Pseudoxanthoma Elasticum with Generalized Retinal Dysfunction, a Common Finding?

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**METHODS.** Four unrelated patients carrying a clinical diagnosis of PXE presented with unexplained visual loss. After ophthalmic examination, retinal and macular function was assessed by full-field electroretinogram (ERG) and pattern ERG, respectively, according to ISCEV (International Society for Clinical Electrophysiology of Vision) recommendations. Molecular analysis of the ABCC6 gene was performed in three patients by dHPLC (denaturing high-performance liquid chromatography) and direct sequencing.

**RESULTS.** Full-field ERG revealed significant reduction of cone and rod responses in all four patients. Fundusscopic appearances varied. Three patients were found to carry ABCC6 mutations. In case 1, a novel nonsense mutation (p.L1474X) was detected in exon 31 paired with a splice-site mutation. Mutation analyses in cases 3 and 4 revealed previously reported ABCC6 mutations.

**CONCLUSIONS.** These findings suggest that retinal dysfunction in PXE may not be uncommon. The mechanism underlying retinal dysfunction is unknown but may result from metabolic disturbance leading to retinal toxicity with a possible role of modifying genetic or environmental factors rather than specific ABCC6 mutations. (Invest Ophthalmol Vis Sci. 2007;48: 4250–4256) DOI:10.1167/iovs.05-1604

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Supported by a Career Development Award from Foundation Fighting Blindness (IA).

Submitted for publication December 16, 2005; revised March 27, 2006; accepted July 12, 2007.

Disclosure: I. Audo, None; O.M. Vanakker, None; A. Smith, None; B.P. Leroy, None; A.G. Robson, None; S.A. Jenkins, None; P.J. Coucke, None; A.C. Bird, None; A. De Paepe, None; G.E. Holder, None; A.R. Webster, None

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Pseudoxanthoma elasticum (PXE, MIM [Mendelian Inheritance in Man] 264800) is a systemic disorder characterized by progressive degeneration and calcification of elastic fibers. The phenotype of the disease is highly variable, partially depending on the age of the patient, and includes mainly skin, ocular, and cardiovascular abnormalities (see Ref. 1 for review). The prevalence of the disease is estimated between 1 in 70,000 and 1 in 100,000.2-3 In the past, both autosomal recessive and dominant transmission have been described. However, recent evidence suggests that most, if not all, familial cases are recessive forms. Previously described dominant pedigrees can be explained by the presence of pseudodominance due to a high carrier rate or consanguinity.3,4 Several groups have identified ABCC6 as the defective gene,3-8 which causes a loss of function resulting in the PXE phenotype. It is a member of the ATP-binding cassette transmembrane transporter family, also assigned to the subfamily of multidrug resistant proteins (MRPs).9 It is highly expressed in human and mouse liver, to a lesser degree in kidneys,10-12 but at only very low levels in the tissues that are clinically most affected.3,10 The precise function of ABCC6 remains unclear, although its location at the basolateral side of the cellular membrane suggests a role in transporting substances involved in connective tissue homoeostasis, normally extruded from liver and kidney. Hence, PXE could be considered a metabolic disease.13,14

The cutaneous phenotype typically consists of discolored (yellowish) papules and plaques on the neck and Moroccan leatherlike skin lesions with redundant folds in flexural areas. Cardiovascular complications that may be associated with the disease are mainly due to accelerated atherosclerosis (e.g., peripheral and coronary artery disease, stroke).1

The ocular phenotype is variable. A “peau d’orange” fundus appearance can be present in childhood as the earliest ophthalmic sign, but it tends to become less distinct with age. This unusual mottled feature corresponds with yellowish lesions of the retinal pigment epithelium (RPE), typically in the midperiphery and particularly on the temporal side. Angioid streaks radiating from the optic disc are commonly reported, and other signs may include optic nerve head drusen and reticular pigmentary dystrophy of the macula. Crystalline bodies may be present in the midperiphery and juxtapapillary area with variable degrees of underlying RPE atrophy. These lesions are referred to as “comets” and may be associated with streaks of RPE thinning in a comet-like tail pattern extending toward the posterior pole.15,16 These focal abnormalities may be the only lesions typical of PXE, according to Gass.15

Visual impairment in patients with PXE can occur because of macular atrophy, choroidal rupture, or choroidal neovascularization, with or without choroidal hemorrhage from angiod streaks, and theoretically from complications of optic nerve head drusen. Visual function is poorly documented in the literature: François and De Rouck17 described mild amplitude
reduction in electroretinograms (ERGs) in 46% of cases, mostly in eyes with advanced disciform macular degeneration suggesting that retinal dysfunction is not an unusual abnormality in PXE. However, et al.18 failed to detect any functional changes associated with the peau d’orange retinal appearance.

In this study, four patients with PXE are reported who had generalized retinal dysfunction involving both cones and rods.

METHODS

Electrophysiology was performed with gold foil recording electrodes according to ISCEV (International Society for Clinical Electrophysiology of Vision) standards.19-20 Molecular analysis was performed in three patients. The ABCC6 gene was amplified by using polymerase chain reaction primers previously described by Wang et al.21 and Le Saux et al.22 For the detection of the common exon 23 to 29 deletion, a PCR-based method with primers described by Le Saux et al.22 was performed on all samples. In this assay, PCR amplification will be observed only in the presence of a multiexon deletion. The coding region and intron–exon boundaries of the whole ABCC6 gene were analyzed with dHPLC (denaturing high performance liquid chromatography; Wave System; Transgenomics, Inc., Omaha, NB) and direct sequencing (model 3100 sequencer with PRISM BigDye Terminator kit, Applied Biosystems, Inc., Foster City, CA).

The protocols used to perform this study adhered to the tenets of the Declaration of Helsinki and were approved by the local Ethics Committee.

RESULTS

Case 1

A 16-year-old Turkish boy had a history of night blindness since the age of 2. Although fit and well at presentation, he had been treated for non-Hodgkin malignant lymphoma at the age of 2 years with chemotherapy, including systemic treatment with endoxan, oncovin, ara-C, methotrexate, and 6-mercaptopurin and intrathecal methotrexate, prednisolone, and alexan. He reported poor color discrimination, worsening of visual acuity, and progressive visual field constriction over a 3-year period. There was no family history of visual problems or systemic diseases. His dizygotic twin brother was asymptomatic with normal vision, and his parents were first cousins (Fig. 1a). At the time of his first visit, his vision was 6/12 in both eyes with an optical correction of $-4(-1.75)20^\circ$ in the right eye and $-3.75(-2.25)165^\circ$ in the left. He had been wearing spectacles since the age of 5. Static perimetry (Fig. 2) showed diffuse loss of sensitivity worse in the central field. On slit lamp examination, his anterior segments were unremarkable, and, in particular, there was no evidence of crystalline deposits in the corneal limbus. Fundus examination (Fig. 3a) revealed bilateral angioid streaks, a peau d’orange aspect on the temporal side of the macula of both eyes and some macular RPE atrophy. Intraretinal crystalline bodies were disseminated over the posterior pole and midperiphery and associated with underlying RPE atrophy. Some of the larger crystalline lesions were associated with a punched-out appearance. Fundus autofluorescence examination (Fig. 3b) showed areas of low density consistent with RPE atrophy associated with the crystals, angioid streaks, and fovea. The punched-out lesions had a distinct autofluorescent appearance with a high-density center surrounded by a hypoautofluorescent ring. Angioid streaks and the crystalline lesions showed areas of window defect on fluorescein angiography congruent with the underlying RPE atrophy (Fig. 3c). On indocyanine green angiography, punched-out lesions correspond to hypofluorescent areas (Fig. 3d). Examination with optical coherence tomography (Fig. 3e) revealed the presence of crystalline bodies at the level of the inner retinal layers.

Color contrast sensitivity was assessed along the protan, deutan, and tritan axes. All thresholds were grossly elevated (data not shown). The patient underwent electrophysiology (Fig. 4a). Pattern ERG was markedly reduced in the right eye and undetectable in the left. The rod-specific ERG was markedly subnormal in both eyes. The maximum responses showed reduced amplitude for both a- and b-waves. The 30-Hz flicker and single flash cone ERGs showed profound delay and profound reduction in amplitude. The findings are those of severe generalized retinal dysfunction involving the cone more than the rod systems, with pattern ERG evidence of macular involvement, worse on the left than the right. Other tests results, including full blood count, ionogram, creatinine level and hemoglobin electrophoresis, were normal. No sickle cell trait was detectable. A dermatologic examination showed no evidence of the skin lesions in the flexural areas, and the patient declined a skin biopsy. Visual acuity dropped to 6/24 bilaterally 32 months after presentation but the fundus appearance was unchanged.

Molecular analysis of the ABCC6 gene revealed two base-changes: a 3507(−3)G→T transition at the splice acceptor site of intron 24 and a p.L1474X nonsense mutation (c.4420 A→T, exon 31; Figs. 5a, 5b).

Case 2

This 45-year-old Moroccan man presented with a 2-year history of gradual visual loss in both eyes and photophobia. He also reported difficulties seeing in the dark and especially adjusting from light to dark. Six years earlier, he had been given a diagnosis of PXE based on typical skin lesions and fundus abnormalities. There was no family history of visual problems or systemic disorders. His parents were first cousins (Fig. 1b). His visual acuity at the time of the referral was hand movements with $+1(-1.50)90^\circ$ on the right and 6/18 to 2 with $+0.50(-1.25)90^\circ$ on the left. Visual fields to confrontation...
showed residual perception in the superior and temporal field of both eyes. A fundus examination (Fig. 3f) showed healthy optic discs, narrow vessels, angioid streaks, and bilateral pigmented fibrovascular scars in the macula, probable sequelae of bilateral choroidal neovascularization. A peripheral examination showed atrophy of the RPE outside the arcade, with pigment migration at the level of the retina.

Electrophysiology was performed (Fig. 4). No pattern ERG was detectable. A full-field ERG showed no rod-specific response, severely subnormal maximal rod–cone responses, and markedly delayed and reduced 30-Hz flicker ERG. The findings were consistent with severe generalized retinal dysfunction affecting both rod and cone systems, with severe bilateral macular involvement. These results suggest advanced photoreceptor dystrophy and cannot be explained on the basis of fibroglial scars present on fundus examination. He was reviewed in the clinic 6 months later and showed a decline in his vision-to-hand movement in the right eye and 6/36 in the left. Two years after presentation, his vision remained stable, and his fundus appearance was unchanged. The patient declined further testing and was unwilling to provide a blood sample for genotype analysis.

Case 3

This 37-year-old white woman with a diagnosis of PXE was referred in May 2003 for follow-up of her retinal status. She reported night blindness and peripheral visual field constriction. She had received a diagnosis of PXE in her early 20s, based on characteristic fundus abnormalities and typical skin lesions. There was no family history of ocular disease or systemic disorder and there was no parental consanguinity. Her visual acuity was 6/9 in the right eye with −1.50 D and 6/24 with −1 D in the left eye. Visual fields showed concentric constriction in her right eye and diffuse loss of sensitivity in the left eye. Fundus examination (Fig. 3g) revealed multiple angioid streaks, a temporal appearance of peau d’orange, punched-out lesions in the midperiphery, and RPE atrophy in the periphery with pigment migration into the neural retina. There was no evidence of choroidal neovascularization. One of the angioid streaks passed through the foveola in the left eye, with additional RPE atrophy explaining the poor left visual acuity. Pattern ERG and full-field ERG abnormalities were severe bilaterally, in keeping with generalized retinal dysfunction affecting rod more than cone photoreceptors with evidence of severe bilateral macular involvement (Fig. 4). Her vision remained stable, and her fundus appearance was unchanged at the 1-year follow-up.

On mutation analysis, this patient was homozygous for the p.R1141X nonsense mutation (c.3421C>T) in exon 24 (Fig. 5c).

Case 4

This white man was first seen at the age of 26. He carried a diagnosis of PXE based on fundus changes (angioid streaks and peau d’orange), typical skin lesions, and a history of digestive complications with gastrointestinal hemorrhage. The diagnosis was confirmed by skin biopsy, which showed degeneration and calcification of the elastic fibers.

He had visual field loss that initially had been attributed to bilateral complicated optic nerve head drusen. He also had three affected siblings with variable manifestations of PXE as well as an affected mother, maternal aunt, and a cousin. There was a possible low degree of consanguinity in the family, which raised the suspicion of pseudodominant PXE (Fig. 1c).

His vision at the time of the first referral was 6/18 in the right eye and 6/9 in the left. Over the course of follow-up, his visual acuity gradually deteriorated consequent to choroidal...
neovascularization in the right eye, which resulted in a disciform scar, and atrophic changes in the left eye, in addition to the optic nerve drusen. By the age of 55, his vision was hand movements in the right eye and 1/60 in the left eye. Fundus examination (Fig. 3h) showed a waxy appearance of both optic nerves with nerve head drusen, angioid streaks, a fibroglial scar on the right macula and an area of geographic atrophy on the left. The severity of visual impairment was unusual for PXE and electrodiagnostic tests were performed to distinguish between optic nerve and retinal dysfunction (Fig. 4). No pattern ERG was detectable, indicating severe macular dysfunction bilaterally. Rod specific ERG was subnormal. Maximum ERGs, 30-Hz flicker, and transient photopic ERGs were mildly subnormal bilaterally. These findings were consistent with severe bilateral macular dysfunction with evidence of mild generalized retinal involvement affecting the rod more than the cone system.

The patient was found to be compound heterozygous for the known p.R1114P missense mutation (c.3341G→C) in exon 24 and a multiexon deletion of exons 23 through 29 (Figs. 5d, 5e).

**DISCUSSION**

The present study characterizes the molecular and clinical features of a heterogeneous group of four patients with PXE who underwent comprehensive assessment of generalized retinal function. Full-field ERG revealed three functional pheno-
FIGURE 4. Electrophysiology (a) Case 1: scotopic rod-specific ERGs are markedly subnormal from both eyes. The maximum responses show reduced amplitude for both a- and b-waves. Both 30-Hz flicker and single flash cone ERGs show profound delay and profound reduction in amplitude. Pattern ERG is markedly reduced in the right eye (RE) and undetectable in the left (LE). The findings are those of severe generalized
types; cone–rod dystrophy (case 1), rod–cone dystrophy (cases 3 and 4), and severe photoreceptor dystrophy involving rods and cones equally (case 2). Uniquely in PXE, pattern ERGs were recordable, allowing objective assessment of macular function.25–27

Funduscopic appearances also varied. Diffuse changes extending beyond the arcades were seen (cases 1 and 2) with numerous intraretinal crystals in case 1. One possibility is that the crystalline retinopathy is due to the chemotherapeutic agents used during his childhood illness, although there is no precedent for this with any of the agents listed. Alternatively, this appearance has been reported in the retinas of persons with PXE,1,15 whereas no association between the chemotherapeutic drugs taken by the subject and crystals can be found in the literature. In case 1, a novel nonsense mutation (p.L1474X) was detected in exon 31. A further base change was detected, 3507(−3)C→T, which is predicted to affect splicing and has been detected previously in PXE patients (Olivier Vanackker, personal communication, 2005). Mutation analysis in cases 3 and 4 revealed known mutations in exon 24 and a multiexon deletion which is frequently observed in PXE. There have been few reports in the literature describing retinal function in PXE. Francois and De Rouck17 described 15 patients. Slightly subnormal dark adaptation was present in 18 eyes. Nonstandard ERG recordings suggested mild retinal dysfunction in 14 eyes that involved only the cone system (2 cases), only the rod system (4 cases), or both rod and cone systems (8 cases). Most of these patients had advanced disciform degeneration, although normal ERGs were also recorded in cases of terminal macular degeneration. These findings suggest that moderate retinal dysfunction, similar to that in case 4 (Fig. 4d) may commonly be present and could explain the night vision difficulties reported frequently by PXE patients in our experience.

Yoshida et al.24 described an association between PXE and severe retinal dysfunction in a single Japanese pedigree.24 The proband was homozygous for a novel mutation in ABCC6 (nonsense mutation in exon 5), and his two affected daughters were heterozygous for this mutation with clinical signs of PXE and ERG evidence of rod–cone dystrophy (RP). The authors suggested either a coincidental association or the possibility of a combination of ABCC6 alleles, which can give rise either to PXE or RP. ABCC6 may also behave like a modifier gene contributing to the pathogenesis of RP.24

A coincidental association between PXE and retinal dystrophy in the current series could occur, with co-inheritance of the ABCC6 mutations with those in an as yet unknown gene with a role in retinal function. This scenario would be more likely in cases 1 and 2, for whom parental consanguinity theoretically increases the likelihood of an association of two separate and independent recessive disorders. The generalized retinal dysfunction seen in these four patients may have been due to the effect of specific alleles of the ABCC6 gene. However, the genetic analysis of three patients suggests not. Five of the six mutant alleles are known to contribute to PXE in other studies and are considered to represent null alleles. The one novel mutation detected in this study, p.L1474X, would cause a premature-truncating codon and would be likely to succumb to nonsense-mediated decay and hence also to be null. It is more likely therefore that the determination of whether PXE will be accompanied by generalized retinal dysfunction will depend on modifying factors, either environmental or genetic, that affect the way retinal tissue tolerates the metabolic abnormalities of the disorder.

Why retinal tissue should be affected at all by ABCC6 disruption is not clear. Only low levels of ABCC6 mRNA have been detected in frequently affected tissues5,10,12,19 although conflicting results have been reported in the literature regarding immunohistochemical analysis of the ABCC6 protein.5,9,12,19 Conversely, high levels of ABCC6 mRNA and protein were found in liver, which is unaffected in PXE.7,10–15 Gorgels et al.15 observed a decrease in HDL cholesterol levels in Abcc6−/− mice and suggested that alteration of plasma lipid composition may be implicated in the systemic changes observed in PXE. Retinal toxicity could be secondary to changes in Bruch’s membrane precluding proper nutrient supply to photoreceptors leading to their secondary dysfunction and degeneration. The further investigation of Abcc6−/− mice might give insights into the mechanisms that lead to the pathologic changes observed in PXE.15

Figure 5. Electropherograms of the sense strand of genomic DNA from the patients investigated (a–d) and an agarose gel electrophoresis picture of patient 4 (e). A heterozygous splice-site mutation (a, 3507(−3)C→T) and heterozygous nonsense mutation (b, c.4420A→T) in case 1, a homozygous nonsense mutation c.3421C→T in case 3, and a heterozygous missense mutation (d, c.3414G→C) in case 4. Note that only the undeleted allele amplified. The presence of a multiexon deletion (e, del23-29) with specific primers with positive controls is shown in case 4. Arrows: position of the mutation.
When considering the influence of \textit{ABCC6} mutations on retinal function, electrophysiological assessment provides important insight into the underlying causes of visual failure and most accurately describes the functional phenotype. It should probably be considered more often, although it would normally be indicated if any discrepancy exists between the patient’s visual complaints, visual acuity, and clinical examination.

In conclusion, the present study highlights an underreported but disabling association of PXE—generalized retinal dysfunction—and describes the detailed phenotype of these patients. It appears that this specific complication of PXE is not due to the inheritance of specific alleles, which suggests the action of modifiers. It is possible that the generalized retinal dysfunction in PXE is more common than is recognized.

\textbf{References}


