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Mutation Analysis (*ABCC6*) in a Family with Pseudoxanthoma Elasticum - Presymptomatic Testing with Prognostic Implications

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Sir, Pseudoxanthoma elasticum (PXE) is a multi-system disorder characterized by ectopic mineralization of extracellular matrix of connective tissues (For review, see ref 1). The disease primarily affects the skin, the eyes, and the cardiovascular system, with considerable mortality and occasional morbidity. The characteristic skin manifestations, *i.e.*, yellowish papules, which tend to coalesce into larger inelastic plaques on the predilection sites, are primarily of cosmetic concern, but they imply the risk for development of serious ocular and vascular complications.

PXE is caused by mutations in the *ABCC6* gene which encodes transmembrane transporter expressed primarily in the liver and kidneys². The precise function of this transporter under physiologic conditions and its ligand molecules *in vivo* are currently unknown. It has been suggested, however, largely based on animal studies utilizing an *Abcc6* “knock-out” mouse as a model system, that PXE is a metabolic disorder which in the absence of functional *ABCC6* transporter activity results in deficiency of anti-mineralization factors in the circulation³. This situation then allows progressive mineralization of the peripheral connective tissues to ensue.

Well over 300 mutant *ABCC6* alleles have been identified in families with PXE, and mutation analysis has established that PXE is an autosomal recessive disorder^{4,5}. The utility of mutation detection in PXE is emphasized by the fact that the onset of the disease is delayed, and definitive diagnosis in patients with PXE is often not established until late

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teens or twenties, or even later in life. In families with history of an affected individual with known *ABCC6* mutations, genotyping can be used to determine if other members of the family are at risk for developing PXE by presymptomatic DNA testing. In this brief report, we illustrate this utility of mutation analysis in a family with history of PXE.

The proband, a 19-year old female, had been diagnosed for PXE on the basis of characteristic cutaneous finding and the presence of angioid streaks at the age of 14 years (Fig. 1A, B). Skin biopsy revealed accumulation of mineralized elastotic material in the mid-dermis by histopathologic examination using Hematoxylin & Eosin and von Kossa stains (Fig. 1C, D). The proband's parents did not have any evidence of PXE, consistent with autosomal recessive inheritance of this disease (Fig. 2A). The proband had an older sister who refused to participate in this study, and a younger, clinically unaffected brother of 13 years of age, who was concerned of the potential of developing PXE later in his life.

After appropriate counseling and having obtained an informed consent, DNA was isolated from peripheral blood of the proband, her parents, and the younger brother. The analysis of the *ABCC6* gene was performed by PCR amplification of all exons and flanking intronic sequences, followed by automated nucleotide sequencing, as described previously². Two homozygous sequence variants were identified in the *ABCC6* gene of the proband: p.R1221H and p.R1268Q. Both parents were heterozygous carriers of these mutations in their DNA (Fig. 2B). Previously, p.R1221H has been identified as pathogenic mutation in a patient with PXE⁶. Furthermore, the arginine in the position 1221 is evolutionarily highly conserved (Fig. 2C). In contrast, the p.R1268Q sequence variant has been suggested to be a relatively common polymorphism with an allelic frequency of ~30% in healthy Caucasian and Japanese populations^{7,8}. Collectively, it appears that p.R1221H is the pathogenic mutation resulting in PXE, when present in both alleles, as in the case of the proband of this study.

Analysis of the *ABCC6* gene in the proband's younger brother revealed that he was also heterozygous carrier of both sequence variants, analogous to the clinically unaffected parents (Fig. 2A). On the basis of these analyses, we concluded, therefore, that the younger brother was not at risk for developing PXE later in his life.

In summary, in the pedigree examined in this study, the proband had unequivocal diagnosis of PXE on the basis of clinical findings and histopathology, and this was confirmed by demonstration of homozygous mutations in the *ABCC6* gene. The proband's younger brother, 13-years of age, was found to be heterozygous carrier of the same mutation in one allele only, similar to the unaffected parents. These findings suggest that the younger brother is not at risk for development of PXE, thus alleviating concerns of his future health status with respect to PXE. In this context it should be noted that heterozygous carriers of *ABCC6* mutations have been suggested to be at an increased risk of premature coronary artery disease^{9,10}. Thus, the younger brother may benefit from careful monitoring of the cardiovascular disease early on and initiation of prevention therapy as necessary. The latter considerations regarding the risks of a heterozygous individual, such as the proband's younger brother and the parents, should be included in the pretest counseling process.

What's already known about this topic?

- Mutations in the *ABCC6* gene have been identified in patients with PXE, an autosomal recessive disorder
- The onset of PXE is late, often during the 2nd or 3rd decade of life

What does this study add?

- The family illustrates the potential of presymptomatic diagnosis for this, currently intractable disorder
- In the present family, heterozygous carrier status suggests that a family member of the proband is not at risk for PXE

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Figure 1. Diagnosis of PXE in the proband of the family. Note the characteristic cutaneous lesions on the side of the neck (A) and angioid streaks in the eye (B) (arrow). Histopathology reveals accumulation of mineralized elastotic material in mid-dermis when examined by Hematoxylin and Eosin (C) or von Kossa (D) stains (arrows).

Figure 2.

Mutation analysis of the *ABCC6* gene in the family. (A) The proband (arrow; II-2) has a clinically unaffected older sister and a younger brother, and the parents (I-1, I-2) show no clinical signs of PXE. (B) Sequencing of the proband's *ABCC6* gene (II-2) revealed that the arginine in position 1221 in control DNA is replaced by homozygous substitution by histidine (p.R1221H) reflecting G-to-A nucleotide substitution (arrow) and confirming the diagnosis of PXE. The 13-year old younger brother (II-3) is a heterozygous carrier of the p.R1221H substitution and is consequently predicted not to develop PXE, similar to his parents. The mutation p.R1221H co-segregates in the family with an allelic polymorphism, p.R1268Q (A). (C) Note the evolutionary conservation of arginine 1221 in the *ABCC6* gene in different species. The alignment was performed with ClustalW2 Program (www.ebi.ac.uk/Tools/clustalW2).