Heterozygosity for a Single Mutation in the \textit{ABCC6} Gene May Closely Mimic PXE

\textbf{Consequences of This Phenotype Overlap for the Definition of PXE}

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\textbf{Objectives:} To illustrate a phenotypic overlap consisting of usual, but limited, or atypical manifestations of pseudoxanthoma elasticum (PXE) between heterozygous carriers of a single \textit{ABCC6} mutation and patients diagnosed with PXE, carriers of homozygous or compound heterozygous mutations.

\textbf{Design:} Evaluation for full and typical, incomplete, mild, or overlooked PXE during a 5-year period (2001-2005) based on the following 1992 expert consensus conference items: (1) yellowish papular skin eruption, (2) dermal elastorrhexis and mineralization of elastic fibers in lesional skin, and (3) angioid streaks. Testing for \textit{ABCC6} mutations was performed in all cases after informed consent.

\textbf{Setting:} French multidisciplinary outpatient clinic for patients with PXE.

\textbf{Participants:} Patients prospectively referred for PXE and first-degree relatives.

\textbf{Main Outcome Measure:} Prevalence of PXE with a limited or atypical phenotype and manifesting heterozygosity.

\textbf{Results:} Ninety-four patients were diagnosed as having PXE. Fifty-eight relatives were also examined, and none displayed the characteristic signs of the disease. Despite the histoclinical items and \textit{ABCC6} genotyping, we were unable to establish a definitive diagnosis in 5 additional referred cases, ie, to distinguish between PXE with a limited or atypical phenotype and heterozygosity with skin and/or ophthalmologic and/or cardiovascular manifestations suggestive of PXE.

\textbf{Conclusions:} We assume that all categories established at the 1992 consensus conference correspond to PXE, but that the 5 patients reported herein also have PXE. Homozygous, compound heterozygous, or heterozygous individuals may fulfill only some of the clinical and/or histopathologic consensus criteria of PXE. They cannot be placed into any category. Expressivity is highly variable in carriers of 1 or 2 \textit{ABCC6} mutations, and the disease manifestations overlap between both genotypes. Physicians should thus be more cautious with respect to the prognosis when faced with heterozygous relatives of a patient diagnosed with undisputable PXE. Indeed, heterozygotes may uncommonly experience severe ophthalmologic complications. Whether they may also have cardiovascular complications related to or worsened by PXE remains to be determined.

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\textbf{PSEUDOXANTHOMA ELASTICUM} (PXE) is an inherited disorder of connective tissue characterized histologically by mineralized and fragmented elastic fibers. Pseudoxanthoma elasticum primarily affects the skin, retina, and cardiovascular systems with high (and still unexplained) clinical heterogeneity in the extent and severity of the phenotype.\textsuperscript{1,2} The manifestations of PXE result from mutations in the gene encoding the ATP-binding cassette subfamily C6 (\textit{ABCC6}) (GenBank U91318).\textsuperscript{3-6} To date more than 150 \textit{ABCC6} mutations have been described.\textsuperscript{7} The identification of \textit{ABCC6} as the causative gene for PXE has significantly improved our understanding of the disease: (1) PXE is recessively inherited (pseudodominance is occasionally demonstrated); (2) there is no clear phenotype-genotype correlation; and (3) the prevalence of PXE and the heterozygous carrier state are probably underestimated in the general population.\textsuperscript{2,7,8} Extrafamilial and intrafamilial phenotype heterogeneity suggests that the contributions of factors other than \textit{ABCC6} variants affect the expression of the phenotype. Interestingly, phenotype heterogeneity is not limited to patients with PXE but is also relevant to the phenotype of obligate carriers of \textit{ABCC6} mutations.\textsuperscript{1,2,9} Indeed, some heterozygous individuals may display slight skin or eye involvement,\textsuperscript{8} and the heterozygous carriers for the mutation \textit{p.Arg1141X} might be at risk of coronary artery disease.\textsuperscript{9} It has therefore been suggested that clinical (and in some
cases histologic) evaluation may be useful in relatives who are obligate heterozygous carriers, to detect mild and often neglected manifestations or occult PXE. In this report we describe heterozygous individuals with symptoms suggestive of PXE. Despite clinical evaluation, skin biopsy, and ABCC6 genotyping, it was impossible in 5 cases to distinguish PXE individuals with an incomplete phenotype from heterozygous individuals with PXE symptoms and/or severe complications. Such situations may be uncommon but a phenotype overlap appears to exist between heterozygous carriers and patients with definite PXE.

**METHODS**

**PATIENTS AND PATHOLOGIC EVALUATION**

Our group is a French multidisciplinary outpatient clinic to which individuals known or suspected to have PXE are referred for expert medical evaluation. The diagnosis of PXE in the study cohort was made according to the usual major histoclinical criteria for category 1 PXE: (1) yellowish popular skin eruptions, (2) dermal elastorrhexis and mineralization of elastic fibers demonstrated in lesional skin, and (3) angioid streaks in adults. First-degree relatives of patients with PXE were also evaluated whenever possible to look for mild or overlooked manifestations of PXE. For all individuals, the evaluation consisted of skin, vascular, and general examinations and funduscropy.

A skin biopsy was performed on skin lesions suggestive of PXE or when other arguments for the diagnosis of PXE were present (eg, angioid streaks). Skin biopsy specimens were most often obtained from lesional skin of the antecubital fossa, which is sun protected and free of solar elastosis. In the absence of lesional skin on the antecubital fossa, specimens were taken from the axillary or cervical skin. The elastic network, the morphologic characteristics of dermal elastic fibers, and the extent of mineralization were examined with hematoxylin-eosin, orcein, and von Kossa staining. The search for calcification of dermal elastic fibers was repeated when necessary, since such changes can be present in some areas and not in others.

Angioid streaks and other ocular signs of PXE (eg, peau d’orange) were evaluated by funduscopy. Doppler examination was performed in heterozygous individuals when intermittent claudication was present or when peripheral pulses were absent. Hemoglobin electrophoresis and coagulation evaluation were done in all subjects suspected to have PXE to rule out PXE associated with β-hemoglobinopathies or PXE-like disorder with a clotting disorder.

**MOLECULAR DIAGNOSIS**

Testing for ABCC6 mutations was performed systemically using previously described methods. DNA was isolated from peripheral blood using standard procedures. Briefly, ABCC6-specific primers were designed to amplify all 31 exons and the exon-intron boundaries. Intron-derived primers specific for polymerase chain reaction (PCR) amplification of ABCC6 exons 1 through 9 were synthesized using previously reported sequences. Intron-derived primers for PCR amplification of other ABCC6 exons were synthesized using sequence information from bacterial artificial chromosome clone CIT9875K-A-96284 containing ABCC6. Polymerase chain reaction fragments were subsequently purified with the QIAquick gel extraction kit (Qiagen, Courtaboeuf, France) and sequenced using the Big Dye DNA sequencing kit (Applied Biosystems, Courtaboeuf, France). Reactions were analyzed in an ABI 3100 sequencer (Applied Biosystems), and sequence chromatograms were analyzed using Sequence Analysis and Seqscape v2.1 software (Applied Biosystems). The recurrent large deletion of exons 23 to 29 (EX23_29del) was screened by PCR using a previously described set of nested primers. Finally, large gene rearrangements were investigated using quantitative multiplex PCR of short fragments.

ABCC6 molecular analyses were performed in a laboratory approved by the French Ministry of Health for molecular diagnosis. Written informed consent was obtained from all participants. This study followed the principles of the Declaration of Helsinki and was approved by the appropriate ethics committee of the Angers University Hospital.

**RESULTS**

Since 2001, PXE has been prospectively diagnosed or confirmed by our group in 94 patients displaying the 3 characteristic histoclinical criteria. The detection rate of mutated alleles (ratio between the number of mutant alleles found and the number of mutated alleles expected) was 90%. Fifty-eight relatives of patients with PXE were also examined, and none showed evidence of the disease. In 5 additional cases reported herein, we were unable to give a definite diagnosis. Four of these 5 individuals had been previously referred with an uncertain diagnosis of PXE. One subject was evaluated as a heterozygous obligate carrier because she had 2 children with unambiguous PXE. Patients 1, 3, and 4 were of Maghrebian ancestry, while patients 2 and 5 were of European origin.

**CASE 1**

Patient 1 was a 70-year-old woman who had 2 children with unambiguous PXE. Skin examination revealed recently noticed periumbilical yellowish confluent papules suggestive of cutaneous PXE (Figure 1A). Pathologic evaluation of a periumbilical skin biopsy sample demonstrated unequivocal dermal elastorrhexis and mineralization (Figure 1B and C). Other skin areas were spared. No angioid streaks or other ophthalmologic changes suggestive of PXE were found on funduscopy. Sequencing of ABCC6 coding regions revealed a single mutation, c.3712G→C (p.Asp1238His). Her 2 affected children shared this mutation. The daughter carried the variant p.Gln363_Arg373del/p.Asp1238His, and the son had the alleles p.Thr1130Met/p.Asp1238His. Since the mother did not carry the p.Gln363_Arg373del and p.Thr1130Met mutations, these alleles were most likely paternally transmitted, indicating that she bore only 1 mutant allele. She died from a thrombotic ischemic stroke at age 75 years. The limited manifestations of PXE in this elderly patient did not fit any category of PXE defined in the 1992 consensus conference. We speculate that PXE-related arterial occlusive changes, albeit non-specific, could have contributed to the pathogenesis of the stroke.

**CASE 2**

A 58-year-old man presented with bilateral angioid streaks complicated by neovascularization. Central visual loss in the left eye necessitated vitrectomy and surgical ablation of the juxtafoveal neovascular membrane. His ophthalm-
mologic history was somewhat atypical for PXE: the angioid streaks were straight; peau d’orange was absent; and postsurgical evolution was unexpectedly favorable, with rapid recovery of significant visual acuity (7/10) without relapse after 4 years. This patient had no clinically visible skin lesions of PXE. Four skin biopsies were performed in usually affected areas in PXE (antecubital fossa, neck, and axilla) and in an abdominal scar, as suggested by Lebwohl et al.16 No elastorrhexis or mineralization was found. No heart or arterial involvement was present. However, the patient’s sister had typical PXE with complete and severe skin, eye, and vascular involvement, associated with 2 disease-causing variants (c. ABCC6del and c.496C→T [p.Arg166Cys]). ABCC6 molecular analysis of patient 2 revealed the same 2 mutations detected in his sister’s DNA. According to the histoclinical criteria, the male patient possibly had PXE type 2c with atypical angioid streaks.

CASE 3

A 46-year-old woman had experienced 2 episodes of pulmonary edema. The cause of those events was unclear, but she had mild arterial hypertension and demonstrated diastolic heart dysfunction. Skin examination revealed atypical skin lesions and no yellowish papules. The patient had widespread areas of yellowish lax skin on the neck, trunk, and axillary folds (Figure 2A). The histologic examination of 2 lesional skin biopsy samples failed to demonstrate elastorrhexis. In only some dermal areas, elastic fibers were somewhat dystrophic or fragmented (Figure 2B), and unusual calcifications were present featuring large dark deposits (Figure 2C). Ophthalmologic evaluation demonstrated bilateral and uncomplicated small angioid streaks (Figure 2D). A single ABCC6 mutation was found (c. 1424A→T, p.His475Leu) in this sporadic case. Several diagnoses were possible, including late and atypical category 1 or 2B PXE or symptomatic heterozygous carriage of a single ABCC6 mutation. Although heart diastolic dysfunction has been described in PXE,17 this feature was not helpful for the diagnosis of this case.

CASE 4

A 63-year-old man was referred to us because of loss of central vision related to neovascular complications of angioid streaks affecting the left eye (not shown). Uncomplicated angioid streaks were present in the other eye. He had no clinical skin manifestations, and no occult dermal elastorrhexis or mineralization was found after examination of 2 skin biopsy samples from the antecubital fossa and axilla. Arteriosclerosis was absent. He had a single ABCC6 mutation: c. 3701A→T (p.Glu1234Val). The clinical features of this sporadic case did not fit any category of the 1992 conference,10 but were suggestive of a severe presentation of a heterozygous carriage.

CASE 5

A 47-year-old woman was referred following the fortuitous finding of bilateral angioid streaks on ophthalmologic examination. The streaks were thick but short and temporal and therefore not fully typical of PXE...
Clotting disorder, β-thalassemia, and sickling syndrome was absent in all 5 cases. The possibility of digenism was excluded. In these cases, the PXE phenotype did not result from the deleterious combination of 1 mutation in \textit{ABCC6} and of 1 mutation in the \( \beta \)-globin gene (data not shown).

**COMMENT**

The histoclinical criteria established in 1992 for the diagnosis and categorization of PXE are widely used.\textsuperscript{10} The combination of the 3 major diagnostic criteria for category 1 provides undisputable diagnosis of PXE. Christen-Zach et al\textsuperscript{18} have recently reported the good sensitivity and specificity levels of the combination in 10 Swiss PXE families with notable consanguinity. In their study, all individuals \textit{“haplotypic homozygous”} for PXE manifested the 3 major criteria, while none of the heterozygous carriers studied had any. One should note that category 1 criteria provide PXE diagnosis but not prognosis. Some individuals with all 3 criteria may have a benign evolution of the disease, while others can develop severe ophthalmologic and/or cardiovascular complications. The onset and evolution of PXE manifestations remain unpredictable and suggest that other genetic or environmental determinants have a role in the phenotype severity.\textsuperscript{2}

Lebwohl et al\textsuperscript{10} also defined categories 2A through 2D for patients who do not have the typical clinical skin lesions of PXE. These categories are defined by variable combinations of angioid streaks, elastic fiber mineralization in nonlesional skin, and family history of PXE. The authors of the 1992 report acknowledged that the definition of these additional categories was somewhat controversial and that the diagnosis of PXE in individuals with only minor criteria was questionable. The usefulness of these categories has never been evaluated.

The 1992 criteria were valid in most of our patients and allowed us confirmation of the diagnosis of PXE (category 1) in 94 individuals from the prospective series we started in 2001. Fifty-eight related individuals had no manifestations suggestive of PXE. However, we were unable to establish a definite diagnosis for another 5 individuals despite complete histoclinical evaluation complemented by mutation analysis. We were not able to show a clear clinical difference between PXE patients with a mild or incomplete phenotype and heterozygous carri-
ers with manifestation(s) of PXE. Our 5 patients were older than 45 years, and the onset of additional histoclinical category 1 PXE diagnostic criteria seemed unlikely because penetrance of the major criteria is usually complete by age 30 years.1,2

Patient 2 had an incomplete PXE phenotype with no skin involvement. Yet he carried 2 *ABCC6* mutations, while a sibling had a complete phenotype. In this setting, the diagnosis of PXE is certain, but the patient can only be placed in category 2C of the consensus criteria, emphasizing that he had only atypical angioid streaks. Four of the 5 patients described herein are presumed to be heterozygous carriers of a single *ABCC6* mutation. We acknowledge that we cannot fully ascertain the absence of a second mutant allele because of limitations of the current techniques and a mutation detection rate of 90%, as reported in several published studies.7,13,15 Genotyping and examination of affected siblings were of no help to clarify 3 situations because these patients represented sporadic cases. Familial history and molecular results for patient 1 indicated a heterozygous status of this patient. One must consider that some patients with PXE in whom a single mutant allele was identified may in fact just be heterozygous carriers with high expressivity of the clinical manifestations. Of note, even if the 4 present individuals were compound heterozygotes with 1 unidentified mutation, they remained outside the categories defined in 1992, and the diagnosis of PXE could not be definitely affirmed.

Previous biological and histologic findings support moderate phenotypic expression in heterozygous carriers. Using 2 different genetic approaches, Bacchelli et al19 as well as our own group20 demonstrated that carriers of 1 *ABCC6* mutation can have histologic and immunohistochemical changes of skin elastic fibers midway between those of PXE and wild-type relatives. Furthermore, Hendig et al21 recently demonstrated in heterozygous carriers that serum levels of fetuin-A (a systemic inhibitor of mineralization) were intermediate between those of PXE individuals and unaffected relatives. These findings support the hypothesis that heterozygosity creates a deleterious environment favorable to the development of clinical symptoms of PXE. In addition, Trip et al8 found in a large Dutch series that carriers of the frequently occurring p.Arg1141X mutation had a significantly higher risk of coronary artery disease than controls.8

Autosomal recessive diseases are classically those in which the heterozygous state is indistinguishable from the healthy state. In practice, however, there may be subtle (or even significant) distinctions noted in heterozygous carriers of a disorder conventionally regarded as recessive. In some instances, the distinctions do not belong to the usual spectrum of the disease. Interestingly, some examples of manifesting heterozygosity exist in disorders that are related to ABCC transporter dysfunctions. Heterozygosity for a single gene mutation in *ABCC7* (CTFR), the gene causing cystic fibrosis, has been associated in adults with an increased incidence of various clinical features of cystic fibrosis, eg, bronchopulmonary aspergillosis, obstructive azoospermia, or sinusitis.22,23 Heterozygous mutation in the drug transporter gene *ABCC2*, causing Dubin-Johnson syndrome, alters methotrexate elimination with clinical relevance.23

The possibility that mild or uncommon manifestations of PXE also occur in heterozygous carriers of *ABCC6* mutations is suggested by our clinical data and by previously published biochemical and histological works.10-21 If heterozygotes have manifestations of the disease, some would consider this disorder dominant. However, dominance is not likely in PXE. If it were, many more heterozygous individuals should display a clinical phenotype. Symptomatic heterozygous individuals should display a clinical phenotype. The other genetic or environmental factors that de-
termine the occurrence of their symptoms cannot be determined in our small series.

Three patients in the present study experienced at least 1 severe clinical event: lethal stroke (patient 1), acute pulmonary edemas related to heart dysfunction (patient 3), and central blindness (patient 5). Occurrence of severe events is of clinical relevance because heterozygous carriers were often believed to have only rare and mild manifestations suggestive of PXE (eg, uncomplicated angioid streaks and sporadic skin papules).1,9 We illustrate here that heterozygosity may be responsible for significant clinical ophthalmologic events.

Cardiovascular manifestations are absent from both minor and major 1992 criteria.10 The authors must have assumed that arteriosclerosis in PXE was not specific and indistinguishable from arteriosclerosis of other causes and was therefore of little value for the diagnosis of PXE. However, heart and/or arterial events that occur in association with PXE are undoubtedly linked to elastic fiber dystrophy in arterial walls and represent the main (if not the sole) involvement that will determine a fatal prognosis in patients with PXE.1,2 The direct responsibility of suspected elastic changes of PXE in arterial walls cannot be confirmed in heterozygous patients 1 and 3. Nonetheless, it is tempting to speculate that such changes took part in the vascular pathologic processes and clinical consequences.

Our observations support 2 conclusions that are relevant for clinical practice. First, some heterozygous individuals may have some uncommon but severe ophthalmologic and, more speculatively, cardiovascular manifestations of PXE. We believe that these individuals should be diagnosed as having PXE. There is, therefore, an overlap in the PXE phenotype between heterozygous carriers and patients with an undisputable diagnosis of PXE. This phenotypic overlap may render the clinical diagnosis of the less severe forms of PXE equivocal. Expressivity appears to be highly variable in both genotypes. Physicians should thus be more cautious regarding prognosis when faced with heterozygous relatives of a patient with PXE. Such an approach would also make possible the initiation of more appropriate clinical follow-up of these individuals for the detection of complications.

Second, some carriers of 1 or even 2 mutations of ABC6, or some individuals with clinical manifestations suggestive of or compatible with PXE, cannot be placed into any 1992 category. The sensitivity of both the major and minor histoclinical and molecular criteria might be too low, and some cases of PXE may be overlooked. We assume that all 1992 categories correspond to PXE and that our present 5 patients had PXE. Extending the definition of PXE to heterozygous individuals with significant manifestations of the disease should be considered.

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