

9-27-2024

Inorganic Pyrophosphate Plasma Levels in Patients with GGCX-Associated PXE-Like Phenotypes

Qiaoli Li


Catherine Troutman

Mary Peckiconis

Tamara Wurst

Sharon Terry

Follow this and additional works at: <https://jdc.jefferson.edu/bmpfp>

 Part of the [Biochemistry Commons](#), [Diseases Commons](#), [Genetics Commons](#), and the [Molecular Biology Commons](#)

[Let us know how access to this document benefits you](#)

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Biochemistry and Molecular Biology Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.



OPEN ACCESS

EDITED BY

Dimitra Kiritsi,
University of Freiburg Medical Center, Germany

REVIEWED BY

Amir Hossein Saeidian,
Children's Hospital of Philadelphia Research
Institute, United States
Flora Szeri,
Research Centre for Natural Sciences, Hungary

*CORRESPONDENCE

Qiaoli Li,
✉ Qiaoli.Li@Jefferson.edu

RECEIVED 29 May 2024

ACCEPTED 16 September 2024

PUBLISHED 27 September 2024

CITATION

Li Q, Troutman C, Peckiconis M, Wurst T and
Terry SF (2024) Inorganic pyrophosphate
plasma levels in patients with GG CX-associated
PXE-like phenotypes.
Front. Genet. 15:1429320.
doi: 10.3389/fgene.2024.1429320

COPYRIGHT

© 2024 Li, Troutman, Peckiconis, Wurst and
Terry. This is an open-access article distributed
under the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Inorganic pyrophosphate plasma levels in patients with GG CX-associated PXE-like phenotypes

Qiaoli Li^{1,2*}, Catherine Troutman³, Mary Peckiconis³,
Tamara Wurst³ and Sharon F. Terry³

¹Department of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, United States, ²PXE International Center of Excellence in Research and Clinical Care, Thomas Jefferson University, Philadelphia, PA, United States, ³PXE International, Inc., Damascus, MD, United States

Introduction: Pseudoxanthoma elasticum (PXE) is an autosomal recessive ectopic calcification disorder clinically affecting the skin, eyes, and vascular system. Most cases of PXE are caused by inactivating pathogenic variants in the *ABCC6* gene encoding a hepatic transmembrane efflux transporter, which facilitates the extracellular release of ATP, the precursor of inorganic pyrophosphate (PPi), a potent endogenous inhibitor of calcification. Pathogenic variants in *GGCX*, encoding γ -glutamyl carboxylase required for activation of vitamin K-dependent coagulation factors as well as matrix Gla protein (MGP) and Gla-rich protein (GRP), two inhibitors of ectopic calcification, have also been reported to cause cutaneous changes like those seen in PXE. While ectopic calcification in *ABCC6* deficiency has been associated with reduced plasma levels of PPi due to loss of *ABCC6* transport activity in the liver, plasma PPi levels have not been reported in patients with *GGCX*-associated phenotypes.

Methods: We analyzed five patients from three unrelated families on their clinical, laboratory, and molecular findings who carry biallelic variants in *GGCX* and present with phenotypic characteristics associated with PXE. The variants were identified using a next-generation sequencing panel consisting of 29 genes associated with ectopic calcification.

Results and conclusion: This study demonstrates that in addition to *ABCC6*, *GGCX* variants can cause the PXE phenotype, expanding PXE and perhaps other heritable ectopic calcification disorders' clinical and genetic heterogeneity. The results also show that the plasma concentrations of PPi in these patients are not reduced compared to healthy control individuals, suggesting that plasma PPi does not govern ectopic calcification in *GGCX* deficiency.

KEYWORDS

ABCC6, ectopic calcification, GG CX, inorganic pyrophosphate, Pseudoxanthoma elasticum

1 Introduction

Pseudoxanthoma elasticum (PXE; OMIM #264800) is an autosomal recessive multisystem disorder clinically affecting the skin, the eyes, and the arterial blood vessels (Neldner, 1988). The phenotypic hallmark of PXE is ectopic calcium mineral deposition in non-skeletal connective tissues, particularly the elastic structures. In the skin, the primary lesions are small, yellowish papules with a predilection for flexural areas, and these lesions progressively coalesce into larger plaques of inelastic, leathery, and loose skin with a yellowish hue and loss of recoil. The characteristic eye manifestations consist of angioid streaks and peau d'orange. Calcification of the elastin-containing retinal layer, the Bruch's membrane, causes fractures, which may eventually lead to neovascularization and retinal bleeding. Before the advent of antiangiogenesis therapies for macular bleeding, this could cause progressive loss of visual acuity. Calcification of the elastin-rich mid-lamellar layer of peripheral arteries causes intermittent claudication. It occasionally results in gastrointestinal bleeding, and there is some suggestion that it might lead to hypertension and, rarely, myocardial infarction and stroke. The clinical manifestations of PXE are usually not recognized until early adulthood or adolescence, either diagnosed by finding yellowish papules of the skin that progressively coalesce to make a leathery plaque on flexor areas, the incidental finding of angioid streaks on fundoscopy in an asymptomatic individual or in a patient presenting with visual distortion secondary to subretinal hemorrhage.

PXE is usually caused by inactivating pathogenic variants in the *ABCC6* gene (Ringpfeil et al., 2000; Le Saux et al., 2000; Bergen et al., 2000). The gene encodes ABCC6, an efflux transporter protein expressed predominantly on the basolateral surface of hepatocytes in the liver and not in peripheral tissues clinically affected by PXE (Belinsky and Kruh, 1999; Scheffer et al., 2002). PXE is a metabolic disease, and recent studies have identified ABCC6 as a critical player in the generation of extracellular inorganic pyrophosphate (PPi), a potent endogenous inhibitor of calcification (Ralph et al., 2022). Specifically, ABCC6 facilitates the extracellular release of adenosine triphosphate, which is rapidly hydrolyzed by ENPP1 to generate PPi (Jansen et al., 2014; Jansen et al., 2013). As a result of nonfunctional ABCC6 transport activity, plasma PPi levels in patients with PXE and *Abcc6* knockout murine models of PXE are reduced to approximately 30%–40% of controls (Li et al., 2017; Jansen et al., 2014; Kowal et al., 2021; Saeidian et al., 2022). It is now generally accepted that deficiency of plasma PPi is the underlying cause of ectopic calcification in PXE, with low PPi levels in circulation allowing calcification of the peripheral tissue to occur. The extent of reduction of plasma PPi also correlates with the onset and disease severity in genetic and acquired conditions of ectopic calcification (Ralph et al., 2022).

Several genetically distinct clinical conditions display signs and symptoms similar to those found in PXE, with aberrant calcification of elastic structures in the skin. One such distinct condition exhibits retinal and cutaneous lesions similar to PXE with or without vitamin K-dependent coagulation factor deficiency caused by inactivating pathogenic variants in the *GGCX* gene (De Vilder et al., 2017). The gene encodes a carboxylase catalyzing the γ -glutamyl carboxylation of Gla-proteins, including clotting factors required for blood coagulation (Zhang and Ginsburg, 2004). These patients

demonstrate cutaneous and retinal lesions that are similar and, in some cases, indistinguishable from those found in PXE. While PPi is a strong determinant of ectopic calcification in several genetic and acquired diseases of ectopic calcification (Ralph et al., 2022), the plasma PPi concentrations in these phenotypes apparently caused by pathogenic variants in *GGCX* have not been reported. It is unknown if plasma PPi deficiency would also contribute to ectopic calcification in *GGCX* deficiency.

This study examined five affected individuals from three unrelated families with phenotypes consistent with PXE, with or without vitamin K-dependent coagulation factor deficiency, who were negative for variants in *ABCC6* but carried biallelic pathogenic or likely pathogenic variants in the *GGCX* gene. The aim of this study was to measure concentrations of PPi in these patients to better understand the pathogenesis of ectopic calcification in this PXE-like disorder.

2 Materials and methods

2.1 Study participants

All patients were enrolled with written informed consent into this study with approval from the Genetic Alliance Institutional Review Board (Approval number PXE001). These patients have a clinical diagnosis of PXE confirmed by a dermatologist and/or an ophthalmologist, based on the diagnostic criteria proposed by the PXE International Research Consortium at the 2014 Annual Meeting (Uitto et al., 2014). They had either a skin biopsy, which by histopathological stains revealed fragmented elastic fibers and calcium phosphate deposition in the lesional skin, and/or retinal changes, including peau d'orange and angioid streaks. Patients were registered in the databases of PXE International, an advocacy organization for PXE. We used the Phenodex score, a severity index created by PXE International and adopted internationally to assess phenotypes in the five common organ systems associated with PXE: skin (S), eyes (E), cardiac (C), vasculature (V), and gastrointestinal (GI), to determine the clinical severity of PXE (Pfundner et al., 2007).

2.2 Variant detection and bioinformatics

Genomic DNA was obtained from PXE International Biobank, extracted from saliva (Qiagen, Valencia, CA), or from peripheral blood samples (DNA Genotek Inc., Ontario, Canada) of affected individuals. Variant detection was performed using a next-generation sequencing (NGS) panel containing 29 ectopic mineralization-associated genes, including *ABCC6* and *GGCX* (Saeidian et al., 2022). The panel includes *ABCC6*, *ADIPOQ*, *AHSG*, *ANKH*, *APOE*, *ATF4*, *CASR*, *ENPP1*, *FAM20A*, *FGF23*, *GALNT3*, *GGCX*, *KL*, *MGP*, *NT5E*, *SAMD9*, *SLC20A2*, *SLC29A1*, *SPPI*, *TRIM24*, *TNFRSF11B*, *A2AP*, *ALPL*, *ENTPD1*, *PAI-1*, *PLAT*, *PLAU*, *PLAUR*, and *PLG*. The TruSeq Custom Amplicon kit (Illumina Inc., San Diego, CA) and DesignStudio (Illumina, Inc., San Diego, CA) were used for target enrichment and library design, respectively. All coding exons, at least 20 bp of the intron at each intron-exon boundary, and up to 50 bp of 3'- and 5'-untranslated

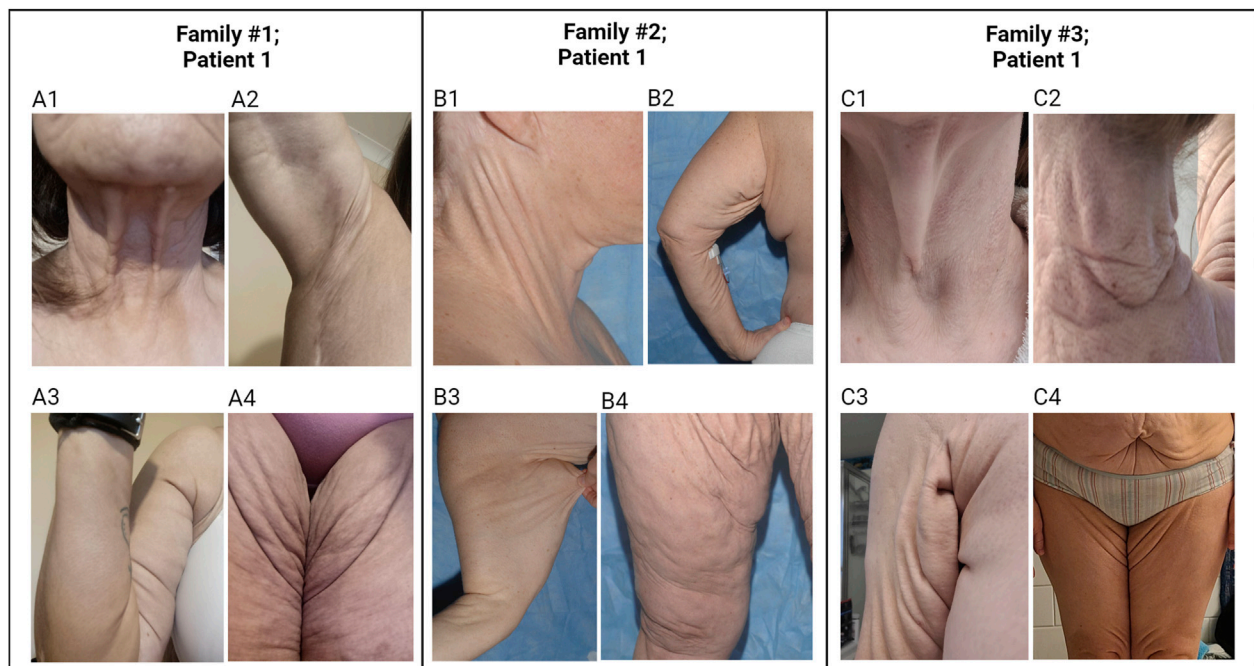


FIGURE 2

Cutaneous features in individuals with PXE-like phenotypes. Patient 1 in Family #1: The patient's skin is loose and redundant in the anterior neck (A1), axillary areas (A2, A3), and groin areas (A4). Patient 1 in Family #2: The patient's skin is loose and sagging in the lateral neck (B1), axillary areas (B2, B3), and groin areas (B4). Patient 1 in Family #3: The patient's skin is loose and sagging in the lateral neck (C1), posterior neck (C2), axillary area (C3), and abdomen and groin areas (C4). The yellowish papules and plaques in the skin of these patients demonstrate characteristic features of PXE. Note that pictures in (B1, B4) were published previously (Li et al., 2009b).

antecubital fosse, and inguinal region. A dermatologist diagnosed her with PXE when she was 32. At age 33, she was diagnosed with angioid streaks in both eyes, but has not progressed to have any retinal neovascularization or bleeding. Her Phenodex scores (an index specific to detailing the common manifestations of PXE) are reported in [Supplementary Table S1](#).

Family #2 has two affected individuals of Irish descent, 1 and 2 ([Figure 1](#)). The proband 1 is a 65-year-old female who was initially diagnosed with an autosomal recessive disorder of vitamin K-dependent coagulation factor deficiency at age 13. Her bleeding disorder was extensively studied, and has been reported previously ([Goldsmith et al., 1982](#)). At the time of her initial hematological evaluation, both prothrombin and factor X activities were severely reduced: prothrombin time (s) 17.8–20.3 (normal range 12–16), prothrombin (% normal) 20 (normal limits 65–150), factor X (% normal) 20%–22% (normal range 65–185). She responded to 5 mg vitamin K1 administered daily without further significant bleeding problems. The proband had a younger brother, 2, who also had a similar bleeding diathesis with similar laboratory findings.

At age 20, patient 1 was evaluated by dermatologists for cutaneous changes and she was diagnosed with PXE. At age 49, the proband exhibited loose, sagging, and redundant skin with loss of recoil, particularly affecting the neck, axillary, and groin areas ([Figure 2](#)). Her physical examinations also revealed small yellowish papules coalescing into larger plaques in the flexor areas; these lesions demonstrated characteristic features of PXE. At age 30, the proband's ophthalmologist reported evidence of

very faint angioid streaks and no choroidal neovascularization. At the time of this writing, her angioid streaks are still faint and there have been no retinal bleeds. The proband also complained of intermittent claudication. She had extensive plastic surgery including her neck, face, axilla, and abdomen. The brother of the proband also had co-existent PXE and vitamin K-dependent coagulation factor deficiency. It is unknown if he had peau d'orange or angioid streaks. He had no retinal bleeds or vision loss at the time of his death, at age 55 of an abdominal aortic aneurysm. The dermatological and ophthalmological features of this family were reported previously ([Li et al., 2009b](#)). Their skin, eyes, and vascular systems are scored using the Phenodex ([Supplementary Table S1](#)).

Family #3 had two affected subjects of Irish/German descent, 1 and 3 ([Figure 1](#)). The proband 1 is a female aged 45 years. Around age 13, she noticed lesions, papules, and loose skin on her axillae, neck, and under the chin. In her 20s, the loose and sagging skin spread to other areas of the skin, and she reports it is now severe in all areas. Histopathology of the lesional skin revealed calcification in the dermis, and a dermatologist diagnosed her with PXE at age 26. By age 35, she had sagging skin in her anterior and posterior neck, axillary, and inguinal areas ([Figure 2](#)). At the age of 26 years, the ophthalmologist reported faint angioid streaks in both eyes, and an unspecified "retinopathy" in the periphery of both eyes. At age 40, she was diagnosed with Grade 1 mitral valve prolapse. The proband's brother 3 noticed similar skin changes at age 22 and was diagnosed with PXE at age 31. The other organs were not affected except for skin manifestations ([Supplementary Table S1](#)).

TABLE 1 Bioinformatics analyses of *GGCX* variants.

Variant ^a	Domain	ClinVar ID	Population data No. of homozygous; MAF (%)		Prediction outcome by			
			gnomAD (Aggregated) ^b	BRAVO ^c	Aggregated outcome ^d	ACMG/ AMP ^e	ClinGen ^f	CADD score ^g
c.1426C>G, p.Arg476Gly	ER lumen	3335889	0; 0.00068	0; 0.0020	Deleterious	LP	LP	34.00
c.247C>T, p.Arg83Trp	ER lumen	2203111	0; 0.0025	0; 0.0023	Deleterious	P	P	23.70
c.1120C>T, p.Gln374Ter	Membrane	16202	—	—	Deleterious	P	P	40.00
c.763G>A, p.Val255Met	ER lumen	16206	0; 0.0139	0; 0.0050	Deleterious	LP	LP	25.70
c.938_939del, p.Pro313Argfs*33	Membrane	3023945	0; 0.0060	0; 0.0066	Benign	P	P	29.1

^aVariant nomenclature was based on NM_000821.7.

^bThe Genome Aggregation Database (gnomAD v4.1) consists of over 800,000 unrelated individuals sequenced in population genetic studies. The number of individuals carrying the specific variant as homozygous and the minor allele frequency (MAF) are provided.

^cBRAVO, is a variant browser containing allele frequencies for 868 million variants observed in 150,899 deeply sequenced genomes from the TOPMed, data Freeze 18.

^dWhile there are over ten different prediction algorithms, only the aggregated outcome is provided (<https://franklin.genoox.com/clinical-db/home>).

^eThe American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) classifies variants as benign (B), likely benign (LB), VUS (variants of unknown significance), likely pathogenic (LP), and pathogenic (P).

^fClinGen is a National Institutes of Health (NIH)-funded resource dedicated to building a central resource that defines the clinical relevance of genes and variants for use in precision medicine and research. ClinGen variant pathogenicity curation utilizes the ACMG/AMP, guideline for sequence variant interpretation.

^gThe Combined Annotation Depletion score (CADD) is provided for each variant. CADD, score 20 of a variant suggests top 1% most deleterious to the human genome.

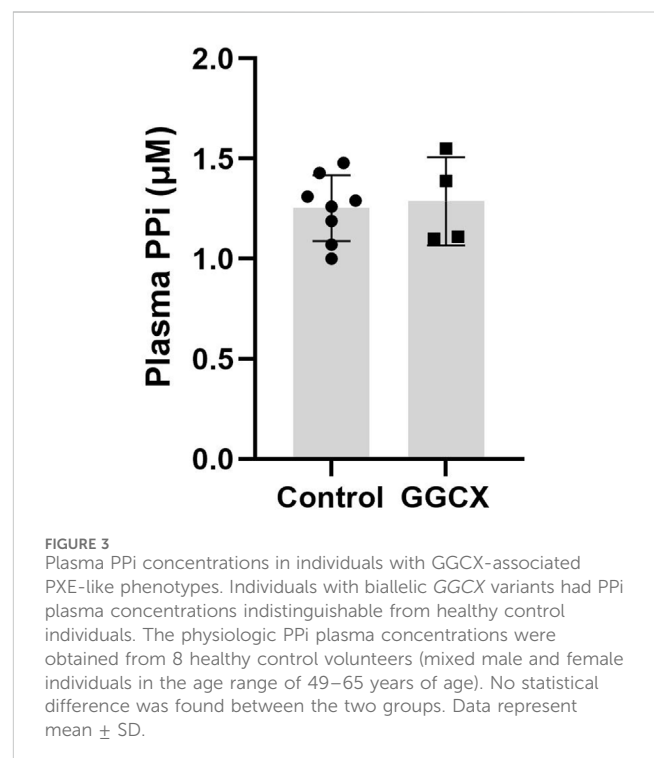
There was no evidence of blood clotting problems for either individual.

3.2 Identification of *GGCX* variants

The proband 1 in Family #1 had a homozygous c.1426C>G, p.Arg476Gly variant in the *GGCX* gene (Figure 1). This variant was not previously described. Two of her siblings and her son are heterozygotes of this variant and clinically healthy. Patients 1 and 2 in Family #2 were compound heterozygous for c.247C>T, p.Arg83Trp and c.1120C>T, p.Gln374Ter in the *GGCX* gene (Figure 1). The proband's parents and three children are heterozygotes. Both variants have been previously reported (Li et al., 2009b). Patients 1 and 3 in Family #3 were compound heterozygous for c.763G>A, p.Val255Met and c.938_939del in *GGCX* (Figure 1). The p.Val255Met variant was previously reported (Li et al., 2009a) and the c.938_939del variant was reported in ClinVar. Parents and an older sibling are heterozygotes. This inheritance is consistent with autosomal recessive mode. No pathogenic sequence variations were found in these individuals in other genes in the panel, including *ABCC6*.

3.3 Bioinformatics analyses of *GGCX* variants

Five *GGCX* variants were identified in three families (Table 1). These variants are rare, with minor allele frequencies lower than 0.015% in the general population genetics databases – gnomAD and BRAVO. The aggregated predictions were deleterious for four of them: c.1426C>G (p.Arg476Gly), c.247C>T (p.Arg83Trp),



c.1120C>T (p.Gln374Ter), and c.763G>A (p.Val255Met) While the aggregated prediction was benign for c.938_939del, individual prediction algorithms such as Mutation Taster, PolyPhen2, and SIFT predict it as disease-causing. The ClinGen and American College of Medical Genetics and Genomics/Association for

Molecular Pathology (ACMG/AMP) classifies all variants as likely pathogenic (LP) or pathogenic (P). All variants had CADD scores above 20, predicted to be among the top 1% most deleterious to the human genome.

3.4 Circulating concentrations of PPI in PXE-like patients carrying biallelic *GGCX* variants

Plasma PPI concentrations were determined in patient 1 in Family #1, patient 1 in Family #2, patients 1 and 3 in Family #3. These individuals harbor biallelic pathogenic variants in *GGCX*. The results demonstrated that these individuals, despite the diagnosis of PXE and being registered in PXE International, had PPI plasma concentrations indistinguishable from healthy controls ($p > 0.5$) (Figure 3).

4 Discussion

Considering the genetic heterogeneity of heritable ectopic calcification disorders, our variant detection employed a next-generation sequencing panel consisting of 29 genes associated with ectopic calcification (Saeidian et al., 2022). The gene panel includes *ABCC6*, the gene discovered to be associated with most cases of PXE in 2000 (Ringpfeil et al., 2000; Le Saux et al., 2000; Bergen et al., 2000). The panel also has *GGCX*, whose pathogenic variants were first linked to multiple vitamin K-dependent clotting factor deficiency (VKCFD1; OMIM #277450), an autosomal recessive disorder characterized by a mild to severe bleeding tendency and a predisposition to thrombotic events (Brenner et al., 1998). Recently, variants in *GGCX* were associated with a new clinical entity including some similarities to the PXE phenotype (catalogued as “PXE-like”) and also with multiple coagulation factor deficiency (PXE/VKCFD1; OMIM #610842) (Vanakker et al., 2007). All five subjects in the study were clinically diagnosed with PXE and referred by PXE International. The sequencing panel failed to detect pathogenic variants in *ABCC6*. Instead, we identified biallelic variants in the *GGCX* gene in all five individuals. We also identified a previously unreported *GGCX* variant, c.1426C>G, p.Arg476Gly. While p.Arg476Gly is new, two variants at the same amino acid, c.1426C>T, p.Arg476Cys, and c.1427G>A, p.Arg476His, have been previously reported in patients with phenotypes similar to PXE (Vanakker et al., 2007; Watzka et al., 2014). Functional studies of the variant proteins at the same amino acid, p.Arg476Cys and p.Arg476His, demonstrate abolished carboxylation of vitamin K-dependent proteins, which is critical for blood coagulation, vascular calcification, and bone metabolism (Ghosh et al., 2021; Hao et al., 2021). The c.938_939del variant is expected to cause an out-of-frame translation followed by a premature termination codon (p.Pro313Argfs*33), resulting in a truncated and non-functional *GGCX* protein. All the variants are rare, with minor allele frequencies lower than 0.015% in the general population.

The *GGCX* gene encodes a carboxylase required for γ -glutamyl carboxylation of Gla-proteins, including clotting factors in the liver, enabling them to control blood coagulation (Zhang and Ginsburg, 2004; Berkner and Runge, 2022). Thus, γ -glutamyl carboxylase deficiency explains the coagulation disorder. In our study, Family #2 exhibits two discrete phenotypic features: a co-existence of

deficiency of vitamin K-dependent coagulation factors and cutaneous features similar to the abnormal skin elasticity, skin laxity, and ectopic skin calcification of PXE. However, Family #1 and #3 have what appears to be a far more severe cutaneous PXE phenotype without bleeding issues. The types of variants in the *GGCX* gene influence the severity of the coagulopathy. This notion is reflected by observations that not all patients with *GGCX* variants have vitamin K-dependent coagulation factor deficiency (De Vilder et al., 2017), which functional studies could explain that distinct variants in the *GGCX* gene can reduce the corresponding carboxylase activity to a different degree in different substrates (Tie et al., 2016; Hao et al., 2021; Rishavy et al., 2022; Ghosh et al., 2021).

While plasma PPI concentrations in PXE patients with *ABCC6* deficiency were reduced to approximately 30%–40% of controls, we found that patients with *GGCX* deficiency had similar plasma PPI levels to controls. The results point to different pathomechanisms of ectopic calcification in these genetic diseases caused by variants in distinct genes. *ABCC6* is a hepatic efflux transporter mediating the extracellular release of ATP, which is cleaved to PPI to prevent ectopic calcification. The current prevailing view for the development of ectopic calcification in patients with *GGCX* deficiency relies on the ability of matrix Gla protein (MGP) and Gla-rich protein (GRP) to prevent premature calcification when modified by γ -glutamyl carboxylation (Ghosh et al., 2022; De Vilder et al., 2017). If the carboxylation reaction does not occur due to deficient carboxylase enzyme activity, the undercarboxylated forms of MGP and GRP are inactive and cannot prevent ectopic calcification.

In conclusion, we identified five individuals who carry *GGCX* variants and develop signs and symptoms similar to PXE, expanding the *GGCX* variant landscape and genetic spectrum responsible for PXE. Despite their associated gene being *GGCX*, all five individuals in the study were diagnosed through a positive von Kossa stain and either peau d'orange or angioid streaks, hallmarks of the PXE diagnostic criteria. They all receive support from PXE International. The ectopic calcification in patients with *GGCX* deficiency cannot be explained by low plasma concentrations of PPI. The results suggest that although PPI is a significant determinant of ectopic calcification, it does not govern ectopic calcification in the context of *GGCX* deficiency. One of the limitations of the study relates to the small number of individuals analyzed. Further work is necessary to examine PPI homeostasis in patients with *GGCX* deficiency.

Data availability statement

The previously unreported *GGCX* variant, c.1426C>G, p.Arg476Gly, presented in the study, is deposited in ClinVar, accession number SCV005092011.1.

Ethics statement

The studies involving humans were approved by Genetic Alliance Institutional Review Board (Approval number PXE001). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written

informed consent was obtained from the individual(s), and minor(s); legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

QL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Supervision, Writing—original draft, Writing—review and editing. CT: Data curation, Formal Analysis, Writing—review and editing. MP: Data curation, Formal Analysis, Writing—review and editing. TW: Data curation, Formal Analysis, Writing—review and editing. ST: Data curation, Formal Analysis, Writing—review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by PXE International and NIH/NIAMS grant R01AR072695 (QL).

Acknowledgments

We thank all the affected individuals and families for their participation. We thank Douglas Ralph for technical assistance.

References

- Belinsky, M. G., and Kruh, G. D. (1999). MOAT-E (ARA) is a full-length MRP/cMOAT subfamily transporter expressed in kidney and liver. *Br. J. Cancer* 80, 1342–1349. doi:10.1038/sj.bjc.6690527
- Bergen, A. A., Plomp, A. S., Schuurman, E. J., Terry, S., Breuning, M., Dauwerse, H., et al. (2000). Mutations in ABCG6 cause pseudoxanthoma elasticum. *Nat. Genet.* 25, 228–231. doi:10.1038/76109
- Berkner, K. L., and Runge, K. W. (2022). Vitamin K-dependent protein activation: normal gamma-glutamyl carboxylation and disruption in disease. *Int. J. Mol. Sci.* 23, 5759. doi:10.3390/ijms23105759
- Brenner, B., Sanchez-Vega, B., Wu, S. M., Lanir, N., Stafford, D. W., and Solera, J. (1998). A missense mutation in gamma-glutamyl carboxylase gene causes combined deficiency of all vitamin K-dependent blood coagulation factors. *Blood* 92, 4554–4559. doi:10.1182/blood.v92.12.4554
- De Vilder, E. Y., Debacker, J., and Vanakker, O. M. (2017). GGCX-associated phenotypes: an overview in search of genotype-phenotype correlations. *Int. J. Mol. Sci.* 18, 240. doi:10.3390/ijms18020240
- Ghosh, S., Kraus, K., Biswas, A., Muller, J., Buhl, A. L., Forin, F., et al. (2021). GGCX mutations show different responses to vitamin K thereby determining the severity of the hemorrhagic phenotype in VKCFD1 patients. *J. Thromb. Haemost.* 19, 1412–1424. doi:10.1111/jth.15238
- Ghosh, S., Oldenburg, J., and Czogalla-Nitsche, K. J. (2022). The role of GRP and MGP in the development of non-hemorrhagic VKCFD1 phenotypes. *Int. J. Mol. Sci.* 23, 798. doi:10.3390/ijms23020798
- Goldsmith, G. H., JR., Pence, R. E., Ratnoff, O. D., Adelstein, D. J., and Furie, B. (1982). Studies on a family with combined functional deficiencies of vitamin K-dependent coagulation factors. *J. Clin. Invest* 69, 1253–1260. doi:10.1172/jci110564
- Hao, Z., Jin, D. Y., Chen, X., Schurgers, L. J., Stafford, D. W., and Tie, J. K. (2021). γ -Glutamyl carboxylase mutations differentially affect the biological function of vitamin K-dependent proteins. *Blood* 137, 533–543. doi:10.1182/blood.2020006329
- Jansen, R. S., Duijst, S., Mahakena, S., Sommer, D., Szeri, F., Varadi, A., et al. (2014). ABCG6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation—brief report. *Arterioscler. Thromb. Vasc. Biol.* 34, 1985–1989. doi:10.1161/ATVBAHA.114.304017

Gratitude to Lionel Bercovitch, MD, PXE International's Medical Director for reviewing and editing the manuscript.

Conflict of interest

Authors CT, MP, TW, and ST were employed by PXE International, Inc.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2024.1429320/full#supplementary-material>

- Jansen, R. S., Kucukosmanoglu, A., De Haas, M., Saptho, S., Otero, J. A., Hegman, I. E., et al. (2013). ABCG6 prevents ectopic mineralization seen in pseudoxanthoma elasticum by inducing cellular nucleotide release. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20206–20211. doi:10.1073/pnas.1319582110

- Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nat. Genet.* 46, 310–315. doi:10.1038/ng.2892

- Kowal, L., Huang, J., Luo, H., Singh, J., Snook, A. E., Uitto, J., et al. (2021). Functional assessment of missense variants in the ABCG6 gene implicated in pseudoxanthoma elasticum, a heritable ectopic mineralization disorder. *J. Invest. Dermatol.* 142, 1085–1093. doi:10.1016/j.jid.2021.08.435

- LE Saux, O., Urban, Z., Tschuch, C., Csiszar, K., Bacchelli, B., Quaglino, D., et al. (2000). Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. *Nat. Genet.* 25, 223–227. doi:10.1038/76102

- Li, Q., Grange, D. K., Armstrong, N. L., Whelan, A. J., Hurley, M. Y., Rishavy, M. A., et al. (2009a). Mutations in the GGCX and ABCG6 genes in a family with pseudoxanthoma elasticum-like phenotypes. *J. Invest. Dermatol.* 129, 553–563. doi:10.1038/jid.2008.271

- Li, Q., Kingman, J., VAN De Wetering, K., Tannouri, S., Sundberg, J. P., and Uitto, J. (2017). Abcc6 knockout rat model highlights the role of liver in PPI homeostasis in pseudoxanthoma elasticum. *J. Invest. Dermatol.* 137, 1025–1032. doi:10.1016/j.jid.2016.11.042

- Li, Q., Schurgers, L. J., Smith, A. C., Tsokos, M., Uitto, J., and Cowen, E. W. (2009b). Co-existent pseudoxanthoma elasticum and vitamin K-dependent coagulation factor deficiency: compound heterozygosity for mutations in the GGCX gene. *Am. J. Pathol.* 174, 534–540. doi:10.2353/ajpath.2009.080865

- Neldner, K. H. (1988). Pseudoxanthoma elasticum. *Clin. Dermatol* 6, 1–159. doi:10.1016/0738-081x(88)90003-x

- Nykamp, K., Anderson, M., Powers, M., Garcia, J., Herrera, B., Ho, Y. Y., et al. (2017). Sherlock: a comprehensive refinement of the ACMG-AMP variant classification criteria. *Genet. Med.* 19, 1105–1117. doi:10.1038/gim.2017.37

- Pfendner, E. G., Vanakker, O. M., Terry, S. F., Vourthis, S., McAndrew, P. E., McClain, M. R., et al. (2007). Mutation detection in the ABCG6 gene and genotype-phenotype analysis in a large international case series affected by pseudoxanthoma elasticum. *J. Med. Genet.* 44, 621–628. doi:10.1136/jmg.2007.051094

- Ralph, D., VAN De Wetering, K., Uitto, J., and Li, Q. (2022). Inorganic pyrophosphate deficiency syndromes and potential treatments for pathologic tissue calcification. *Am. J. Pathol.* 192, 762–770. doi:10.1016/j.ajpath.2022.01.012
- Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., and Kircher, M. (2019). CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res.* 47, D886–D894. doi:10.1093/nar/gky1016
- Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and Genomics and the association for molecular Pathology. *Genet. Med.* 17, 405–424. doi:10.1038/gim.2015.30
- Ringpfeil, F., Lebwohl, M. G., Christiano, A. M., and Uitto, J. (2000). Pseudoxanthoma elasticum: mutations in the MRP6 gene encoding a transmembrane ATP-binding cassette (ABC) transporter. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6001–6006. doi:10.1073/pnas.100041297
- Rishavy, M. A., Hallgren, K. W., Wilson, L. A., Hiznay, J. M., Runge, K. W., and Berkner, K. L. (2022). GGXX mutants that impair hemostasis reveal the importance of processivity and full carboxylation to VKD protein function. *Blood* 140, 1710–1722. doi:10.1182/blood.2021014275
- Saeidian, A. H., Youssefian, L., Huang, J., Touati, A., Vahidnezhad, H., Kowal, L., et al. (2022). Genetic heterogeneity of heritable ectopic mineralization disorders in a large international cohort. *Genet. Med.* 24, 75–86. doi:10.1016/j.gim.2021.08.011
- Scheffer, G. L., Hu, X., Pijnenborg, A. C., Wijnholds, J., Bergen, A. A., and Scheper, R. J. (2002). MRP6 (ABCC6) detection in normal human tissues and tumors. *Lab. Invest* 82, 515–518. doi:10.1038/labinvest.3780444
- Tie, J. K., Carneiro, J. D., Jin, D. Y., Martinhago, C. D., Vermeer, C., and Stafford, D. W. (2016). Characterization of vitamin K-dependent carboxylase mutations that cause bleeding and nonbleeding disorders. *Blood* 127, 1847–1855. doi:10.1182/blood-2015-10-677633
- Uitto, J., Jiang, Q., Varadi, A., Bercovitch, L. G., and Terry, S. F. (2014). Pseudoxanthoma elasticum: diagnostic features, classification, and treatment options. *Expert Opin. Orphan Drugs* 2, 567–577. doi:10.1517/21678707.2014.908702
- Vanakker, O. M., Martin, L., Gheduzzi, D., Leroy, B. P., Loeys, B. L., Guerci, V. I., et al. (2007). Pseudoxanthoma elasticum-like phenotype with cutis laxa and multiple coagulation factor deficiency represents a separate genetic entity. *J. Invest Dermatol* 127, 581–587. doi:10.1038/sj.jid.5700610
- Watzka, M., Geisen, C., Scheer, M., Wieland, R., Wiegering, V., Dorner, T., et al. (2014). Bleeding and non-bleeding phenotypes in patients with GGXX gene mutations. *Thromb. Res.* 134, 856–865. doi:10.1016/j.thromres.2014.07.004
- Zhang, B., and Ginsburg, D. (2004). Familial multiple coagulation factor deficiencies: new biologic insight from rare genetic bleeding disorders. *J. Thromb. Haemost.* 2, 1564–1572. doi:10.1111/j.1538-7836.2004.00857.x