Late-onset Pseudoxanthoma Elasticum Associated with a Hypomorphic *ABCC6* Variant

PETER CHARBEL ISSA, CAROLYN TYSOE, AND RICHARD CASWELL

• PURPOSE: **To describe patients with late-onset pseudoxanthoma elasticum (PXE) associated with a likely hypomorphic *ABCC6* variant.**
• DESIGN: **Retrospective observational case series.**
• METHODS: **Clinical evaluation, multimodal retinal imaging, genetic testing, and molecular modeling.**
• RESULTS: **Three patients, in whom vision symptoms first arose at 80 years of age or later, showed age-related macular degeneration (AMD)-like fundus changes. However, features characteristic of PXE, including discrete angioid streaks and reduced fluorescence on late-phase indocyanine green angiography, prompted genetic testing which revealed the c.1171A > G variant in combination with a large deletion in the *ABCC6* gene in each case. None of the patients had obvious skin changes or cardiovascular disease atypical for their age. Comparative molecular modeling supported the hypothesis that the c.1171A > G_{ABCC6} variant acted as a hypomorphic variant.**
• CONCLUSIONS: **Late-onset PXE extends the spectrum of ectopic calcification disorders caused by mutations in *ABCC6* and may clinically be limited to the eye, mimicking AMD. Patients may be identified based on specific ocular changes, whereas skin and cardiovascular changes may remain ambiguous. The study provides evidence for a role for hypomorphic *ABCC6* variants in the pathogenesis of PXE. (Am J Ophthalmol 2020;218: 255–260. © 2020 Elsevier Inc. All rights reserved.)**

PSEUDOXANTHOMA ELASTICUM (PXE) (ONLINE MENdelian Inheritance in Man [OMIM] entry 264800) is a multisystem disease, characterized by progressive calcification of tissue rich in elastic fibers.[1] The diagnosis is based on characteristic changes of the skin and/or the eye, usually in combination with bi-allelic mutations in the *ABCC6* gene[2] which result in alterations of a metabolic pathway inhibiting ectopic tissue calcification.[3]

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Disease expression in patients with PXE may vary considerably.[4–6] Clinically relevant manifestation may initially remain limited to the skin, the eyes, or both. Many patients do not have cardiovascular symptoms, at least not early in the disease course. Moreover, the age at which first signs and/or symptoms are reported is also highly variable, with a reported age-of-onset between childhood and the fifth or sixth decade of life.[4,5,7] An initial diagnosis of PXE even later in life may be challenging because of the increasing frequency of cardiovascular disease and degenerative skin changes with ageing, and the high prevalence of age-related macular degeneration (AMD), which may mimic many features of the ocular PXE phenotype.[6,8]

This study describes the cases of 3 patients with late-onset PXE who had not noted any signs or symptoms of the disease before 80 years of age and whose diagnoses were based on specific findings upon retinal examination. In silico structural analysis of the mutated protein indicated an associated hypomorphic *ABCC6* variant.

SUBJECTS AND METHODS

THIS IS A RETROSPECTIVE OBSERVATIONAL CASE SERIES OF patients with first PXE-related symptoms after the age of 80 seen at a single academic center. All clinical investigations and diagnostic genetic testing were performed as part of routine clinical care, and therefore this work did not require ethical review.[9] Ophthalmic assessment was carried out according to standard departmental practice, including visual acuity measurements, fundus autofluorescence (AF), and spectral-domain optical coherence tomography (OCT) imaging (Spectralis HRA-OCT; Heidelberg Engineering, Heidelberg, Germany), fluorescein and indocyanine green (ICG) angiography (HRA2, Heidelberg Engineering), and fundus color photography (TRC-50DX, Topcon, Tokyo, Japan).

The coding regions and 50 nucleotides of flanking intronic sequences of the *ABCC6* (GenBank accession number NM_001171.5), *ENPP1* (accession number NM_006208.2), and *NT5E* (accession number NM_002526.3) genes were analyzed by targeted next-generation sequencing using a customized capture run on an Illumina NextSeq500 machine (Agilent, Santa Clara, California), which detects both base substitutions and partial or whole-gene deletions. Partial gene deletions were confirmed by dosage analysis of the *ABCC6* gene using

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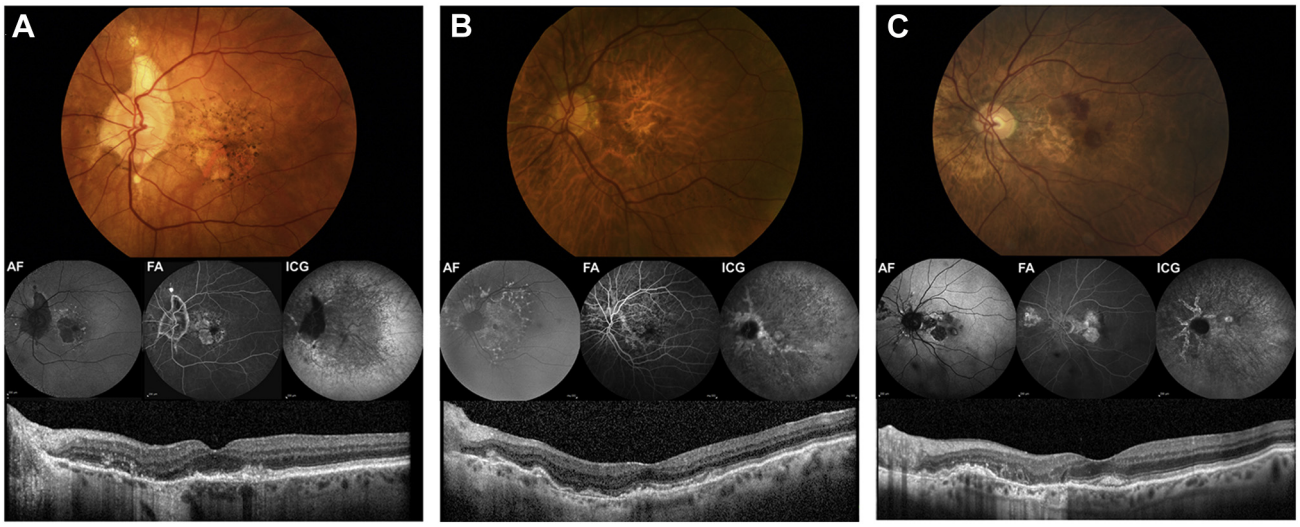

FIGURE 1. Retinal phenotype of patients 1 (A), 2 (B), and 3 (C) with late-onset pseudoxanthoma elasticum. Only 1 eye is shown per patient because both eyes were similar. (Upper) Fundus color images. (Middle) AF, fluorescein angiography (FA), late phase (> 30 minutes) ICG angiography. (Bottom) OCT. OCT of patients 2 and 3 show no sub- or intraretinal fluid despite neovascular disease because of previous treatment with an intravitreally injected VEGF inhibitor.

multiplex ligation-dependent probe amplification (kit P092-B3; MRC-Holland, Amsterdam, the Netherlands). Variant pathogenicity was determined using the 5-tier classification system according to American College of Medical Genetics guidelines.[10]

Comparative molecular modeling was performed using the Swiss-Model server (University of Basel, Basel, Switzerland),[11] which identified existing structures of bovine ABCC1 as suitable templates. Variants were introduced in silico using the FoldX modeling suite.[12] All structures were visualized in PyMOL (Schrödinger, New York, New York).

RESULTS

PATIENT CHARACTERISTICS AND GENETIC FINDINGS ARE summarized in Figure 1 and the Table. Patient 1 (80 years old) reported a recent onset of distorted vision in his left eye. He had no previous sight problems, no history or symptoms indicating cardiovascular disease, and did not regularly take any medication. Fundoscopy revealed bilateral peripapillary atrophy, subtle angioid streaks, reticular pseudodrusen, pigmentary macular changes, and small paracentral atrophic areas. Fundus AF imaging showed flecks of increased AF in the macula. Areas of atrophy were well visualized on AF imaging and fluorescein angiography, whereas late-phase ICG angiography accentuated angioid streaks and showed a reduced fluorescence at the posterior pole. OCT imaging revealed mild paracentral cystoid changes in the right eye, likely associated with evolving outer retinal atrophy. The patient had no skin changes characteristic of PXE.

Patient 2 (82 years old), without previous vision problems or ocular surgery, reported reduced vision for about 6 months. An aneurysm of the ascending aorta had recently been diagnosed, but he was otherwise healthy, particularly without any additional cardiovascular symptoms or medication. Funduscopy revealed macular pigmentary changes and angioid streaks radiating from the peripapillary area. Fundus AF imaging showed areas of increased AF and adjacent fleck-like lesions, whereas small areas with atrophic retinal pigment epithelium appeared with a low signal. Angiography and OCT imaging confirmed an active choroidal neovascularization (CNV) in both eyes, and bilateral intravitreal treatment with a vascular endothelial growth factor (VEGF) inhibitor was initiated. Late-phase ICG angiography showed extensive angioid streaks and reduced fluorescence at the posterior pole. The patient had no characteristic skin lesions that would have allowed a clinical diagnosis of PXE.

Patient 3 (95 years old) had been diagnosed elsewhere with neovascular AMD when he was 83 years old, and since then, he had received bilateral intravitreal injections due to recurrent neovascular activity. He also had cataract surgery at the age of 85. He had been treated for arterial hypertension for 26 years and for type 2 diabetes mellitus for 18 years. Examination of the ocular fundus raised suspicion of angioid streaks radiating from around the optic disc, and there were reticular pseudodrusen, patches of atrophy, and subretinal hemorrhages at the posterior pole. Retinal imaging, including fluorescein and ICG angiography, OCT, and fundus AF imaging better defined the angioid streaks, areas of atrophy, and CNV. Late-phase ICG angiography revealed an abnormally low signal throughout the 55-degree image. His skin showed mild papular appearance

TABLE. Patient Characteristics and Genetic Findings of 3 Patients With Late-Onset Pseudoxantoma Elasticum

Patient	Age (y)	Sex	Visual Acuity		*ABCC6* Variants[a]	
			OD	OS	Nucleotide	Protein
1	80	M	20/25	20/20	c.1171A>G	p.(Arg391Gly)
					c.(475-23_794+35)_(794+35_795-1)	p.? [deletion of exon 7]
2	82	M	20/200	20/40	c.1171A>G	p.(Arg391Gly)
					c.(2995+1_c.2996-1)_(c.4208+1_c.4209-1)	p.? [deletion of exons 23-29]
3	95	M	20/80	20/25	c.1171A>G	p.(Arg391Gly)
					c.(2995+1_c.2996-1)_(c.4208+1_c.4209-1)	p.? [deletion of exons 23-29]

M = male; OD = right eye; OS = left eye.

[a]Genetic diagnosis was ascertained through analysis of all the coding regions and exon/intron boundaries of the *ABCC6* and *ENPP1* genes by targeted next-generation sequencing. Dosage analysis of the *ABCC6* gene was performed by multiplex ligation-dependent probe amplification. Nomenclature is based on GenBank accession NM_001171.5 and the Human Genome Variation Society recommendations for describing the genotypes.

in the flexural creases which was considered nonspecific at his age.

In all 3 patients, genetic testing revealed the same heterozygous missense variant in *ABCC6*, c.1171A>G p.(Arg391Gly) (accession NM_001171.5), in combination with a partial gene deletion (Table). The c.1171A>G p.(Arg391Gly) allele has previously been reported as a pathogenic variant in PXE (Human Genome Mutation Database accession CM040969).[13] However, it also occurs in the Genome Aggregation Database (https://gnomad.broadinstitute.org; accessed January 23, 2020) at a carrier frequency of ~1 in 366 and was present as a homozygous variant in 5 individuals in this cohort. There are thus conflicting possible interpretations as to the pathogenicity of this variant.

Comparative molecular modeling was performed using structures of bovine ABCC1, which shares significant homology with ABCC6, as templates. Notably, structures for ABCC1 have been solved in 3 conformations: an unliganded (apo-) form, with the substrate channel open to the cytoplasm ("inward-facing"); the inward-facing form bound to the substrate leukotriene-c4 (LTC_4); and the ATP-bound, outward-facing form.[14,15] Modeling of the ABCC6 sequence of these structures yielded models covering residues ~200-1500 of ABCC6, with 47% sequence identity to the template. Predicted models show Arg391 is situated near the cytoplasmic end of a helix in transmembrane domain (TMD) 1 of the protein, lying between the lasso region and TMD2 (Figure 2A). These 2 parts of the protein move together and toward each other as first ligand, and then ATP binding induces the conformational switch from the inward-facing to the outward-facing conformation. In this movement, the Arg391 sidechain appears to act as a link between the lasso region and the TMD2 domain (Figure 2B, Supplemental Figure). In the outward-facing form, Arg391 would be able to form interactions with residues in both the lasso region, specifically Glu253 which likely forms a salt bridge with the Arg391 sidechain, and residues Val1147 and Asn1151 in TMD2, thus contributing to the stability of this conformation. However, the absence of a sidechain in the variant glycine residue precludes such interactions likely leading to reduced efficiency of the transition from the inward-to-outward facing forms upon ligand and ATP binding and thus impairment of the activity cycle of ABCC6. This hypothesis was supported by thermodynamic analysis in silico, which indicated a moderately destabilizing effect of the Arg391Gly substitution in the unliganded, inward-facing form of the channel, becoming progressively stronger in the ligand-bound, inward-facing, and outward-facing forms respectively (data not shown).

DISCUSSION

THIS STUDY REPORTS LATE-ONSET PXE IN 3 PATIENTS WHO were asymptomatic and hence undiagnosed before the age of 80 years. Their condition was diagnosed based on characteristic ocular findings and the presence of 2 pathogenic or likely pathogenic variants in *ABCC6*, hence fulfilling diagnostic criteria of PXE.[2] Concurrent mild cardiovascular disease (asymptomatic aortic aneurysm, arterial hypertension) in 2 of the 3 patients would unlikely have allowed the diagnosis because these findings are not PXE-specific at older ages. None of the patients had obvious PXE-related skin changes, possibly because of the overall mild disease manifestation and/or the possible masking of very subtle signs by age-related skin changes.

Late-onset PXE extends the phenotypic spectrum of *ABCC6*-related disease at the mild end, with the most severe manifestation in the form of generalized arterial calcification of infancy at the other end of the spectrum.[7] The

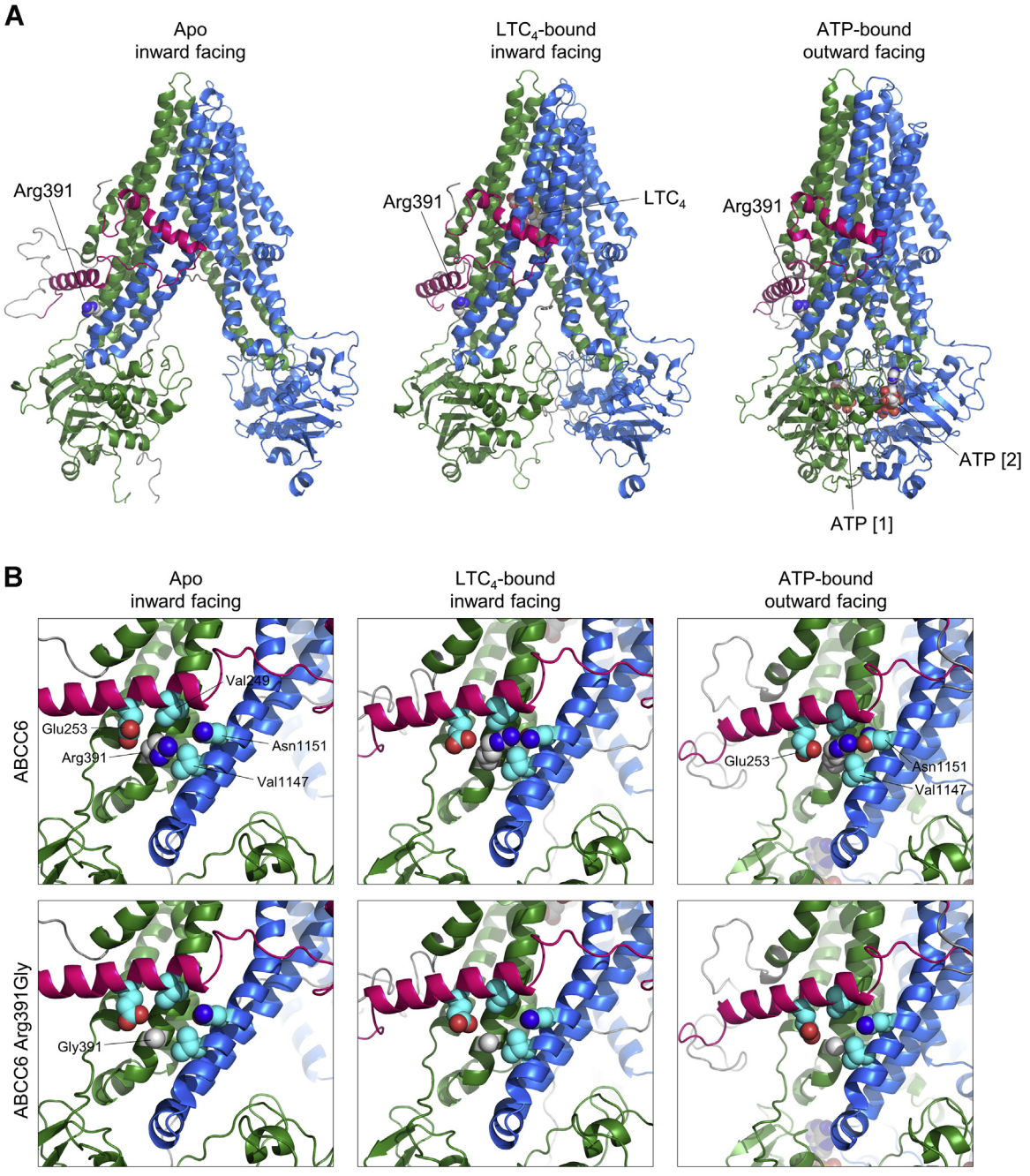

FIGURE 2. Predicted structure of ABCC6 and the Arg391Gly variant. (A) ABCC6 was modeled on Protein Data Bank (PDB) entries 5uj9, 5uja, and 6bhu of bovine ABCC1, representing the apo- (inward facing), ligand-bound (inward facing), and ATP-bound (outward facing) conformations, respectively, as labeled; in each part the Arg391 sidechain and ligand molecules are shown as space-filling spheres, colored by atom type (carbon = pale gray; nitrogen = blue; oxygen = red; phosphorus = orange). ABCC6 domains are colored as pink = lasso (or L_0) domain (residues 204-268); green = TMD1 (302-867); and blue = TMD2 (residues 932-1489). The molecule is oriented to show the cytoplasmic ATP-binding regions at the bottom of the figure. (B) Upper row, same as (A) but enlarged to show detail around Arg391 and rotated 45° rightward around the vertical axis. In addition to Arg391, sidechains are shown in space-filling format for near neighbors, with carbon atoms colored cyan. The lower row shows the same view for predicted structures of the Arg391Gly variant.

enormous range of age-of-onset and disease severity in a monogenic disease indicates variable mutational effects and/or presence of significant genetic disease modifiers. Although milder PXE-like disease may also be observed secondary to hemoglobinopathies[16] or as part of the syndromic spectrum in other rare heritable ectopic

mineralization disorders, such association appears unlikely in the reported patients of British descent with no other clinical findings indicating such a disorder.

Three of these patients had reticular pseudodrusen in combination with a CNV or outer retinal atrophy. These fundus features may occur in PXE and AMD and hence would not allow differentiation between the 2 disease entities in the elderly patient.[17,18] However, the presence of angioid streaks led to further investigations including late-phase ICG angiography, which identified decreased staining of the posterior pole with enhanced visibility angioid streaks, both findings typically seen in PXE patients.[19,20] The absence of obvious *peau d'orange* and peripheral comet lesions indicates that these features are not necessary for diagnosing ocular manifestations of PXE.

The same missense variant in combination with a partial gene deletion in all 3 patients indicated that c.1171A>G_{ABCC6} may be a hypomorphic variant associated with milder, late-onset disease. Such a dosage effect would be in keeping with previous observations that monoallelic *ABCC6* mutations may be associated with a mild, late-onset ocular phenotype,[21] and with a generally milder PXE phenotype in patients with combination of a missense with a truncating variant.[5] Younger PXE patients carrying the c.1171A>G_{ABCC6} variant would then require additional (genetic) modifiers that aggravate the phenotype.

Although the 5 apparently healthy individuals listed in the Genome Aggregation Database (gnomAD) carrying the variant in the homozygous state would generally be a strong argument against a variant's pathogenicity, c.1171A>G_{ABCC6} has repeatedly been reported to be associated with PXE and hence is usually interpreted as pathogenic. It remains to be shown if c.1171A>G_{ABCC6} homozygosity results in disease at all or if the 5 homozygous individuals in GnomAD could be in a presymptomatic stage of disease and were consequently included in the "healthy" control cohort.

From a functional perspective, Arg391 appears to play a role in the structural transition from inward-facing to outward-facing conformations of the transporter by acting as a link between the lasso region and TMD2. The predicted effect of the c.1171A>G_{ABCC6} variant, which substitutes arginine for glycine, would be to negate this role, likely impairing the dynamic interchange between the different states of ABCC6. The limited magnitude of the effects of the variant on thermodynamic stability would be unlikely to cause global loss of function but could be sufficient to affect local structural changes, consistent with the hypothesis that c.1171A>G_{ABCC6} acts as a hypomorphic variant.

Limitations of this study include that detailed cardiovascular investigations were not performed and that compound heterozygosity could not be confirmed because no family members were available for segregation. Moreover, although the diagnosis of another disease with potential PXE-like presentation appears unlikely, this possibility was not fully explored (extended genetic testing, blood electrophoresis).

In summary, 3 patients with late-onset PXE were diagnosed based on ocular and genetic findings, indicating that specific retinal changes may be sensitive for detecting mild disease. Late-onset PXE should be considered a differential diagnosis to AMD.

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