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# A single nucleotide polymorphism in the *Abcc6* gene associates with connective tissue mineralization in mice similar to targeted models for pseudoxanthoma elasticum

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## TO THE EDITOR

Pseudoxanthoma elasticum (PXE; OMIM#264800) is characterized by progressive, late-onset, ectopic mineralization of elastic fibers, clinically affecting skin, retina, and the cardiovascular system with considerable morbidity and occasional mortality (Neldner, 1988). It is an autosomal recessive disorder with a slight female preponderance and an estimated prevalence of ~1 in 50,000-70,000. The clinical diagnosis is usually made through recognition of characteristic skin lesions, *i.e.*, small, yellow papules on flexural areas progressively coalescing into plaques of inelastic, leathery skin. The cutaneous findings are associated with angioid streaks in the retina and mineralization of arterial blood vessels. Adding to the diagnostic difficulty is the considerable phenotypic heterogeneity in age of onset and the extent and severity of organ system involvement. Since identification of mutations in the ATP binding cassette, subfamily C, member 6 gene (*ABCC6*) as the genetic basis in the overwhelming majority of families with PXE, tremendous progress has been made in understanding the molecular genetics, clinical phenotypes, and pathogenesis of this disease (Uitto *et al.*, 2010).

Mutations in the *ABCC6* gene underlie the classic form of PXE and over 300 distinct mutations representing over 1000 mutant alleles have been reported (Uitto *et al.*, 2010). However, no apparent correlation has been established between PXE phenotypes and the



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nature or the position of the mutations in *ABCC6* (Pfendner *et al.*, 2007). Genetic variations in gamma-glutamyl carboxylase (*GGCX*) (Li *et al.*, 2009; Vanakker *et al.*, 2007), secreted phosphoprotein 1 (*SPP1*) (Hendig *et al.*, 2007), and xylosyltransferase I (*XYLT*) (Schon *et al.*, 2006) genes, together with environmental risk factors such as diet (LaRusso *et al.*, 2009), appear to modify the phenotype with respect to the age of onset and the extent and severity of organ involvement in PXE.

Understanding of the mechanisms underlying PXE has been advanced by development of targeted mutant mice with genetic, histopathological, and ultrastructural features similar to those in patients with PXE (Gorgels *et al.*, 2005; Klement *et al.*, 2005). A characteristic finding in the targeted mutant mouse (B6;129S1/SvImJ-*Abcc6*$^{tm1Jfk}$) is the mineralization of the vibrissae dermal sheaths, the principal components of which were calcium and phosphate (Kavukcuoglu *et al.*, 2012). This specialized hair follicle type is not found in humans, and mineralization of the vibrissae dermal sheath is very unusual in mice. The genetic background of mice can modify phenotypes associated with a single gene mutation as is the case with this *Abcc6*$^{tm1Jfk}$ null mouse (Li and Uitto, 2010). Dystrophic cardiac calcinosis (DCC) in C3H/HeJ mice is also associated with a polymorphism in the *Abcc6* gene (Aherrahrou *et al.*, 2008) but these mice lack vibrissa mineralization (Fig. 1). These observations suggested that spontaneous mutations in *Abcc6* may exist in inbred mouse strains and such mice would then provide novel models to study detailed mechanisms of PXE, especially for defining the role of modifier genes.

In a large scale aging study of 31 inbred strains (Table S1), mineralization of vibrissae dermal sheaths was routinely diagnosed histologically in KK/H1J mice 6 months of age and older and in very old 129S1/SvImJ mice (Fig. 1, Tables 1, S1). One case out of 68 RIIIS/J mice examined had vibrissae mineralization at the age of 20 months. Mineralization of various internal organs was observed with distinct strain distributions (Table S2). Vibrissae mineralization and other PXE-like lesions (mineralization of medium-sized arteries, retina, lung, and dermis) were particularly severe in the KK/H1J strain. KK/H1J mice also had a number of other pathological findings, including severe fibro-osseous lesions of bones (Fig. 1) as well as epicardial mineralization and fibrosis (i.e., dystrophic cardiac calcification (DCC)) (Table 1)). The fibroosseous bone lesion was found in a number of strains without PXE-like mineralization and was not associated with mineralization of elastic fibers, indicating that it most likely represents an unrelated disease or that *Abcc6* was a strong modifier gene in KK/HIJ mice when mutated (Berndt and Sundberg manuscripts in preparation).

Haplotype analysis of *Abcc6* revealed that one non-synonymous single nucleotide polymorphism (SNP) was associated with tissue mineralization. Specifically, the SNP in exon 14 (rs32756904) showed an A allele at base pair position 53,257,951 on chromosome 7 in KK/HlJ and 129S1/SvImJ — strains with vibrissae mineralization — but a G allele in strains without those lesions (Table 1). The phenotypic differences between the strain groups with the various alleles were significant (Table S2). The only exception was one out of 68 RIIIS/J mice, which had vibrissae mineralization. RIIIS/J mice have a G allele at rs32756904 in exon 14. The SNP was confirmed by sequencing in all strains shown in Table 1. This SNP was recently shown to create a novel splice donor site, which results in deletion

of five nucleotides at the end of exon 14, causing a frame shift of the reading frame and a premature termination codon of translation (Li et al., unpublished), similar to previous findings in C3H/HeJ mice (Aherrahron *et al.*, 2008).

Examination of modifier genes for human PXE, including *GGCX* (Vanakker *et al.*, 2007) (i.e., *Ggcx*), *SSP1* (i.e., *Senp6*), and *XYLT* (i.e., *Xylt1* and *Xylt2*), as well as the calcium-sensing receptor (*Casr*) from mouse studies (Hough *et al.*, 2004), did not reveal differential SNP patterns between affected and unaffected strains. DCC, characterized by mineralization and fibrosis of the heart, has been shown to be associated with four quantitative trait loci (*Dyscalc1-4*) from a cross between C3H/HeJ and C57BL/6J mice (Meng *et al.*, 2007). *Dyscalc1* is now listed as *Abcc6* (http://www.informatics.jax.org). Combined with observations that similar lesions occur in strains without the vibrissae mineralization (Tables 1, S2) and that relatively few heart lesions occur in the $Abcc6^{tm1Jfk}$ null mice (Klement *et al.*, 2005), it is likely that the cardiac lesions may not be primarily associated with *Abcc6* mutations but that *Abcc6* may rather be a major modifier of DCC phenotypes.

KK/H1J, and to a lesser extent 129S1/SvImJ mice, provide potential new spontaneous models that may be useful for studying PXE-like phenotypes. Other lesions in these mice with various degrees of mineralization may be part of the overall pathologic process: they may reflect expression of the influence of strain specific modifier genes, or may represent new clinical variations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **PXE** | Pseudoxanthoma elasticum |
| **SNP** | single nucleotide polymorphism |
| **ATP binding cassette** | subfamily C, member 6 (gene symbol: *Abcc6*, mice; *ABCC6*, human; protein symbol for both: ABCC6); secreted phosphoprotein 1 (gene symbol: *SPP1*, human); SUMO/sentrin specific peptidase 6 (gene symbol: *SENP6*, human; *Senp6*, mouse); xylosyltransferase I (gene symbol: *XYLT*, human; *Xylt1* and *2*, mouse) |
| **DCC** | dystrophic cardiac calcinosis |

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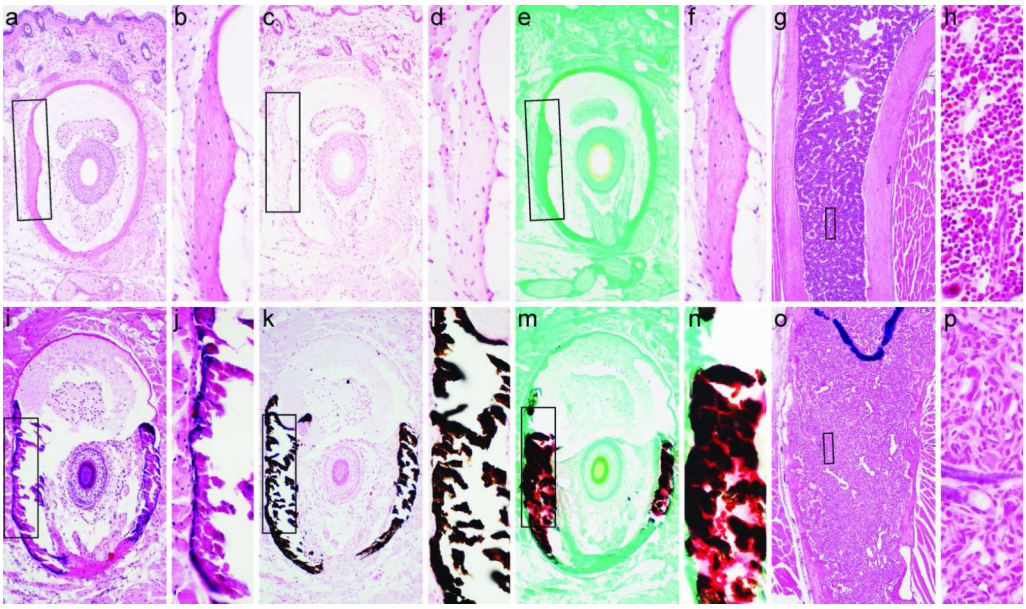


**Figure 1. Vibrissae mineralization and fibro-osseous bone lesions**

Sections of vibrissae from a 375 day old female C3H/HeJ mouse have no mineralization of the connective tissue wall (a-f) while those from a KK/HlJ mouse are mineralized (i-n). This was confirmed by von Kossa (c, d, k, l) and alizarin red (e, f, m, n) stains. Normal cortical bone and bone marrow from a female C3H/HeJ mouse (g, h) is very different from that of a 555 day old female KK/HlJ mouse (o, p) with fibro-osseous lesions. Note the thin cortex and effacement of the bone marrow by fibrovascular stroma in the KK/HlJ mouse (aldehyd fuschin stain).

**Table 1**

*Abcc6* genotype and phenotype differences between strains.

| ***Abcc6* genotype** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | Exon | rs number | Chr | Position (Mb) | Alleles (“-”strand) | 129S1/SvImJ | C3H/HeJ | DBA/2J | KK/HlJ | A | BALB/cByJ | FVB/NJ | PWD/PhJ | C57BL/10J |
| *Abcc6* | 14 | rs32756904 | 7 | 53,257,951 | G/A | A | A | A | A | G | G | G | G | G |
| Lesions | | | | | | | | | | | | | | |
| Diagnosis | | | | | | Severity of lesions | | | | | | | | |
| Epicardial Fibrosis and Mineralization | | | | | | | + | ++++ | +++ | +++ | +++ | | | +++ |
| Fibro-osseous Bone Lesions | | | | | | ++++ | + | | ++++ | | + | ++ | | ++ |
| Vibrissae Mineralization | | | | | | + | | | ++++ | | | | | |
| Systemic Mineralization (lung, retina) | | | | | | | | | ++ | | | | | |
| Arterial Mineralization | | | | | | | | + | ++++ | | | | | |

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