From Clinical Diagnosis to the Discovery of Multigene Rare Sequence Variants in *Pseudoxanthoma elasticum*: A Case Report

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**INTRODUCTION**

*Pseudoxanthoma elasticum* (PXE; OMIM#264800) is an inherited disorder characterised by early cutaneous alterations, and by late clinically relevant ocular, and cardiovascular manifestations. *ABCC6* genetic tests are used to confirm clinical PXE diagnosis, but this strategy may be rather challenging when only one *ABCC6* pathogenic variant is found. A next-generation sequencing approach focusing on 362 genes related to the calcification process and/or to inherited retinal diseases was performed on a patient with clinical PXE diagnosis (skin papules and laxity, angioid streaks, and atrophy) who was carrier of only one *ABCC6* rare sequence variant. Beside *ABCC6*, several rare sequence variants were detected which can contribute either to the occurrence of calcification (*GGCX* and *SERPINF1* genes) and/or to ophthalmological manifestations (*ABCA4*, *AGBL5*, *CLUAP1*, and *KCNV2* genes). This wide-spectrum analysis approach facilitates the identification of rare variants possibly involved in PXE, thus avoiding invasive skin biopsy as well as expensive and time-consuming diagnostic odyssey and allows to broaden and to deepen the knowledge on this complex rare disease and to improve patients’ counselling, also with a future perspective of personalised medicine.

**Keywords:** ABCC6, calcification, PXE, rare disease, skin
PXE patients are typically carriers of two pathogenic variants in the \textit{ABCC6} gene, even though in $\sim$10\% of clinically affected patients only one sequence variant can be detected. Moreover, there are a number of pathologic conditions, overlapping the PXE phenotype, where other genes, as \(\gamma\)-glutamyl carboxylase (GGCX) or ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), have a pathogenic role (6–8).

In addition, the heterogeneity of the PXE phenotype in terms of number of organs involved, of disease onset and severity suggested the involvement of modifier genes (9).

Within this context, PXE diagnosis as well as patients’ counselling can benefit from the use of next-generation sequencing technologies allowing to facilitate and to broaden the identification of genes that can be involved in the disease.

**CASE REPORT**

A 56-year-old female showed papules on neck and axillae as well as marked skin laxity and redundance (i.e., neck, axillae, periumbelliac area, groyne, and inner thighs) (Figure 1A). Ophthalmological examinations revealed angiod streaks and peau d’orange. The right eye exhibited CNV requiring 2 treatments with intravitreal anti-VEGF injections, a large and peau d’orange. The right eye exhibited CNV requiring 2 treatments with intravitreal anti-VEGF injections, a large and peau d’orange. The right eye exhibited CNV requiring 2 treatments with intravitreal anti-VEGF injections, a large and peau d’orange. The right eye exhibited CNV requiring 2 treatments with intravitreal anti-VEGF injections, a large and peau d’orange. The right eye exhibited CNV requiring 2 treatments with intravitreal anti-VEGF injections, a large and peau d’orange. The right eye exhibited CNV requiring 2 treatments with intravitreal anti-VEGF injections, a large and peau d’orange.

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**DISCUSSION AND CONCLUSION**

PXE is a multisystemic disease, although dermatologists, due to early cutaneous manifestations, are frequently the first physicians to be involved, and therefore even single case reports are instrumental to deepen the knowledge on the challenging complexity of this rare disease.

In particular, the \textit{GGCX} gene encodes a \(\gamma\)-glutamyl carboxylase, an enzyme required for the activation of vitamin K-dependent proteins (e.g., blood coagulation factors and matrix Gla protein-MGP). The carboxylated form of MGP is a key factor in preventing ectopic calcification and reduced \(\gamma\)-glutamyl carboxylase activity implies a decreased carboxylation of MGP, thus favouring pathologic mineralization. Mutations in \textit{GGCX} have been described in patients with \textit{retinitis pigmentosa}, \textit{cutis laxa}, and \textit{pseudoaxanthoma elasticum}-like skin manifestations (17). Moreover, RSVs in \textit{ABCC6} and \textit{GGCX} have been reported in a family with PXE without coagulation disorder (6). Blood coagulation tests performed on our patient did not reveal decreased levels of vitamin K-dependent clotting factors, consistently with the observation that a single RSV on \textit{GGCX} gene is not sufficient to reduce the clotting factor activity (18). Our data support the occurrence of a \textit{GGCX} and \textit{ABCC6} PXE digenic inheritance (6).

Interestingly, we found a RSV in \textit{SERPINF1} gene, which encodes PEDF (pigment epithelium-derived factor), a molecule that, depending on cell type and tissue context, can modulate the expression of osteogenic genes (e.g., \textit{ALP}, \textit{Runx2}, \textit{BMP-2}) as well as angiogenesis, being a potent inhibitor of ocular blood vessels’ growth. We have already demonstrated the accumulation of PEDF in the calcified skin (19, 20), and alterations in PEDF expression/secretion have been also correlated to the development of CNV and to the pathophysiology of retinal diseases (21). PEDF is both a secreted extracellular and intracellular protein and mutagenesis studies on Arg residues in position 67 and 69 showed that nuclear import of PEDF is compromised and therefore the anti-angiogenic function of PEDF may be modified (15), further supporting the possible involvement of this molecule in PXE.
FIGURE 1 | (A) Skin plaques and skin laxity in the axillary area. (B,C) Colour fundus photographs of the posterior pole of the right and left eye, respectively. A large peripapillary atrophic area (B) and angioid streak passing through the fovea (C) are evident. (D,E) Optical coherence tomography examinations of the right and left eye, respectively. The atrophy of the retinal pigment epithelium (RPE) is evident in the right eye (arrowheads). Arrows show the abnormalities of the RPE–Bruch’s membrane complex.

TABLE 1 | List of rare sequence variants found in patient in heterozygous state.

| Gene/Chr-Exon | Nucleotide/Amino acid change | Freq ExAC | Freq GnomAD | Freq 1000G | dbSNP | VarSome | Calcification-related genes | RetNet gene | References |
|---------------|-----------------------------|------------|-------------|------------|-------|---------|-----------------------------|-------------|------------|
| ABCA4 Chr1–Ex13 | c.1928T>G p.Val643Gly | 0.001346 0.00172 0.0010 | rs61754024 LP | ✓ | (14) |
| ABCB6 Chr16–Ex29 | c.4196G>A p.Glu1400Lys | 0.000009 / / | rs63751241 P | ✓ | (10) |
| AGBL5 Chr2–Ex12 | c.2227T>G p.Ser743Ala | 0.000058 0.00006 / | rs779635578 VUS | ✓ | This study |
| CLUAP1 Chr16–Ex5 | c.465T>A p.Tyr155Ter | / / / / | P | ✓ | This study |
| GGCX Chr2–Ex2 | c.198A>G p.Met66Val | / / / / | LP | ✓ | This study |
| KCNV2 Chr9–Ex1 | c.349G>A p.Gly117Ser | 0.00017 0.000064 0.0002 | rs200353727 VUS | ✓ | This study |
| SERPINF1 Chr9–Ex3 | c.200G>A p.Arg67Gln | 0.000025 / / | rs753681259 VUS | ✓ | (15) |

The ABCA4 gene encodes an ATP-binding cassette transporter expressed in the RPE and acting as an inward-directed retinoid flipase. RSVs in this gene determine damage to photoreceptors and to the RPE, causing retinal dystrophies (i.e., from mild fundus flavimaculatus to cono-rod dystrophy, age-related macular degeneration-2, retinitis pigmentosa-like phenotypes, up to retinal atrophy) (14) that have been also described in PXE patients. A recent study reported a patient with two rare variants in the ABCC6 gene and homozygosity for ABCA4 RSV suggesting a synergic interaction causing alterations of RPE and/or of photoreceptor function and mineralization of the Bruch’s membrane (22). Although the inheritance of these retinal diseases follows an autosomal recessive pattern, some Authors have suggested that patients with only a single rare ABCA4 variant can represent a subgroup of age-related macular degeneration or of a late-onset Stargardt’s disease (23, 24).

Multigene analysis also detected RSVs in CLUAP1, AGBL5, and KCNV2 genes, which are responsible for Leber congenital amaurosis, retinitis pigmentosa, and cone dystrophy, respectively (https://sph.uth.edu/retnet/). At present, these genes and/or their products have been never related to PXE, nevertheless we cannot rule out the possibility that, in combination with mutations in other genes, these variants may play a role in patients’ ophthalmological manifestations.

In conclusion, rare sequence variants in GGCX and ABCC6 or in ABCA4 and ABCC6 have been sporadically reported in single PXE patients (6, 22), whereas no association has been observed between SERPINF1 and ABCC6 genes. For the first time, all these four genes are described in a patient clinically diagnosed as PXE, reinforcing the hypothesis that RSVs in these genes, but possibly also in other genes, may contribute to the development and progression of multisystemic clinical manifestations and to the
heterogeneity of the PXE phenotype acting in a complementary and/or synergic manner either as causative or as modifier genes.

Physicians may experience difficulties when facing rare diseases with clinical overlapping phenotypes and obstacles may arise for the appropriate recognition of clinical manifestations and for reaching a final diagnosis. Therefore, within this context, PXE patients, as well as their medical doctor, can benefit from the use of multigene analyses which can: (i) facilitate the identification of rare sequence variants involved in the disease, thus avoiding invasive approaches (i.e., skin biopsy required to reveal elastic fibre mineralization when ABCC6 test does not confirm clinical diagnosis), as well as expensive and time-consuming diagnostic odyssey before reaching a final diagnosis; (ii) deepen the knowledge on the disease in order to better understand the heterogeneity of phenotypic features and of diseases progression and (iii) improve patients’ counselling also in a future perspective of personalised medicine approaches.

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**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Ethics Committees (Project ID: 2018/13014). The patient provided her written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**

All authors contributed to the analysis of the data, contributed to manuscript revision, read, and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.726856/full#supplementary-material

**Supplementary Table 1** | List of genes responsible for calcification-related diseases or playing a role in the calcification process.
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