Pseudoxanthoma elasticum:
A clinical, pathophysiological and genetic update
including 11 novel ABCC6 mutations

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Abbreviations: ABCC6, ATP-binding cassette, sub-family C, member 6; MRP6, Multidrug resistance-associated protein 6; NBF, Nucleotide binding fold; PXE, Pseudoxanthoma Elasticum
ABSTRACT

Pseudoxanthoma elasticum (PXE) is an inherited systemic disease of connective tissue primarily affecting the skin, retina, and cardiovascular system. PXE is characterized pathologically by elastic fibers mineralization and fragmentation (so-called “elastorrhexia”), and clinically by high heterogeneity with regards to the age of onset, extent and severity of each organ system involvement. PXE was recently related to mutations in the ABCC6 (ATP-binding cassette subtype C number 6) gene. At least, one ABCC6 mutation is found in about 80% of patients. These mutations are identified in most of the 31 ABCC6 exons and consist in missense, nonsense, frameshift mutations or large deletions. To date no correlation between the nature or the location of the mutations and phenotype severity has been established. Recent findings support exclusive recessive inheritance. The proposed prevalence of PXE is 1/25,000, but this is probably an underestimation. Consequently, the prevalence of heterozygous carriers, and the prevalence of different organ involvement in carriers of one or two ABCC6 mutations are not precisely known.

ABCC6 encodes the protein ABCC6 (also known as MRP6), a member of the large ATP-dependent transmembrane transporter family that is expressed predominantly in the liver and kidneys, and only to a lesser extent in tissues affected by PXE. To date, the physiological substrate(s) of ABCC6 remain(s) to be determined, but the current hypothesis is to consider PXE as a metabolic disease with undetermined circulating molecule(s) interacting with the synthesis, turnover and/or maintenance of elastic fibers. All these areas of concern are reviewed and updated.
INTRODUCTION

Pseudoxanthoma elasticum (PXE; MIM 264800) is an inherited systemic disease of connective tissue primarily affecting the skin, retina, and cardiovascular system.\textsuperscript{1-5} PXE is characterized by elastic fibers mineralization and fragmentation, resulting in a diagnostic pathology picture called “elastorrhexia”.\textsuperscript{1} To a lesser extent, milder ultrastructural alterations of other extracellular matrix (ECM) components (collagen fibrils and glycosaminoglycans) have been demonstrated.\textsuperscript{1, 6-8} Cutaneous PXE was first delineated in 1896 by Darier,\textsuperscript{9} who coined the term “pseudoxanthoma elasticum” stating that the yellowish papules of PXE differed from authentic xanthomas and were related to elastic tissue fragmentation. Angioid streaks, the cardinal ophthalmologic sign of PXE, were also described in the XIXth century. They were independently related to PXE skin changes by Grönblad and Strandberg in 1929.\textsuperscript{10, 11} Precocious atheromatosis was the last sign to be related to the condition. Carlborg reported cardiovascular elastic calcification in 29 Swedish patients with PXE in 1944.\textsuperscript{12} PXE was supposed for a long time to be a primary disorder of the elastic fibers system with candidate genes encoding structural components of the fibers (elastin, fibrillins, other microfibril-associated (glyco)proteins) or related enzymes.\textsuperscript{13, 14} Surprisingly, PXE was eventually related to mutations in the \textit{ABCC6} (ATP-binding cassette subtype C number 6) gene.\textsuperscript{15-18} \textit{ABCC6} encodes ABCC6 a member of the large ATP-dependent transmembrane transporter family. ABCC6 is abundantly expressed in liver and kidney cell membranes, and to a lesser extent in other tissues affected or not by PXE (skin, vessel walls, and retina).\textsuperscript{15, 19-23} Even if the substrate(s) transported by ABCC6 remain(s) to be determined, the association of PXE to ABCC6 efflux transport alterations raises new and exciting pathophysiology hypotheses.\textsuperscript{22, 24, 25} Among them, the current idea is that PXE is a systemic metabolic disease resulting from lack or accumulation over time in the bloodstream of molecules interacting with the ECM synthesis, turnover and/or maintenance.\textsuperscript{26}
METHODS

We searched Medline in November 2004 using the key words “pseudo-xanthoma elasticum”, “PXE”, “ABCC6” and “MRP6” and considered original and bringing progress papers, published after 1988. Characteristic PXE clinical events and pathology findings have been described and reviewed in previous comprehensive large series that will be referred to for tutorial description. Papers updating recent progress will be commented in more detail.

CLINICAL FEATURES AND MANAGEMENT

PXE is found in all populations studied so far. Its prevalence seems higher in Afrikaners of South Africa mainly because of a founder effect. A ubiquitous F/M ratio equal to ~2/1 is recurrently mentioned, with no satisfactory explanation. PXE is characterized by a marked clinical heterogeneity, even between sibs, considering age of onset, extent and severity of each organ system involvement. Most patients have a normal lifespan.

Skin involvement
The skin primary lesion is a yellowish papule of 1 to 5 mm in diameter. Such papular lesions tend to gradually coalesce to form plaques with cobblestone appearance (fig 1). Typical skin lesions of PXE are located on the neck and in flexural areas. Cervical lesions often develop first, appearing on the lateral aspects and commonly respecting, at least initially, the medial part of the nape. They often rise between the age of 8 and 12 years, but may be more precocious. Flexural involvement tends to start in teenage years. The most commonly affected sites are the axillae, but involvement of antecubital and popliteal fossae and groins is also generally noted. Involvement of anterior aspects of wrists, umbilicus or lumbar skin area is less common. At the maximum of papular coalescence, skin loses its elasticity (rather than becoming hyperelastic) and evocative redundant skin folds develop (fig 2). Most patients have a limited skin surface involvement, but generalized “cutis laxa-like” PXE exists, and is of considerable esthetic concern. Recently, Lebwohl et al. emphasized the clinical value of face involvement. The authors showed that horizontal and oblique mental creases are a valuable sign of PXE (fig 3). These creases have a high specificity for the diagnosis of PXE before the age of 30. Less common cutaneous manifestations have been occasionally described: acne-like lesions on the neck or the trunk, featuring comedones or inflammatory papules, elastosis perforans serpiginosa, reticulate pigmented rash. In very rare instances, spontaneous resolution of PXE skin changes has been published. Calcination cutis is rarely associated with PXE, most often in addition to phosphocalcium metabolism disorders. These conditions could refer to particular subgroups of PXE. Mucosal lesions identical to their cutaneous counterparts may be found on the inner aspect of the lower lip (fig 4), on the cheeks or the palate, or on the genitalia, but also all along the digestive tract mucous membrane. A skin biopsy specimen is mandatory for the diagnosis of PXE, in order to demonstrate the cardinal histology features. Aberrant clumped and fragmented elastic fibers are evidenced in the mid-dermis using hematoxylin-eosin-safran stain, or using more specific elastic stains (orcein or Verhoeff’s) which color the fibers in black (fig 5). Calcium and phosphorus depositions may be demonstrated using the von Kossa stain. Characteristic pathology features can be observed in clinically involved skin, but also in apparently normal skin. Lebwohl et al. demonstrated occult axillary PXE in four patients who presented with
premature cardiovascular disease and angioid streaks but no skin changes. The same group previously demonstrated pathological skin findings indicative of PXE on scar biopsy in 6 out of 10 patients with angioid streaks, but without clinically characteristic skin lesions. As an important consequence, the diagnosis of PXE may be suspected and made in patients without any visible skin involvement.

Ultrastructural elastic tissue alterations are seen in both lesional and non-lesional skin, in contrast with other ECM changes that are only evidenced in involved skin, in the vicinity of altered elastic fibers. Prominent abnormalities affect the elastic fibers featuring small or large electron-dense bodies (calcifications) and holes in the core of the fibers (fig 6). Calcifications result in “fractures” of the fibers, which occur during biopsy severing. Collagen bundles display fibrils of irregular diameter and occasional flower-like features. Aggregates of filamentous material composed of elastin and proteoglycans are found close to the surface of elastic fibers. Electron microscopy observations are misleading unless elastorrhexia has been demonstrated by optic microscopy. Indeed, the ultrastructural changes described above are not specific for PXE, and may be encountered in other inherited diseases of the ECM, but also in skin aging.

Surgical reduction of excessive and redundant skin may be envisaged in some instances for cosmetic improvement. The long-term outcome is poorly evaluated since patient series are scarce. Viljoen et al. reported favorable post-surgical outcome in 8 female patients with a mean follow-up of 6 years. Delayed healing and poor scarring occurred in two out of 8 patients, because of transepidermal calcium particles extrusion. The efficiency of collagen or autologous fat injections in the mental creases remain to be evaluated.

Eye involvement

Elastic tissue is also present in the eye, namely in a thin layer between the retinal pigment epithelium and the choriocapillaris, the so-called Bruch’s membrane. The elastic fibre content of Bruch’s membrane has an unique, maze like structure interwoven with collagen fibers on the side. This is different from elastin structures in skin and blood vessels. In PXE, the Bruch’s membrane becomes calcified and brittle. The cracks in the membrane usually occur on the eye-muscle/optic nerve head track forcing lines, so is not caused by calcification of the Bruch’s fibres alone. Elastic fiber alterations are responsible for acquired dehiscences and subsequent cracks in the membrane, ophthalmoscopically resulting in “angioid streaks” (fig 7). Angioid streaks feature greyish irregular lines radiating outward from the optic papilla and grossly resembling vessels, hence the name. They may be optimally visualized using fluorescein and/or indocyanine green angiograms. Angioid streaks are not, in most instances, responsible for any visual symptoms but may be complicated by proliferation of aberrant choroidal neo-vessels into the subretinal space (fig 8). Neo-vessels have brittle walls, and this may result in recurrent, spontaneous or trauma-induced retinal haemorrhages. Neo-vessels and retinal haemorrhages result in macular symptoms (metamorphopsia, scotoma), peripapillary atrophy, disciform macular/foveal scarring and definitive central visual loss. Legal blindness is then not uncommon in patients with PXE. Additional ocular features of PXE, such as the “peau d’orange” appearance (diffuse motting of the fundus), drusen, or comet like streaks are less evocative of PXE. However, they may precede angioid streaks for years and be helpful for the diagnosis of PXE in the presence of atypical or early skin lesions in children.

Visual complications are very difficult to treat and have the greatest impact on disability and quality of life in patients suffering from PXE. Laser therapy is performed in the presence of submacular neovessels. It may be efficient in stopping vessel proliferation or bleeding, but causes retinal burns and subsequent definitive scotoma. Because anatomical lesions are reminiscent of age-related macular degeneration, specialized ophthalmologists have tried to...
perform laser phototherapy coupled with verteporfin infusions. To date, functional results of such regimen remain unclear in PXE. Comprehensive analysis of the results obtained in series of individuals treated is required. Finally, surgical procedures such as macular translocation for subfoveal choroidal neovascularization have occasionally been performed. Use of antiangiogenic drugs in the prevention of choroidal neovascularization and bleeding is still in the domain of pre-clinical research.

**Cardio-vascular involvement**

Elastic fiber-rich arterial walls are also involved in the pathologic process of PXE, resulting in precocious and slow-evolving segmental arterial narrowing. This atheromatosis is histologically indistinguishable from atheromatosis of other causes such as use of tobacco or chronic blood hypertension. Internal elastic laminae of small- and middle-sized arteries are mainly involved. The slow course of vessel narrowing is associated with the development of arterial collaterality. Consequently, severe vascular symptoms are rather infrequent in PXE. Kornet et al. shows that a thicker and more elastic carotid artery was associated with elastin fragmentation and proteoglycans accumulation, in patients with pseudoxanthoma elasticum. Two types of clinical manifestations may result from arterial involvement:

**Occlusive arterial disease** may be responsible for limb arteritis, coronary artery disease, digestive angina and cerebrovascular disease. Absence of peripheral pulses is frequent, and should evoke the diagnosis of PXE in young individuals. Intermittent claudication in the lower limbs and tiredness in the upper limbs are the most common symptoms. Angina pectoris, or silent coronary insufficiency, may be present. Myocardial infarction is rare, but has even been reported in teenagers or young individuals. Therefore, PXE should be discussed in young individuals with precocious coronary artery disease and no cardiovascular risk factors. Apart from very rare reports, surgical coronary revascularization seems valuable. However, the left internal mammary artery may be involved in PXE and is not suitable for bypass grafting. Ischaemic brain infarctions in patients with PXE are caused by small-vessel disease. They are uncommon albeit more prevalent than in the general population. In the same recent large series, association between intracranial aneurysm and PXE has been ruled out. Reno-vascular hypertension is probably also rarer than expected in the literature. We have only seen one case of this complication in our series of more than 60 individuals having PXE (unpublished data).

**Mucosal bleeding** may also occur as the consequence of arterial involvement, although its precise mechanism remains to be determined. Some authors proposed that bleeding could be related to defective submucosal vasoconstriction. Gastrointestinal haemorrhage is by far the most frequent location, but case reports have probably dramatically overestimated this severe complication. The precise source of bleeding may be difficult to identify, and some haemorrhages require radical surgery. Three patients in our series have experienced gastrectomy for haemostatic purpose. Uterine or bladder bleedings have also occasionally been observed. However, it is worth noting that there is no vascular brittleness in PXE. Spontaneous ruptures of vessel walls, as seen in vascular type Ehlers-Danlos, have not been reported in PXE. Therefore, vascular surgery or radiology procedures can be performed if indicated.

Heart involvement is uncommon in PXE. Whether valvular diseases such as aortic or mitral stenoses, or mitral valve prolapse, are significantly associated with PXE remains to be determined using stringent diagnostic criteria. Restrictive cardiomyopathy in relation with diffuse endocardial fibro-elastosis seems to be specific of PXE but is very rare.
Other manifestations
Gheduzzi et al. recently demonstrated that ultrastructural alterations of elastic fibers were present in virtually all organs and tissues obtained post mortem from two PXE patients.\textsuperscript{59} However, only tissues that are rich in elastic fibers are associated with clinical manifestations. The lung is a remarkable exception that has no explanation. Interestingly, calcifications may also be present on mammograms. The former might be of diagnostic value for PXE in women; breast tumoral microcalcifications are often easily ruled out.\textsuperscript{60} Most women with PXE have normal pregnancies and deliveries. Case reports overemphasized the risk of gastric bleeding during pregnancy that remains uncommon. Ophthalmological monitoring is however critical during pregnancy in order to prevent retinal haemorrhage during pushing efforts. A recent review states that there is no basis for advising women with PXE to avoid becoming pregnant, and that most pregnancies in PXE are uncomplicated.\textsuperscript{61}

Positive and differential diagnosis

Positive diagnosis
A classification of PXE into clinical subtypes has been previously proposed.\textsuperscript{62} Its relevance should be re-evaluated in the light of the identification of $ABCC6$ as the causative gene. To date, minimal criteria for the diagnosis of PXE were the association of dermal elastorrhexia (with or without clinically visible changes) and angioid streaks. This is most always true, but we have shown in rare patients with two identified $ABCC6$ mutations, that one or other criterion could be absent. This observation is confirmed by the recent results in two Italian families in which one patient with two identified mutations displayed only ocular symptoms, while another only had skin involvement.\textsuperscript{63} Positive family history, on one hand, and symptomatic atheromatosis, on the other hand, are clearly inconstant and therefore, if absent, should not lead to rule out the diagnosis of PXE. $ABCC6$ genotyping is to date only available in specialized centers, and its usefulness for diagnosis remains to be determined (see below).

Dermatological differential diagnosis
Clinical and pathological recognition of skin changes suggestive of PXE is often easy. Conditions that clinically mimic PXE are summarized in Table I.\textsuperscript{64, 65} In most instances, pathology findings rule out PXE diagnosis by demonstrating increased or decreased elastic tissue, without elastic fiber fragmentation or calcification. Cutaneous and articular hyperlaxity may exist in PXE but are less severe than in Ehlers-Danlos syndromes.

Table I: Clinical differential diagnosis of PXE skin lesions

<table>
<thead>
<tr>
<th>Conditions</th>
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<tbody>
<tr>
<td><strong>Papules</strong></td>
</tr>
<tr>
<td>- « Acquired » pseudo-PXE related to haemoglobinopathy</td>
</tr>
<tr>
<td>- Skin aging (chronological and/or actinic)</td>
</tr>
<tr>
<td>- Elastoma, Buschke-Ollendorff syndrome</td>
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<tr>
<td>- Pseudo-PXE related to salpetor or D-penicillamin</td>
</tr>
<tr>
<td>- Papular elastorrhexia</td>
</tr>
<tr>
<td>- Elastosis perforans serpiginosa</td>
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<tr>
<td>- Perforating peri-umbilical PXE</td>
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<tr>
<td><strong>Plaques or redundant skin</strong></td>
</tr>
<tr>
<td>- Elastoderma</td>
</tr>
<tr>
<td>- Anetoderma</td>
</tr>
<tr>
<td>- Cutis laxa</td>
</tr>
<tr>
<td>- Ehlers-Danlos syndrome (rare)</td>
</tr>
</tbody>
</table>
Ophthalmological differential diagnosis
Angioid streaks are not specific for PXE, and have also been encountered in inherited haemoglobinopathies, Marfan disease, Ehlers-Danlos syndromes or Paget’s disease of bone. An additional and not yet well-classified dominant autosomal condition has recently been described in an Italian three-generation pedigree. In this family, affected individuals displayed mottled fundus, angioid streaks and drusen in various combinations, but no skin involvement suggestive of PXE, and linkage to ABCC6 was excluded.

Recommendations for the care of patients with PXE
Today, no specific treatment exists for PXE. Management focuses on prevention, screening and monitoring of complications. However, standards of follow-up (type and rhythm) do not exist in PXE, and the identification of individuals who will develop serious complications related to PXE is difficult.

Prevention
The role of diet is not clear. One single study stated that early calcium restriction could positively influence the evolution of PXE in reducing the extent of mineralization. However, this work was based on a questionable (and not validated) system of severity grading and retrospective evaluation of calcium intake, which considerably lowered the validity of the author’s conclusions.

Sports using balls, or combat sports are contraindicated to reduce the occurrence of facial traumas and the subsequent development of angioid streaks and/or retinal haemorrhage. Reduction of atheromatosis risk factors has been proposed: avoidance of smoking, control of diabetes, lipid disorders, and hypertension. Aspirin, non-steroidal anti-inflammatory drugs or other hypocoagulant drugs should be avoided because of the risk of mucosal bleeding. The benefit/risk ratio of these drugs in the presence of cardio-vascular complications is unknown in PXE, and caution is advised.
MOLECULAR GENETICS

Identification of the PXE gene

PXE has previously been considered as a heritable connective tissue disease with primary involvement of elastic fibers. Genes encoding elastin and elastin-associated proteins were therefore functional candidate genes. Subsequently, genes encoding elastin (located on chromosome 7)\(^6\) fibrillin 1 and 2 (respectively located on chromosome 15 and 5)\(^13\) were excluded by linkage analysis. Furthermore, other genes encoding members of the microfibrillar protein family and the lysyl oxidase, an enzyme participating in crosslinking of the elastic fibers, were also excluded.\(^14\) More recently, the PXE gene was mapped to chromosome 16p13.1 using positional cloning approaches.\(^69, 70\) Further studies refined this locus to a region of about 500 kb\(^71, 72\) containing five genes with no obvious relation to the extracellular matrix of connective tissue (\(ABCC1, ABCC6, pM5\), and two copies of an unknown gene subsequently identified as gene encoding the Nuclear Pore Interacting Protein (NPIP)). Direct sequencing of these genes identified pathogenic mutations in the \(ABCC6\) gene.\(^15-18\) No mutations were identified in the other positional candidate genes.\(^16, 73\)

The PXE gene: \(ABCC6\)

\(ABCC6\) belongs to the subfamily C of ATP-binding cassette (ABC) genes that include 13 members (\(ABCC1\) to \(ABCC13\)). ABC proteins are active pumps which can transport various substrates, including ions, phospholipids, steroids, polysaccharides, amino acids and peptides against the substrate’s concentration gradient across membranes.\(^74\) They are implicated in drug and antibiotic resistance, signal transduction, protein secretion and antigen presentation.\(^75\)

\(ABCC6\) consists of 31 exons spanning ~73 kb. The \(ABCC6\) mRNA, ~6 kb in size, has an open reading frame of 4.5 kb encoding \(ABCC6\) (multidrug resistance-associated protein 6) a 1503 amino acids protein. \(ABCC6\) is predominantly expressed in human liver and kidneys.\(^21, 76\) Low expression levels of \(ABCC6\) were detected in tissues involved by PXE (skin, vessel walls, and retina), and in other tissues not affected by PXE.\(^15, 21\) Two \(ABCC6\) pseudogenes homologous to the 5’ part of \(ABCC6\) (exon 1 through intron 9, and exon 1 through intron 4) were recently identified and are closely mapped to \(ABCC6\).\(^77, 78\)

The PXE protein: \(ABCC6\)

\(ABCC6\) is composed of three hydrophobic membrane segments comprising 5, 6, and 6 transmembrane spanning domains respectively, and two evolutionary conserved intracellular nucleotide binding folds (NBF) (fig 9). NBFs contain conserved Walker A and B domains, and conserved C motif critical for ATP binding and transmembrane transporter functions. \(ABCC6\) was classified as a multidrug resistance-associated protein because of its homology with MRP1. Indeed, \(ABCC6\) and MRP1 are the most closely related members of the MRP family with 45 % identity.\(^21, 79\) MRP1 is a well characterized transmembrane efflux pump transporting amphipathic anionic conjugates and glucuronidated and sulphated compounds.\(^80, 81\) \(ABCC6\) was associated, under \textit{in vitro} conditions, with the MgATP-dependent transport of the glutathione S-conjugate leukotriene C(4) and S-(2, 4-dinitrophenyl)glutathione and the cyclopentapeptide BQ123.\(^22, 24, 25\) \(ABCC6\) also confers low levels of resistance to several agents, including etoposide, teniposide, doxorubicin, and daunorubicin.\(^24\) However, the role of \(ABCC6\) in drug resistance was questionable from the beginning.\(^21\) Using several polyclonal antibodies, \(ABCC6\) was localized to the basolateral side of human hepatocytes and to the basolateral membranes of kidney proximal tubules,\(^23, 82\) suggesting that \(ABCC6\) extrudes into the blood specific substrate(s) from liver and kidney. However, the exact
function and the physiological metabolite(s) actively transported by ABCC6 have not been yet identified.

**ABCC6 mutations**

Consequent to the recognition of *ABCC6* as the defective gene in PXE, several groups identified mutations in PXE patients. To date 90 different disease-causing mutations have been reported (79 previously published \(^{15, 17, 18, 63, 83-89}\), and 11 new ones in this study) in almost all the 31 *ABCC6* exons (fig 10, Table II). Among them, there are 49 missense mutations, 13 nonsense mutations, 8 splicing mutations, 3 small insertions leading to frameshift, 14 small deletions a majority of which lead to a frameshift, 2 deletions spanning at least one exon, and one deletion of the entire *ABCC6* gene (fig 10, Table II). Although the consequences of splicing mutations have not been investigated, at least one third of the mutations introduce stop codons or frameshift that lead to premature termination of the traduction. Interestingly, among the 49 different missense mutations in *ABCC6* (42 previously published and 7 new ones in the present study) a majority (43) replaces critical amino acids in intracellular domains (7 and 19 mutations are located in NBF1 and NBF2 respectively), 4 are located in transmembrane domains, and only 2 mutations have been identified in extracellular domains. Distribution of the missense mutations through ABCC6 is indicated in figure 9. This distribution of mutations is consistent with the role of NBFs in ATP-driven transport. Functional studies have already shown that ABCC6 transport is abolished by missense mutations located in the NBF2.\(^{25}\) This distribution suggests that intracellular domains different from NBFs are also functionally important, possibly through recognition of the substrate(s).

**Table II:**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Nucleotide Variation</th>
<th>Protein Alteration</th>
<th>Location (Gene)</th>
<th>Location (Protein)</th>
<th>Reference</th>
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<tr>
<td>Missense</td>
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<td></td>
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<td>87, 90</td>
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| Gene | rsID | Mutation | Exon | Chromosome | Population
|------|------|----------|------|------------|--------------|
| M1127T | 3380 | C>T | Exon 24 | 8 | 63, 63, 87, 88
| T1130M | 3389 | C>T | Exon 24 | 8 | 17
| R1138W | 3412 | G>A | Exon 24 | 8 | 90
| R1138P | 3413 | G>A | Exon 24 | 8 | 17, 63, 88, 90
| G1203D | 3608 | C>T | Exon 25 | TS17 | 90
| D1238H | 3663 | C>T | Exon 26 | COOH | 87
| V1298F | 3712 | G>C | Exon 28 | NBF 2 | 90
| G1299S | 3902 | C>T | Exon 28 | NBF 2 | This study
| T1301I | 3904 | G>A | Exon 28 | NBF 2 | 87, 90
| G1321S | 3907 | G>C | Exon 28 | NBF 2 | 87, 90
| L1335P | 3920 | G>C | Exon 28 | NBF 2 | This study
| R1314W | 3940 | C>T | Exon 28 | NBF 2 | 90
| L1335P | 3941 | G>A | Exon 28 | NBF 2 | 90
| G1321S | 3961 | G>A | Exon 28 | NBF 2 | 78, 86
| Q1347H | 4004 | C>T | Exon 28 | NBF 2 | 90
| L1335P | 4015 | G>C | Exon 28 | NBF 2 | 18, 63, 90
| Q1347H | 4036 | G>C | Exon 28 | NBF 2 | 63
| Q1347H | 4041 | C>T | Exon 28 | NBF 2 | 90
| G1354R | 4060 | C>T | Exon 29 | NBF 2 | 78, 86
| D1361N | 4081 | G>A | Exon 29 | NBF 2 | 90
| K1394N | 4182 | C>T | Exon 29 | NBF 2 | 78, 86
| G1399X | 4198 | C>T | Exon 29 | NBF 2 | 63, 88
| I1424T | 4271 | G>A | Exon 30 | NBF 2 | 90
| R1459C | 4377 | C>T | Exon 30 | NBF 2 | 87

### Nonsense

| rsID | Mutation | Exon | Population
|------|----------|------|--------------|
| 595 | C>T | G199X | Exon 5 | 89
| 681 | C>G | Y227X | Exon 7 | 84
| 1132 | G>C | R518X | Exon 9 | 63, 78, 83
| 1552 | T>C | Q378X | Exon 12 | 63, 84, 88
| 2245 | C>T | R518X | Exon 12 | 87
| 2304 | A>C | Q749X | Exon 17 | 90
| 3088 | C>T | R1030X | Exon 23 | 63, 90
| 3421 | C>T | R1141X | Exon 24 | 15, 17, 18, 63, 78, 85, 87, 88, 90, 84, 85, 88
| 3490 | C>T | R1164X | Exon 24 | 88
| 3668 | G>A | W1223X | Exon 26 | 90
| 3709 | C>T | Q1237X | Exon 26 | 63
| 3823 | C>T | R1275X | Exon 27 | 90
| 4192 | C>T | R1398X | Exon 29 | 87

### Splicing Alteration

| rsID | Mutation | Exon | Intron | Population
|------|----------|------|--------|--------------|
| IVS8+2delTG | | | 8 | This study
| IVS13-29 T>A | | | 13 | This study
| IVS14-5 T>G | | | 14 | This study
| IVS17-12delTT | | | 17 | 87
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<td>63</td>
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<tr>
<td>IVS21+1 G&gt;T</td>
<td>Intron 21</td>
<td></td>
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<td>IVS25-3 C&gt;A</td>
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<td>IVS26-1 G&gt;A</td>
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</table>

**Insertion**

<table>
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<td>3544dupC</td>
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<tr>
<td>4220insAGAA</td>
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**Small Deletion**

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<tr>
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<td>179_195del</td>
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<tr>
<td>220_222del</td>
<td>V74del</td>
<td>Exon 3</td>
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<td>960delC</td>
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<tr>
<td>1088_1120del</td>
<td>Q363_R373del</td>
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<td>Exon 16</td>
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<td>3343_3345del</td>
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**Large Deletion**

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<td>Exon 15</td>
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<td>Exons 23_29</td>
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**ABCC6**

CL X: cytoplasmic loop number X, ECL X: extracellular loop number X, TS X: transmembrane segment number X, COOH: C terminal portion, NBF X: Nucleotide Binding Fold number X

Nucleotide numbers are derived from cDNA ABCC6 sequences (GenBank accession no. NM_00171.2).

Although most of the 90 pathogenic mutations have been identified in one or a limited number of families, two variants (Ex23_29del and R1141X) are recurrent mutations. Frequency of these two recurrent mutations differ according to the population studied: mutation Ex23_29del represents ~28 % of the detected mutations in the US population and ~4 % in the European population, whereas mutation R1141X represents ~4 % of the detected mutations in the US population and ~28 % in the European population. Furthermore, frequency of the R1141X differs between European countries (e.g. 30 % in Dutch population, 91, 92 26 % in Italian patients, 63 and 13 % in French population 88). A common founder effect was identified for mutation R1141X in French and Italian populations. 63, 88 We found that arginine codon 518 was a recurrently mutated amino acid in a cohort of 19 French...
families with PXE (11.5% of the detected mutations for each variant R518Q and R518X). These two mutations represent 19% of the mutations detected in the Italian population. In Japanese patients, neither R1141X nor Ex23_29del mutations were identified, whereas mutations 2542delG and Q378X account for 53% and 25%, respectively. In South African families of Afrikaners, mutation R1339C represents more than half of the mutations detected, with a common haplotype indicating a founder effect. These mutations are rarely identified in US or European populations. Detection rate in wide studies varies from 0.55 to 0.83. Lack of mutation detection in some patients could be due to exonic deletions (e.g. deletion of exon 15), splice site mutations distant from the coding sequence, mutations in the gene regulatory sequences, or investigation of patients with acquired PXE-like syndrome not related to ABC6 mutations, such as seen in beta-thalassemia and sickling syndromes (see below). Locus heterogeneity of PXE is unlikely, but could not currently be ruled out.
CLINICAL GENETICS

Mode of inheritance
PXE was first described as a sporadic disorder, but both autosomal recessive and autosomal dominant inheritances have been reported. In fact, no molecular evidence for autosomal dominant inheritance has been established to date, and a corpus of evidence supports a common (probably exclusive) autosomal recessive inheritance of PXE. Firstly, no family with PXE transmitted through three generations has been published. Secondly, both dominant and recessive forms of PXE have been linked to the same chromosomal region 16p13.1, and ABCC6 mutations were identified in families described with autosomal recessive or dominant inheritance. Moreover, no specific dominant mutation has been described, and the same mutations have been identified in recessive and dominant families. Thirdly, identification of ABCC6 mutations established pseudo-dominant inheritance in 2 families. Finally, clinical delineation of the disease may be confusing because limited manifestations can be detected in some heterozygous carriers, and because cutaneous findings mimicking PXE, or cardiovascular manifestations, can be found in the general population, although at an older age. A recent review questioning autosomal dominant PXE concluded that this mode of inheritance might exist, but it would then be marginal.

Genotype-Phenotype Correlation
To date, no correlation has been established between the phenotype and the nature or the position of the mutations. A high degree of allelic heterogeneity makes such an approach difficult. Nevertheless, homozygosity or compound heterozygosity for mutations leading to a premature stop codon is not significantly associated with a more severe phenotype. High intrafamilial phenotypic variation is suggestive of the contribution of factors other than ABCC6 genetic background to phenotype severity, such as nutrition, hormones, life-style, environmental factors, or medical history. Implication of ABCC6-independent modifying genetic factors may also contribute to the phenotype severity.

Epidemiology and allelic frequencies
The prevalence of PXE in the general population is still uncertain in 2004, but its estimation has dramatically increased over the last decades. A few years ago an estimated prevalence of 1 in 10^5 births or less could be found in textbooks. This was undoubtedly underestimated, probably reflecting in part a lack of knowledge of PXE by physicians. In 2000, the American patients support group PXE International proposed a prevalence of PXE ~1/25 000 in New England. According to this prevalence, the calculated frequency of heterozygous using the hardy Weinberg law is 1.25 % (1/80).* In 2002, Trip et al. reported that the prevalence of the sole R1141X mutation was significantly increased in young individuals with coronary artery disease (3.2 %), but was also frequent in the general Dutch population. They identified 8 heterozygous carriers for mutation R1141X among 1057 controls (0.76 %). This mutation represents about one quarter of the mutant alleles in this population. Thus, the prevalence of heterozygous carriers in this population could be estimated to be approximately 3 % (0.76 % x 4). This high rate of

* Hardy Weinberg law: p^2 + 2pq + q^2 = 1, with p and q being the frequency of the wild type and the mutated allele respectively, p^2 the frequency of homozygous for the wild type allele, 2pq the frequency of heterozygotes and q^2 the frequency of the disease.
Here, q^2 = 1/25 000, q = √(1/25 000) = 0.0063; p = 1 - q = 1 - 0.0063 = 0.9937
Therefore, the frequency of heterozygous carriers (2pq) can be calculated as 2 x 0.9937 x 0.0063 ~ 0.0125 (1.25 %)
heterozygous carriers was unexpected. This result should be confirmed by other studies in other countries (even if PXE is an ubiquitous condition), but it may permit the evaluation of the prevalence of PXE individuals (temporarily defined as individuals carrying 2 ABCC6 mutations) to be 1/4 450, assuming this population is in Hardy Weinberg equilibrium.

One critical issue is to know if all individuals carrying 2 ABCC6 mutations display a PXE phenotype. In France (750 000 lifeborn / year), considering a disease prevalence of 1 in 4 450, 169 individuals carrying 2 ABCC6 mutations should be born each year (30 considering a prevalence of one in 25 000). However, far less than 1000 patients are recognized as having PXE in France. Do the remaining individuals have a slightly symptomatic form of PXE that does not prompt them to seek medical advice? Do they have a total absence of symptoms? The consequence of this observation is that the classical PXE phenotype could only represent a small part of the ABCC6 mutation carriers.

**Heterozygous carriers**

Sherer *et al.* have reported on mild ophthalmological and/or cutaneous involvement in heterozygous carriers, but they did not indicate the frequency of that phenomenon in their cohort of patients. A few papers have emphasized the carriage of a sole ABCC6 mutation as a cardio-vascular risk factor. In the study by Trip *et al.*, mutation R1141X in ABCC6 appeared to be an independent risk factor for coronary heart disease in young people. This observation, if confirmed in other similar cohorts, could be of course of considerable concern for public health.

Because some PXE patients sometimes have only moderate symptomates, it can be very difficult to delineate patients with mild PXE from heterozygous carriers with mild expression. The course and prognosis are probably not the same for these two categories of individuals, and this should prompt clinicians to consider molecular diagnosis in members of pedigrees with a patient suffering from PXE.

**Genetic counselling**

PXE mode of inheritance is probably exclusively autosomal recessive. Risk for parents of an affected child is therefore 1/4 for another pregnancy, without possibility to evaluate the phenotype severity. For a PXE patient the risk of having affected children varies, in the absence of consanguinity, with the rate of heterozygous carriers in their population. Given the recent data, that risk could be somewhere between 1/66 (for a rate of 1/33 (3 %) heterozygous carriers) to 1/160 (for a rate of 1/80 heterozygous carriers). This risk is significant, and could explain pseudo-dominant inheritance in some families.

** Here, the frequency of heterozygous carriers (2pq) = 0.03 (3 %).

Therefore, considering p ~ 1, q = 0.03 / 2 = 0.015, and the frequency of the disease (q^2) = 0.015^2 ~ 2.25 10^{-4} ~ 1/4 450
NOVEL ISSUES IN PATHOPHYSIOLOGY AND PERSPECTIVES

Pathophysiology
Both elastin synthesis and degradation are accelerated in the dermis of PXE patients compared with controls, and this seems to correlate with age and extent of the disease. The time-dependent mineralization of elastic fibers has been recurrently studied by several groups from the past 20 years. A critical step in the pathogenesis of PXE was achieved when Baccarani-Contri et al. demonstrated the mechanisms by which PXE elastic fibers become calcified and secondarily fragmented. Using immuno-electron microscopy these authors showed that elastic fibers displayed an enhanced expression of normal constitutive proteins (e.g. vitronectin), but also accumulated aberrant matrix proteins known for their high affinity for calcium and normally involved in mineralization processes (e.g. alkaline phosphatase, bone sialoprotein, osteonectin). The authors therefore stated that PXE was primarily a disorder of the fibroblast, in accordance with their previous results showing arguments for an aberrant fibroblast behavior. The Quaglino et al. study demonstrated that PXE skin fibroblasts had altered cell-cell and cell-matrix interactions and enhanced proliferation, with synthesis capabilities in vitro. Other biochemical reports are also in favor of a role of the fibroblast in PXE since other matrix structural alterations have been demonstrated in addition to elastorrhexia. Aggregates of thread material containing glycosaminoglycans, as well as structural collagen fibrils alterations, are seen in the vicinity of elastic fibers. Abnormalities in the metabolism of glycosaminoglycans have been evidenced.

Most of these observations were made before the identification of ABCC6 as the PXE gene, and the question that remains today is to find the link between the absence or the functional insufficiency of a membrane transporter in liver and kidney, and the mineralization of elastic fibers in distant organs. The hypothesis of PXE being a metabolic condition with impaired and circulating factor(s) responsible for elastic tissue changes has been raised. The metabolic hypothesis does not exclude the possibility of local changes in clinically involved tissues and cells.

Beta globins diseases: another pathway to the phenotype?
One of the most puzzling findings in the field of clinical and basic PXE research is that patients with inherited haemoglobinopathies, most often beta thalassemia, but also sickling syndromes, may have elastic tissue changes closely resembling PXE. Indeed, the so-called “PXE-like” clinical syndrome consists in skin, eye, and cardio-vascular symptoms indistinguishable from those of “classic PXE”. Notably, these manifestations occur later in life than in patients with PXE, and their prevalence increases with advancing age. Their frequency is significant in patients with major or intermediate beta-thalassemia: in a cohort of 100 consecutive patients, 16 had skin changes, 20 had angioid streaks, and 26 at least one of both symptoms. The causative defect is believed to be acquired and related to the primary haemoglobinopathy. Baccarani-Contri et al. showed however that structural elastic changes were strictly identical to those they had described in “classic” PXE. Hence, the “PXE-like” phenotype identified in a number of thalassemia is indistinguishable from “classic PXE”, but with no defect in the ABCC6 gene. This raises the possibility that an additional pathway exists, independent of ABCC6 sequence changes, leading to the PXE phenotype.
ACKNOWLEDGMENTS

We thank our colleagues Drs. Bonicel P, Maître F, Arbeille B for ocular, optic and electronic microscopy pictures respectively and Dr O. Le Saux for critical reading of the manuscript.

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Legends of the figures

Fig 1
PXE primary skin involvement: axillary yellowish papules gradually coalescing to form plaques with cobblestone appearance

Fig 2
Redundant axillary skin folds demonstrating loss of elasticity

Fig 3
Oblique mental creases: a valuable clinical sign for the diagnosis of PXE in young individuals

Fig 4
Mucosal PXE lesions on the inner aspect of the lower lip

Fig 5
Optic microscopy (orcein, x40) of skin biopsy. Clumped and fragmented elastic fibers in the mid dermis (arrowed). By comparison, a single spared fiber is indicated by a star in the middle of the figure.

Fig 6
Electronic Microscopy (x2500) of skin biopsy. Electron-dense bodies (calcifications) in the core of the elastic fibers are indicated by an arrow. Some calcifications result in “fractures” of the fibers (indicated by a star).

Fig 7
Angioid streaks featuring radiate cracks in the Bruch’s membrane (Fluorescein angiogram)

Fig 8
Proliferation of aberrant choroidal neo-vessels into the subretinal space shading the macula and resulting in central blindness (Fluorescein angiogram)

Fig 9
Position of the missense mutations through the ABCC6 protein

Fig 10
Position of the mutations in the ABCC6 gene
REFERENCES

12. Carlborg U. Study of circulatory disturbances, pulse wave velocity, and pressure pulses in larger arteries in cases of pseudoxanthoma elasticum and angioid streaks: a contribution to the knowledge of the function of the elastic tissue and the smooth muscles in larger arteries. Acta Med Scand 1944(151 (suppl)):209.


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Fig. 9
Pseudoxanthoma elasticum: a clinical, pathophysiological and genetic update including 11 novel ABCC6 mutations
Nicolas Chassaing, Ludovic Martin, Patrick Calvas, Marc Le Bert and Alain Hovnanian

J Med Genet published online May 13, 2005

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