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Acquired Pseudoxanthoma Elasticum Presenting After Liver Transplantation

Lionel Bercovitch, MD^{1,7}, Ludovic Martin, MD, PhD², Nicolas Chassaing, MD, PhD³, Timothy W. Hefferon, PhD⁴, Didier Bessis, MD⁵, Olivier Vanakker, MD, PhD⁶, and Sharon F Terry, MA⁷

¹Departments of Dermatology, Rhode Island Hospital and Warren Alpert Medical School of Brown University, Providence, RI, USA

²Department of Dermatology, Angers University Hospital and UMR CNRS 6214/INSERM 771, Integrated Neurovascular Biology, University of Angers, Angers, France

³Dept of Genetics, Toulouse University Hospital, University of Toulouse, Toulouse, France

⁴Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD, USA

⁵Department of Dermatology, Montpellier University Hospital, University of Montpellier, Montpellier, France

⁶Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, University of Ghent, Ghent, Belgium

⁷PXE International, Inc., Washington, DC, USA

Abstract

Background—Pseudoxanthoma elasticum (PXE) is thought to be a metabolic disorder resulting from mutations in the gene encoding the cellular transporter, ABCC6, which is primarily expressed in liver and kidney. We encountered three patients who developed clinical and histopathological evidence of PXE after liver transplantation, suggesting that PXE could have been acquired from the transplanted organ.

Objective—To delineate the clinical features and screen each patient and samples of donor liver for mutations in the ABCC6 gene.

Methods—Each patient underwent full clinical examination, skin biopsy, and ophthalmological examination, as well as whole genome sequencing using standard techniques. Fixed samples of donor liver tissue were available for mutation analysis in two patients and of donor kidney tissue in one.

Results—All 3 patients had unequivocal clinical and histopathologic evidence of PXE. No patient (or family member available for screening) had evidence of mutations in ABCC6. Neither liver specimen nor the single available kidney specimen showed evidence of mutations in ABCC6.

Corresponding author: Lionel Bercovitch, M.D., Department of Dermatology, Rhode Island Hospital, 593 Eddy Street, Providence, RI 02903 Phone: (401)444-7139 FAX (401)444-7105 Lionel_Bercovitch@brown.edu.

Conflict of interest statement: The authors individually have no conflicts of interest to declare. PXE International, Inc. is an assigned co-holder of the United States patent for the PXE gene, *ABCC-6*, from which it derives only a token licensing fee

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Limitations—Liver tissue was not available from one patient and DNA was of poor quality in another, resulting in limited screening. Genetic testing does not detect *ABCC6* mutations in 10% of patients with confirmed PXE.

Conclusion—Although we were unable to demonstrate *ABCC6* mutations in limited screening of fixed donor livers, the absence of any PXE mutations in the affected patients, the timing of onset of PXE, and the known acquisition of other metabolic disorders and coagulopathies from donor liver suggest that PXE was likely acquired via liver transplantation.

Pseudoxanthoma elasticum (PXE) is a genetic disorder characterized by mineralization of dystrophic elastic fibers, especially in the dermis, Bruch's membrane of the retina, and the internal elastic lamina of medium-sized arteries. These changes lead to characteristic skin lesions of the neck and flexural creases, angioid streaks and subretinal choroidal neovascularization and hemorrhage in the eyes, and premature peripheral vascular, coronary artery, and cerebrovascular occlusive disease. The phenotype and severity of PXE are highly variable and may be very mild. PXE is caused by mutations in the *ABCC6* gene, which encodes a membrane transport protein whose exact physiological function and substrate remain unknown(1). Although the phenotype is primarily expressed in the skin, eye, and arteries, the gene is predominantly expressed in liver and kidney, especially liver(2,3). We encountered three cases of PXE that occurred following deceased donor liver (and in one case, subsequent kidney) transplantation, raising the possibility that PXE could be acquired through transplantation of the liver from a donor with unrecognized PXE.

Case 1

A 27-year-old white female with congenital biliary atresia developed progressive jaundice and liver failure necessitating urgent orthotopic deceased donor liver transplantation (OLT) at age 11. Despite postoperative immunosuppression with cyclosporine and prednisone, she developed chronic rejection of the liver transplant and cyclosporine-related renal failure. At age 23, she began hemodialysis and underwent deceased donor renal transplantation one year later. She developed acute rejection of the renal transplant 7 months postoperatively despite immunosuppression with azathioprine and prednisolone. After an unsuccessful attempt to salvage the transplant with muromonab-CD3 (Orthoclone OKT3), she resumed hemodialysis 3 months later. On daily tacrolimus and low dose prednisone, her hepatic and renal function has remained stable over the last three years.

At the time of renal transplant rejection in February, 2006 the patient first noted yellowish papules on the neck (Fig. 1A). Histopathological examination of a lesional skin biopsy specimen showed characteristic elastic fiber changes featuring a considerable accumulation of clumped fibers in the mid-dermis that stained black with von Kossa stain. The epidermis, papillary dermis and subcutaneous fat were unremarkable. Funduscopic examination was completely normal at that time but she progressively developed small peripapillary angioid streaks (Fig. 2). Doppler ultrasound examination of peripheral arteries and the aorta revealed no abnormalities or evidence of calcification. Over the last two years, the patient has developed similar skin changes in the axillary, inguinal, popliteal and antecubital folds and mild laxity of most affected sites.

ABCC6 mutation analysis was performed on peripheral blood leukocytes using denaturing high-performance liquid chromatography (dHPLC). Sequencing of the entire coding region plus flanking intronic sequences revealed nine common polymorphisms but no known deleterious *ABCC6* mutations. Testing was also negative for genomic rearrangements involving *ABCC6* as determined by quantitative multiplex polymerase chain reaction (PCR) of short fluorescent fragments (QMPSF) (4). The family history was negative for PXE and no evidence of PXE was noted on physical examination of both parents and all siblings, but

no family member was screened for mutations in *ABCC6*. Her serum calcium, phosphorus, and vitamin D levels were normal. Hemoglobin electrophoresis showed no evidence of hemoglobinopathy. Prothrombin and partial thromboplastin times were normal, and no mutations were found on sequencing of gamma-glutamyl carboxylase (*GGCX*) and *VKORC1* genes.

Since PXE-like lesions arose after organ transplantation and *ABCC6* is expressed most highly in liver and kidney, we hypothesized that one of the transplanted organs might carry *ABCC6* mutations that led to clinical features of PXE in the patient. Donor liver samples had been fixed in Bouin's solution, from which it is difficult to extract high-quality DNA(5). Despite numerous extraction and amplification attempts, including whole genome amplification, we were unable to obtain quality sequence data. Using very short fragment amplification, we were able to exclude presence of the recurrent p.Arg512Gln, p.Arg512X and p.Arg1141X mutations. We successfully analyzed whole genome amplified DNA from the frozen kidney donor sample. We screened for the most common *ABCC6* mutations but found no mutations in exons 9, 12, 21, 24, or 28.

Case 2

A 29-year-old white female developed chronic autoimmune hepatitis necessitating treatment with prednisone and cyclosporine at age 12. By age 14, she had developed hepatic cirrhosis and progressive portal hypertension. At age 21 she underwent deceased donor OLT and splenectomy because of progressive liver failure and the sequelae of chronic portal hypertension. She has continued to do well 8 years postoperatively on low doses of tacrolimus and prednisone.

She underwent dermatologic examination prior to liver transplantation and no lesions were observed on the neck. A few months after her transplant, yellowish papules were noted on the lateral aspects of the neck (Fig. 1B), then in the axillary folds. Histopathological examination of lesional skin from the neck demonstrated swollen and irregularly clumped elastic fibers in the mid-dermis. Von Kossa staining revealed the presence of calcium deposits within the fibers. Funduscopy examination was normal at the time of diagnosis and remains so eight years post-transplant. Doppler ultrasound examination of peripheral arteries of the upper and lower extremities and aorta showed no abnormalities and no evidence of calcification.

ABCC6 mutation analysis by direct sequencing and QMPSF showed 13 common polymorphisms but no evidence of sequence changes associated with PXE. Family history was negative for PXE and physical examination of both parents and all siblings showed no signs of PXE. Hemoglobin electrophoresis revealed no evidence of hemoglobinopathy. Sequencing of *GGCX* and *VKORC1* genes did not reveal any mutations. No donor liver sample was available for mutation analysis.

Case 3

A 23-year-old white female developed fulminant hepatic failure of unknown etiology at age 6 and underwent unrelated deceased donor liver transplantation. The transplant liver never functioned well; at age 17, it was removed and she underwent elective liver transplantation from a living related donor (her mother). On oral tacrolimus, her transplant functioned well with only mild abnormalities of serum liver enzymes. She had episodic fever, chills, abdominal pain thought to be related to cholangitis. She had never taken penicillamine, and there was no family history of PXE (confirmed by physical examination of both parents and two female siblings). When the patient was 12 years of age, her mother first noted abnormal skin on the posterior and lateral neck. The changes apparently progressed after her second

transplant in 2004 but have been stable since 2006. Histopathological examination of a skin biopsy specimen obtained in 2006 showed characteristic dermal elastic tissue changes of PXE on Verhoeff-Von Giesen stain and von Kossa stain showed calcification of the dystrophic elastic fibers. Examination of the skin in 2009 showed no clinical signs of progression (Figure 1C) and ophthalmologic examination in January 2009 was completely normal with no evidence of peau d'orange or angioid streaks.

Mutation analysis was performed on DNA derived from peripheral blood leukocytes of the patient, both parents, and two female siblings. No mutations associated with PXE were detected in any family member. Both the patient and her mother had identical genotypes for all the known nucleotide polymorphisms in *ABCC6*.

DNA from the liver explant was screened for mutations by dHPLC and sequencing (Transgenomic Labs, Gaithersburg, MD, USA). Analysis disclosed amino acid changing variants A158V (c.437C>T) in exon 4, and HapMap polymorphism M848V (c.2542A>G) in exon 20. A158V is a pseudogene variant that may have been detected as a result of non-specific PCR product, while V848 is the vastly more common allele at this position. Thus, we were unable to identify any disease-associated variants in DNA from the donated liver.

Discussion

The liver is the site of numerous biosynthetic and metabolic processes and it is not surprising that genetic disorders resulting from mutations causing abnormal protein synthesis can be transmitted by liver transplantation, especially in comparison to other solid organ transplants. Several coagulation and metabolic disorders have been acquired following liver transplantation in which the donor liver unknowingly harbored causative mutations(6-12).

No collagen or elastic tissue disorders have been described as a direct consequence of liver transplantation or immunosuppression. A post mortem study of 25 patients who underwent allogeneic liver transplantation (13) disclosed ectopic microcalcification in 84% (8% in the skin). The mechanism was postulated to be metastatic secondary to elevation of calcium-phosphate solubility product during intraoperative calcium chloride infusion, unrelated to the mechanism for ectopic calcification postulated for PXE(14).

Acquired PXE is very uncommon. Our cases cannot be attributed to exposure to penicillamine nor do they clinically resemble acquired PXE as seen in obese multiparous females with cardiovascular risk factors. None of the patients had evidence of beta-thalassemia. Acquired PXE is not a known manifestation of chronic kidney or liver disease. Therefore, none of the known forms of acquired PXE accounts for the clinical presentation in these patients.

Each of the present cases has developed unequivocal clinical and histopathologic cutaneous features of PXE following deceased donor liver transplantation. In case 1, small peripapillary angioid streaks were noted while in the remaining cases, no funduscopy changes of PXE were present as long as 8 years post transplant. In inherited PXE, angioid streaks are rarely visible before the second decade of life although they are usually visible after the age of 20. None of the patients had cardiovascular or other systemic changes, but all three were young.

Cutaneous PXE developed within one year of liver transplantation in Case 2. This is a shorter latency than one usually sees in PXE, which almost never presents clinically before age 3 or 4. In Case 1, the skin findings were noted 13 years following liver transplantation and one year following kidney transplantation.

PXE is currently presumed to be a metabolic disorder(15). There is ample experimental evidence, particularly in the mouse model, to support the metabolic hypothesis (15,16). Parabolic pairing of wild type mice and *Abcc6*^{-/-} knockout mice halts connective tissue mineralization in the knockout animals(16), while the engraftment of wild-type hepatocytes onto *Abcc6*^{-/-} mice leads to up to 90% reduction in mineralization of muzzle skin in comparison with untreated controls(17). If the first transplanted liver in case 3 was responsible for development of acquired PXE, it is tempting to speculate that receipt of the second liver from her normal mother might have stopped the progression of her disease.

Two *ABCC6* mutations are found in only about 70% of patients with PXE, while in less than 10% of PXE patients, no mutation can be found using direct sequencing and screening for exonic rearrangements (18,19). The exact reasons for this are unknown. Thus, the absence of PXE mutations in the peripheral blood leukocytes of the three affected individuals, the complete absence of PXE in any family members, and the family genotype data in Case 3 makes it less likely that they had genetic PXE. Similarly, the absence of known disease-causing mutations in the donor liver in Case 3 does not exclude the possibility that the disease was acquired as a result of the liver transplant. Since we were unable to obtain donor liver for DNA analysis in Cases 1 and 2, no conclusion can be drawn regarding whether PXE was acquired as a result of transplantation of a liver containing PXE mutations.

Due to a combination of lack of availability of donor tissue and degradation of DNA from available samples, a comprehensive search for mutations was impossible. Thus we were unable to identify *ABCC6* mutations in DNA from transplanted organs. In Case 1, screening of mutation hot spots was completed on the kidney; however, the DNA was quite degraded, and comprehensive attempts to sequence the whole gene would require more than 40 overlapping PCR products, with a low likelihood of amplification and sequencing success. Since no properly fixed liver tissue was available for sequencing, neither organ can be excluded as the cause of PXE in the patient; however the absence of any common mutations in the kidney points to the liver as the likely culprit.

In Case 2, no liver sample was available and the donor was deceased. In Case 3, we were able to screen mutation hotspots in the first liver explant, but degradation of the DNA again made comprehensive *ABCC6* sequencing prohibitive, if not impossible. Furthermore, in these authors' experience, the dHPLC method, by which the DNA was analyzed, may miss up to 35% of mutations that would be detected by sequencing. The second liver sample was from the patient's mother, and thus was effectively screened by proxy when her peripheral blood was analyzed. If and when additional cases of acquired PXE after liver and/or kidney transplantation are identified, care will hopefully have been taken to adequately preserve tissue samples for subsequent DNA analysis.

In summary, PXE developed in three young female patients following deceased donor liver transplantation. None of the patients had known *ABCC6* mutations in peripheral blood leukocytes, and in the only case in which donor liver tissue was of sufficient quality to perform mutation analysis, no PXE associated mutations were found. The immunosuppression regimens were different in each patient, excluding a specific immune suppressant as a cause. One could assume that PXE acquired post-liver transplant in a recipient known to harbor no deleterious *ABCC6* mutations was likely acquired through transplantation of donor liver containing such mutations. The fact that this rare disorder has not been reported previously following liver transplantation despite the high number of these procedures performed would argue for a genetic mechanism. Whether or not transplantation of liver or kidney from an individual with PXE can lead to acquired PXE in a recipient who harbors no PXE mutations is open to speculation, but the availability of a knockout mouse model for PXE should eventually allow testing of this hypothesis *in vivo*.

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Abbreviations and Acronyms

PXE	Pseudoxanthoma elasticum
OLT	Orthotopic liver transplantation
dHPLC	Denaturing high-performance liquid chromatography
QMPSF	Quantitative multiplex polymerase chain reaction of short fluorescent fragments

Figure 1.
Yellowish papules observed on the lateral neck of cases 1(A), 2 (B), and 3 (C) (Montage courtesy of Dr. Antonio P. Cruz).

Figure 2.
Fundus photographs of case 1, showing subtle peripapillary angiod streaks (arrowheads).
(Courtesy of Dr Frédéric Sonnenmoser, Montpellier, France)