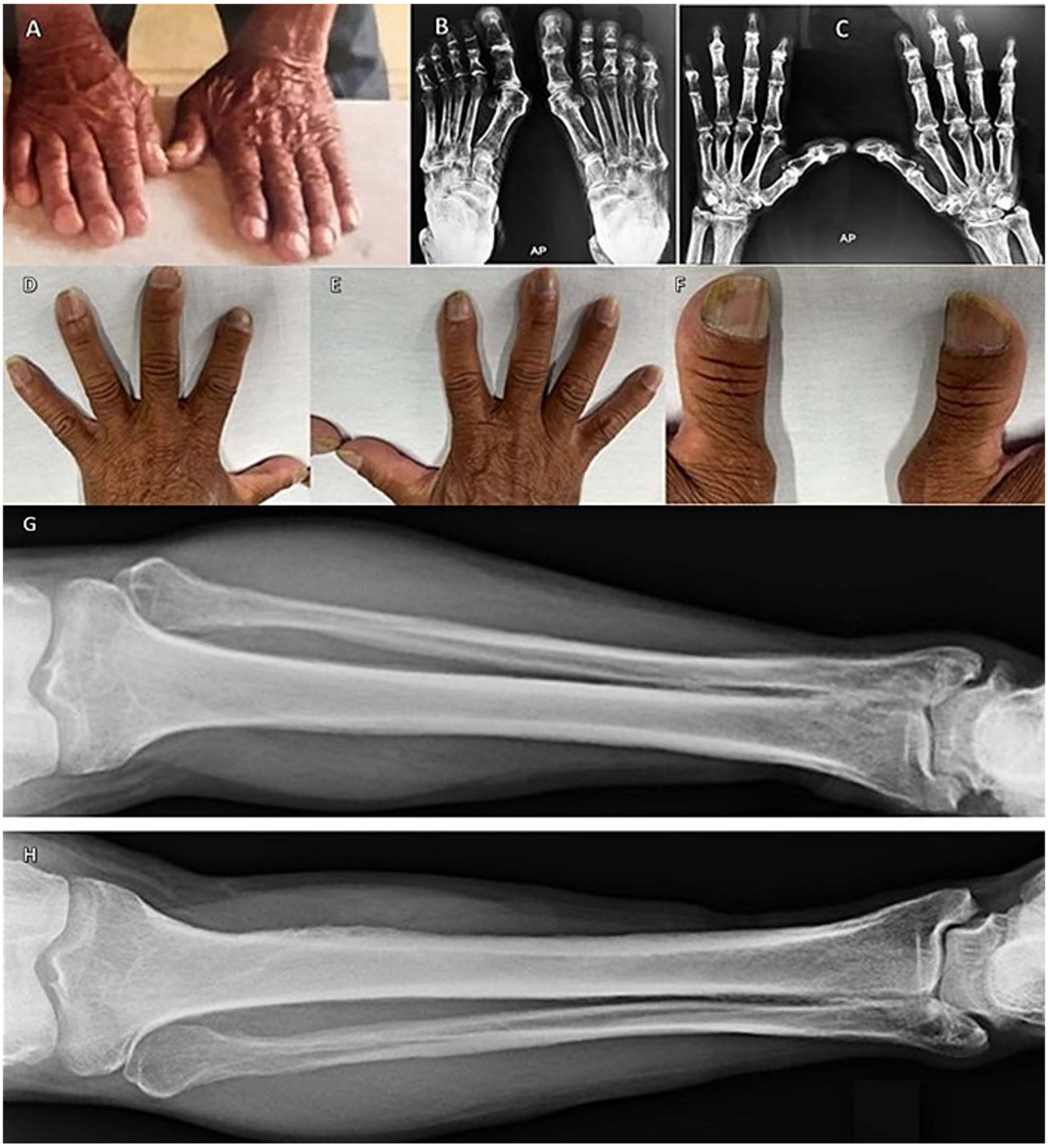

Figure 2. A: Index case 2's father showing moderate digital clubbing of the third, fourth, and fifth digits of the right hand and mild clubbing of the second and third digits of the left hand. B and C: Index case 2's mother: Radiographic image showing mild periosteal reaction present in the hands and feet associated with pachydermia. D–H: Index case 3's father showing mild clubbing of the second and third digits of the left hand (D); mild digital clubbing of the third, fourth, and fifth digits of the right hand (E); enlargement of the first digit of both hands, suggesting periostosis (not confirmed by radiography) (F); and evident periostosis in both tibias and fibulas (G and H).

documented in the patient's parents and in 1 sibling (Figure 1A) (Table 2). The electropherograms of the index case, his parents, and his brother are shown (Figure 1B). In the genealogy, there were no other PHO cases despite reports of at least 2 consanguineous marriages in the family, including the index case's parents who were first cousins. On physical examination, there was clinical suspicion that the index case's father, an obligate heterozygote, exhibited a mild and clinical fruste form of PHO. Indeed, the index case's father, aged 73 years old, had mild digital clubbing of the third, fourth, and fifth digits of the right hand and mild clubbing of the second and third digits of the left hand, as well as distal enlargement of the first digit of both hands, suggesting periostosis (Figure 2D–F). However, radiological examination did not confirm hand periostosis, although periosteal reactivity was evident in both tibias and fibulas (Figure 2G and H). The patient did not have *cutis verticis gyrata*. The index case's mother and brother also did not have *cutis verticis*

gyrata, pachydermia, or digital clubbing, and radiological examinations were negative for periostosis (Table 2).

Case 4

After identification of the PV c.310G > T (p.G104*), the family of index case 4 was invited to receive genetic counseling. Upon history taking, consanguinity was documented as the index case's parents were third-degree cousins (Figure 1A). The presence of the variant c.310G > T, in heterozygosity, was verified in the following evaluated family members: mother (I-1), father (I-2), brother (II-1), sister (II-2), and son (III-1) (Table 2, Figure 1A). The electropherograms of the index case and the relatives are presented in Figure 1B. Radiological examinations of his 74-year-old father, the 72-year-old mother, as well as of 2 brothers (39 and 52 years old) and the son (22 years old) did not reveal any periosteal reactivity in the hands, feet, and long bones. On physical examination, minimal digital clubbing of the second digit of both hands was identified in the father and of the second and third digits in the mother (Table 2).

Estimated penetrance of PHO in *SLCO2A1* heterozygotes

Overall, 14 *SLCO2A1* heterozygotes were identified from 3 families (2–4) genetically screened from our cohort and 5 of them manifested mild clinical forms of PHO, with an estimated penetrance of 36%. Aiming to refine the PHO penetrance rate in *SLCO2A1* heterozygous carriers, we compiled our casuistic with the cohort of 19 *SLCO2A1* heterozygous individuals reported by Xu et al.[19] and with 4 heterozygous probands reported by Bloch et al.[8] The former cohort proved the existence of the autosomal dominant inheritance model, documenting 7 families who were genetically screened whose affected members segregated *SLCO2A1* PVs, in heterozygosity, as well as by another 5 families whose probands, homozygotes, or compound heterozygotes for *SLCO2A1* had obligate heterozygous parents manifesting PHO disease.[19]

By this approach, we identified 47 heterozygous *SLCO2A1* carriers reported so far. The estimated overall penetrance was 70%, being higher in men (males: 24/29 [82.8%]; females: 9/18 [50%]; p = .024) (Table 3). The estimated penetrance rate of 12 heterozygous family members (probands excluded) belonging to 7 PHOAD families reported by Xu et al.[19] was 92% (11/12), while the PHO penetrance rate was 46% (11/24), including the 14 heterozygotes from our casuistic with 10 *SLCO2A1* heterozygous carriers from 5 PHOAR2 families reported by Xu et al. (Table S3).

All our 5 affected heterozygous carriers were elderly individuals (septuagenarians and octogenarians), and parents of homozygous *SLCO2A1* probands (Table 2). Thus, PHO was identified in 5 of the 6 members of the parental generation, with a mean age of 77 ± 6 years (72–86 years) (Tables 2 and 4), while clinical manifestation was absent in all 9 younger cases aged 39.4 ± 18 years (13–65 years).

Age at the PHO diagnosis of the 4 probands reported by Bloch et al.[8] was available as well as for 8 cases (probands/relatives) from Xu et al.[19] (Table 4). Combining all 17 affected *SLCO2A1* heterozygous cases, the mean age at PHO diagnosis was 49.3 ± 22.5 years (14–86 years) (Table 4). The youngest case was an adolescent proband aged 14 years old reported by Bloch et al.,[8] who presented the complete form of PHO, while the oldest case was an 86-year-old woman in the present study who presented minimum pachydermia and mild periostosis (Table 4, Figure 2).

Overall, 19 different *SLCO2A1* PVs were associated with affected heterozygotes, including patients of the present study and those previously reported as *SLCO2A1* heterozygotes by Xu et al.[19] and by Bloch et al.[8] (Figure 3). Concerning variant types, 10 of these were missense, another 5 were frameshift, with 2 nonsense and 2 intronic variant types. Three of these were recurrent (Figure 3). Considering all 47 *SLCO2A1* heterozygotes, most individuals harbored missense PVs (64%, 30/47). However, there was a tendency for higher PHO penetrance rates in individuals harboring truncated PVs (missense PVs: 18/30 [53%] vs truncated PVs: 15/17 [88%]; p = .053) (Table 3).

Phenotype of *SLCO2A1* heterozygotes

The phenotypes of each of the 33 affected *SLCO2A1* heterozygous cases reported so far are shown in Table 4. Overall, the prevalences of periostosis, digital clubbing, pachydermia, and joint swelling were, respectively, 75.8% (25/33), 66.7% (22/33), 36.4% (12/33), and 21% (7/33) (Table 4).

At diagnosis, the prevalence of the different clinical forms in these 33 heterozygous *SLCO2A1* cases was, respectively: complete form (24%, 8/33), incomplete form (30%, 10/33), fruste form (18%, 6/33), and IDC (27%, 9/33). Isolated digital clubbing was the predominant phenotype in the subset of *SLCO2A1* heterozygous relatives of probands of PHOAD and PHOAR2 families (41%, 9/22), with an equal prevalence of incomplete and fruste forms (27.3%, each). This higher prevalence of IDC in this subset was due to predominance in males (53.8%, 7/13) as most of the females had the fruste form (55.6%, 5/9). In contrast to screened heterozygous cases, probands of PHOAD families had the PHO complete form (63.6%, 7/11) as their main clinical manifestation, followed by the incomplete one (36.4%, 4/11). Overall, males clinically manifested only the PHO complete and incomplete forms while milder clinical forms were more prevalent in females (Table 5).

Discussion

Combining our 4 unrelated families harboring *SLCO2A1* PVs and the existing families previously reported in the literature, the data reveal a pattern of incomplete PHO penetrance in *SLCO2A1* heterozygotes, which is modulated by sex, age, and genotype. Notably, penetrance rates were higher in elderly individuals, males, and those carrying truncated *SLCO2A1* variants. In parallel, a marked phenotypic variability was documented with more pronounced clinical manifestations in males. Importantly, penetrance rate and periostosis are likely to occur more frequently in heterozygous women than previously documented in women harboring *SLCO2A1* biallelic variants from PHOAR2 families, and IDC may represent a distinct clinical feature in *SLCO2A1* heterozygotes.

Homozygous *SLCO2A1* PVs found in our 3 Brazilian and 1 Palestinian index cases were concordant with autosomal recessive inheritance. Two novel *SLCO2A1* PVs were reported in our study, which expands the panel of *SLCO2A1* PVs occurring in PHOAR2 families (Figure 1). All index cases had the complete form of PHO (Table 1; **Supplementary data**, Figures S4–S7).

Table 3. Estimated penetrance of PHO based on the 14 *SLCO2A1* heterozygous individuals (present study) combined with the 33 heterozygous *SLCO2A1* carriers compiled from the literature.

Estimated PHO penetrance based on all 47 heterozygous *SLCO2A1* individuals (14 from the actual study plus 33 from the literature)									
Study and families	**Gender (M:F)**	**Affected/ total (male)**	**Affected/ total (female)**	**Affected carriers/total carriers (only heterozygous carrier)**	**Mutation (NM_005630.2)**	**Protein (NP_005621.2)**	**Mutation type**	**ACMG/ AMP**	**Inheritance model**
Xu et al.[19]									
Family 1	4:1	4/4	0/1	4/5	c.1660G > A	p.G554R[a]	Missense	PV	PHOAD
Family 2	2:0	2/2	0/0	2/2	c.664G > A	p.G222R[a]	Missense	PV	PHOAD
Family 3	1:1	1/1	1/1	2/2	c.1065dupA	p.Q356TfsX77	Frameshift	PV	PHOAD
Family 4	1:1	1/1	1/1	2/2	c.1293delT	p.S432AfsX48	Frameshift	PV	PHOAD
Family 5	1:1	1/1	1/1	2/2	c.1106G > A	p.G369D	Missense	PV	PHOAD
Family 6	1:2	1/1	2/2	3/3	c.1807C > T	p.R603X[a]	Nonsense	PV	PHOAD
Family 7	2:1	2/2	1/1	3/3	c.1807C > T	p.R603X[a]	Nonsense	PV	PHOAD
Family 8[b]	0:1	0/0	1/1	1/1	c.621C > A	p.Y207X	Nonsense	PV	PHOAR2/PHOAD
Family 8[b]	1:0	1/1	0/0	1/1	c.664G > A	p.G222R[a]	Missense	PV	PHOAR2/PHOAD
Family 9[b]	1:0	1/1	0/0	1/1	c.541G > C	p.G181R	Missense	LPV	PHOAR2/PHOAD
Family 9[b]	0:1	0/0	0/1	0/1	c.983 T > C	p.F328S	Missense	LPV	PHOAR2/PHOAD
Family 10[b]	1:0	1/1	0/0	1/1	c.1121C > T	p.P374L	Missense	LPV	PHOAR2/PHOAD
Family 10[b]	0:1	0/0	0/1	0/1	c.763G > A	p.G255R	Missense	LPV	PHOAR2/PHOAD
Family 11[b]	1:0	1/1	0/0	1/1	c.1660G > A	p.G554R[a]	Missense	PV	PHOAR2/PHOAD
Family 11[b]	0:1	0/0	0:1	0/1	c.1814 + 1G > A	NA	Splicing	PV	PHOAR2/PHOAD
Family 12[b]	1:1	1/1	0:1	1/2	c.1807C > T	p.R603X[a]	Nonsense	PV	PHOAR2/PHOAD
Bloch et al.[8]									
Index case 7	1:0	1/1	0/0	1/1	c.234 + 1G > A	NA	Splicing	PV	PHOAD
Index case 8	1:0	1/1	0/0	1/1	c.1523_ 1524delCT	p.(P508Rfs*69)	Frameshift	PV	PHOAD
Index case 9	1:0	1/1	0/0	1/1	c.1625G > A	p.(R542H)	Missense	LPV	PHOAD
Index case 10	1:0	1/1	0/0	1/1	c.31del	p.(Q11R*66)	Frameshift	PV	PHOAD
Present study									
Family 2[b]	3:3	1/3	1/3	2/6	c.1259G > T	p.C420F	Missense	PV[c]	PHOAR2/PHOAD
Family 3[b]	2:1	1/2	0/1	1/3	c.526G > A	p.A176T	Missense	PV[c]	PHOAR2/PHOAD
Family 4[b]	3:2	1/3	1/2	2/5	c.310G > T	p.G104[a]	Missense	PV[c]	PHOAR2/PHOAD
Total	29:18	24/29 (82.8%)[d]	9/18 (50%)[d]	33/47 (70%)					

[a]Recurrent *SLCO2A1* variants in unrelated families. [b]Probands from families 8-12 (Xu et al., 2021) and from families 2-4 (present paper) are not represented here as they harbor compound heterozygous *SLCO2A1* variants or in homozygosity (PHOAR2). [c]Criteria applied to classify each one of variants of the present paper are informed, including proband of the family 1, *a*. p.Q188R (family 1): PM2, supporting; PM3, supporting; and PP4, very strong; *b*. p.C420F (family 2): PS3, strong; PM2, moderate; PM3, strong; PP3, moderate; and PP4, very strong; *c*. p.A176T (family 3): PM2, supporting; PM3, moderate; and PP4, very strong; *d*. p.G104* (family 4): PVS1, very strong; PS4, moderate; PM3, moderate; PM2, supporting; and PP4, very strong. [d]$p = .024$. [e]$p = .053$. Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathologists; F, female; LPV, likely pathogenic variant; M, male; NA, not available; PHO, primary hypertrophic osteoarthropathy; PHOAD, primary hypertrophic osteoarthropathy, autosomal dominant, caused by monoallelic *SLCO2A1* deficiency; PHOAR2, primary hypertrophic osteoarthropathy, autosomal recessive 2, caused by biallelic *SLCO2A1* deficiency; PV, pathogenic variant; *SLCO2A1*, solute carrier organic anion transporter family member 2A1.

SLCO2A1 heterozygotes

Importantly, segregation analysis in 3 Brazilian PHO families revealed heterozygous *SLCO2A1* variants in 14 relatives (Table 2). Detailed clinical and radiological assessments of these heterozygotes identified previously overlooked mild symptoms of PHO in 5 elderly individuals (septuagenarians and octogenarians) who were parents of homozygous *SLCO2A1* probands with the complete form of PHO (Table 2). Thus, our present findings confirm recent literature data in 5 Chinese families, demonstrating the occurrence of both autosomal recessive (PHOAR2) and dominant (PHOAD) inheritance patterns within families harboring *SLCO2A1* mutations.[19]

Our genetic and clinical data, combined with those reviewed from the literature,[8,19] compiled 47 *SLCO2A1* heterozygous carriers from 11 PHOAD families (23 carriers) and 8 pedigrees (24 carriers) with both coexisting inheritance patterns (PHOAR2/PHOAD) (Table 3). The integrated clinical data from this analysis expanded our knowledge of the PHO phenotype and penetrance in *SLCO2A1* heterozygous carriers.

Estimated penetrance of PHO in *SLCO2A1* heterozygotes (age, sex, and genotype)

It is noteworthy that an overall PHO penetrance of 70% (33/47), male-related penetrance of 83% (24/29), and female-related penetrance of 50% (9/18) in the analyzed cases was estimated (Table 3). With the exclusion of 11 PHOAD probands, penetrance in 36 heterozygous *SLCO2A1* relatives from 7 PHOAD and 8 PHOAR2 families (Table S3) was as follows: overall penetrance (61%; 22/36), male-related penetrance (72.2%; 13/18), and female-related penetrance of (50%; 9/18).

Age

All heterozygous *SLCO2A1* probands from 11 previously reported PHOAD families[8,19] were diagnosed with PHO

Table 4. Clinical features of affected heterozygous *SLCO2A1* carriers from PHOAD and PHOAR2 families, including previously reported cases and cases of the present study.

Study/all families	Identification	Sex	Main clinical features				Affected heterozygous *SLCO2A1* carriers	Inheritance pattern	Phenotype	Age,[a] yr
			Digital clubbing	Pachydermia	Joint swelling	Periostosis	Screened or probands			
Xu et al.[19]										
Family 1	II-3	M	Yes	_	_	_	Screened	PHOAD	IDC	NA
	III-1	M	Yes	Yes	Yes	Yes	Proband	PHOAD	CF	43
	III-4	M	Yes	_	_	_	Screened	PHOAD	IDC	NA
	IV-1	M	Yes	_	_	_	Screened	PHOAD	IDC	23
Family 2	I-1	M	Yes	_	_	_	Screened	PHOAD	IDC	64
	II-2	M	Yes	_	_	Yes	Proband	PHOAD	IF	30
Family 3	I-2	F	Yes	_	_	Yes	Screened	PHOAD	IF	NA
	II-1	M	Yes	Yes	Yes	Yes	Proband	PHOAD	CF	NA
Family 4	I-2	F	_	_	_	Yes	Screened	PHOAD	FF	NA
	II-2	M	Yes	Yes	Yes	Yes	Proband	PHOAD	CF	NA
Family 5	II-4	F	_	_	_	Yes	Screened	PHOAD	FF	NA
	III-1	M	Yes	_	Yes	Yes	Proband	PHOAD	IF	NA
Family 6	I-2	F	_	Yes	_	_	Screened	PHOAD	FF	NA
	II-1	M	Yes	Yes	Yes	Yes	Proband	PHOAD	CF	NA
	II-2	F	_	Yes	_	_	Screened	PHOAD	FF	22
Family 7	II-2	F	_	_	_	Yes	Screened	PHOAD	FF	NA
	II-5	M	Yes	_	_	Yes	Screened	PHOAD	IF	NA
	III-3	M	Yes	_	_	Yes	Proband	PHOAD	IF	NA
Family 8	I-1	M	Yes	Yes	Yes	Yes	Screened[b]	PHOAR2/PHOAD	CF	49
	I-2	F	Yes	_	_	_	Screened[b]	PHOAR2/PHOAD	IDC	49
Family 9	I-1	M	Yes	_	_	Yes	Screened[b]	PHOAR2/PHOAD	IF	NA
Family 10	I-1	M	Yes	_	_	_	Screened[b]	PHOAR2/PHOAD	IDC	NA
Family 11	I-1	M	_	_	_	Yes	Screened[b]	PHOAR2/PHOAD	FF	55
Family 12	I-1	M	_	Yes	_	Yes	Screened[b]	PHOAR2/PHOAD	IF	NA
Bloch et al.[8]										
Case 7	_	M	Yes	Yes	Yes	Yes	Proband	PHOAD	CF	33
Case 8	_	M	Yes	Yes	_	Yes	Proband	PHOAD	CF	14
Case 9	_	M	Yes	_	_	Yes	Proband	PHOAD	IF	40
Case 10	_	M	Yes	Yes	_	Yes	Proband	PHOAD	CF	30
Present study										
Family 2	I-1	M	Yes	_	_	_	Screened[b]	PHOAR2/PHOAD	IDC	81
	I-2	F		Yes	_	Yes	Screened[b]	PHOAR2/PHOAD	IF	86
Family 3	I-1	M	Yes	_	_	Yes	Screened[b]	PHOAR2/PHOAD	IF	73
Family 4	I-1	M	Yes	_	_	_	Screened[b]	PHOAR2/PHOAD	IDC	74
	I-2	F	Yes	_	_	_	Screened[b]	PHOAR2/PHOAD	IDC	72
Total			25/33 (75.8%)	12/33 (36.4%)	7/33 (21%)	22/33 (66.7%)	Proband (11/33; 33.3%) Screened (22/33; 66.7%)			49.3 ± 22.5 (14-86)

"NA" indicates that exact ages at the PHO diagnosis are not informed by Xu et al.,[19] but indirect data about ages are informed: *a*. probands (family [F] 1–F7): 18–43 y (all male); *b*. screened family member (F1–F7): 38–67 y (except cases with 22 and 23 y informed in the table); *c*. screened family member (F8–F12): 49–71 y (5 fathers, 1 mother). Acro-osteolysis was evident in 3 heterozygous probands from 7 PHOAD families reported by Xu et al.; chronic watery diarrhea was present in 5 heterozygous probands from 7 PHOAD families reported by Xu et al. [a]Age at PHO diagnosis. [b]Heterozygous *SLCO2A1* parents of probands with biallelic *SLCO2A1* mutations (homozygous or compound heterozygous). Abbreviations: CF, complete form; F, female; FF, fruste form; IDC, isolated digital clubbing; IF, incomplete form; M, male; NA, not available; PHO, primary hypertrophic osteoarthropathy; PHOAD, primary hypertrophic osteoarthropathy, autosomal dominant, caused by monoallelic *SLCO2A1* deficiency; PHOAR2, primary hypertrophic osteoarthropathy, autosomal recessive 2, caused by biallelic *SLCO2A1* deficiency; *SLCO2A1*, solute carrier organic anion transporter family member 2A1; –, absent.

between 14 and 43 years old and most of them had onset of symptoms/signs between ages 10 and 20 years (8/11; 72%), similar to that previously seen in patients with PHO carrying biallelic *SLCO2A1* PVs (PHOAR2).[3–5,7] This outcome clearly differs from 11 affected heterozygous *SLCO2A1* relatives from the 7 PHOAD probands who were mainly middle-aged or elderly individuals at the diagnosis (only 3 cases were younger than 40 years: 22, 23, and 38 years).[19] Among the 10 heterozygotes of the parental generation from 5 PHOAR2 probands, 6 of them were diagnosed with PHO between ages 49 and 71 years, 5 affected males and only 1 female[19] (Table 4, families 8–12). Indeed, the 5 affected heterozygous *SLCO2A1* members of the parental generation from 3 Brazilian homozygous probands of the present study were elderly (72–86 years) (Tables 2 and 4). Taken together, these data demonstrate an age-related penetrance in *SLCO2A1* heterozygous carriers, with disease manifestations occurring at a younger age in PHO (PHOAD) probands and later in their PHOAD relatives, as well as in the *SLCO2A1* heterozygous parents of PHOAR2 probands.

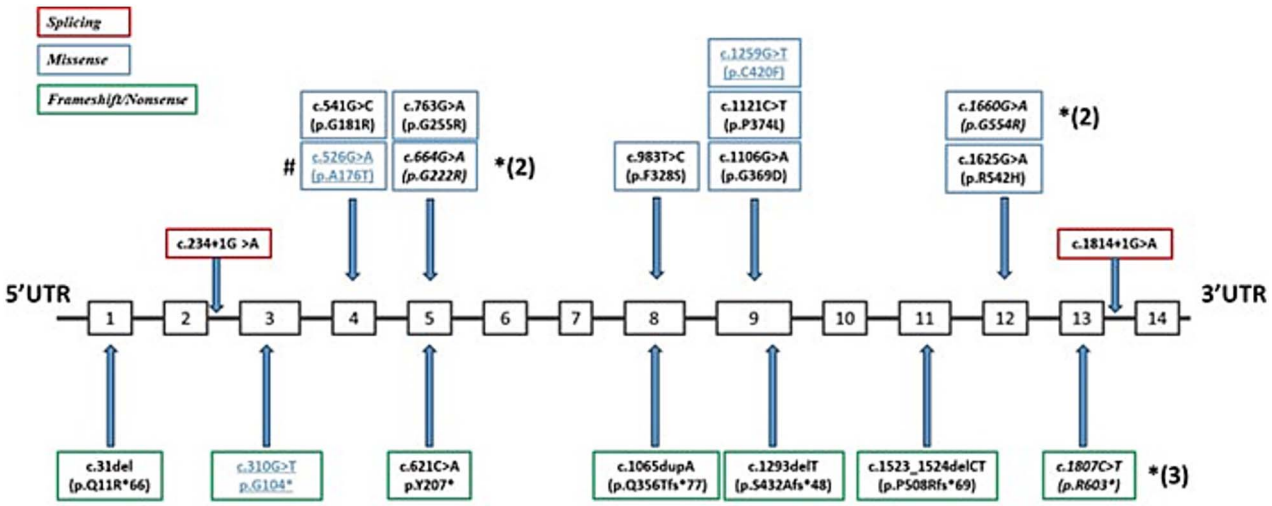

Figure 3. Schematic representation of the *SLCO2A1* gene illustrating all germline pathogenic allelic variants associated with autosomal dominant inheritance characterized by the presence of affected cases harboring *SLCO2A1* monoallelic variants. Black boxes represent each 1 of the 14 exons of the *SLCO2A1* gene, while lines between them correspond to the introns. Blue boxes, missense mutations; red boxes, splicing mutations; green boxes, frameshift or nonsense mutations; text written in blue and underlined, mutations found in 3 families of the present study with affected *SLCO2A1* heterozygote carriers: p.420F (family 2), p.A176T (family 3), and p.G104* (family 4). #Novel *SLCO2A1* variant described in the present study (the other novel variant reported in this study, p.Q188R, was not included as segregation analysis was not available to the at-risk family members). *Recurrent variants documented in unrelated PHO families. Numbers in parentheses "()" indicate the number of unrelated PHO families reported with the same *SLCO2A1* variant, in heterozygotes. Note: Only studies published after the original description by Xu et al.[19] on the association of *SLCO2A1* heterozygous variants with autosomal dominant inheritance are represented (Xu et al.,[19] Bloch et al.,[8] and present study). Thus, heterozygous *SLCO2A1* variants of all 11 PHOAD families (7 by Xu et al. and 4 by Bloch et al.) and 8 PHOAR2 families (5 by Xu et al. and 3 by the present study) are compiled. Abbreviations: PHO, primary hypertrophic osteoarthropathy; PHOAD, primary hypertrophic osteoarthropathy, autosomal dominant, caused by monoallelic *SLCO2A1* deficiency; PHOAR2, primary hypertrophic osteoarthropathy, autosomal recessive 2, caused by biallelic *SLCO2A1* deficiency; *SLCO2A1*, solute carrier organic anion transporter family member 2A1.

Table 5. Prevalence of the different PHO clinical forms verified in 33 affected heterozygous *SLCO2A1* carriers from PHOAD and PHOAR2 families, including aggregated data of previously reported families and the present study.

Phenotype	Complete form, *n* (%)	Incomplete form, *n* (%)	Fruste form, *n* (%)	Isolated digital clubbing, *n* (%)
All heterozygous cases (33)	8/33 (24)	10/33 (30)[a]	6/33 (18)	9/33 (27)
Probands (11) (all males)	7/11 (63.6)[a]	4/11 (36.4)	0	0
Screened cases (22)	1/22 (4.5)	6/22 (27.3)	6/22 (27.3)	9/22 (41)[a]
Male, only screened (13)	1/13 (7.7)	4/13 (30.8)	1/13 (7.7)	7/13 (53.8)[a]
Female, only screened (9)	0	2/9 (22)	5/9 (55.6)[a]	2/9 (22)
Male, screened + probands (24)	8/24 (33.3)[a]	8/24 (33.3)[a]	1/24 (4)	7/24 (29)

[a]The dominant phenotype in each one of the groups of affected heterozygous *SLCO2A1* carriers. Abbreviations: PHO, primary hypertrophic osteoarthropathy; PHOAD, primary hypertrophic osteoarthropathy, autosomal dominant, caused by monoallelic *SLCO2A1* deficiency; PHOAR2, primary hypertrophic osteoarthropathy, autosomal recessive 2, caused by biallelic *SLCO2A1* deficiency; *SLCO2A1*, solute carrier organic anion transporter family member 2A1.

Sex

In contrast to the predominance reported in PHOAR1 (32:57), there was an intriguing and marked predominance of PHO in males with PHOAR2.[2,20,38–41] Thus, in more than 55 PHOAR2 families, PHO was documented only in 1 Chinese elderly woman (67 years), which was first investigated by recurring arthralgia.[7,42] She had a mild incomplete form of PHO (minimum pachydermia, finger clubbing, swelling of the large joints, and periostosis) and carried (compound heterozygous) biallelic *SLCO2A1* variants.[42] Thus, female patients with homozygous or compound heterozygous *SLCO2A1* mutations mostly have first manifestations other than the classical PHO phenotype.[7,9,16,39,43–45] In fact, *SLCO2A1* biallelic PVs were associated with severe anemia and hypoalbuminemia in 2 sisters,[39] severe transfusion-dependent anemia (suggesting myelofibrosis) and mild finger clubbing in a 34-year-old woman,[9] chronic enteropathy in 14 females,[16] isolated congenital nail clubbing in 2 Pakistani sisters,[43] and even premature menopause in a young female (37 years).[45] Notably, in the present study all probands were males, as no females harboring biallelic *SLCO2A1* PVs were detected.

With regard to heterozygous *SLCO2A1* carriers, all affected probands from PHOAD and PHOAR2 families were males (Tables 4 and 5). Overall, the PHO penetrance was significantly higher in heterozygous males (82.8% vs 50%; p = .024), shown by the higher frequency of affected heterozygous *SLCO2A1* males (72.7%; 24/33) (Table 3). However, in contrast to the extreme rarity of periostosis in females by *SLCO2A1* biallelic mutations,[42] 4 heterozygous women from PHOAD families[19] and 1 octogenarian mother of a PHOAR2 proband in the present study had periostosis, leading to an estimated penetrance of 28% (5/18) in *SLCO2A1* heterozygous females (Tables 2 and 4). These data suggest that mild periostosis is likely more frequent in PHOAD families and heterozygous women from PHOAR2

families. With regard to asymptomatic disease, the phenotype of apparently healthy heterozygous *SLCO2A1* individuals of PHOAR2 families may have been previously neglected and therefore needs careful reassessment to rule out the fruste forms of PHO.

Genotype

Recently, in a comprehensive review, Lu et al.[41] compiled all *HPGD* and *SLCO2A1* PVs associated with the PHO phenotype reported so far, including PHOAR1, PHOAR2, and PHOAD. In the present study, we extensively reviewed all previously reported *SLCO2A1* variants, including those found in our cases, with a focus on *SLCO2A1* heterozygous individuals (Figure 3).

Overall, 19 different *SLCO2A1* variants (10 missense, 9 truncated variants) were associated with the 47 heterozygous carriers reported so far (Table 3, Figure 3), 14 of them from the present study, and penetrance rate was higher in individuals carrying truncated *SLCO2A1* PVs (15/17; 88%) than missense variants (18/30; 53%) (p = .053) (Table 3). Additional studies, incorporating larger series of *SLCO2A1* heterozygotes, are needed to validate a potential genotype impact on PHO penetrance in *SLCO2A1* heterozygotes.

Phenotype in *SLCO2A1* heterozygotes

Overall, digital clubbing, periostosis, pachydermia, and joint swelling were present in 25 (75.8%), 22 (66.7%), 12 (36.4%), and 7 (21%) of 33 affected heterozygous *SLCO2A1* cases, respectively (Table 4). Notably, *cutis verticis gyrata* was present in only 1 heterozygous *SLCO2A1* patient (3%; 1/33) reported by Xu et al.[19] To date, none of the 33 affected heterozygous cases have been documented with *SLCO2A1*-linked chronic enteropathy, although chronic watery diarrhea was present in 5 heterozygous PHOAD probands. In turn, acro-osteolysis was evident in only 3 affected heterozygous cases (9%; 3/33) (Table 4).[8,19]

Importantly, overt clinical disease was more frequent in the heterozygous male probands (PHOAD) than in clinically screened heterozygous males from PHOAD or PHOAR2 families.[19] In addition, screened heterozygous *SLCO2A1* females had milder phenotypes than heterozygous males. As an example, all 8 heterozygous cases with the complete form of PHO were males and most of them were index cases (7/8 cases) (Tables 4 and 5). Also, the PHO incomplete form was more frequent in males (8/10 cases), while the fruste form of disease was prevalent in females (5/6 cases) (Table 5). Importantly, the clinical severity of pachydermia and periostosis was more pronounced in heterozygous *SLCO2A1* males than in females, especially in male probands.[19]

Nearly one-third of affected *SLCO2A1* heterozygotes had the incomplete form (30%; 10/33), while one-quarter manifested as the complete form (24%; 8/33). Considering both groups, most were represented by males (89%; 16/18) (Table 5).

It is noteworthy that IDC, in different degrees, was the only clinical manifestation in 27% of the affected heterozygous *SLCO2A1* carriers (27%; 9/33) (Table 5). Notably, IDC associated with biallelic mutations has been reported in the Pakistani population: 2 families related to *SLCO2A1* mutations and another to *HPGD* mutations, comprising both sexes.[38,43,46] In addition, Seifert et al.[4] reported 1 PHO family with 3 affected siblings harboring homozygous *SLCO2A1* mutations whose father was a heterozygous carrier and had IDC. In addition, they reported 1 young heterozygous *SLCO2A1* male with IDC and a healthy heterozygous father. Guda et al.[15] characterized an IDC family and early-onset colon neoplasia carrying the same nonsense heterozygous *SLCO2A1* mutation identified in our family 4 proband, whose couple of the parental generation, heterozygote to this PV, had IDC (Tables 2 and 4). These data reinforce the presence of IDC as a possible phenotype in heterozygous *SLCO2A1* cases documented in our study and in Xu et al.[19] (Tables 4 and 5).

Overall, the autosomal dominant pattern of inheritance noticed in PHOAD families and in the parental generation of PHOAR2 families is characterized by marked intrafamilial and interfamilial clinical variability. However, this variable phenotype is likely intrinsically modulated by sex and aging. Thus, a phenotypic signature of *SLCO2A1* heterozygotes may be abstracted, characterized by a decreasing pattern of clinical severity from index cases to screened family members, and from men to women. It has been postulated that differences in sex hormones between women of reproductive age and menopausal women influence the prostaglandin metabolism, justifying the lower PHO penetrance and the presence of milder, late-onset disease in females.[7,42,45]

Despite the mild phenotype observed in the elderly individuals and the lack of penetrance in the younger individuals in our study, reports of heterozygotes from the 2 previous studies[8,19] revealed individuals with clinical PHO manifestation at younger ages. Thus, genetic testing should be offered to all at-risk family members, and clinical surveillance could be suggested for heterozygous relatives.

SLCO2A1 variants

We identified 2 novel *SLCO2A1* PVs. The c.563A > G (p.Q188R) missense variant (case 1) is located in a protein domain that interacts with the sodium-independent organic anion transporter between residues 1 and 630. Notably, 2 nearby variants (residue 181), associated with the PHO phenotype, were deposited in the HGMD (records CM126914 [Gly181Asp] and CM126908 [Gly181Ala]) and described by Diggle et al.,[9] reinforcing their pathogenicity. As this patient was a native of Palestine, we searched for PHO cases reported in neighboring countries and can confirm it as a novel pathogenic variant.[9,46–49] This case had 2 siblings with PHO and 9 other siblings who may benefit from molecular diagnosis. However, his 2 siblings, living in the West Bank, with clinical symptoms compatible with a typical history of PHO are thus far not undergoing follow-up for the underlying disease. Thus, despite the apparent classical picture of PHO in his siblings, revealed through photos by our patient, we could not affirm them as homozygous since *SLCO2A1* heterozygotes may have a heterogeneous clinical presentation, including the complete form. The information given by our patient is that all other siblings are free of PHO-associated symptoms or signs, although the clinical and radiologic evaluations were unavailable in the heterozygous cases of the family.

The second novel *SLCO2A1* missense PV, c.526G > A p.Ala176Thr, present in homozygosity in index case 3 (IV-6) was found, in heterozygosity, in 3 tested relatives (Figure 1). The phenotype analysis of the parental generation, obligate heterozygous carriers, revealed a mild/moderate clinical PHO presentation, characterized by moderate digital clubbing and periostosis in the father, contrasting with the typical

phenotype of the complete form seen in the index case. Although other PVs have not been reported at codon 176, PVs near this region affecting codons 170, 179, and 181 have been described, indicating that alterations in this region could impact protein function (www.hgmd.cf.ac.uk).

The other 2 *SLCO2A1* PVs were previously described in association with the PHO phenotype.[9,37] Thus, the missense variant c.1259G > T (p.C420F), present in index case 2 in homozygosity (Figure 1), was found in 6 at-risk relatives in heterozygosity. The presence of a moderate form of digital clubbing in the father (81 years old) and mild periostosis and pachydermia in the mother (86 years old) indicates that heterozygotes may present with a milder form of PHO (Figure 2). The patient's siblings, who are approximately 50 years old, and his children ages 13 and 17 years had no clinical or radiological PHO manifestations. Interestingly, the absence of a history of consanguinity in the parental generation associated with an initial history of mild PHO noticed in his father and grandfather suggested autosomal dominant inheritance with intrafamilial phenotypic variability. However, the index-case homozygosity defined the autosomal recessive inheritance. In turn, the PV segregation, in heterozygosity, with a milder phenotype in the parental generation and, potentially, in his paternal grandfather confirms recent *SLCO2A1* data on the predominantly mild phenotype in heterozygotes, which supports autosomal dominant transmission running parallel to recessive inheritance in this family.

Intriguingly, this variant found was first reported in 4 PHO brothers from a consanguineous family with Colombian ancestry, and also recently reported in a young homozygosis Portuguese proband from heterozygous non-consanguineous parents.[9,37] Furthermore, this variant was not found in all genomic databanks investigated, except for the exceptionally rare presence in 3 heterozygous individuals of an admixed American gnomAD cohort (MAF: 0.009%). From the aggregated data and considering the historical Portuguese and Spanish colonization of Latin America, one can hypothesize the origin of the *SLCO2A1* p.C420F variant in Iberian Peninsula populations. In this context, a founding *SLCO2A1* mutation was previously reported in the Japanese population.[5] Notably, a homozygous woman with the p.C420F variant, reported by Diggle et al.[9] had severe transfusion-dependent anemia not found in both our women heterozygotes and in a previous study reported by Nicolau et al.[37]

Finally, index case 4, with the classic PHO phenotype, was homozygous to nonsense variant c.310G > T (p.Gly104*) (Figure 1), as previously reported by Diggle et al.[9] This variant was found in heterozygosity in 5 at-risk relatives (Figure 1). A mild/moderate form of PHO was verified in the parental generation characterized by the presence of mild/moderate and incomplete finger clubbing without any evidence of periostosis or pachydermia. Conversely, the siblings and son of the index case had no clinical presentation suggestive of PHO.

Diggle et al.[9] first described this nonsense variant, in homozygosity, in an Italian patient whose parents were consanguineous.[9] The patient had a classical clinical presentation of PHO with digital clubbing, periostosis, and *cutis verticis gyrata*. This mutation, in heterozygosity, was previously described in the same year in a Japanese patient who had the incomplete form characterized by digital clubbing and periostosis, in addition to a negative family history for PHO.[5] Furthermore, Guda et al.[15] reported a family with male-restricted digital clubbing, without PHO, who had this same variant in heterozygosity.[15] Moreover, our proband's 72-year-old mother had mild and incomplete digital clubbing of the hands, in contrast to moderate digital clubbing observed in her husband. The absence of digital clubbing in 3 heterozygous women reported by Guda et al.[15] (aged 54, 57, and 87 years) and its presence in the proband's 72-year-old mother suggests that age, in addition to sex, may influence penetrance modulation.

Genetic disorders with autosomal dominant inheritance are associated with varying rates of penetrance. For instance, penetrance is nearly 100% in multiple endocrine neoplasia type 1 (MEN1), frequently very high (60%–100%) in multiple endocrine neoplasia type 2 (MEN2), but variable and influenced by specific *RET* mutations. In contrast, lower penetrance rates, estimated at approximately 20%, are observed in individuals carrying mutations in the *AIP* gene from kindreds diagnosed with familial isolated pituitary adenoma syndrome (FIPA) or in 33% of *TMEM127* carriers predisposed to develop pheochromocytoma in later life. Low penetrance is often characterized by asymptomatic carriers and generations unaffected by the disease. Phenotypic variability is common in these syndromes, except for MEN2, which exhibits a strong genotype–phenotype correlation.[50–54]

Classically, compound heterozygous or homozygous germline mutations in the calcium-sensing receptor (CaSR) may cause severe neonatal hyperparathyroidism in its autosomal recessive form, whereas heterozygous mutations are associated with familial hypocalciuric hypercalcemia (FHH), an autosomal dominant disorder. Despite full penetrance in both forms, the clinical manifestations and outcomes differ significantly.[55,56] This model, where a single gene causes both Mendelian inheritance patterns, was only recently documented in *SLCO2A1* mutations, which also give rise to a dominantly inherited form.[19] Although FHH is typically painless and asymptomatic, there are reports of cases with moderate hypercalcemia requiring treatment, as well as symptoms such as fatigue, weakness, and an increased risk of chronic kidney disease, coronary heart disease, pancreatitis, femoral fractures, and chondrocalcinosis with advancing age. The long-term benefits of calcimimetics remain unclear.[57] Similarly, *SLCO2A1* heterozygotes exhibit diverse phenotypes, although milder than those observed in homozygotes. The benefits of treatment with cyclooxygenase-2 inhibitors have been promising but their long-term use requires further investigation.[19,44]

In conclusion, our data reinforce the existence of autosomal dominant transmission caused by *SLCO2A1* mutations. In addition, we originally demonstrated that PHO penetrance in *SLCO2A1* heterozygotes is incomplete, and modulated by sex, age, and genotype. Importantly, a marked phenotypic variability was verified here, with *SLCO2A1* heterozygotes clinically manifesting from complete forms to fruste forms or IDC. Furthermore, more pronounced phenotypes were documented in males and a higher prevalence of periostosis in *SLCO2A1* heterozygous women than previously reported in women harboring biallelic *SLCO2A1* variants from PHOAR2 families.

Currently, genetic counseling for patients with PHO and their family members should be conducted considering the risk of patients having developed clinically evident disease and transmitting the disease to their offspring through an autosomal dominant or recessive inheritance model (PHOAD or PHOAR2). Importantly, penetrance and the phenotypic

spectrum are strongly influenced by inheritance (monoallelic or biallelic transmission), sex, and age. Future studies are needed to elucidate the underlying mechanisms responsible for variable penetrance and the different clinical presentations of PHO associated with *SLCO2A1* mutations.

Author contributions

Adriano Miguel Arcanjo (Formal analysis, Investigation, Methodology, Visualization [equal]), Amanda Fernandes de Souza (Formal analysis, Investigation, Methodology, Visualization [equal]), Elisangela Pereira de Souza Quedas (Formal analysis, Investigation, Methodology, Supervision), Joya Emilie de Menezes Correia-Deur (Formal analysis, Methodology), Dalton Libanio Ferreira (Formal analysis, Investigation, Methodology), Sergio Pereira de Almeida Toledo (Formal analysis), Delmar Muniz Lourenço Jr (Conceptualization, Funding, Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Visualization). All authors read and approved the final manuscript. Adriano Miguel Arcanjo and Amanda Fernandes de Souza equally contributed.

Supplementary material

Supplementary material is available at *JBMR Plus* online.

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Conflicts of interest

None declared.

Data availability

All authors agree to make available, upon request from the corresponding author, all data generated, analyzed, and/or used during the current study.

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